Small molecules as inhibitors of streptococcal hyaluronidase: a computer-assisted and multicomponent synthesis approach

Dissertation

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„Das schönste Glück des denkenden Menschen ist: das Erforschliche erforscht zu haben und das Unerforschliche ruhig zu verehren.“

Johann Wolfgang von Goethe

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Publications and Poster presentations

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Poster presentations:

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Contents

1 Introduction

1.1 Hyaluronic acid

1.1.1 Structure and physicochemical properties

In 1934, John Palmer and Karl Meyer were the first to isolate a novel polysaccharide from the vitreous humor of bovine eyes. They named this biopolymer hyaluronic acid, from hyaloid (vitreous) and the constituent uronic acid.[1] However, the chemical structure of hyaluronic acid was determined by Meyer and coworkers not before the 1950s.[2] Today, hyaluronic acid (HA) is classified as a non-sulfated member of the glycosaminoglycan family. Hyaluronic acid is a high molecular weight linear polysaccharide containing alternating D-glucuronic acid (GlcUA) and *N*-acetyl-D-glucosamine (GlcNAc) residues linked by β-1,4 and β-1,3 glycosidic bonds (Figure 1.1).[3]

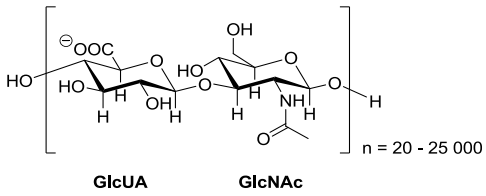

Figure 1.1 Chemical structure of hyaluronic acid.

The polysaccharide consists of 2000 – 25000 disaccharide units, corresponding to a contour length of 2 – 25 µm.[4] Its molecular mass in human normal synovial fluid has been estimated to be 6 – 7 x 10^6 and in rheumatoid fluid 3 – 5 x 10^6 Dalton.[5, 6] HA is able to incorporate a large volume of water into its solvent domain, which is more than 1000 times greater than the volume of the original material.[7] At physiological pH, the carboxylic groups of the glucuronic acid residues (pK_a 3-4) are dissociated.[8] To maintain charge neutrality, exchangeable cation counterions associate to the polyanion. In biochemical literature, the term hyaluronate was commonly used to designate the polyanion character. Nowadays, in order to emphasize its polysaccharide nature and in agreement with the recommendations on polysaccharide nomenclature, the macromolecule is most frequently referred to as hyaluronan, regardless of the degree of dissociation.[9]

The three-dimensional organization of hyaluronan is a matter of continuous investigations. An extended 2-fold helical structure of hyaluronan in solution has been proposed on the basis of NMR measurements in dimethylsulfoxide and X-ray fiber diffraction at low pH.[10-13] Newer techniques, using molecular dynamics simulations and high-field NMR experiments with ^{15}N-enriched oligosaccharides, propose a fairly more complex secondary structure including a 4-fold helix model of hyaluronan.[14] In general, the backbone of a hyaluronan molecule is stiffened by a combination of the chemical structure of the disaccharide,

internal hydrogen bonds, and interactions with the solvent.[15] Consequently, in solutions a hyaluronan molecule forms a voluminous expanded random coil structure, but this structure reflects a very rapid inter-converting set of conformational states.[8, 16]

Hyaluronan is essentially present in all tissues and body fluids of vertebrates as well as in some bacteria.[17] It is a major constituent of the extracellular matrix in most tissues. The concentration of hyaluronan is particularly high in rooster comb, in the synovial fluid, in the umbilical cord, in the vitreous humor of the eye and in the skin and other connective tissues. The lowest concentration is found in blood serum.[17, 18] Intracellular locations of hyaluronan have also been documented in the cytoplasm, the nucleus and the nucleolus, but the intracellular function of the biopolymer is not yet understood.[19, 20]

1.1.2 Hyaluronan metabolism and physiological functions

Hyaluronan is synthesized at the inner surface of the plasma membrane as an unbound linear polymer, which is different from other glycosaminoglycans (GAGs) that are synthesized by resident Golgi enzymes and covalently attached to core proteins.[3, 4, 21] The biosynthesis of HA is regulated by hyaluronan synthases HAS1, HAS2 and HAS3 isoenzymes.[22, 23] During synthesis, HA is extruded through the plasma membrane into the pericellular space.[23] Postsynthetic modification, e.g. sulfatation or epimerization, do not occur. In humans, HA metabolism is a very fast process compared to the turnover of other polysaccharides.[17] One third of the HA in a human body is converted every day. [24]

The relatively simple chemical composition of hyaluronan lacks the structural complexity of the other GAGs. However, HA is involved in complex processes including cell migration, differentiation or wound healing. HA fragments of different size interact with cell surface receptors and binding proteins such as CD44 and RHAMM, which are involved in signal transduction processes.[25-27] Abnormalities in hyaluronan metabolism have been implicated in many diseases, such as inflammation, cardiovascular diseases and growth and metastasis of tumor cells.[28]

1.2 Hyaluronidases

1.2.1 Prevalence and classification of hyaluronidases

Hyaluronidases represent a class of enzymes that predominantly degrade hyaluronan. The corresponding enzymes are widely distributed in nature, being found in mammals,

invertebrate animals (crustaceans, leeches, and insects), pathogenic fungi (*Candida*), bacteria e.g. *Streptomyces* and bacteriophages.[29] Today, the term “hyaluronidase”, which was coined by Karl Meyer in 1940, might be regarded as imprecise, as these enzymes also degrade chondroitin and chondroitin sulfates, albeit at a slower rate. Hence, absolute specificity of bacterial and mammalian hyaluronidases for hyaluronan is not given.[30] According to their catalytic mechanism, hyaluronidases are classified into three distinct families.[31, 32]

The first group of hyaluronidases includes endo-β-*N*-acetyl-D-hexosaminidases (EC 3.2.1.35, type I hyaluronidases). Type I hyaluronidases are enzymes that cleave the β-1,4 glycosidic bond of the high-molecular weight substrate with tetrasaccharides as main products. Such enzymes are present in mammalian spermatozoa, lysosomes, venoms of hymenoptera and snakes. For the prototypic enzyme of this group, the bovine testicular enzyme (BTH), it has been shown that it also catalyzes transglycosylation reactions, so that di-, hexa-, and octasaccharides are also formed during hydrolysis of hyaluronan.[29, 33, 34] The second group includes endo-β-glucuronidases (EC 3.2.1.36, type II hyaluronidases). Type II hyaluronidases degrade the β-1,3 glycosidic bond of hyaluronan. Since these enzymes utilize a hydrolysis mechanism, their mode of action resembles that of eukaryotic or vertebrate enzymes (type I) more closely than that of the bacterial enzymes (type III). Such enzymes have been isolated from leech and may also be present in hookworms.[35] The third group includes bacterial hyaluronidases (EC 4.2.2.1, type III hyaluronidases). Different from the two other groups of hyaluronidases, type III hyaluronidases do not simply catalyze hydrolysis. They degrade hyaluronan via a *β*-elimination process to yield 4,5-unsaturated oligosaccharides of various lengths, often as small as disaccharides. With respect to the catalytic reaction, these enzymes were also named hyaluronate lyases.

1.2.2 Hyaluronidases from eukaryotes

The human genome contains six known DNA sequences encoding hyaluronidase-like proteins. They include Hyal-1, Hyal-2, Hyal-3, Hyal-4 and PH-20 (SPAM 1). A pseudogene, HYALP1, is not translated.[36] According to current research, the enzymatically active enzymes Hyal-1 and Hyal-2 are regarded as the most important hyaluronidases for the degeneration of hyaluronan in somatic tissues, where they act in a complementary manner on the degradation of HA.[37-39] The human sperm adhesion molecule 1 (SPAM 1) or PH-20 is a membrane bound testicular hyaluronidase with a key

role during the fertilization process. The enzyme is bound via a GPI-anchor on the sperm surface and is necessary to penetrate the hyaluronan-rich follicle cell layer.[40-42]

The bovine enzyme BTH, which is extracted from bull testis, has been used as a spreading factor in several medical fields for many years.[43] For example, BTH was used to study effects on coronary blood flow after myocardial infections.[44, 45]

The identity between all sequences of human hyaluronidases varies from 33.1 % (Hyal-3 and Hyal-4) to 41.2 % (Hyal-4 and PH-20).[46, 47] To date, three-dimensional information for type I and II hyaluronidases is only available from the crystal structures of the bee venom hyaluronidase, a major allergen present in bee venom (PDB-codes: 1FCQ, 1FCU, 1FCV) and the mammalian Hyal-1 enzyme (PDB-code: 2PE4). [48, 49]

1.2.3 Hyaluronidases from prokaryotes

Hyaluronidase has been found as a surface protein in a number of gram-positive and gram-negative bacteria. Human infections caused by gram-positive microorganisms are increasingly difficult to treat, predominantly due to emerging resistant strains, not only against penicillin and penicillin-like antibiotics but even against novel antibiotics such as vancomycin.[50-52] Such bacteria are involved in various diseases such as gas gangrene, meningitis, synovitis, hyperplasia, nephritis, mycoplasmosis, periodontal disease, mastritis, pneumonia, septicemia, syphilis and toxic shock syndrome.[52-57] In this context, hyaluronate lyases are suggested to play a major role in pathogenesis and disease progression of gram-positive bacteria such as *Streptococcus*, *Staphylococcus*, *Peptostreptococcus*, *Propionibacterium*, *Streptomyces* and *Clostridium* species.[58-62] The hyaluronidase protein potentially acts as a virulence factor; the depolymerization of HA *in vivo* breaks down the physiological barrier of the host connective tissue and exposes the host cells to various bacterial toxins. Therefore, the enzymatic degradation of tissue-hyaluronan would reflect an important step for bacterial invasion and spreading. However, due to the lack of experimental data, the specific role of hyaluronidase, e.g. in streptococcal pathogenesis, is controversially discussed as the degradation of HA in tissue may also be driven by nutritional purposes.[32, 54, 63-68] In contrast, hyaluronate lyases produced by gram-negative bacteria, are less likely to play a role in pathogenesis.[69-71]

In particular, hyaluronate lyases from pathogenic strains of *Streptococci*, e.g. from *S. pneumoniae* and *S. agalactiae*, reflect a versatile target for the development of novel therapies to investigate and putatively to treat bacterial disease.[72] *Streptococcus pneumoniae* is a human pathogen that colonizes the upper respiratory tract and causes

life-threatening diseases such as pneumonia, bacteremia, and meningitis throughout the world.[73-76] The currently licensed pneumococcal vaccine is only moderately effective, and it is not prescribed for children younger than 2 years due to poor antibody responses.[52] In addition, during the last decades, antimicrobial resistance has increased both in the USA[77-86] and worldwide.[87-91] Another member of the *Streptococcus* genus, *S. agalactiae*, can cause serious disease, especially in neonates.[92-94] This reinforces the need for improved vaccines and/or the development of small molecules as effective drugs to combat pneumococcal infections.

Moreover, among the bacterial hyaluronidases, the enzymes from *Streptococcus pneumoniae* (*Spn*Hyl) and *Streptococcus agalactiae* (*Sag*Hyal) are best characterized. Remarkably, these lyases were found to share substantial sequence identity with regard to the active site and thus are likely to use a similar catalytic mechanism.[95] Three-dimensional structural information (X-ray diffraction analysis) for both enzymes is available of the native enzyme and of complex structures with hyaluronan substrates (cf. Table 1.1).

Table 1.1 Crystal structures of bacterial hyaluronidases.

Bacterium	PDB code	Ligand	Comment	Resolution (Å)	Citation
S. pneumoniae	1EGU	none		1.56	ref[95]
	1LOH	HA hexasaccharide		2.00	ref[96]
	1LXK	HA tetrasaccharide		1.53	
	1C82	HA disaccharide		1.70	ref[97]
	2BRV	none	complex from 70% sat. malonate	3.30	ref[98]
	2BRW	none	complex from 30% PEGME	2.80	
	1OJM	unsulfated chondroitin disaccharide		1.78	ref[30]
	1OJN	6-sulfated chondroitin disaccharide	Y408F mutant	1.60	
	1OJO	4-sulfated chondroitin disaccharide	Y408F mutant	1.75	
	1OJP	6-sulfated chondroitin disaccharide		1.90	
	1N7N	none	W292A mutant	1.55	ref[99]
	1N7O	none	F343V mutant	1.50	
	1N7P	none	W292A/F343V double mutant	1.55	
	1N7Q	HA hexasaccharide	W291A/W292A double mutant	2.30	
	1N7R	HA hexasaccharide	W291A/W292A/F343V mutant complex	2.20	
S. agalactiae	1F1S	none		2.10	ref[100]
	1I8Q	HA hexasaccharide		2.20	
	1LXM	HA hexasaccharide		2.20	ref[101]

Bacterial hyaluronate lyases are generally composed of four distinct domains, a substrate-binding module, a spacer domain, a major catalytic domain (α-domain), followed by a C-terminal domain (β-domain) that regulates HA access to the cleft.[102] The N-terminal substrate-binding module and the C-terminal domain are connected to the rest of the protein by peptide linkers.[98] The stable and fully catalytically active enzyme structure of *S. pneumoniae* hyaluronate lyase (PDB code 1LXM) is a globular protein, which contains two distinct structural domains; the α-helical domain and the β-sheet domain on the C-terminal region. The full-length four-domain pneumococcal enzyme, recombinantly expressed in *Escherichia coli*, is unstable and undergoes degradation by a (auto)-proteolytic process.[103, 104] The hyaluronate lyases from *S. pneumoniae* and *S. agalactiae* (strains 3502, 4755) share a sequence homology of approximately 68 %. As illustrated in Figure 1.2, in contrast to the pneumococcal lyase, the catalytically active enzyme from *Sag*Hyal (PDB code 1LOH) is a protein with three domains (spacer domain, α-domain, β-domain).

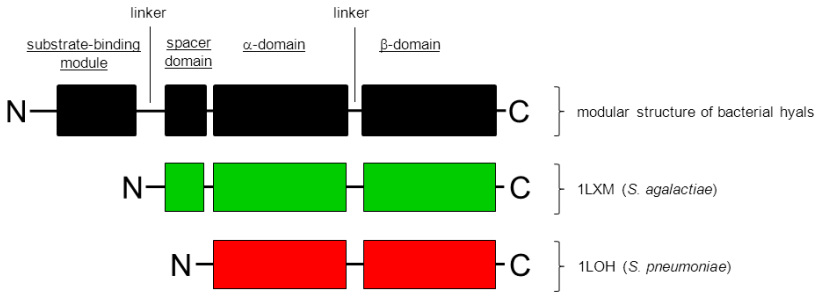

Figure 1.2 Modular structure of bacterial hyaluronidases and sequences of 1LXM (from *S. agalactiae*) and 1LOH (from *S. pneumoniae*).

Structural differences between *Spn*Hyl and *Sag*Hyl are not primarily due to low sequence homology, but rather correspond to specific domain motions, as those enzymes represent highly flexible structures.[98] As a fact, the architecture, as well as the geometry of the active site of hyaluronate lyases is highly conserved (cf. Figure 1.3).

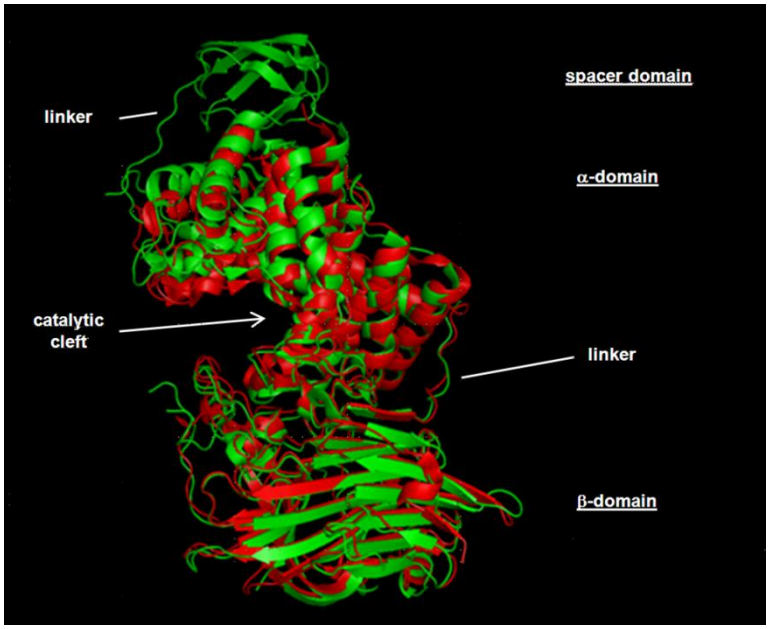

Figure 1.3 Superposition of crystal structures of *Spn*Hyl (red, PDB code 1LOH) and *Sag*Hyal (green, PDB code 1LXM).

Based on X-ray crystallography followed by structural, biochemical and molecular biology studies, a mechanism of catalysis for the bacterial enzyme and its mode of action during degradation of hyaluronan substrates has been proposed.[98] Accordingly, the helical domain contains a large, deep and elongated cleft, which is responsible for substrate binding and degradation (Figure 1.4).[52, 95, 97]

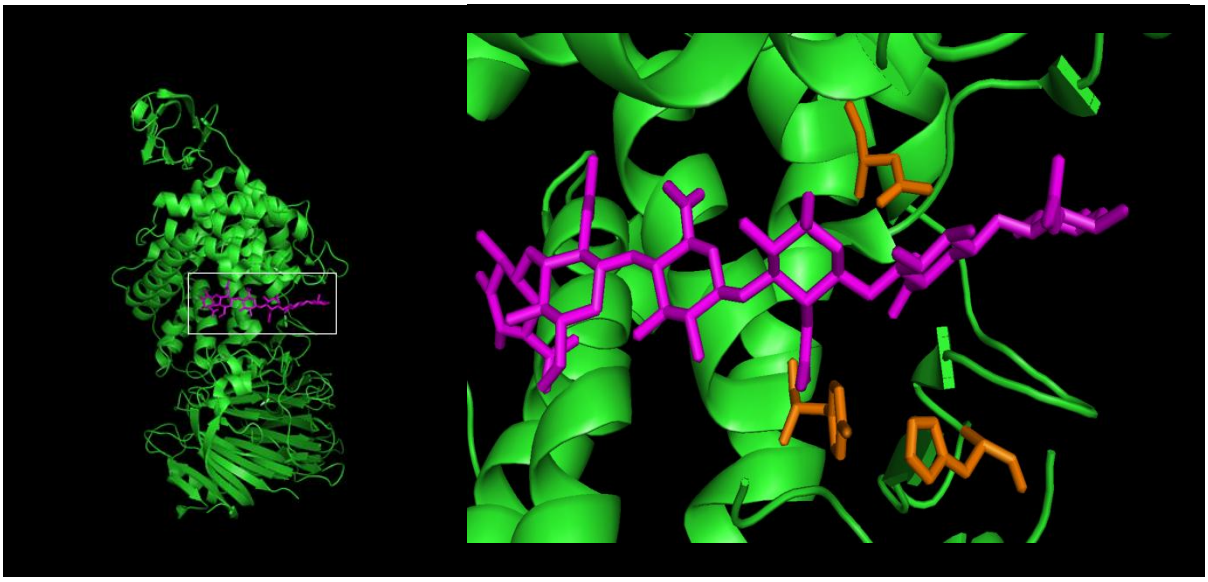

Figure 1.4 Three-dimensional view of the crystal structure of *Sag*Hyal (PDB code 1LXM) with HA hexasaccharide bound to the catalytic cleft (α-domain). A zoomed section of the substrate binding cleft is shown on the right, displaying HA on the active site.

Most of the amino acid residues along the cleft are either charged (positive patch, negative patch) or aromatic. In the narrowest part of the cleft, three catalytic residues, Asn[429], His[479], and Tyr[488] (*Sag*Hyl numbering[101]) are located, which degrade the substrate

through a proton acceptance and donation (PAD) mechanism.[95, 105] The aromatic patch, located beneath the catalytic site, is regarded to assist the cleavage of hyaluronan by a precise positioning of disaccharide units of the polymeric substrate. Following a progressive process of degradation, the enzyme moves continuously forward to the nonreducing end of the hyaluronan chain with disaccharides being the smallest degeneration product. The PAD mechanism involves five steps: (1) enzyme binding to hyaluronan; (2) catalytic cleavage of the glycosidic bond; (3) hydrogen exchange with the microenvironment; (4) dissacharide product release; (5) translocation of the substrate and the processive mode of action of the enzyme.[52, 106] The role of the β-sheet domains is probably only supportive in maintaining the structure of the catalytic cleft as well as modulating access to the cleft.[106] The catalytic mechanism of cleavage of the ß-1,4 glycosidic bond of hyaluronan is illustrated in more detail in Figure 1.5.

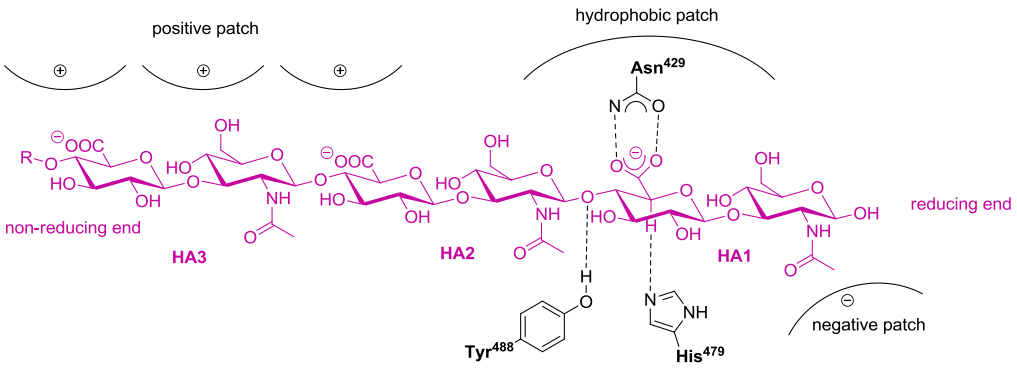

Figure 1.5 Mechanism of hyaluronan degradation by hyaluronate lyase from *S. agalactiae* (*Sag*Hyal$_{3502}$); schematic presentation of hyaluronic acid with HA1, HA2 and HA3 as disaccharide units and the position of the sidechains of Tyr^{488}, His^{479} and Asn^{429} relative to the substrate (modified from Li and Jedrzejas).[52]

1.2.4 Hyaluronidase inhibitors

In general, the role of hyaluronan and hyaluronan degrading enzymes, the hyaluronidases, is far from being understood. To overcome this misery, potent and selective inhibitors might give the ability to characterize and understand the (patho-) physiological role of hyaluronidases. Furthermore, from a therapeutic perspective, inhibitors might be useful in developing anti-bacterial[54], anti-venom/toxin[107], anti-tumor[108], antiallergic agents[109] or even as contraceptives.[110] Documented hyaluronidase inhibitors are of various chemical forms. In most cases, these inhibitors were discovered incidentally or by random screening.[62] Few inhibitors of streptococcal hyaluronidases were detected in complex with the target enzyme (c.f. Table 1.2). However, until today, only very little attention has been paid on the rational development of synthetic molecules as hyaluronidase inhibitors.

Table 1.2 Crystal structures of bacterial hyaluronidases in complex with small molecule inhibitors.

Bacterium	PDB code	Ligand	Resolution (Å)	Citation
S. pneumoniae	2BRP	Sulfamic acid 1-decyl-2-(-sulfamoyloxyphenyl)-1*H*-indole-6-yl ester	2.00	ref[111]
	1W3Y	(2*E*, 4*R*, 5*S*)-2,3,4,5-Tetrahydroxy-6-(palmitoyloxy)hex-2-enoic acid	1.65	ref[112]
	1F9G	Ascorbic acid	2.00	ref[72]

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2 Scope and objectives

Enzymes cleaving hyaluronic acid (hyaluronan), the hyaluronidases, are widespread in nature. They are not only present in the animal kingdom, but are also considered as virulence factors of microorganism, including several pathogenic strains of *Streptococci*. Weak hyaluronidase inhibitors have been identified among natural products and synthetic molecules. However, with respect to drug discovery or a rational design of inhibitors, hyaluronidases still represent a class of neglected enzymes. Inhibitors of bacterial hyaluronate lyases could serve as pharmacological tools or even could be used as new adjuvants in the chemotherapy of drug-resistant bacteria.

In previous studies, hyaluronidase inhibitors among vitamin C derivatives, indoles and benzoxazoles were identified in our workgroup. The most potent derivatives showed IC_{50} values in the lower micromolar range for bacterial hyaluronidases. Characteristics of the most active inhibitors are acidic functional groups and a high degree of lipophilicity, resulting in very high plasma protein binding. Hence, such agents represent pharmacological tools for *in vitro* studies rather than for *in vivo* investigations.

Focusing on a streptococcal hyaluronidase (*Sag*Hyal$_{4755}$), the aim of this thesis was the discovery of novel lead compounds and the elucidation of the structure-activity relationships of inhibitors of the bacterial enzyme. From the beginning, only small molecules predicted to possess drug-like properties, were accepted as target molecules. From the methodological point of view, the project comprises two different approaches:

(1) In a collaborative project (Origenis GmbH, Martinsried, Germany), a combination of innovative computer-assisted methods, of drug design and synthesis technologies should be applied to identify novel structural motifs. Initially, a compound library of inhibitors of the target enzyme had to be established for the generation of suitable templates in a ligand-based drug design approach. By implementation of the methodological repertory of the collaboration partner, the structure-activity relationships should be analyzed *in silico*. Accordingly, a selection of predicted inhibitors should be synthesized in parallel by multicomponent synthesis, analyzed and tested for biological activity in medium-throughput and verified as new inhibitors. Successive hit to lead development was intended, in order to obtain drug-like inhibitors of the target enzyme.

(2) As previously shown for *Sag*Hyal$_{4755}$, the introduction of alkyl groups in position 1 of hydroxylated 2-phenylindoles (scaffold: 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol) led to the discovery of inhibitors with activities in the lower micromolar range. To further elaborate the structure-activity relationships of 2-phenylindoles, a series of substances should be optimized in terms of inhibitory activity and selectivity for the target enzyme *Sag*Hyal$_{4755}$. In this context, less lipophilic compounds were aimed at to improve drug-like

properties. Furthermore, depending on the *N*-substituent, compounds derived from the 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol scaffold are known for antiestrogenic activity at low nanomolar concentrations. Hence, minimization of this off-target effect had to be considered in case of 2-phenylindole-type hyaluronidase inhibitors. To cope with this problem, structural modifications on the indole scaffold and bioisosteric replacements should be elaborated.

3 Methods for the determination of hyaluronidase activity

3.1 Introduction

Along with the discovery of hyaluronan and hyaluronan degrading enzymes a variety of methods have been developed to determine the enzymatic activity of hyaluronidases. An overview of the previously developed and applied test systems was given by Hynes and Ferretti in 1994.[1] Meanwhile, primarily spectrophotometric,[2-7] fluorogenic,[8, 9] enzymoimmunological,[10-12] radio-chemical[13, 14] and physicochemical[15-17] methods have been described. In addition, quantitative hyaluronidase assays are also performed by chromatography,[18-20] capillary zone electrophoresis,[21] zymography[22] and in agarose plate assays.[23] Although a lot of methods and assay formats are known today, only very few are embedded in routine analyses, e.g., for the identification of hyaluronidase inhibitors. The reasons are most commonly low sensitivity or laborious and technically complex workup procedures. The most widely used quantitative hyaluronidase assays are colorimetric such as the Morgan-Elson assay developed by Reissig et al.[2], the “stains-all” assay which was developed and investigated by Benchetrit[3] and Homer[6] or assays based on the change in viscosity[24] and turbidity.[25] In this thesis, a colorimetric and a turbidimetric assay are routinely exploited to determine the inhibitory effect of the synthesized compounds. Inhibitory activity was elucidated for hyaluronidase inhibitors on bacterial hyaluronate lyases from *Streptococcus agalactiae* strain 4755 (*Sag*Hyal$_{4755}$) and *Streptococcus pneumoniae* (*Spn*Hyl) and the bovine testicular enzyme BTH (formerly commercially available, e.g., under the trade name Neopermease®). The principles of both routinely applied methods, namely the Morgan-Elson assay and the turbidimetric assay, are briefly explained in the following sections.

3.2 Morgan-Elson assay

The Morgan-Elson assay has been classified as a chemical assay by Hynes and Ferretti.[1] In the incubation mixture enzymatic degradation of the substrate hyaluronan (HA) results in HA-fragments of different size and molecular weight. The reducing ends of hyaluronic acid fragments bear a characteristic 2-(acetylamino)-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine; GlcNAc) moiety. *N*-acetyl-D-glucosamine residues give colored products when treated with an acidic solution of 4-(dimethylamino)benzaldehyde (Ehrlich´s reagent; DMAB). Determination of hyaluronidase enzymatic activity is based on the formation of a red colored product that can be photometrical detected at a wavelength of 586 nm.

The reaction was investigated in more detail and described by Muckenschnabel et al. (Figure 3.1).[7] At 100 °C under alkaline conditions, the furanose form of *N*-acetyl-D-glucosamine residues is converted to the chromogens I (α-configuration) and II (β-configuration) after elimination of ROH.[26, 27] Subsequent treatment with a mixture of concentrated hydrochloric acid and glacial acetic acid results in elimination of water yielding chromogen III. In the final step of the Morgan-Elson reaction chromogen III reacts with 4-(dimethylamino)benzaldehyde (Ehrlich´s reagent) generating mesomeric forms of N^3-protonated 3-acetylimino-2-(4-dimethylaminophenyl)methylidene-5-(1,2-dihydroxy-ethyl)furane (red colored product).

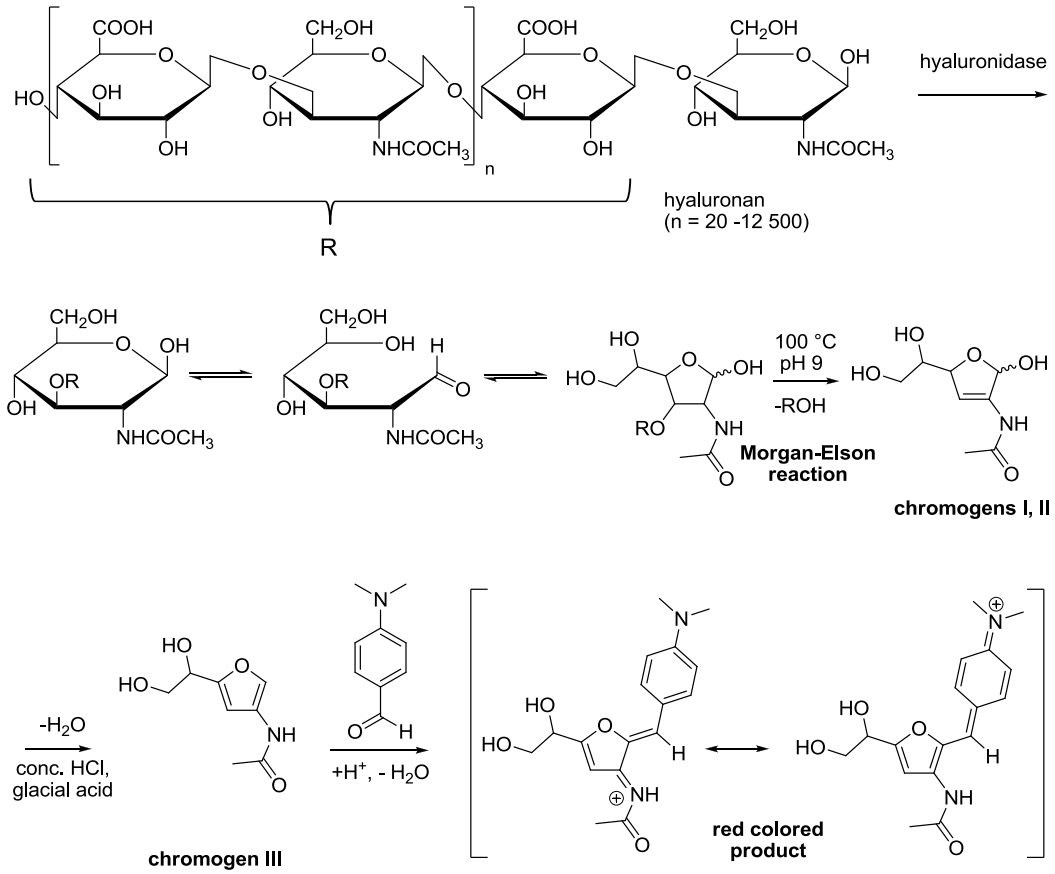


Figure 3.1 Mechanism of the Morgan-Elson reaction resulting in red colored products. Adopted from Muckenschnabel et al.[7]

The Morgan-Elson assay is a useful, broadly applicable method for the determination of hyaluronidase activity in the presence of inhibitors. However, the examination of putative inhibitors in the Morgan-Elson assay hides some chemical restrictions. Side-reactions of the acidic 4-(dimethylamino)benzaldehyde-solution with inhibitors in the incubation mixture might occur and falsify the photometric readout at λ = 586 nm. For example, we previously identified potent inhibitors of *Sag*Hyal$_{4755}$ among indoles and phenylindoles.

There has been evidence for such heterocycles to form colored products when treated with acidic 4-(dimethylamino)benzaldehyde under assay conditions.[28] Moreover, related reactions of indole derivatives with 4-(dimethylamino)benzaldehyde were reported in the literature. Pindur and coworkers studied the reaction of 3-methyl-1*H*-indole (**1**) with 4-(dimethylamino)benzaldehyde (Figure 3.2).In this case, according to the mechanism proposed by van Urk, the elimination of water under acidic conditions yields a colored product (cyanine, **2**). Subsequent oxidation leads to the formation of a cationic bisindolylphenylmethane (**3**).[29, 30]

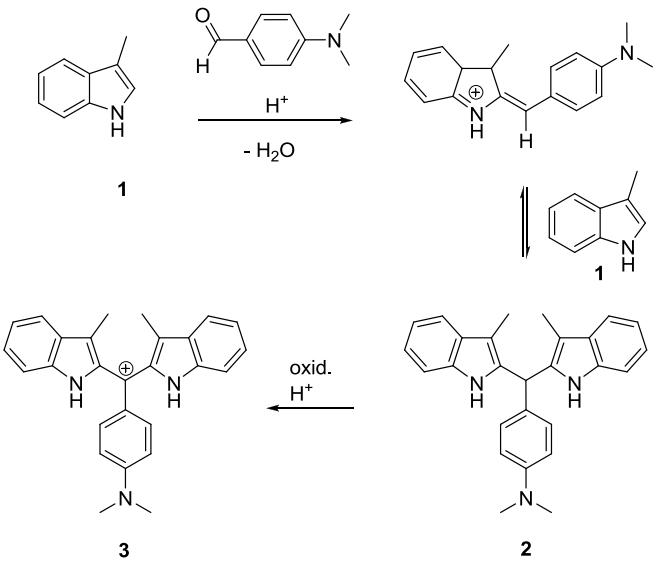


Figure 3.2 Proton catalyzed reaction of 3-methyl-1*H*-indole (**1**) with DMAB (Ehrlich's reagent) according to Pindur. A pH dependent equilibrium of bisindolylarylmethane leads to the formation of cyanine (**2**). Oxidation of the latter compound yields a trinuclear cyanine (**3**), representing the dominating coloring components.[29, 30]

Extensive investigations of 1,2,3-trimethyl-1*H*-indole (**4**) by Pindur and coworkers revealed an electrophilic aromatic substitution of 4-(dimethylamino)benzaldehyde (DMAB) in position 5 or 6 of the indole scaffold resulting in more intensly colored molecular species (cf. **5**, **6**; Figure 3.3).[31]

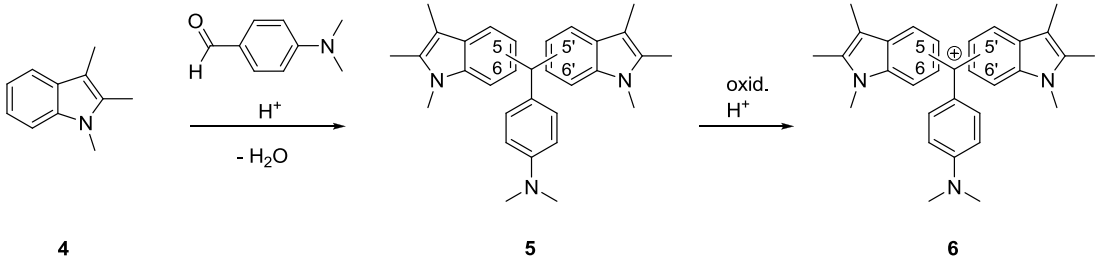


Figure 3.3 Proton catalyzed reaction of 1,2,3-trimethyl-1*H*-indole (**4**) with DMAB (Ehrlich's reagent) according to Pindur. The formation of colored bisindolylarylmethanes (5,5'- and 6,6'-regioisomeres, **5**) and subsequent oxidation (cf. **6**) was observed.[31]

Moreover, for indomethacin, a non-steroidal anti-inflammatory drug, also known as an inhibitor of *Sag*Hyal$_{4755}$, an analytical procedure is based on the van Urk-reaction and providing colored products in the same manner.[32]

Due to these considerations, the Morgan-Elson colorimetric assay was not applied as a routine assay for the determination of inhibitory activity on bacterial and mammalian hyaluronidases, since false-negative results could not be excluded for novel chemical entities.

Another severe limitation of the Morgan-Elson assay results from the rather harsh workup procedures (cf. heating to 100 °C, addition of a solution of concentated hydrochloric acid and glacial acetic acid). As we considered these conditions incompatible with an automated assay for high-throughput procedures, a transfer of this approach to 96-well or 384-well MTP assay formats was not attempted.

3.3 Turbidimetric assay

The turbidimetric assay represents a physicochemical assay according to the classification given by Hynes and Ferretti.[1] Enzymatic degradation of hyaluronan (HA) results in HA-fragments of different size and molecular weight. Non-degraded, high-molecular hyaluronan (mw > 8 kDa) form insoluble complexes when treated with a solution of alkaline cetrimonium bromide (cetyltrimethylammonium bromide, CTAB) according to the method initially described by Di Ferrante. In contrast, oligosaccharides and smaller hyaluronan fragments (mw < 6 –8 kDa) are not precipitated under these conditions. Determination of hyaluronidase activity is based on the loss of transmitted light intensity at wavelengths between 580 and 600 nm due to the scattering effect of suspended particles. Therefore, hyaluronidase activity is directly proportional to the intensity of transmitted light. This fast and highly reproducible assay can be easily performed using cuvettes and microtiter plates and represents the standard method for the determination of inhibitory activities of the synthesized compounds in this thesis.

The sensitivities of the aforementioned assays were determined by Hofinger: the limits of quantitation were 2 U/mL (Morgan-Elson assay) and 5 U/mL (turbidimetric assay), respectively.[33] In addition to the previously established 96-well microtiter plate (MTP) assay, a modified 96-well MTP assay and a novel 384-well MTP assay format were established with respect to (automated) screenings of large compound libraries of potential hyaluronidase inhibitors.

3.4 Adaptation of turbidimetric assay formats

3.4.1 General requirements

3.4.2 Selection of organic solvent

The effects of commonly used organic solvents (DMF, DMSO, EtOH, MeCN, MeOH) on the activity of BTH and *Sag*Hyal$_{4755}$ were previously investigated in our workgroup.[28, 34] DMSO up to a final assay concentration of 4 % (v/v) was identified as the most suitable solvent, with regard to negligible effects on enzymatic activity as well as sufficient solubility of inhibitors. Therefore, DMSO was used as the standard solvent for the preparation of stock solutions of (purified) inhibitors for pharmacological investigations.

3.4.3 Adjusting the pH value for the study of various hyaluronidases

Hoechstetter and Hofinger studied the pH dependent activity profiles of BTH and *Sag*Hyal$_{4755}$.[33, 35] They found highest activity for the bacterial hyaluronate lyase at pH 5.0, but it is not possible to select a pH where all investigated enzymes show sufficient high activity. A pH value of 6 was reported by Jedrzejas as the optimum pH of *Spn*Hyl.[36] To obtain comparable results concerning the IC_{50} values of the investigated inhibitors which also depend on pH, BTH and *Spn*Hyl were tested at pH 5.0 where these enzymes show considerably high activity in the turbidimetric assay. Thus, the received IC_{50} values are comparable to previously gained data from our workgroup (cf. investigations by Binder, Braun, Salmen and Spickenreither).[28, 34, 37, 38]

The turbidimetric assay was used in four different standardized formats to identify inhibitors of hyaluronidases. The measurements were performed in cuvettes, in 96-well flat-bottomed transparent microtiter plates using either manual (“manual 96-well MTP”) or automated dispensing (“auto 96-well MTP”) and in 384-well flat-bottomed transparent microtiter plates with automatical dispensing (“384-well MTP”).

Enzymatic activity of BTH, *Sag*Hyal$_{4755}$ and *Spn*Hyl was investigated in all assay formats. The Morgan-Elson assay was performed in cuvettes without exception.

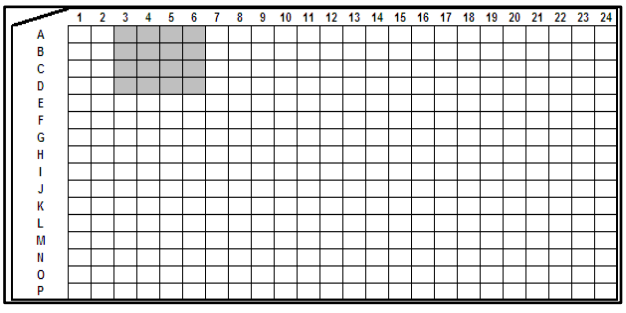
3.4.4 Plate layout

Modification of the existing 96-well assay format became necessary as the liquid handling system was restricted to provide a minimum volume of 10 µL. Hence, the composition and dilution of assay ingredients had to be modified in order to meet these requirements. The final concentrations of the assay components remained unchanged to ensure comparable results. A representative plate-layout of the auto 96-well is displayed in Figure 3.4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	H	#1	#9	#17	#25	#33	#41	#49	#57	#65	A, 1	B, 1
B	H	#2	#10	#18	#26	#34	#42	#50	#58	#66	A, 2	B, 2
C	H	#3	#11	#19	#27	#35	#43	#51	#59	#67	A, 3	B, 3
D	H	#4	#12	#20	#28	#36	#44	#52	#60	#68	A, 4	B, 4
E	L	#5	#13	#21	#29	#37	#45	#53	#61	#69	A, 5	B, 5
F	L	#6	#14	#22	#30	#38	#46	#54	#62	#70	A, 6	B, 6
G	L	#7	#15	#23	#31	#39	#47	#55	#63	#71	A, 7	B, 7
H	L	#8	#16	#24	#32	#40	#48	#56	#64	#72	A, 8	B, 8

Figure 3.4 Plate layout for automated dispensing into 96-well microtiter plate. Maximum (“H”) and minimum (“L”) samples are placed in column 1, references samples in columns 11 (“A”, concentrations 1-8) and column 12 (“B”, concentrations 1-8), respectively; 72 screening compounds (“#1”-“#72”) are placed in columns 2 to 10.

In principle, the 96-well format was designed to hold a total of 72 screening compounds at a final assay concentration of 200 µM. Each compound was added at a single concentration. Maximum and minimum signals (column 1) and reference samples (columns 11, 12) were embedded on each plate. To receive additional data for each compound from single-concentration measurements, the experiments were performed in duplicate. Therefore, a second microtiter plate was prepared in the same way and investigated under identical conditions. A representative plate-layout of the auto 384-well is displayed in Figure 3.5.



	3	4	5	6
A	#1 0.2 µM	#1 2 µM	#3 0.2 µM	#3 2 µM
B	#1 20 µM	#1 200 µM	#3 20 µM	#3 200 µM
C	#2 0.2 µM	#2 2 µM	#4 0.2 µM	#4 2 µM
D	#2 20 µM	#2 200 µM	#4 20 µM	#4 200 µM

Figure 3.5 Plate layout for 384-well microtiter plate. The MTP accommodates maximum and minimum samples in columns 1, 2 and reference compounds in columns 23, 24. 80 screening compounds are placed in 4 concentrations (z-pattern alignment) from columns 2 to 22. The segment on the right illustrates an enlargement of the 384-well MTP (rows A-D, columns 3-6) to display the dilution pattern of 4 random compounds (compounds #1 to #4).

For the automated 384-well MTP assay format, a total of 80 screening compounds were located on each plate. The layout was designed to allow dispensing of every compound in four concentrations: 0.2 µM, 2 µM, 20 µM and 200 µM (final assay concentration). Replicates were not accomplished for the 384-well assays.

3.5 Experimental procedures

3.5.1 Materials and methods

Hyaluronic acid from *Streptococcus zooepidemicus* was purchased from Aqua Biochem GmbH (Dessau, Germany). Stabilized hyaluronate lyase, i.e. 200,000 units (according to the supplier, 0.572 mg from *S. agalactiae* strain 4755 plus 2.2 mg of BSA and 37 mg of Tris-HCl per vial) of lyophilized hyaluronate lyase, was kindly provided by id-Pharma (Jena, Germany). Lyophilized hyaluronidase from bovine testis (Neopermease®) (200,000 units, according to the supplier; 4 mg plus 25 mg of gelatin per vial) was a gift from Sanabo (Vienna, Austria). BSA was purchased from Serva (Heidelberg, Germany). Ascorbic acid 6-palmitate (Vcpal), diclofenac and 4-(dimethylamino)benzaldehyde (DMAB) were purchased from Sigma-Aldrich (Munich, Germany). If not indicated otherwise all chemicals were of analytical or HPLC grade and obtained from Merck (Darmstadt, Germany). Water was purified by a Milli-Q system (Millipore, Eschborn, Germany).

3.5.2 Morgan-Elson assay

3.5.2.1 Reagents and solutions

The following reagents and solutions were prepared immediately before usage. McIlvaine's buffer[39] was prepared from solution 1 (0.2 M Na_2HPO_4, 0.1 M NaCl) and solution 2 (0.1 M citric acid, 0.1 M NaCl) by mixing appropriate portions to adjust the required pH value (pH = 5.0). The alkaline borate solution was prepared from a borate solution (solution A: 17.3 g H_3BO_4 and 7.8 g KOH in 100 mL water) and a potassium carbonate solution (solution B: 8.0 g K_2CO_3 in 10 mL water) by combining 10 volumes of solution A and 1 volume of solution B. For the solution of Ehrlich's reagent, 1 volume of concentrated solution (20.0 g 4-(dimethylamino)benzaldehyde dissolved in 25 mL of concentrated hydrochloric acid and 75 mL of glacial acetic acid; storage conditions: 4 °C,

light protection) was diluted with 4 volumes of glacial acid. Stock solutions containing 5 mg/mL hyaluronic acid in water and 0.2 mg/mL BSA in water were prepared and stored at 4 °C.

3.5.2.2 Incubation of test compounds

For the incubation mixture, test compounds dissolved in 18 µL DMSO (final DMSO concentration: 3.9 % (v/v)) were incubated at 37 °C in the assay mixture containing 200 µL buffer, 50 µL of BSA solution (0.2 mg/mL in water), 50 µL of HA solution (5 mg/mL in water), 82 µL of H_2O and 50 µL of enzyme solution. The enzymatic reaction was stopped after 60 minutes by addition of 110 µL of alkaline borate solution and subsequent heating for 4.5 minutes at 100 °C on a heating block. After cooling on ice for at least 2 minutes, 1250 µL of reagent solution (Ehrlich's reagent) was added and the mixture was incubated at 37 °C for 20 minutes. The samples were transferred into acryl cuvettes and the absorbance of the colored product was measured photometrically at a wavelength of 586 nm using a Cary 100 UV-Vis spectrometer (Varian, Darmstadt, Germany). As reference for 100 % enzymatic activity the formation of the red colored product of a sample without inhibitor (replaced by 18 µL of DMSO) was quantified. In absence of both, enzyme and inhibitor (replaced by 50 µL of BSA solution and 18 µL of DMSO, respectively), a reference for 0 % enzymatic activity was determined.

3.5.2.3 Calculation of enzyme inhibition and IC_{50} values

The highest concentration of the test compounds was adjusted to 1 mM. Regularly, a dilution series covering a concentration range from 0.5 µM to 1 mM was used for the determination of IC_{50} values. The effect of the test compounds on the enzymatic activity was calculated according to Equation 3.1:

$$A\,(\%) = \frac{\mu_{\mathrm{sample}} - \mu_{\mathrm{blank}}}{\mu_{\mathrm{max}} - \mu_{\mathrm{min}}} \cdot 100 \qquad \text{Equation 3.1}$$

A: calculated enzymatic activity in %

μ_{sample}: mean extinction of the incubation mixture containing inhibitor (containing enzyme)

μ_{blank}: mean extinction of the incubation mixture containing inhibitor (without enzyme)

μ_{max}: mean extinction of the incubation mixture without inhibitor (containing enzyme)

μ_{min}: mean extinction of the incubation mixture without inhibitor (without enzyme)

The enzymatic activities were plotted against the logarithm of the inhibitor concentration and IC_{50} ± SEM values were calculated by curve fitting of the experimental data with

Sigma Plot 11.0 (SPSS Inc., Chicago, USA) and are the means of at least two independent experiments performed in duplicate.

3.5.2.4 Determination of hyaluronidase activity

Enzymatic activity was quantified according to the definition of the International Union of Biochemistry, i.e. 1 unit (U). Hyaluronidase catalyzes the liberation of 1 mmol *N*-acetyl-D-glucosamine (GlcNAc) at the reducing ends of sugars per minute under specified conditions. For this purpose, references of known *N*-acetyl-D-glucosamine (GlcNAc) concentration were used. By plotting the absorbance resulting from the formation of the red colored product measured at λ = 586 nm against a dilution series of GlcNAc a linear calibration graph is given. The GlcNAc concentrations typically covered a range from 0.1 mM to 2 mM, incubation time was set to 30 minutes. The incubation mixture containing 200 µL buffer, 50 µL BSA solution (0.2 mg/mL in water), 50 µL of the respective GlcNAc dilution (replacing HA), 82 µL H_2O and 50 µL BSA (replacing the enzyme solution) was treated as previously described. Additionally a blank probe, with 50 µL HA solution (instead of GlcNAc) was added. For the quantification of hyaluronidase activity 50 mL of the respective enzyme solution was incubated with 50 µL HA (5 mg/mL in water). Enzymatic activity was calculated according to Equation 3.2:

$$1U = \frac{1\ \mu mol\ \mathrm{GlcNAc}}{min} \qquad \text{Equation 3.2}$$

U: enzymatic activity (U)

GlcNAc: *N*-acetyl-D-glucosamine at the reducing ends of sugars

According to this definition, a hyaluronidase activity of 0.1 mU (0.1 nmol GlcNAc/min) is equivalent to approximately 1 international unit (IU), 1 (relative) turbidity reducing unit ((r)TRU), 1 national formulatory unit (NFU), 1.5 Benger units and 3.3 viscosity units, respectively.[7]

3.5.3 Turbidimetric assay

3.5.3.1 Reagents and solutions

The following dilutions were prepared immediately before usage: McIlvaine's buffer[39] was used throughout the measurements as incubation buffer, unless otherwise indicated. For BTH, *Sag*Hyal$_{4755}$ and *Spn*Hyl a pH value of 5.0 was applied. The stop reagent was prepared from a solution of 2.5 % of cetrimonium bromide (cetyltrimethylammonium

bromide, CTAB) in 0.5 N NaOH (w/v). Stock solutions containing 2 mg/mL hyaluronan in water and 0.2 mg/mL BSA in water were prepared and stored at 4 °C. Table 3.1 summarizes the enzymatic activities, pH values and periods of incubation employed in the different assay formats.

Table 3.1 Variants of the the turbidimetric assay, depending on the format.

Assay format	Enzyme	Enzyme solution prepared (mU/mL)	Enzyme solution added to incubation mixture (total volume) (µL)	Final enzymatic activity in the incubation mixture (mU)	pH	Incubation time (min)
cuvette	BTH	9.0	30 µL (270 µL)	0.27	5.0	30
cuvette	*Sag*Hyal$_{4755}$	3.3	30 µL (270 µL)	0.1	5.0	30
96-well[a]	BTH	27.0	10 µL (73 µL)	0.27	5.0	30
96-well[a]	*Sag*Hyal$_{4755}$	10.0	10 µL (73 µL)	0.1	5.0	30
96-well[b]	*Sag*Hyal$_{4755}$	10.0	10 µL (75 µL)	0.1	5.0	30
384-well[c]	*Sag*Hyal$_{4755}$	10.0	10 µL (40 µL)	0.1	5.0	30
96-well[a]	*Spn*Hyl	10.0	10 µL (75 µL)	0.1	5.0	30

[a] turbidimetric assay performed in manually handled 96-well MTP format; [b] turbidimetric assay performed in 96-well MTP format with automated dispension; [c] automated dispension.

3.5.3.2 Incubation of hyaluronidase inhibitors

The measurement of enzymatic activity in the presence of inhibitors was performed in cuvettes, in 96-well flat-transparent microtiter plates with manual (“manual 96-well MTP”), or automated dispensing (“auto 96-well MTP”), and in 384-well flat transparent microtiter plates (“384-well MTP”) using an automated dispensing system. The composition of the assay ingredients in the incubation mixture of the differing assay formats are shown in Table 3.2.

Table 3.2 Composition of incubation mixtures for cuvette, manual 96-well, auto 96-well and 384-well format employed in the turbidimetric assay.

Assay format	Buffer (pH = 5.0)	H_2O	BSA	HA	Inhibitor solution	Enzyme solution	Incubation volume
cuvette	120 µL	50 µL	30 µL	30 µL	10 µL[c]	30 µL	270 µL
96-wella	31 µL	12.8 µL	8.1 µL	8.1 µL	3 µL[c]	10 µL	73 µL
96-wellb	31 µL	7.8 µL	8.1 µL	8.1 µL	10 µL[d]	10 µL	75 µL
384-well	16 µL	---	---	4 µL	10 µL[e]	10 µL	40 µL

[a] manually handeled assay format; [b] automated dispensing assay format; [c] inhibitor dissolved in 100 % DMSO; [d] inhibitor dissolved in a solution of 30 % DMSO in H_2O (v/v); [e] inhibitor dissolved in a solution of 16 % DMSO in H_2O (v/v).

3.5.3.2.1 Cuvette assay

The indicated amounts of buffer, H_2O, BSA solution and HA solution were provided to Eppendorf reaction vessels (cf. Table 3.2). Subsequently, 10 µL of inhibitor solution (inhibitor dissolved in 100 % DMSO) was added. After addition of the enzyme solution the mixture was immediately incubated at 37 °C for 30 minutes (cf. Table 3.1). The enzymatic reaction was stopped by addition of 700 µL alkaline CTAB solution (2.5 % CTAB in 0.5 N NaOH (w/v)). Then, the mixture was incubated at room temperature for additional 20 minutes to allow sufficient development of turbidity. Subsequently, the incubation mixture was transferred to cuvettes. The turbidity, quantified as optical density (OD), was measured at a wavelength of 580 nm using a Cary 100 UV-Vis spectrometer (Varian, Darmstadt, Germany) or at a wavelength of 578 nm using a Dr. Lange LP700 spectrophotometer (Dr. Bruno Lange GmbH, Berlin, Germany). For the investigation of potential inhibitors, samples without inhibitor (minimal signal response) and without both inhibitor and enzyme (maximal signal response) were measured as references under assay conditions.

3.5.3.2.2 Manually handled 96-well microtiter plate assay

The indicated amounts of buffer, H_2O, BSA solution and HA solution were provided to a 96-well microtiter plate (cf. Table 3.2). Afterwards, 3 µL of inhibitor solution (inhibitor dissolved in 100 % DMSO) were added. Enzyme solution was added and the assay was immediately incubated at 37 °C for 30 minutes (cf. Table 3.1). The enzymatic reaction was stopped by addition of 200 µL alkaline CTAB solution (2.5 % CTAB in 0.5 N NaOH (w/v)). Subsequently, the plates were incubated for 20 minutes at room temperature to allow sufficient development of turbidity. The turbidity, quantified as optical density (OD), was measured at λ =580 nm (absorbance mode) using a Tecan Genios Pro microtiter plate reader (Tecan Deutschland GmbH, Crailsheim, Germany) with XFluor Genios Pro software (version 4.55). The plates were shaken in the reader for 10 seconds; the OD was measured after 2 seconds of settling time by 10 flashes at the center of each well. For the investigation of potential inhibitors, samples without inhibitor (minimal signal response) and without both inhibitor and enzyme (maximal signal response) were measured as references under assay conditions.

3.5.3.2.3 96-well Microtiter plate format using an automated dispensing system

The indicated amounts of buffer, H_2O, BSA solution and HA solution were provided with a liquid handling system (Tecan TeMo automated liquid handling system, Tecan Deutschland GmbH, Crailsheim, Germany; Multidrop liquid dispenser, Mtx Lab systems Inc., USA) to 96-well microtiter plates (cf. Table 3.2). Afterwards, 10 µL of inhibitor solution (inhibitor dissolved in 30 % DMSO in H_2O (v/v)) were added. The enzyme solution was dispensed and the assay was immediately incubated at 37 °C for 30 minutes (cf. Table 3.1). The enzymatic reaction was stopped by addition of 200 µL CTAB solution (2.5 % CTAB in 0.5 N NaOH (w/v)). Subsequently the plates were incubated for 20 minutes at room temperature. The turbidity, quantified as optical density (OD), was measured at λ = 590 nm (absorbance mode) using a Tecan Ultra microtiter plate reader (Tecan Deutschland GmbH, Crailsheim, Germany) with Magellan Data Analysis Software (version 5). The plates were shaken in the reader for 10 seconds; the OD was measured after 2 seconds of settling time by 10 flashes at the center of each well. For the investigation of potential inhibitors, samples without inhibitor (minimal signal response) and without both inhibitor and enzyme (maximal signal response) were measured as references under assay conditions.

3.5.3.2.4 384-well Microtiter plate assay using an automated dispensing system

The indicated amounts of buffer, H_2O, BSA solution and HA solution were provided with a liquid handling system (Tecan TeMo automated liquid handling system, Tecan Deutschland GmbH, Crailsheim, Germany; Multidrop liquid dispenser, Mtx Lab systems Inc., USA) to 384-well microtiter plates (Table 3.2). Accordingly, 10 µL of inhibitor solution (dissolved in 16 % DMSO in H_2O (v/v)) was dispensed. Enzyme solution was added and the assay was immediately incubated at 37 °C for 30 minutes. The enzymatic reaction was stopped by addition of 200 µL CTAB solution (2.5 % CTAB in 0.5 N NaOH (w/v)). Subsequently, the plates were incubated for 20 minutes at room temperature to allow sufficient development of turbidity. The turbidity, quantified as optical density (OD), was measured at λ = 590 nm (absorbance mode) using a Tecan Ultra microtiter plate reader (Tecan Deutschland GmbH, Crailsheim, Germany) with Magellan Data Analysis Software (version 5). The plates were shaken in the reader for 10 seconds; the OD was measured after 2 seconds of settling time by 3 flashes at the center of each well. For the investigation of potential inhibitors, samples without inhibitor (minimal signal response) and without both inhibitor and enzyme (maximal signal response) were measured as references under assay conditions.

3.5.3.3 Calculation of enzyme inhibition and IC_{50} values

The effect of test compounds on the enzymatic activity was calculated via Equation 3.3:

$$A\ (\%) = \frac{\mu_{max} - \mu_{sample}}{\mu_{max} - \mu_{min}} \cdot 100 \qquad \text{Equation 3.3}$$

A: calculated enzymatic activity in %

μ_{max}: mean extinction of the incubation mixture without inhibitor (without enzyme)

μ_{sample}: mean extinction of the incubation mixture containing inhibitor (containing enzyme)

μ_{min}: mean extinction of the incubation mixture without inhibitor (containing enzyme)

The highest final concentration of the test compounds in the particular assay format was adjusted to 1 mM. A dilution series covering a concentration range from 0.5 µM to 1 mM was used regularly for the determination of IC_{50} values using cuvettes and manual 96-well assay format. The activities were plotted against the logarithm of the inhibitor concentration and IC_{50} ± SEM values were calculated by curve fitting of the experimental data with Sigma Plot 11.0 (SPSS Inc., Chicago, USA) and are the means of at least two independent experiments performed in duplicate.

For measurements performed in the “auto 96-well assay” single concentrations (e.g. 200 µM) of the test compounds were incubated. The compounds were measured as replicates located on two identical 96-well plates. For the auto 384-well assay a dilution series of 4 concentrations, covering 0.2 µM, 2.0 µM, 20 µM and 200 µM, was commonly used. In both cases blank measurements (without enzyme and HA) were performed per MTP. From data obtained from measurements at single concentrations the enzyme inhibition was calculated according to Equation 3.4.

$$B\ (\%) = 100 - \left\{\frac{\mu^*_{max} - \mu^*_{sample}}{\mu^*_{max} - \mu^*_{min}} \cdot 100\right\} \qquad \text{Equation 3.4}$$

B: calculated enzyme inhibition in %

μ^*_{max}: corrected mean extinction of the incubation mixture without inhibitor (without enzyme)

μ^*_{sample}: corrected mean extinction of the incubation mixture containing inhibitor (containing enzyme)

μ^*_{min}: corrected mean extinction of the incubation mixture without inhibitor (containing enzyme)

note: µ* values were achieved via subtraction of appropriate blank measurements

If possible, the IC_{50} values were determined by a 4-parameter logistic fit of percentage activity versus log concentration in the 384-well format.

3.5.3.4 Checking of compound solubility

Since poor solubility of the tested compounds could lead to false positive results in the turbidimetric assay, prior to the investigation of hyaluronidase inhibition the solubility was determined, taking the concentration range from 0.5 µM to 1 mM into consideration. To verify the solubility of the test compounds, a sample containing 920 µL citrate phosphate buffer (pH = 5.0), 120 µL BSA solution (0.2 mg/mL) and 40 µL of a solution of the respective test compound (compound dissolved in 100 % DMSO) was measured at a wavelength of 580 nm using a Cary 100 UV-Vis spectrometer (Varian, Darmstadt, Germany). A cuvette filled with 920 µL of buffer, 120 µL of BSA solution and 40 µL DMSO served as reference. Precipitation of the compounds at the investigated concentrations (≤ 1 mM) with alkaline CTAB solution (2.5 % CTAB in 0.5 N NaOH (w/v)) was not observed and can be excluded.

3.5.3.5 Blank measurements

Turbidity of screening compounds was quantified at a wavelength of 590 nm using a Tecan microplate reader. However, substances obtained from multicomponent synthesis frequently appear as colored products (cf. Figure 3.6). Those compounds were also able to absorb light at a wavelength of λ = 590 nm in the screening for hyaluronidase inhibition.

Figure 3.6 96-well deep well plate containing molecules synthesized via one-pot multicomponent synthesis.

Examples of colored compounds in 96-well and a 384-well microtiter plates are given in Figure 3.6. Here, the sample compounds were already diluted to the final assay concentration.

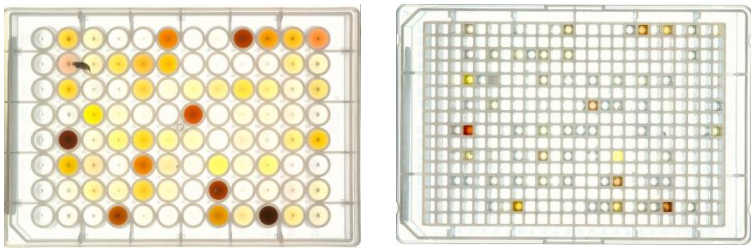


Figure 3.7 Screening plates containing colored compounds.

The investigation at a wavelength of 590 nm in the absence of further assay ingredients revealed strong extinction for individual colored substances. Hence, such substances were able to pretend turbidity which could be interpreted as enzyme inhibition. To avoid false-positive results, blank measurements (“blank plates”), i.e. incubation mixtures devoid of hyaluronan and enzyme solution (replaced by H_2O and BSA solution, respectively), became obligatory for routine operations.

3.6 References

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4 Assessment and validation of screening hits using a medium throughput assay format with automated dispensing

4.1 Introduction

The ability to identify true active compounds depends on the high quality of assays and proper analysis of data. The Z' factor, presented by Zhang et al. in 1999, provides an easy and useful summary of assay quality and has been a widely accepted standard.[1]

In this thesis, for the first time, it became possible to test several hundred compounds in parallel and to characterize them with regard to inhibition of *Sag*Hyal$_{4755}$. These primary screens are less elaborated than conventional assays.[2-5] Compounds are only tested in duplicate used at a single concentration (200 µM). When a positive result or "hit" is discovered in this primary screen, a more accurate and precise secondary screening should be performed to allow quantification and the calculation of IC_{50} values.

Aiming at a fail-safe discovery of hit compounds, a reliable discrimination between molecules with significant biological effects and inactive compounds among a library of substances became necessary. As outlined in Table 4.1, special attention was paid to key parameters for the validation of assay performance and sensitivity in an automated assay format.

Table 4.1 Criteria to determine assay performance or sensitivity.[6]

Parameter	Equation	Comment
Coefficient of variation	$\%CV = \frac{\frac{\sigma}{\sqrt{n}}}{\mu} \cdot 100$	A measure of the precision relative to the mean value, calculated for the maximum and minimum signals. An acceptable limit is < 20 %
Signal window	$\mathrm{SW} = \frac{\mu_{\max} - \mu_{\min} - 3(\sigma_{\max} + \sigma_{\min})}{\sigma_{\max}}$	The significant signal between max and min controls, acceptable value > 2 fold
Z' factor	$\mathrm{Z}' = 1 - \frac{3(\sigma_{\max} + \sigma_{\min})}{\lvert\mu_{\max} - \mu_{\min}\rvert}$	Representation of the SW using a score where a value of > 0.5 indicates an acceptable assay. Z' is measured in the absence of library compounds

σ: standard deviation of the assay signal; n: wells per test compound; µ: mean of the assay signal; max, maximum signal; min, minimum signal.

Parameters and methods of high throughput assay validation were adopted from the "guidance for assay development and HTS manual", an open access manual which was elaborated by coworkers of the Eli Lilly company.[7] Prior to a screening campaign of compounds (pre-screening), assay validation involved two aspects. The first aspect referred to assay ingredients. Here, it is necessary to provide equal composition of the ingredients across all formats (cuvette, 96-well MTP, 384-well MTP) of the turbidimetric

assay.[7] In addition, the percentage of the components in the incubation mixture had to be kept constant. The second aspect of the pre-screening assay validation referred to the responses to biologically active compounds. In particular, the span from minimum to maximum assay response was monitored over time.[7]

4.2 Validation of the automated hyaluronidase assay

For medium-throughput screening, the automated 96-well microtiter plate assay was used. Prior to the screening its accuracy and sensitivity had to be determined. To validate the reliability of the automated assay parameters such as signal window (SW) and Z' factor, were evaluated (cf. Table 4.1).

4.2.1 Pre-screen assay validation

4.2.1.1 Assay ingredients

In view of uniformity, suppliers of chemicals and materials were not changed. Reagents were stored in aliquots suitable for consumption on a single day. Leftover reagents were not saved or used for following assays. Solutions of reference compounds were prepared and diluted shortly before use.[7] The percentual composition of the ingredients in the different formats of the assay is summarized in Table 4.2. There was only a marginal variation in the concentration of buffer components, BSA and HA. The final DMSO concentration varied from 3.7 % to 4.1 % (v/v).

Table 4.2 Percentual composition of assay ingredients in the different formats of the turbidimetric assay.

Assay format	Buffer (pH 5.0)	H_2O	BSA (0.2 mg/mL)	HA (2 mg/mL)	DMSO	Enzyme solution[a]
cuvette	44.5 %	18.5 %	11.1 %	11.1 %	3.7 %	11.1 %
manual 96-well	42.5 %	17.5 %	11.1 %	11.1 %	4.1 %	13.7 %
auto 96-well	41.4 %	19.7 %	10.8 %	10.8 %	4.0 %	13.3 %
auto 384-well	40.0 %	21.0 %	---	10.0 %	4.0 %	25.0 %

[a] enzyme solution was diluted with 0.2 mg/mL BSA.

4.2.1.2 Signal uniformity and signal separation

To investigate the variability of standard assay signals, a “plate uniformity assessment” study was run for the automated 96-well microtiter plate turbidimetric assay, i.e. the uniformity and separation of signals of reference samples was analyzed. For this purpose, three different types of signals (maximum, minimum and mid-point signals (diclofenac, Vcpal)) were recorded on three consecutive days.[7] Maximum (“max”) assay response was obtained for incubation mixtures without inhibitor and without enzyme. Minimum (“min”) assay response was recorded for incubation mixtures without inhibitor. Mid-point (“mid”) signals referred to incubation mixtures containing enzyme in combination with a standard inhibitor (Vcpal) at a final assay concentration of 20 µM. The arrangement of standards and samples is shown in Figure 4.1.

A

	1	2	3	4	5	6	7	8	9	10	11	12
A	H	M	L	H	M	L	H	M	L	H	M	L
B	H	M	L	H	M	L	H	M	L	H	M	L
C	H	M	L	H	M	L	H	M	L	H	M	L
D	H	M	L	H	M	L	H	M	L	H	M	L
E	H	M	L	H	M	L	H	M	L	H	M	L
F	H	M	L	H	M	L	H	M	L	H	M	L
G	H	M	L	H	M	L	H	M	L	H	M	L
H	H	M	L	H	M	L	H	M	L	H	M	L

B

	1	2	3	4	5	6	7	8	9	10	11	12
A	L	H	M	L	H	M	L	H	M	L	H	M
B	L	H	M	L	H	M	L	H	M	L	H	M
C	L	H	M	L	H	M	L	H	M	L	H	M
D	L	H	M	L	H	M	L	H	M	L	H	M
E	L	H	M	L	H	M	L	H	M	L	H	M
F	L	H	M	L	H	M	L	H	M	L	H	M
G	L	H	M	L	H	M	L	H	M	L	H	M
H	L	H	M	L	H	M	L	H	M	L	H	M

C

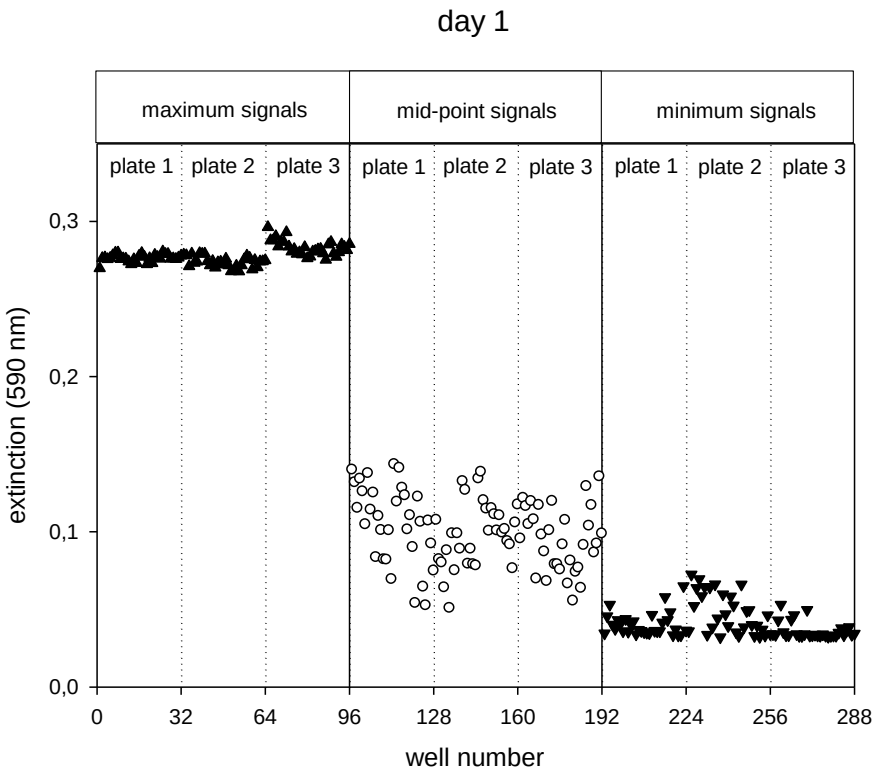
	1	2	3	4	5	6	7	8	9	10	11	12
A	M	L	H	M	L	H	M	L	H	M	L	H
B	M	L	H	M	L	H	M	L	H	M	L	H
C	M	L	H	M	L	H	M	L	H	M	L	H
D	M	L	H	M	L	H	M	L	H	M	L	H
E	M	L	H	M	L	H	M	L	H	M	L	H
F	M	L	H	M	L	H	M	L	H	M	L	H
G	M	L	H	M	L	H	M	L	H	M	L	H
H	M	L	H	M	L	H	M	L	H	M	L	H

Figure 4.1 Arrangement of standards and samples on 96-well assay plates (A, B, C); “H” = maximum, “M” = mid-point, “L” = minimum signal.

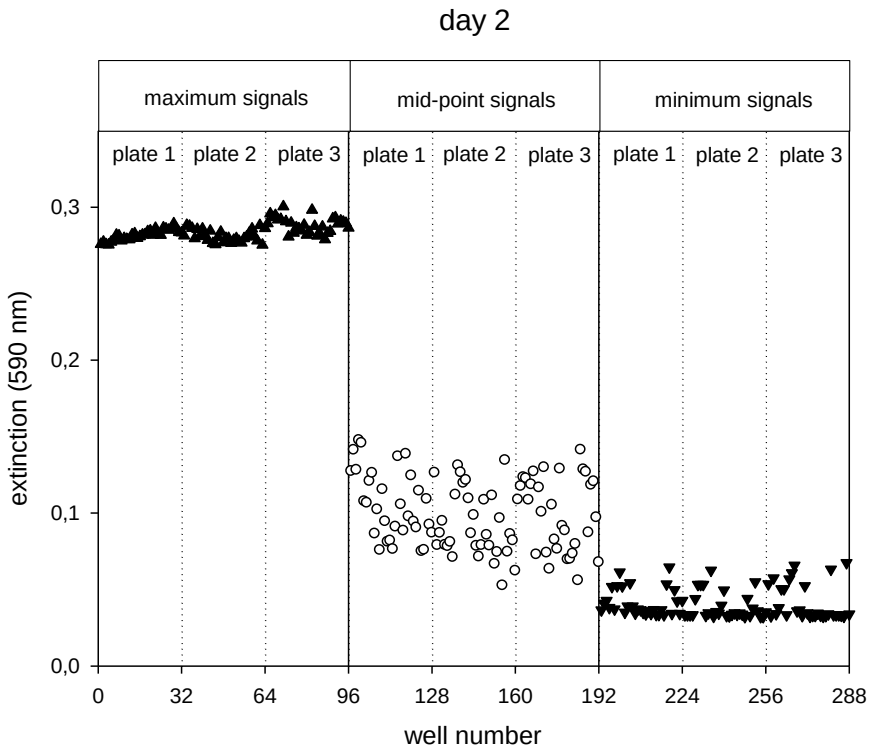
Plate uniformity, i.e. SW value, was computed from the separation of maximum, mid-point and minimum signals. Every plate contained incubation mixtures for the determination of maximum, mid-point and minimum signals. On three consecutive days, the procedure was repeated to obtain raw data for subsequent plate uniformity study.

A scatter plot was used to illustrate signal variation between maximum, mid-point and minimum signals. The inhibition of hyaluronidase was detected as extinction measured at a wavelength of 590 nm. In Figure 4.2, the corresponding signal variation plots for days 1 to 3 are illustrated.

A



B



C

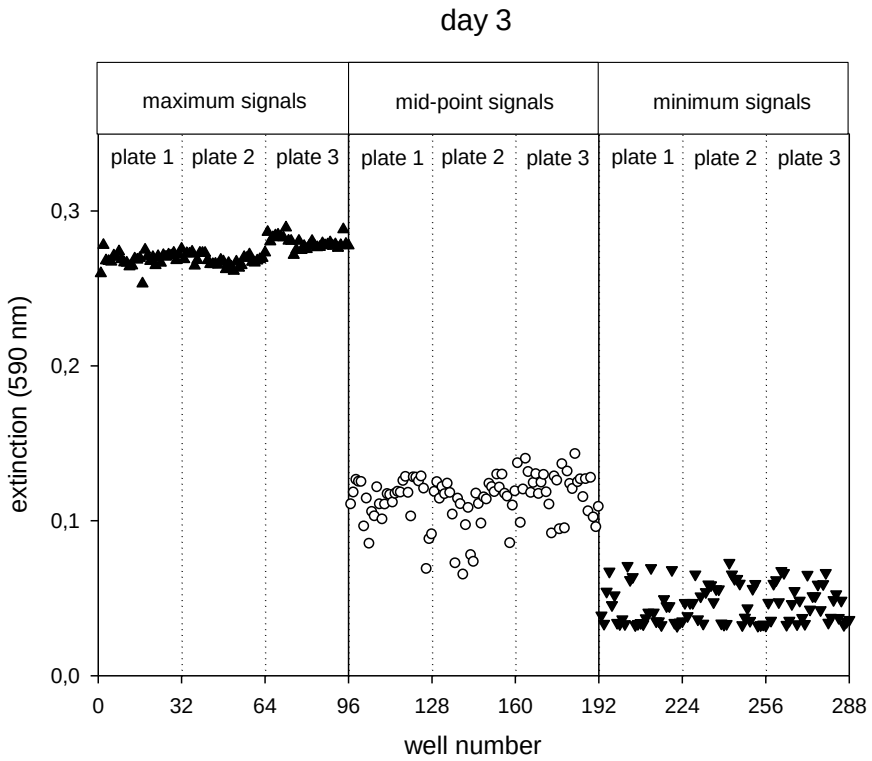


Figure 4.2 Signal variation graph for all plates (A: day 1; B: day 2 and C: day 3). Signal responses for maximum, mid-point and minimal signals were plotted versus extinction at 590 nm. Max, mid and min signals were sorted “plate wise”, e.g. max signals derived from plate 1 (wells 1-32), plate 2 (wells 33-64) and plate 3 (wells 65-96) were plotted as ensemble in graph A.

By graphical evaluation, the plots displayed an appropriate differentiation between maximum and minimum signals. Moreover, maximum signals showed a high degree of reproducibility. Despite of several outliers of the minimum signal, the baseline did not indicate drift patterns. The mid-point signals, as shown in Figure 4.2, were less uniform.

From the collected raw data, the coefficient of variation (% CV) for each type of signal (maximum, mid-point, minimum) on each plate was computed as follows (Equation 4.1).[6, 8]

$$\%CV = \frac{\frac{\sigma}{\sqrt{n}}}{\mu} \cdot 100 \qquad \text{Equation 4.1}$$

% CV: (percentual) coefficient of variation

σ: standard deviation of the assay signal (max, mid, min signal)

n: wells per sample (max, mid, min signal)

μ: mean of the assay signal (max, mid, min signal)

The results are summarized in Table 4.3.

Table 4.3 Coefficients of variation in percent (% CV).

Run	% CV_{max}	% CV_{min}	% CV_{mid}
day 1, plate 1	0.2	3.3	3.2
day 1, plate 2	0.3	4.9	3.7
day 1, plate 3	0.3	2.6	3.9
day 2, plate 1	0.2	3.7	3.5
day 2, plate 2	0.3	3.9	4.2
day 2, plate 3	0.3	5.1	4.3
day 3, plate 1	0.3	4.8	2.4
day 3, plate 2	0.2	4.7	2.8
day 3, plate 3	0.3	4.3	2.1

According to Eastwood et al. (2007), an acceptable limit for the coefficient of variation (% CV) calculated for maximum and minimum signals is less than or equal to 20 %.[7] As shown in Table 4.3, this was fulfilled for maximum (% CV_{max}), mid-point (% CV_{mid}) and minimum (% CV_{min}) values.

Signal window (SW) and Z' factor were calculated according to Equation 4.2 and Equation 4.3, respectively.[6-8]

$$\mathrm{SW} = \frac{\mu_{\max} - \mu_{\min} - 3(\sigma_{\max} + \sigma_{\min})}{\sigma_{\max}} \qquad \text{Equation 4.2}$$

$$Z' = 1 - \frac{3(\sigma_{max} + \sigma_{min})}{|\mu_{max} - \mu_{min}|}$$ Equation 4.3

SW: signal window

Z': Z' factor

μ_{max}: mean of the maximum reference signal

μ_{min}: mean of the minimum reference signal

σ_{max}: standard deviation of the maximum reference signal

σ_{min}: standard deviation of the maximum reference signal

Signal window (SW) and Z' data were computed for each microtiter plate every day. The results are displayed in Table 4.4.

Table 4.4 Signal windows (SW) and Z' factors of days 1 to 3 from plates 1 to 3.

Run	SW	Z'
day 1, plate 1	85.3	0.9
day 1, plate 2	36.5	0.8
day 1, plate 3	45.3	0.9
day 2, plate 1	56.5	0.8
day 2, plate 2	52.6	0.8
day 2, plate 3	37.4	0.8
day 3, plate 1	37.3	0.8
day 3, plate 2	50.6	0.8
day 3, plate 3	46.9	0.8

The published acceptance criteria for SW and Z' are summarized in Table 4.5.

Table 4.5 Acceptance criteria for signal window (SW) and Z' factor (Z'); adopted from Iversen et al.[8]

SW		Z'	
recommended:	SW > 2	excellent:	Z' > 0.5
acceptable:	SW > 1	do-able:	0 < Z' < 0.5
unacceptable:	SW < 1	yes/no assay:	Z' = 0
		unacceptable:	Z' < 0

The 96-well microtiter plate hyaluronidase assay with automated dispensing fulfilled all required assay performance criteria. Consequently, this assay was used for medium-throughput screening.

4.2.2 On-screen assay validation

By analogy to the pre-screen validation, sample giving maximum mid-point and minimum signals were positioned on every plate as shown in section 3.4.4. Vcpal and diclofenac served as reference compounds. Both inhibitors were measured at 8 concentrations. Vcpal, one of the most potent hyaluronidase inhibitors, served as primary control. The dilution series covered a range from to 0.8 µM to 800 µM (final concentrations). Diclofenac, as secondary control, shows over 20 fold less potent inhibition than the primary control. The dilution series covered a range from 8 µM to 800 µM (final concentrations).

To determine the robustness of each screening campaign, the IC_{50} values of Vcpal and diclofenac were analyzed for each 96-well screening plate. In addition to the primary and secondary control, the correlating Z' factor was calculated.[9] An example is shown in Figure 4.3.

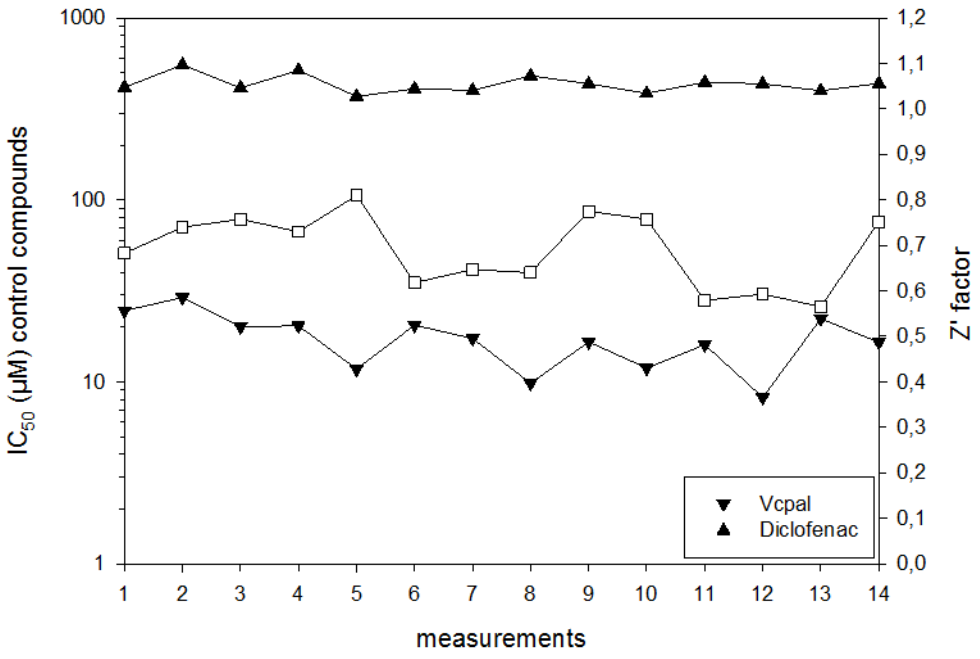


Figure 4.3 Robust screening performance evidenced by Z' factor (white squares) and stable IC_{50} values for Vcpal (▼) and Diclofenac (▲) used as primary and secondary control in each screening plate.

Here, the screening performance of screening plates **ori.hya.47**-**54** is exemplified. For each experiment on a particular plate ("measurement"), IC_{50} values of the primary reference Vcpal (▼), secondary reference Diclofenac (▲) as well as Z' factor (□) are documented. This was performed for every screening plate throughout the testing

procedures. Acceptance criteria were Z' factor > 0.5 (cf. Table 4.5) and no significant drift effect concerning IC_{50} values of the reference compounds across plates. On-screening assay validation of screening plates is documented in section B.8 (appendix II).

4.3 Hit validation

To classify positive screening results as hits, a weight function was applied.

4.3.1 Hit assessment

The standard error of the mean of an individual screening compound (SEM_i) was calculated as follows (Equation 4.4).

$$SEM_i = \frac{\sigma_i}{\sqrt{n}} \qquad \text{Equation 4.4}$$

SEM_i: standard error of the mean of the extinction measured for an individual screening compound (i)
σ_i: standard deviation of the extinction measured for an individual screening compound (i)
n: number of samples (sample size)

A weight function of an individual screening compound (w_i) was calculated from the SEM_i according to Equation 4.5. Weighted effect of an individual screening compound (w_i*) was computed according to Equation 4.6.

$$w_i = \frac{1}{(SEM_i)^2} \qquad \text{Equation 4.5}$$

$$w_i^* = \mu_{E_i} * w_i \qquad \text{Equation 4.6}$$

w_i: weight function of an individual screening compound (i)
SEM_i: standard error of the mean of an individual screening compound (i)
w_i*: weighted effect of an individual screening compound (i)
E_i: extinction in the presence of an individual screening compound (i)
μ_{Ei}: mean extinction in the presence of an individual screening compound (i)

4.3.2 Representation of screening data

To illustrate screening data, two different plots, denoted as “effect plot” and “weighted effect plot” were used. In the “effect plot”, each compound of the screening plate is plotted versus the corresponding mean percentual enzymatic activity derived from duplicate measurements. An example is given in Figure 4.4, representing screening data of plate **ori.hya.scr.3**.

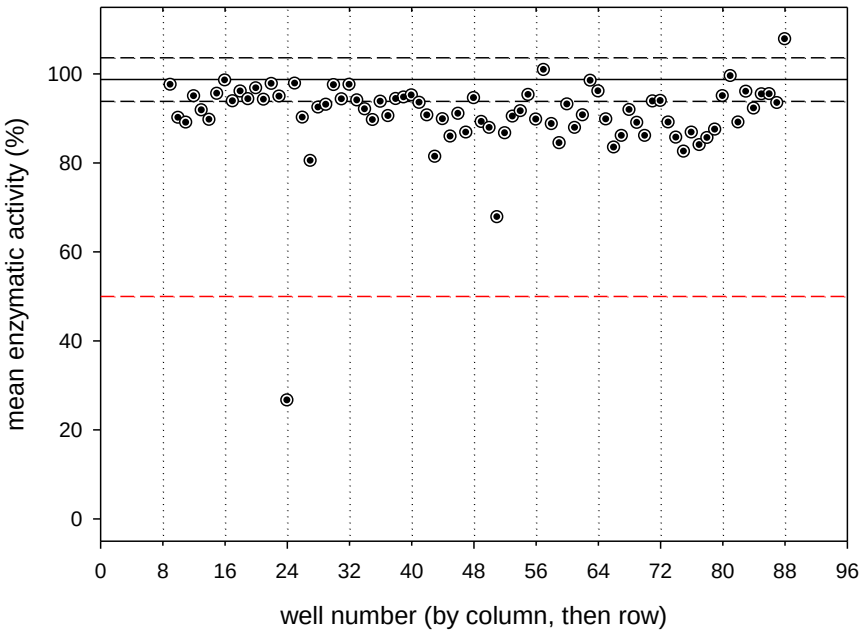


Figure 4.4 Effect plot of screening plate **ori.hya.scr.3**.

In Figure 4.4, the mean value for the references of maximal enzymatic activity is indicated as a black solid line. Upper and lower limits (threefold standard deviation of maximum reference) are indicated as dashed lines. The 50 % effect is indicated as a dashed red line. The compounds are listed according to their well number first by column (i.e. A-H) then by row (i.e. 1-12) in the graph. Each screening compound was investigated in a single-concentration (200 µM). Among 80 compounds, one substance reduced enzymatic activity by more than 50 %.

An additional “effect plot” is shown in Figure 4.5.

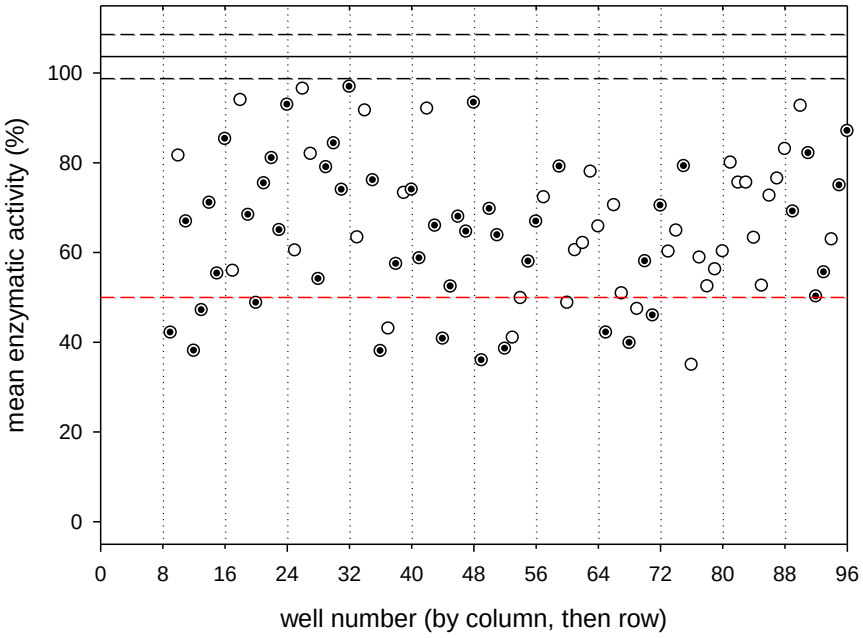


Figure 4.5 Effect plot of screening plate **ori.hya.44**.

As an example, the screening results for plate **ori.hya.44** (88 compounds) are given. Different from the previous example, all substances were tested irrespective of identification of the target mass. Accordingly, compounds without affirmed target mass are labeled as “○“. Test compounds with target mass are marked as “●“. In summary, 16 substances, including four compounds which were not confirmed by mass spectrometry, reduced enzymatic activity to below 50 %.

To assess the relevance of inhibition data, for each “effect plot” the corresponding “weighted effect plot” was additionally computed. In detail, the calculated weighted effect w_i^* (cf. Equation 4.6) was plotted versus mean percentual enzymatic activity. To accept a screening compound as “hit”, the weighted effect w_i^* was recommended to be ≥ 1 and the enzymatic activity < 50 %. Confirmed hits (“●“) within these limits (“hit window”) were subjected to preparative synthesis in order to certify the proposed hyaluronidase inhibition.

The corresponding “weighted effect plot” of plate **ori.hya.scr.3** (cf. Figure 4.4) is given in Figure 4.6.

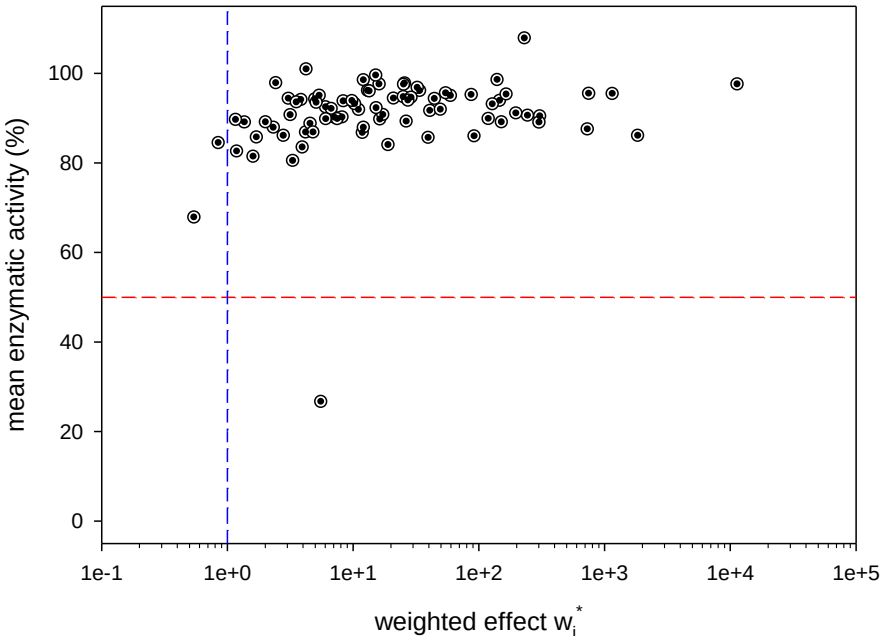


Figure 4.6 Weighted effect plot of screening plate **ori.hya.scr.3**.

As illustrated, only a single substance fulfills both recommended criteria: enzymatic activity < 50 % (dashed red line) and weighted effect $w_i^* \geq 1$ (dashed blue line). Subsequently, this compound was termed as hit.

The “weighted effect plot” of plate **ori.hya.44** (cf. Figure 4.5) is displayed in Figure 4.7.

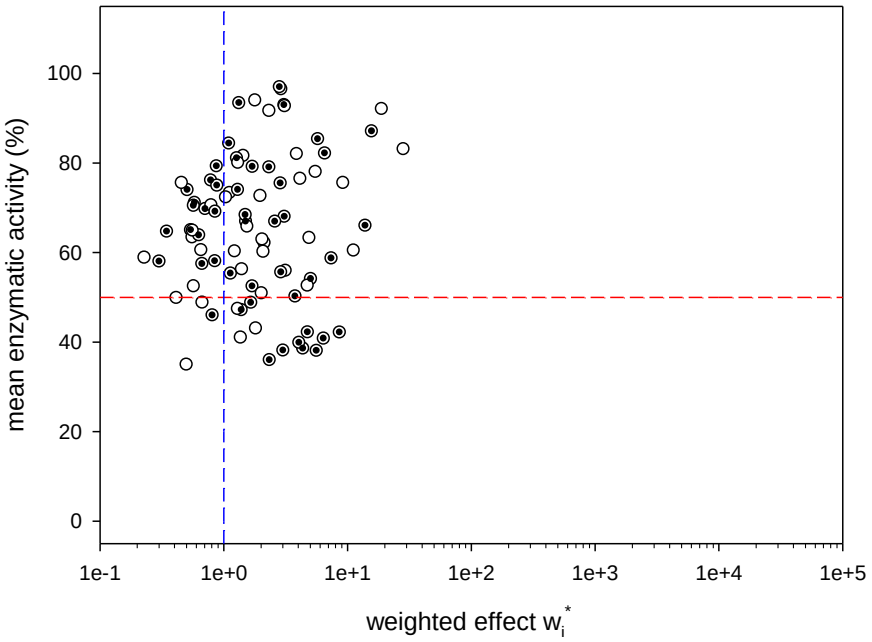


Figure 4.7 Weighted effect plot of screening plate **ori.hya.44**.

As shown in Figure 4.7, 16 substances reduced enzymatic activity to less than 50 %. Different from the effect plot, the weighted effect plot revealed only 10 compounds with confirmed molecular mass in the hit window (enzymatic activity < 50 %; weighted effect $w_i^* \geq 1$). Accordingly, these compounds were termed as hits.

The “effect plots” and “weighted effect plots” for all screening plates are documented in section B.9 (appendix II).

The hit criteria for the screening campaign are summarized in Table 4.6.

Table 4.6 Acceptance criteria for determination of screening hits.

Parameter	**Requirement**
Z’ factor	> 0.5
primary reference (Vcpal)	no drift or edge effects visible for the IC_{50} value
secondary reference (diclofenac)	no drift or edge effects visible for the IC_{50} value
inhibitory effect	> 50 %
weighted effect w_i^*	≥ 1

All identified hit compounds were re-tested as purified compounds for the determination of IC_{50} values. Synthesis and pharmacological characterization of the corresponding compounds will be discussed in chapter 5.

4.4 Summary

According to high-throughput (HTS) techniques, a screening method was devised for liquid handling systems, allowing automated dispensing of all assay ingredients. Validation according to standard parameters of quality control according to procedures established in the pharmaceutical industry proved that the new microplate assay is robust and efficient for the screening of large libraries of potential hyaluronidase inhibitors.

4.5 References

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5 4-Amino-1*H*-imidazole-2(5*H*)-thiones as inhibitors of bacterial hyaluronidase: a computer-assisted and multicomponent synthesis approach

5.1 Introduction

Since the first description of hyaluronan in the early 1930s[1] and hyaluronan degrading enzymes, the hyaluronidases, in the 1940s[2], academic research on this topic represents a controversial and challenging field. The hyaluronidases have been a group of less extensively studied glycosidases.[3] Hence, there is little knowledge about the physiological role of isoforms of hyaluronidases and their involvement with a variety of diseases. Potent and selective hyaluronidase inhibitors are not available so far. However, such compounds are required as molecular tools to perform pharmacological studies aiming at more detailed insights into the complex functions of hyaluronidases in hyaluronan metabolism. Moreover, hyaluronidase inhibitors are of potential therapeutic value. As an example, inhibitors of bacterial lyases might be useful as adjuvants to enhance the chemotherapy of drug-resistant bacteria. However, in terms of drug research including rational design of inhibitors, hyaluronidases have been a class of neglected enzymes. At present, small drug-like molecules with sufficient inhibitory potency are not available, although (weak) hyaluronidase inhibitors of bacterial enzymes have been discovered among natural products and synthetic molecules.[3-7]

Over the past decade, research in our laboratories has led to the discovery and validation of potent inhibitors of hyaluronidases with a preference for the bacterial enzymes. The identification and development of these substances was mainly driven by conventional medicinal chemistry approaches. Inhibitors were designed as substrate analogs or synthesized according to an iterative optimization process, in several cases starting from incidentally discovered inhibitors. For example, lipophilic derivatives of L-ascorbic acid (vitamin C) were developed, which are among the most potent inhibitors of the bacterial hyaluronidase *Sag*Hyal$_{4755}$ known today.[8-11] Moreover, potent hyaluronidase inhibitors were identified among a variety of heterocycles such as benzimidazoles, benzoxazoles, indoles, phenylindoles, gluconolactones and some miscellaneous substances.[12-20] In general, the potency of hyaluronidase inhibitors correlates with lipophilicity, e.g. conferred by fatty acid-like moieties, and the presence of negatively charged groups. The drug-like properties of highly lipophilic compounds are often strongly affected, for example due to low bioavailability or very high plasma protein binding. In fact, the previously identified inhibitors are almost completely bound to plasma proteins, which precludes their application in studies using protein-rich biological material, e.g. serum, and in *in vivo* investigations.

In this context, the rational design of drug-like low molecular weight hyaluronidase inhibitors is a difficult challenge. To cope with this problem, a target-based approach for the design, synthesis and pharmacological characterization of novel lead inhibitors of a streptococcal hyaluronidase (*Sag*Hyal$_{4755}$) is described in this chapter.

Crystal structures of bacterial hyaluronidases incorporating substrate fragments as well as structures in complex with inhibitors were previously published by our workgroup.[18, 21-29] Furthermore, suitable protein structures of the target enzyme were available from the protein data bank. A collaborative project with Origenis GmbH, a specialized biotech company for computer-assisted drug-design in combination with multicomponent reaction synthesis of compound libraries, was launched to expand the range of methods. Modus operandi of the venture was in accordance to a ligand-based drug design cycle for the identification of lead inhibitors (cf. Figure 5.1).

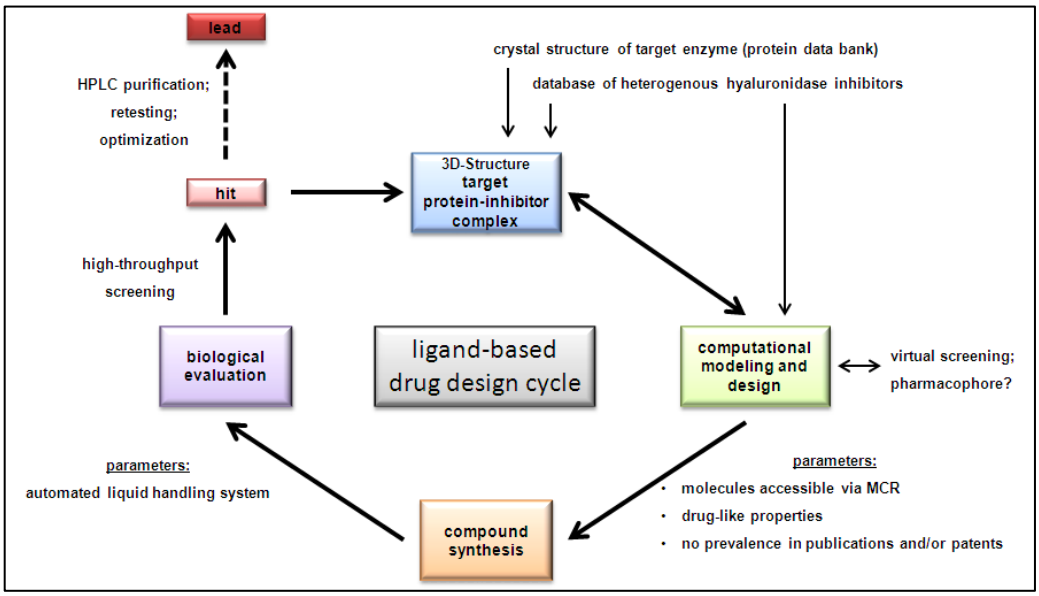


Figure 5.1 Scheme of the pursued ligand-based drug design cycle for the identification of novel lead inhibitors of bacterial hyaluronidase *Sag*Hyal$_{4755}$.

This concept was inspired by standard operation procedures from pharmaceutical industry, but was customized for the own purpose and adjusted with regard to the applied methodology. The combination of innovative computer-assisted methods for drug design and modern synthesis technologies was used to identify novel structural motifs. Initially, a compound library comprising 347 selected inhibitors of the target enzyme was analyzed (cf. section B.1.1., appendix II). The corresponding structure-activity relationships (SAR) were elucidated with the aid of the repertoire of methods of the collaboration partner and models of the enzyme in complex with potential inhibitors were generated *in silico*. For this

purpose, only small molecules, predicted to possess drug-like properties and to be accessible by multi-component synthesis were taken into consideration.

A variety of substances was selected for parallel synthesis and biological tests. As outlined in Figure 5.1, following an iterative process, the suggested compounds were synthesized via parallel synthesis, analyzed and investigated for hyaluronidase inhibition in the medium throughput range. In accordance with the chemical parameters, molecular structures were synthesized in one-pot reactions miniaturized down to 96 deep-well plates. Moreover, automated synthesis was used to synthesize vast compound libraries.

Virtual screening and docking experiments suggested compounds having a 1*H*-pyrrol-2(5*H*)-one- or a 4-amino-1*H*-imidazole-2(5*H*)-(thi)one scaffold as potential hyaluronidase inhibitors (Figure 5.2).

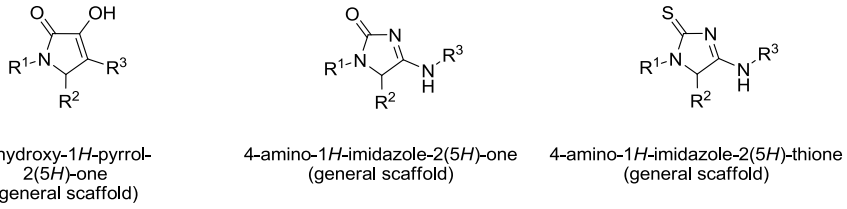


Figure 5.2 General structures of the predicted 1,5-dihydropyrrol-2-one-, 4-amino-1*H*-imidazole-2(5*H*)-one- and 4-amino-1*H*-imidazole-2(5*H*)-thione-type hyaluronidase inhibitors.

These results prompted us to synthesize two generations of compounds predicted to have hyaluronidase inhibitory activity. Several hundred substances were prepared by parallel synthesis, analyzed by means of HPLC-MS and investigated for inhibition of the target hyaluronate lyase *Sag*Hyal$_{4755}$.

5.2 Synthesis and characterization of first generation inhibitors

5.2.1 Parallel synthesis of 1*H*-pyrrol-2(5*H*)-ones

The 1*H*-pyrrol-2(5*H*)-ones (3-pyrrolin-2-ones, 1,5-dihydro-2*H*-pyrrol-2-ones) described in this section were synthesized by one-pot multicomponent reactions. Several methods have been reported for the preparation of 1*H*-pyrrol-2(5*H*)-ones in literature.[30-38] A convenient parallel synthesis was carried out as a “Doebner-type” approach as three component condensation (Figure 5.3).

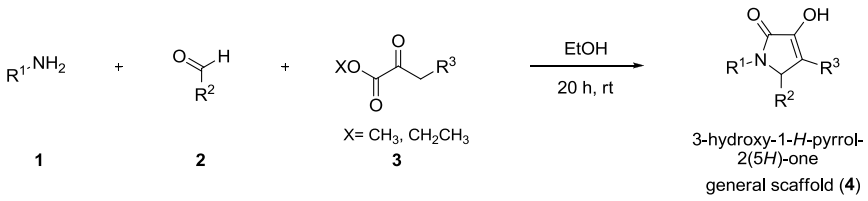


Figure 5.3 Multicomponent synthesis of substituted 3-hydroxylated 1*H*-pyrrol-2(5*H*)-ones (**4**). Substitutions patterns of starting materials **1**, **2** and **3** cf. section B.3 (appendix II). Reagents and conditions: anhydrous EtOH, 20 h, rt.

The reaction of amines **1**, aldehydes **2** and suitable pyruvic acid esters (methyl and ethyl pyruvates) **3** was utilized for the construction of a series of substituted 3-hydroxy-1*H*-pyrrol-2(5*H*)-ones of general structure **4**. In detail, after the generation of the corresponding imine species, pyruvic acid esters were added. The mixture was allowed to react for 20 hours at ambient temperature to give the target molecules.[39-44]

For parallel synthesis, starting materials in anhydrous ethanol were transferred to 96-deep well plates with an automated liquid handling system (Figure 5.4).

Figure 5.4 Images of technical equipment used for parallel synthesis (Tecan Genesis RSP 200/8 parallel synthesizer).

A compound library consisting of 880 substances was obtained from a combinatorial reaction matrix of 11 amines, 16 aldehydes and 5 pyruvic acid esters. The compounds were left on 10 deep well plates (**ori.hya.10**-**19**). After HPLC mass spectral analysis (ESI-TOF), the solvent was evaporated and the crude substances were dispensed in dimethylsulfoxide, adjusting to a concentration of 20 mM. Deep-well plates were sealed and stored at -20 °C. Starting materials (amines **1**, aldehydes **2**, pyruvic ester **3**) and mass spectral analysis of target molecules **4** on plates **ori.hya.10**-**19** are documented in section B.3 (appendix II).

5.2.2 Parallel synthesis of 4-amino-1*H*-imidazole-2(5*H*)-ones and -thiones

The reaction of amines **1**, aldehydes **2** and isocyanides **5** in the presence of potassium cyanate (**6**) lead to 4-amino-1*H*-imidazole-2(5*H*)-ones (**7**) via incorporation of the cyanate counterion (Figure 5.5).[45]

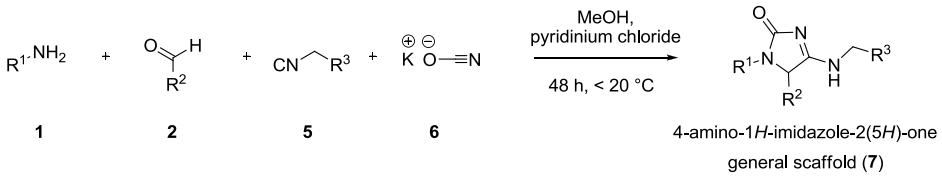


Figure 5.5 Multicomponent synthesis of substituted 4-amino-1*H*-imidazole-2(5*H*)-ones (**7**); substitutions patterns of starting materials **1**, **2** and **5** cf. section B.4 (appendix II). Reagents and conditions: anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

The reactions were carried out as one-pot syntheses under mild conditions. The potential of this four-component condensation reaction, which is also referred to as Ugi four-component condensation (Ugi 4CC), was elucidated for the preparation of a combinatorial library of 4-amino-1*H*-imidazole-2(5*H*)-ones.[46-49]

The mechanism of this condensation reaction involves at first the formation of iminium ions, which are formed from carbonyl compounds and amines in solutions of suitable acidity.[50] Iminium ions in combination with cyanate anions are ideal reaction partners for the acid-sensitive isocyanides, which react by electrophilic α-additions. The participation of cyanate and thiocyanate results in an intramolecular acylation. The nitrogen atom of the α-aminoalkyl group in the α-adduct is acylated by means of a cyclic mechanism, provided that a N-H bond is available. This leads to the formation of five-membered, non-aromatic

heterocycles, the 4-amino-1*H*-imidazole-2(5*H*)-ones and, by analogy, to 4-amino-1*H*-imidazole-2(5*H*)-thiones.[50]

A selection of alkyl- and aryl-substituted amines, aldehydes and isocyanides was arranged from commercially purchased chemicals. Miniaturized parallel synthesis was performed on a liquid handling system (solvent: anhydrous methanol) and was started by the addition of amine and aldehyde. After 30 min, the reaction mixtures on the 96-deep-well plates were cooled to 0 °C. Accordingly excess of pyridinium chloride, potassium cyanate and equimolar amounts of isocyanide were added. Product formation was observed after 48 hours when stored at temperatures below < 20 °C.

Similarly, 4-amino-1*H*-imidazole-2(5*H*)-thiones **9** were synthesized by analogy with the protocol for the preparation of **7**, except that potassium cyanate was replaced by potassium thiocyanate **8** (Figure 5.6). [45, 46, 51, 52]

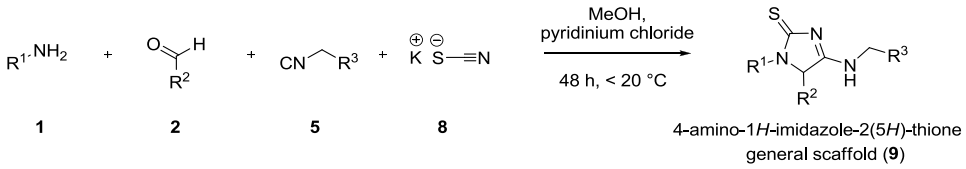


Figure 5.6 Multicomponent synthesis of substituted 4-amino-1*H*-imidazole-2(5*H*)-thiones (**9**); substitutions patterns of starting materials **1**, **2** and **5** cf. section B.4 (appendix II). Reagents and conditions: KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

A screening library of 1408 substances was synthesized from combinatorial variation of 11 amines, 16 aldehydes, 4 isocyanides, potassium cyanate and thiocyanate, respectively. The compounds were loaded on to 16 deep-well plates (**ori.hya.20**-**35**). After ESI-TOF mass spectrometry, the plates were processed as described before. Starting materials (amines **1**, aldehydes **2**, isocyanides **5**) and mass spectral analysis of target molecules **7**, **9** on plates **ori.hya.20**-**35** are documented in section B.4 (appendix II).

One-pot synthesis from both campaigns yielded a total of 2288 compounds. The uneconomic and time-consuming purification of all these substances was not performed. Instead, the crude compounds were analyzed by HPLC-MS (ESI-TOF) and categorized. For biological investigations only those wells were selected in which, according to mass spectometric analysis, the respective target compound represented the major ingredient. A collection of 549 compounds with certified mass spectra and obtained in sufficient quantities was subjected to screening. These substances were picked automatically and put on 7 deep-well screening plates (**ori.hya.scr.1**-**7**, for detais see section B.5

(appendix II)). The remaining 1739 compounds were not investigated for hyaluronidase inhibitory activity.

5.3 Pharmacological results and discussion of first generation inhibitors

5.3.1 General conditions and screening mode

Determination of inhibitory activity was assessed using the automated 96-well microtiter plate turbidimetric assay. Enzymatic activities of test compounds were computed for an assumed final assay concentration of 200 µM. Duplicates were performed on separate plates. *Sag*Hyal$_{4755}$ was dispensed with a final enzymatic activity of 0.1 mU. Test compounds, buffer ingredients and enzyme solution as well as stopping reagent (CTAB) were dispensed with automated liquid handling systems (Tecan TeMo, Tecan Deutschland GmbH, Crailsheim, Germany; Multidrop liquid dispenser, Mtx Lab systems Inc., USA). Incubation time of screening plates was set to 30 min. A total of 209 substituted 1*H*-pyrrol-2(5*H*)-ones and 340 alkylated and arylated 4-amino-1*H*-imidazole-2(5*H*)-ones and –thiones were tested for hyaluronidase inhibition. Effect of screening compounds on enzymatic activity of *Sag*Hyal$_{4755}$ is illustrated by “effect plots” and “weighted effect plots”.

5.3.2 Inhibitory activities of screening compounds on *Sag*Hyal$_{4755}$

Among the 549 investigated substances, 3 compounds (**5.1**, **5.2** and **5.3**) were identified as screening hits. Relative to the total volume of test compounds this represents a hit rate of 0.5 % (0.1 % for 2288 substances). A detailed on-screen assay validation and representation of screening data is given in appendix II (sections B.8.2, B.9.3). Chemical structures of the identified hits are shown in Figure 5.7.

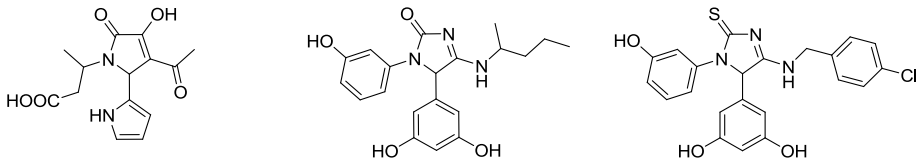


Figure 5.7 Structures of identified hits (5.1-5.3) from the first screening campaign.

At this stage, the criteria to denote a compound as hit (inhibitory effect > 50 %, weighted effect ≥ 1 (cf. section 4.3.2)) were loosened to some extent (inhibitory effect 20 % - 50 %, weighted effect ≥ 1). Accordingly, 8 additional substances (**5.4**-**5.11**, Figure 5.8) with certified mass were pharmacologically characterized. These molecules were investigated to gain new ideas for novel inhibitors.

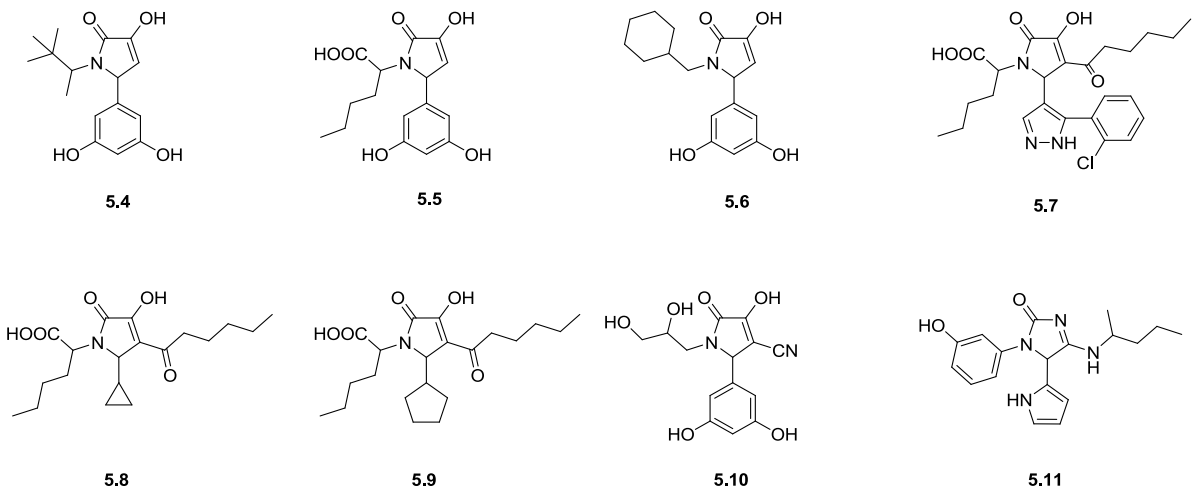


Figure 5.8 Structures of additional compounds of the first screening campaign displaying inhibitory activity between 20 % and 50 % (5.4-5.11).

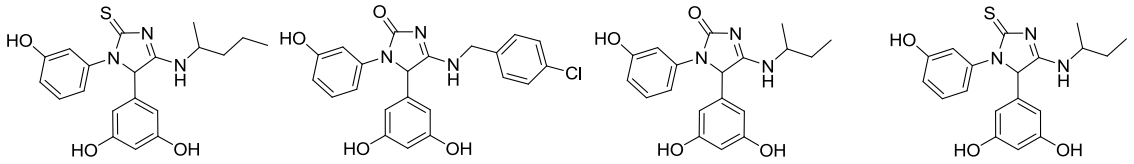
The inhibitory activities of compounds **5.1**-**5.11** are summarized in Table 5.1.

Table 5.1 Inhibitory activity of compounds 5.1-5.11 on *Sag*Hyal$_{4755}$ and BTH.

Compound	*Sag*Hyal$_{4755}$ % inhibition[a]	BTH % inhibition	Comment
5.1	73 ± 6	inactive	hit
5.2	53 ± 7	inactive	hit
5.3	52 ± 0	inactive	hit
5.4	24 ± 3	inactive	missed hit criteria
5.5	28 ± 3	inactive	missed hit criteria
5.6	38 ± 10	inactive	missed hit criteria
5.7	21 ± 2	inactive	missed hit criteria
5.8	13 ± 7	inactive	missed hit criteria
5.9	26 ± 5	inactive	missed hit criteria
5.10	32 ± 11	inactive	missed hit criteria
5.11	26 ± 3	inactive	missed hit criteria

[a] mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were set to an assumed final assay concentration of 200 µM; percent inhibition determined at pH 5.0 in the automated 96-well turbidimetric assay.

Compounds **5.2** and **5.3** were structurally modified by synthesis of the corresponding thioxo- (**5.12**) and oxo- (**5.13**) analogs (Figure 5.9). Additionally, the pentan-2-ylamino moiety in the eastern part of **5.2** was replaced by a *sec*-butylamino moiety in the oxo- (**5.14**) and thioxo- (**5.15**) derivative.



5.12 **5.13** **5.14** **5.15**

Figure 5.9 Structures of newly designed and synthesized 4-amino-1*H*-imidazole-2(5*H*)-ones (5.13, 5.14) and –thiones (5.12, 5.15).

5.3.3 Inhibitory activities of purified compounds on *Sag*Hyal$_{4755}$

To determine inhibitory activities of purified substances, screening hits **5.1**-**5.3** and **5.6**, **5.10** were synthesized in conventional manner. In addition, **5.12**-**5.15** were synthesized by one-pot reactions. In the case of **5.1** and **5.6** the preparation of the target compounds failed. Unfortunately, with **5.1** the most active inhibitor of the screening campaign was lost at this stage. The inhibitory activities of the remaining purified substances (RP-HPLC) are shown in Table 5.2.

Table 5.2 Inhibitory activity of compounds 5.2, 5.3, 5.10, 5.12-5.15 on *Sag*Hyal$_{4755}$ and BTH.

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (µM)[a]**	**BTH IC$_{50}$ (µM)**
5.2	inactive	inactive
5.3	150 ± 20	inactive
5.10	inactive	inactive
5.12	> 1500	inactive
5.13	980 ± 50	inactive
5.14	1100 ± 55	inactive
5.15	200 ± 25	inactive

[a] mean values ± SEM (N = 2, experiments performed in duplicate; IC$_{50}$ values determined at pH 5.0 in the 96-well turbidimetric assay.

As shown in Table 5.2, IC$_{50}$ values of 150 µM and 980 µM were determined for **5.3** and **5.13**, respectively. Compound **5.3** (4-(4-chlorobenzylamino)-5-(3,5-dihydroxyphenyl)-1-(3-

hydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione) represented the most potent confirmed hit. Compared to **5.13**, the replacement of sulfur by oxygen led to 6.5-fold decrease in potency. Pure substance **5.10** revealed a total loss of inhibitory activity, although the screening of the raw product suggested moderate inhibition. Concentration dependent activity of *Sag*Hyal$_{4755}$ in the presence of **5.3**, **5.10** and **5.13** is illustrated in Figure 5.10.

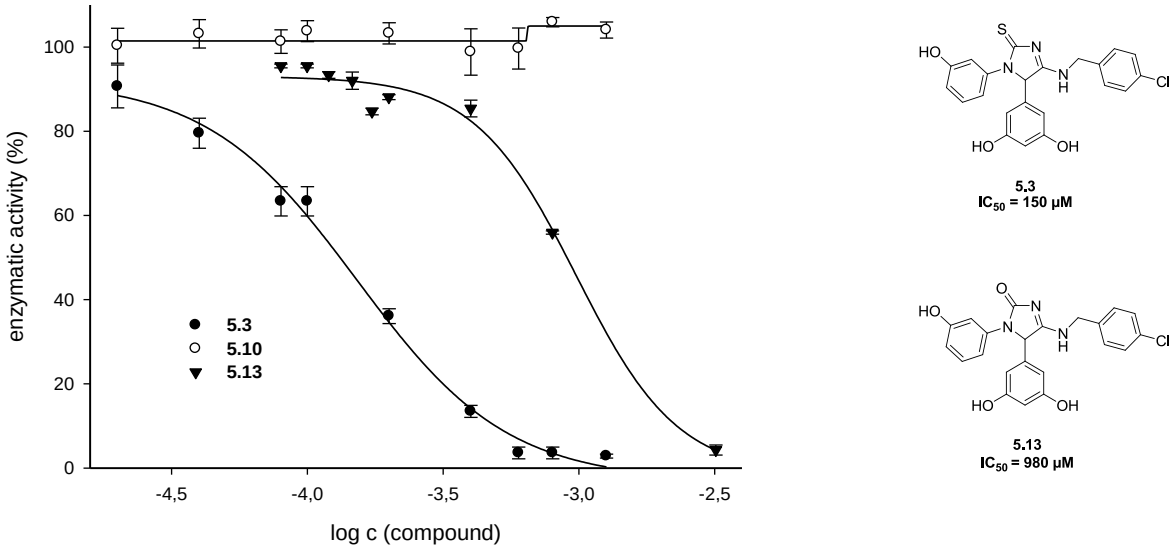


Figure 5.10 Concentration dependent activity of *Sag*Hyal$_{4755}$ in the presence of **5.3**, **5.10** and **5.13**.

For compounds **5.2** (4-amino-1*H*-imidazole-2(5*H*)-one) and **5.12** (4-amino-1*H*-imidazole-2(5*H*)-thione) the aromatic substituent at the 4-amino group was replaced by a pentan-2-yl moiety. Both derivatives showed a (total) loss of inhibitory activity. However, for analogs bearing a *sec*-butyl residue, IC_{50} values of 1100 µM (**5.14**) and 200 µM (**5.15**) were calculated. Concentration dependent activity of *Sag*Hyal$_{4755}$ in the presence of **5.2**, **5.12** and **5.14**, **5.15** is shown in Figure 5.11.

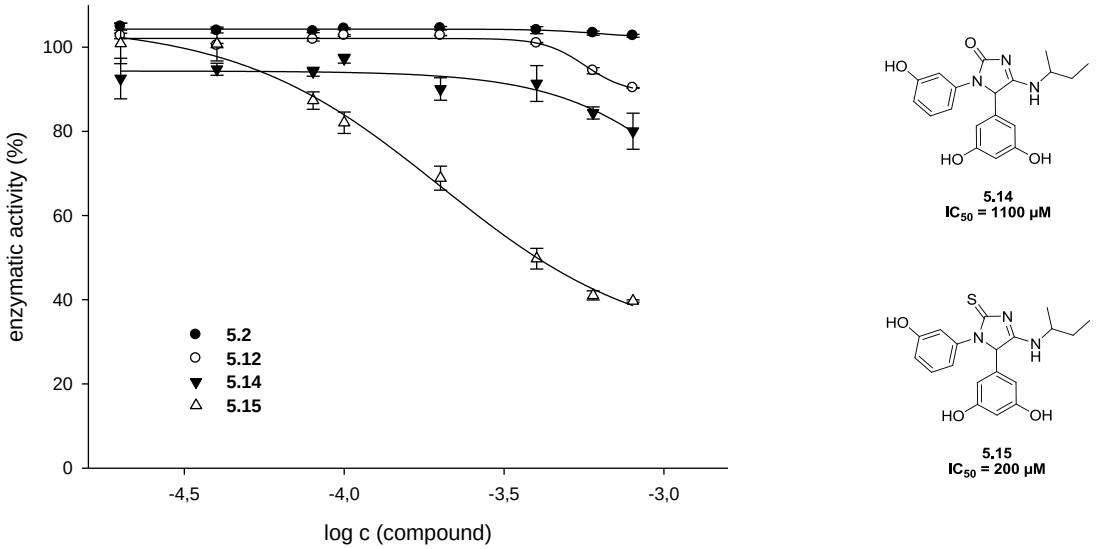


Figure 5.11 Concentration dependent activity of *Sag*Hyal$_{4755}$ in the presence of 5.2, 5.12 and 5.14, 5.15.

Taken together, regardless of the observed dramatic discrepancies between inhibitory activities of raw and purified compounds, the biological activity at the streptococcal lyase *Sag*Hyal$_{4755}$ was confirmed for 4-amino-1*H*-imidazole-2(5*H*)-thione **5.3**. By trend, substituted 4-amino-1*H*-imidazole-2(5*H*)-thiones (cf. **5.2**, **5.3**, **5.14**) showed a stronger inhibition on *Sag*Hyal$_{4755}$ than the structurally related 4-amino-1*H*-imidazole-2(5*H*)-ones (cf. **5.12**, **5.13**, **5.15**). Therefore, 4-amino-1*H*-imidazole-2(5*H*)-thiones bearing aromatic moieties were regarded as a promising starting point for further development of hyaluronate lyase inhibitors. Hence, a daughter generation of 4-amino-1*H*-imidazole-2(5*H*)-thiones with suitable substitution pattern was prepared by parallel synthesis and subjected to evaluation for hyaluronidase inhibition. The procedures and results will be presented in the following section.

5.3.4 Inhibitory activities of purified compounds on BTH

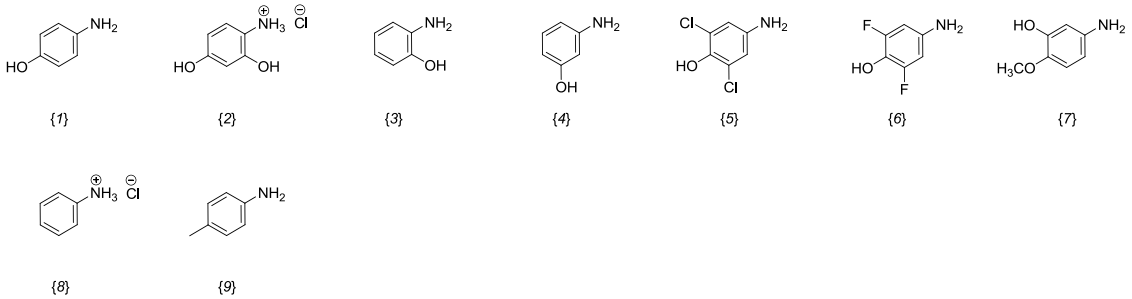
There are major structural differences between streptococcal lyase *Sag*Hyal$_{4755}$ and the bovine testicular hyaluronidase BTH. To ensure that potential activities at mammalian hyaluronidases are not overlooked, all substances were also tested for inhibitory activity on BTH as a typical hyaluronan hydrolase. However, there was no inhibition of this enzyme detected in the investigated concentration range (cf. Table 5.2).

5.4 Synthesis and characterization of second generation inhibitors

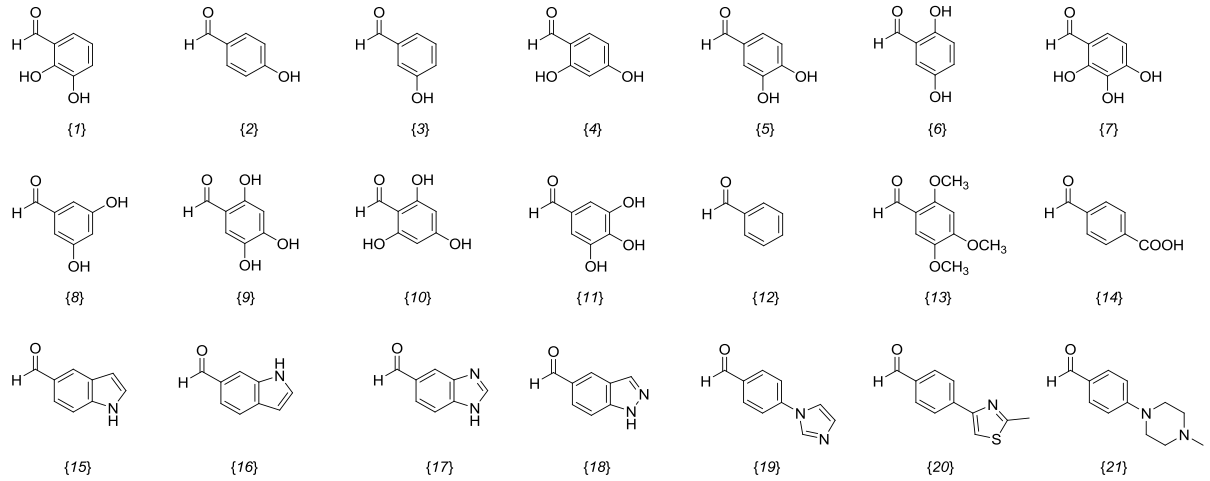
5.4.1 Parallel synthesis of 4-amino-1*H*-imidazole-2(5*H*)-thiones

The compounds in this section offer a 4-(benzylamino)-1,5-diphenyl-1*H*-imidazole-2(5*H*)-thione core structure with varying substituents. For screening, only aromatic starting materials were selected. A combinatorial library of 352 substances was synthesized from 8 amines (**1**{*1*}-{*8*}), 11 aldehydes (**2**{*1*}-{*11*}) and 4 isocyanides (**5**{*1*}-{*4*}) (Figure 5.12).

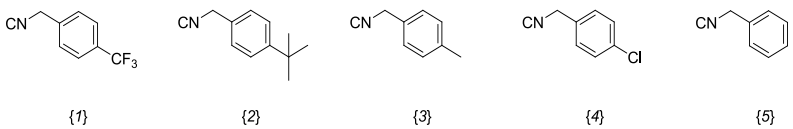
amines **1**{*1*}-{*9*}:



aldehydes **2**{*1*}-{*21*}:



isocyanides **5**{*1*}-{*5*}:



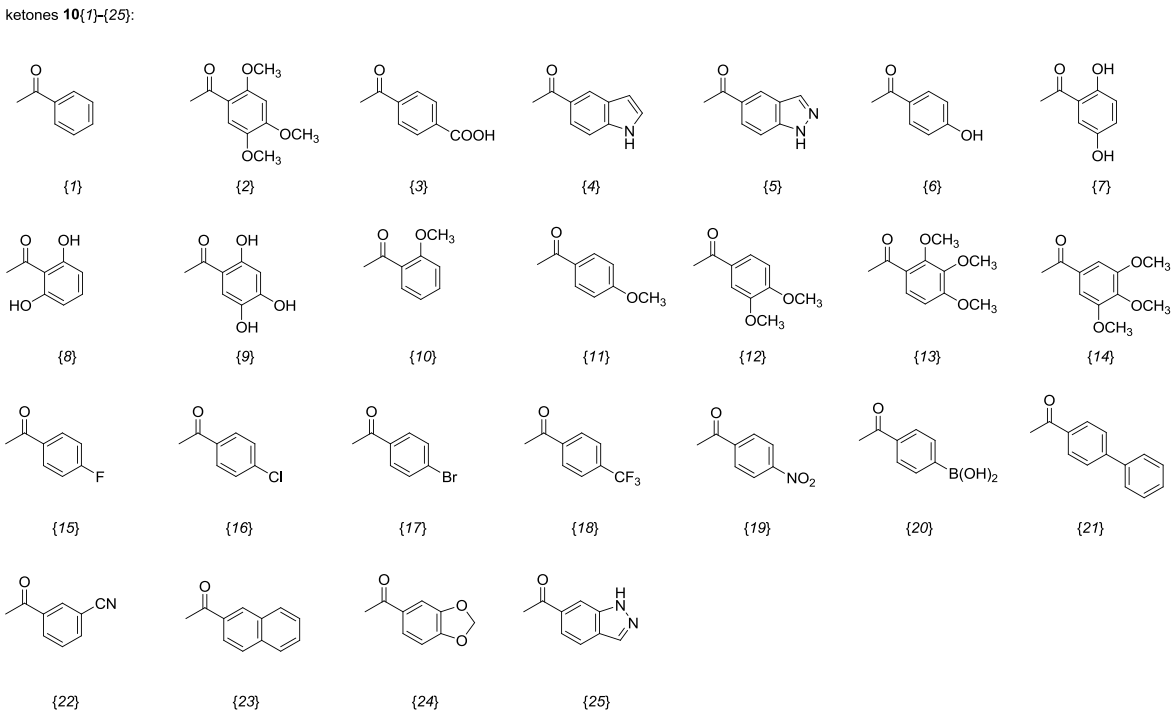


Figure 5.12 Structures of starting materials (amines **1**{*1*}-{*9*}, aldehydes **2**{*1*}-{*21*}, isocyanides **5**{*1*}-{*5*} and ketones **10**{*1*}-{*25*}) for the multicomponent synthesis of 4-amino-1*H*-imidazole-2(5*H*)-thiones.

By analogy with the synthesis outlined in Figure 5.6, equimolar amounts of aromatic amines, aldehydes and isocyanides were mixed in anhydrous methanol containing pyridinium chloride. As a representative example, the synthesis of 1-(3,5-dichloro-4-hydroxyphenyl)-4-(4-(trifluoromethyl)benzylamino)-5-(2,4,5-trihydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (**5.22**) is shown in Figure 5.13.

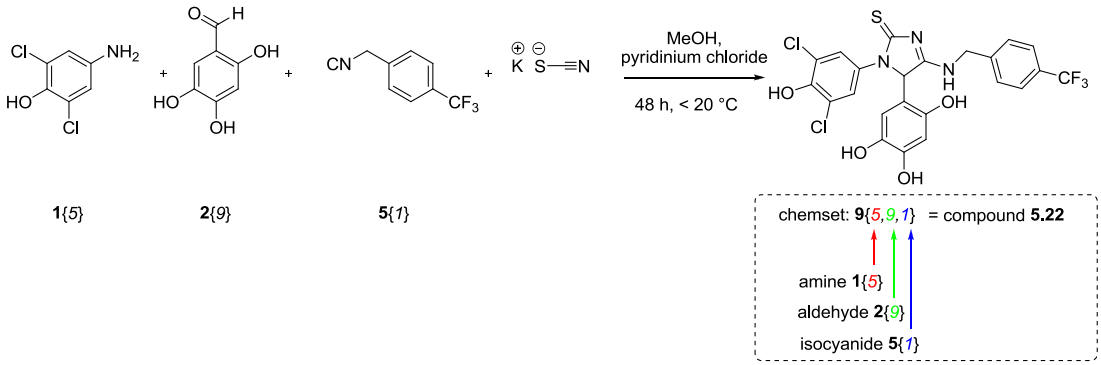


Figure 5.13 Synthesis of 1-(3,5-dichloro-4-hydroxyphenyl)-4-(4-(trifluoromethyl)benzylamino)-5-(2,4,5-trihydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (**5.22**). Reagents and conditions: KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

All starting materials were commercially available. The MCR-derived compounds (352 substances, location: **ori.hya.44**-**47**) were analyzed by ESI-TOF mass spectrometry.

Details are documented in section B.6 (appendix II). Unexpectedly, these derivatives turned out to be unstable, as the target compounds, detected by molecular peaks $[M + H]^+$, disappeared during storage of the samples. This was observed for all molecules on all plates of this screening campaign. However, the time span until complete disappearance of the $[M + H]^+$ varied and took up to 14 days for individual molecules. Data analysis (mass spectrometric analysis by LC-MS and ESI-TOF) proposed an increase by *m/z* +16 generating $\{[M + H]^+ +16\}$ target mass signals. Most likely, the observed rise in molecular weight, corresponding to the atomic mass of oxygen, was caused by an oxidation process. This unexpected finding prompted us to elucidate the chemical properties of such molecules and, eventually, to design more stable compounds for the determination of hyaluronidase inhibitory activity.

For chemical studies, the 4-(benzylamino)-1,5-diphenyl-1*H*-imidazole-2(5*H*)-thione core structure **9** remained unchanged. A repertory of suitable aromatic amines, aldehydes and isocyanides was picked for the preparation of additional molecules on the preparative scale. Besides, suitable ketones (cf. Figure 5.12), enabling the synthesis of compounds with similar substitution patterns or new scaffolds, were provided to prepare 5-methylated 4-amino-1*H*-imidazole-2(5*H*)-thiones. The results are described in the following sections.

5.4.2 Synthesis of 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thiones

Aromatic amines **1**{*1*}, **1**{*4*}-{*7*}, hydroxylated aldehydes **2**{*1*}, **2**{*6*}, **2**{*8*}, **2**{*9*} and isocyanides **5**{*1*}-{*4*} were used for the formation of target molecules **5.16**-**5.25** (Figure 5.14).

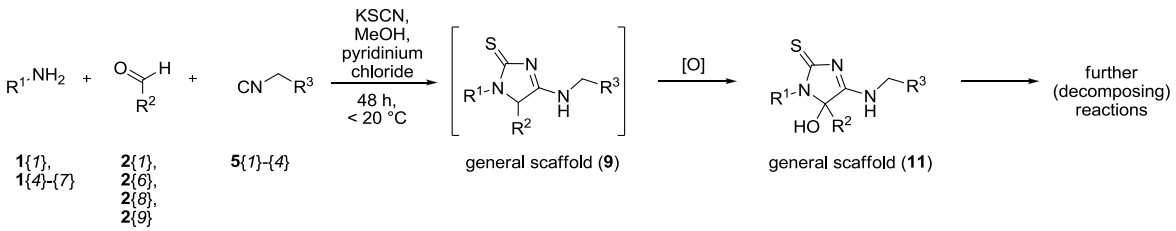
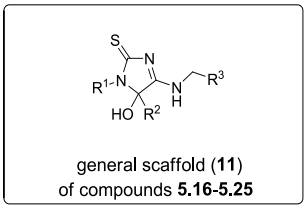


Figure 5.14 Multicomponent synthesis of substituted 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thiones (**11**). Reagents and conditions: KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

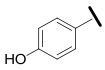
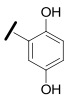
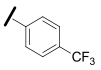
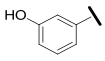
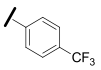
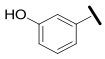
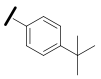
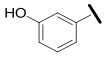
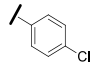
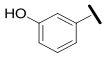
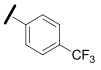
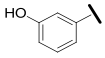
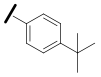
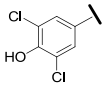
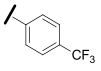
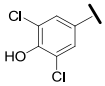
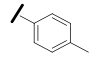
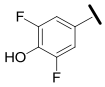
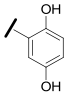
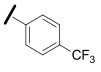
The crude products were isolated as yellowish viscous oils and tediously purified using RP-HPLC. As observed before, the mass spectrum of the desired products (general scaffold **9**, Figure 5.14) indicated the formation of compounds corresponding to a molecular mass of $\{[M + H]^+ + 16\}$ (general scaffold **11**, Figure 5.14). A total conversion to

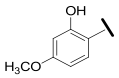
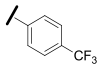
the "*m/z* +16 adducts" was usually observed between 96 and 192 hours after completion of the experiment. For a period of 7 days, *m/z* +16 adducts were found to represent the predominant molecular species of the investigated compounds. When the storage period was extended, besides the {$[M + H]^+ + 16$} ion, MS analysis revealed additional molecular species, suggesting fragmentation and decomposition. Therefore, substances **5.16**-**5.25** were synthesized, stored in solution for 8 days, then purified by RP-HPLC and subjected to pharmacological investigations.

Table 5.3 Substitution pattern for 4-amino-1*H*-imidazole-2(5*H*)-thiones **5.16**-**5.25**.



general scaffold (**11**)
of compounds **5.16**-**5.25**

Chemset	Compound	R^1	R^2	R^3
11{*1,6,1*}	**5.16**			
11{*4,1,1*}	**5.17**			
11{*4,1,2*}	**5.18**			
11{*4,8,4*}	**5.19**			
11{*4,9,1*}	**5.20**			
11{*4,9,2*}	**5.21**			
11{*5,9,1*}	**5.22**			
11{*5,9,3*}	**5.23**			
11{*6,6,1*}	**5.24**			

11{*7,6,1*}	**5.25**			

Aiming at higher chemical stability of the oxidized species, new structures and substitution patterns of the aldehydes (**2**{*12*}-{*21*}) became necessary. For this purpose, hydroxyl-moieties were strictly excluded. Amine (**1**{*5*}) and isocyanide component (**5**{*5*}) remained unchanged in this approach (Figure 5.15).

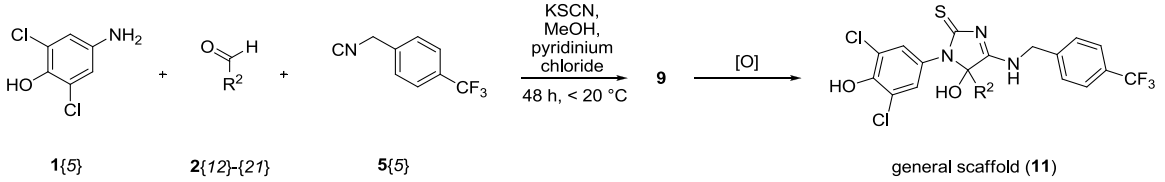
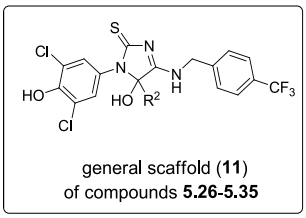
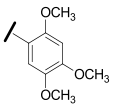
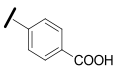


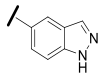
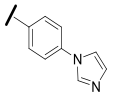
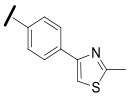
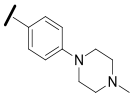
Figure 5.15 General synthesis (multicomponent synthesis) of 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thione derivatives (**11**). Reagents and conditions: KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

Following this strategy, molecules **5.26**-**5.35** were synthesized (Table 5.4).

Table 5.4 Substitution pattern for 4-amino-1*H*-imidazole-2(5*H*)-thiones **5.26**-**5.35**.



Chemset	Compound	R^2
11{*5,12,1*}	**5.26**	
11{*5,13,1*}	**5.27**	
11{*5,14,1*}	**5.28**	
11{*5,15,1*}	**5.29**	
11{*5,16,1*}	**5.30**	
11{*5,17,1*}	**5.31**	

11{*5,18,1*}	**5.32**	
11{*5,19,1*}	**5.33**	
11{*5,20,1*}	**5.34**	
11{*5,21,1*}	**5.35**	

Again, due to a fast oxidation process, almost complete conversion of the crude products to {$[M + H]^+$ + 16} species within the first 48 hours was observed. For all compounds of this series the $[M + H]^+$ molecular peak was no longer detectable after 14 days. Besides that, no major molecular fragments or decomposition could be observed. Purification by preparative RP-HPLC yielded exclusively the {$[M + H]^+$ + 16} derivatives. As a conclusion, substances derived from aldehydes **2**{*12*}-{*21*} (Figure 5.12) were generally unstable toward oxidation in the reaction medium and yielded 5-hydroxylated derivatives. In contrast to compounds **5.16**-**5.25** further decomposition processes were not observed. Substances **5.26**-**35** were obtained as stable 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thiones.

5.4.3 Synthesis of 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones

In order to avoid oxidation on the C-H acidic position 5 of the 4-amino-1*H*-imidazole-2(5*H*)-thiones scaffold, additional modifications for synthesis were investigated. For this purpose aldehydes were replaced by suitable aromatic ketones (methylketones) as building blocks. To retain the chemical properties of the target compounds, comparable aromatic substitution pattern were selected (cf. Figure 5.12). The synthetic protocol was slightly modified by extending the reaction time for amine and ketone to at least 90 minutes to allow sufficient formation of the intermediate ketimine (Figure 5.16).

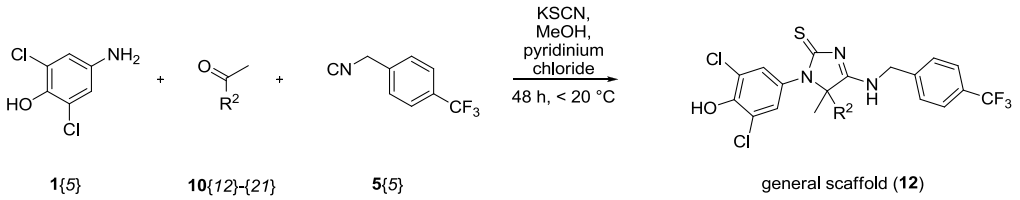
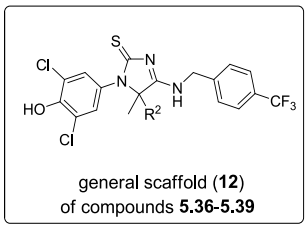
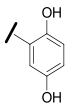
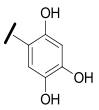


Figure 5.16 Multicomponent synthesis of substituted 1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-(4-(trifluoromethyl)benzylamino)-1*H*-imidazole-2(5*H*)-thiones (**12**). Reagents and conditions: KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

Ketones bearing hydroxyl groups as presented in Table 5.5, displayed only marginal yields (**5.36**) or no formation of target molecules (**5.37**-**5.39**).

Table 5.5 Substitution pattern for 1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-(4-(trifluoromethyl)-benzylamino)-1*H*-imidazole-2(5*H*)-thione derivatives **5.36**-**5.39**.



Chemset	Compound	R^2
12{*5,2,1*}	**5.36**	
12{*5,7,1*}	**5.37**	
12{*5,8,1*}	**5.38**	
12{*5,9,1*}	**5.39**	

In the case of target molecule **5.37**, MS and NMR data suggested the formation of the formimidamide derivative instead of the expected product. The same derivative associated with no formation of the target substance was observed for **5.38** and **5.39**. With a proposal by Cereda[53], a potential reaction scheme is shown in Figure 5.17.

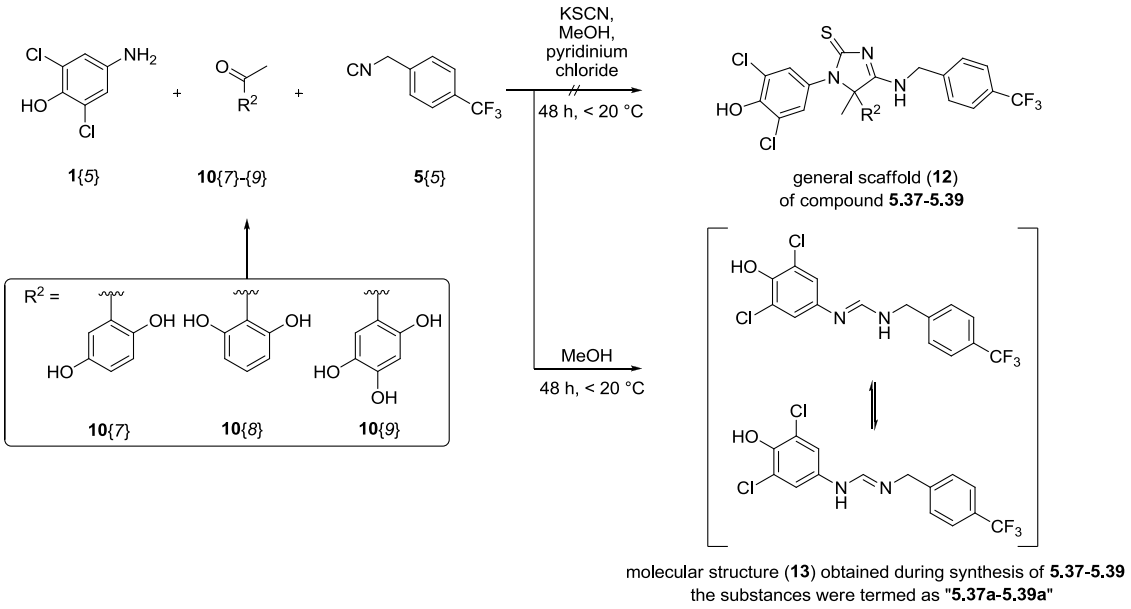


Figure 5.17 Proposed formation of (*E*)-N'-(3,5-dichloro-4-hydroxyphenyl)-N-(4-(trifluoromethyl)benzyl) formimidamide (general scaffold **13**, compound **5.37a**-**5.39a**).

Possibly, the intermediate ketamine was formed only to a minor extend from 4-amino-2,6-dichlorophenol **1**{*5*} in combination with the ketones **10**{*7*}, **10**{*8*} and **10**{*9*}. Subsequently, the unbound amine species could react with the added isocyanide, resulting in the formation of the formimidamide substance **13**. The corresponding compounds were termed as **5.37a**-**5.39a** in the experimental section.

The presence of hydroxyl substituents proved to be detrimental to the synthesis of 5-methylated 4-amino-1*H*-imidazole-2(5*H*)-thiones. Whereas mono-hydroxylated ketones (cf. **5.36**) eventually led to target molecules in poor yields, an adequate formation of the desired products from ketones bearing two or more hydroxylated residues (cf. **5.37**-**5.39**) was not observed.

In the light of experience, a new approach was initiated, taking into account aromatic ketones devoid of hydroxyl groups (Figure 5.18).

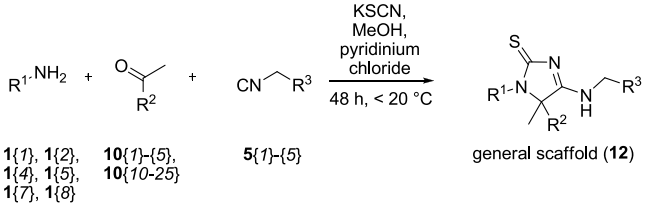
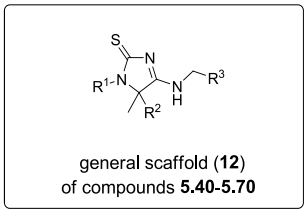


Figure 5.18 Multicomponent synthesis of 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones derivatives **12**. Reagents and conditions: KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

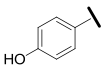
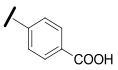
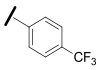
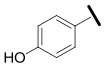
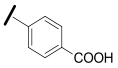
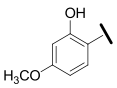
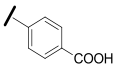
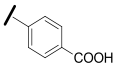
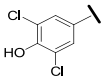
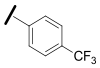
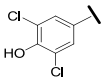
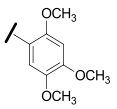
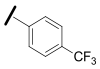
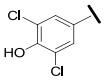
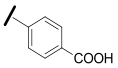
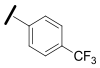
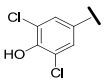
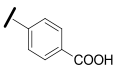
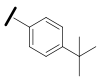
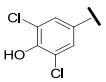
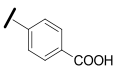
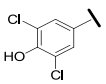
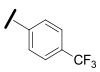
The pertinent products bearing a methyl group in position C-5 were accessible in good yields. Moreover, methylated compounds of the general scaffold **12** did not show oxygen sensitivity. This is in accordance with the literature in so far as C-5 autoxidation of 5,5-disubstituted 4-imino thiohydantoines has not been documented.[54-60]

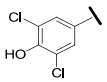
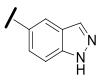
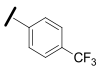
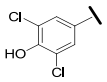
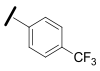
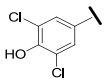
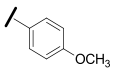
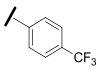
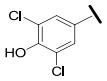
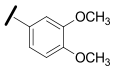
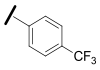
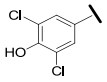
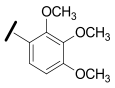
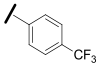
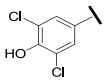
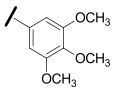
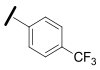
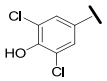
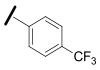
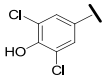
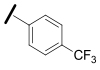
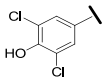
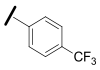
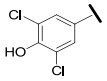
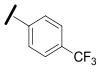
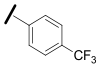
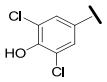
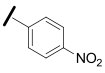
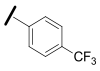
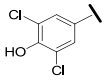
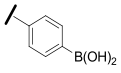
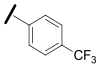
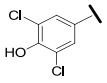
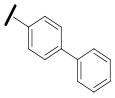
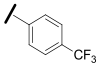
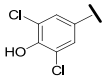
Based on this protocol, substances **5.40**-**5.70** were obtained as stable 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones after RP-HPLC purification (Table 5.6).

Table 5.6 Substitution pattern for 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones derivatives **5.40**-**5.70**.



general scaffold (**12**)
of compounds **5.40-5.70**

Chemset	Compound	R^1	R^2	R^3
12{*1,3,1*}	**5.40**			
12{*1,3,4*}	**5.41**			
12{*2,3,5*}	**5.42**			
12{*4,3,3*}	**5.43**			
12{*5,1,1*}	**5.44**			
12{*5,2,1*}	**5.45**			
12{*5,3,1*}	**5.46**			
12{*5,3,2*}	**5.47**			
12{*5,3,5*}	**5.48**			
12{*5,4,1*}	**5.49**			

12{*5,5,1*}	**5.50**			
12{*5,10,1*}	**5.51**			
12{*5,11,1*}	**5.52**			
12{*5,12,1*}	**5.53**			
12{*5,13,1*}	**5.54**			
12{*5,14,1*}	**5.55**			
12{*5,15,1*}	**5.56**			
12{*5,16,1*}	**5.57**			
12{*5,17,1*}	**5.58**			
12{*5,18,1*}	**5.59**			
12{*5,19,1*}	**5.60**			
12{*5,20,1*}	**5.61**			
12{*5,21,1*}	**5.62**			
12{*5,22,1*}	**5.63**			
12{*5,23,1*}	**5.64**			
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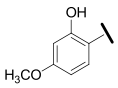
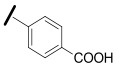
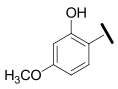
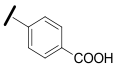
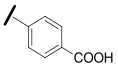
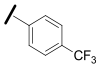
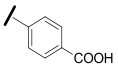
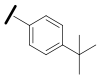
12{*5,25,1*}	**5.66**			

12{*7,3,3*}	**5.67**			
12{*7,3,4*}	**5.68**			
12{*8,3,1*}	**5.69**			
12{*8,3,2*}	**5.70**			

As mentioned above (cf. Table 5.5), multicomponent synthesis of **5.38** failed. In an experiment, **5.45** was treated with BBr_3 to form the corresponding trihydroxylated derivative **5.38** by ether cleavage. However, this approach failed, most likely due to harsh workup procedures (Figure 5.19).

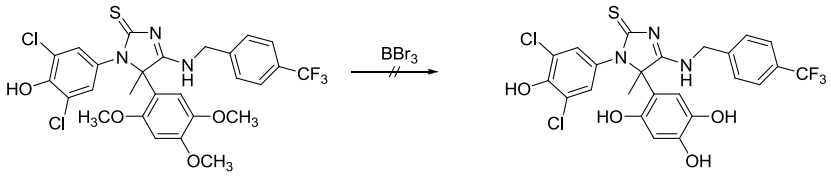


Figure 5.19 The synthesis of **5.38** via deprotection of **5.45** with BBr_3 failed.

Using the ketones shown in Figure 5.20 as building blocks did not result in the required products under the specified conditions. Besides, multicomponent synthesis with 2,2,2-trifluoro-1-phenylethanone and 1-(3,4-dichlorophenyl)-2,2,2-trifluoroethanone was not successful (Figure 5.21).

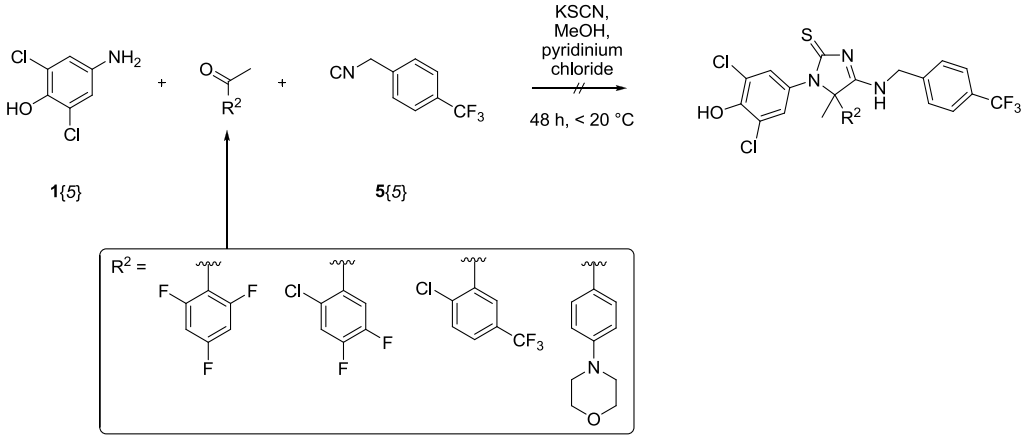


Figure 5.20 Several ketones (see boxed substituents) proved to be inappropriate as building blocks in multicomponent synthesis.

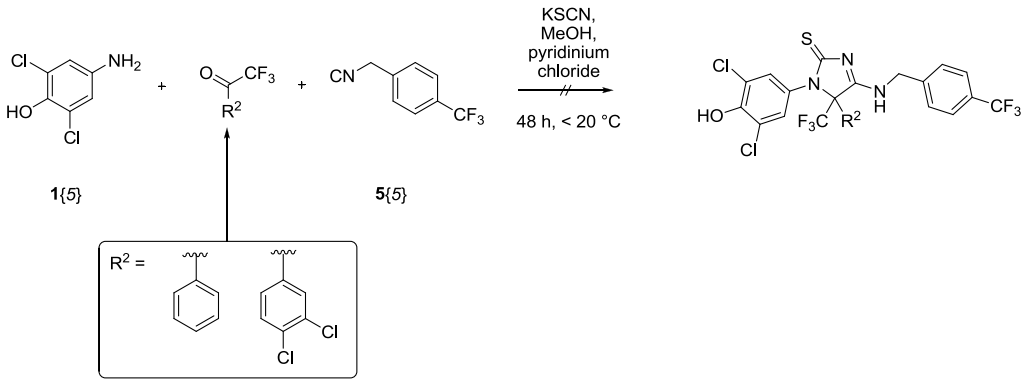
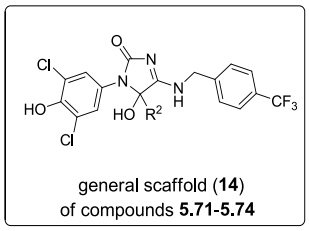


Figure 5.21 2,2,2-trifluoro-1-phenylethanone and 1-(3,4-dichlorophenyl)-2,2,2-trifluoroethanone proved to be inappropriate as building blocks in multicomponent synthesis.

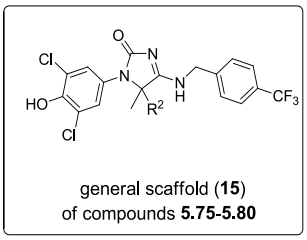
5.4.4 Synthesis of 4-amino-1*H*-imidazole-2(5*H*)-ones

A set of 10 4-amino-1*H*-imidazole-2(5*H*)-ones, oxo analogs of 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thiones and 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones, was synthesized by analogy with the protocol presented in sections 5.4.2 and 5.4.3 (potassium thiocyanate replaced by potassium cyanate) and investigated for inhibition of the streptococcal lyase *Sag*Hyal$_{4755}$. The design of these compounds was inspired by the (moderate to poor) activity of 4-amino-1*H*-imidazole-2(5*H*)-ones, which were among the first generation of inhibitors (see 5.3.2). The structures are summarized in Table 5.7.

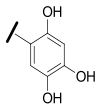
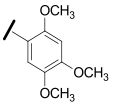
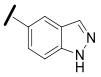
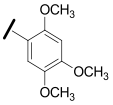
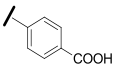
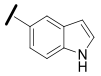
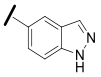
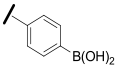
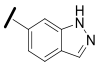
Table 5.7 Substitution pattern of 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-ones derivatives **5.71**-**5.74** and 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-ones derivatives **5.75**-**5.80**.



general scaffold (**14**)
of compounds **5.71-5.74**



general scaffold (**15**)
of compounds **5.75-5.80**

Chemset	Compound	Thio-analogue	R^2
14{*5,9,1*}	**5.71**	**5.22**	
14{*5,12,1*}	**5.72**	**5.26**	
14{*5,13,1*}	**5.73**	**5.27**	
14{*5,18,1*}	**5.74**	**5.32**	
15{*5,2,1*}	**5.75**	**5.45**	
15{*5,3,1*}	**5.76**	**5.46**	
15{*5,4,1*}	**5.77**	**5.49**	
15{*5,5,1*}	**5.78**	**5.50**	
15{*5,20,1*}	**5.79**	**5.61**	
15{*5,25,1*}	**5.80**	**5.66**	

As expected, autoxidation was observed for compounds **5.71**-**5.74**, presumably following the same mechanism as the corresponding 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thiones. In the literature the conversion of 4-imino-1,5-dimethyl-3-(4-nitrophenyl)imidazolidin-2-one to the 5-hydroxy derivative is described to occur in solution.[61] However, when comparing the oxo- with the corresponding thioxo-compounds, the insertion of oxygen in position C-5 was slower in case of substances **5.71**-**5.74**. Nevertheless, autoxidation resulted in a full conversion to derivatives with a molecular

mass of {[M + H]$^+$ +16}. Purification via HPLC and measurements of inhibitory activity were not performed, mass spectral analysis of crude products **5.71**-**5.74** are documented in section B.10 (appendix II). The attempt to apply an analogous protocol to the synthesis of **5.75**-**5.80** failed.

Interestingly, Ugi pointed out that 4-imino-2-thiohydantoins are formed in high yields only from ketones and not from aldehydes, whereas 4-iminohydantoins are easily accessible from aldehydes but not very well from ketones.[62, 63] Taking into account the experience with compounds **5.16**-**5.80**, Ugi's conclusion might be specified as follows. 2-imino-4-thio-hydantoins are formed in high yield from both aromatic ketones and aldehydes. However, products formed from aldehydes are prone to oxidation. Further decomposition is fostered depending on the substitution pattern of the aromatic aldehyde (e.g. (poly-)hydroxylated aromatic substituents). Stable products are accessible from aryl ketones unless they are hydroxylated. 4-Iminohydantoins are accessible from aromatic aldehydes, and autoxidation occurrs by analogy with the decomposition of the corresponding thiohydantoines. In accordance to Ugi's observations, aromatic ketones gave low yields under the specified conditions.

5.4.5 Oxidation of 4-amino-1*H*-imidazole-2(5*H*)-thiones

Compound **5.22** (cf. Table 5.3) was selected to monitor the autoxidation process over a period of 192 hours. In a qualitative experiment, samples containing **5.22** were analyzed by HPLC-MS in 24 hour intervals. Conditions of synthesis and storage such as temperature, exposure to light and composition of the solvent were systematically varied. A detailed description of the parameters is given in Table 5.8.

Table 5.8 Experimental settings for samples containing molecule **5.22**.

Measurement		**Experiment**	
No.	Description	No.	Description
1	completion of synthesis	1	daylight; ambient temperature; addition of sodium sulfate
2	+ 24 h	2	light (24 h); ambient temperature
3	+ 48 h	3	UV-light (254 nm); ambient temperature; addition of sodium sulfate
4	+ 72 h	4	daylight; ambient temperature
5	+ 96 h	5	light (24 h); ambient temperature; addition of sodium sulfate
6	+ 120 h	6	UV-light (λ = 254 nm); ambient temperature
7	+ 144 h	7	light protection; ambient temperature; addition of sodium sulfate
8	+ 168 h	8	light protection; +7 °C
9	+ 192 h	9	light protection; -20 °C; addition of sodium sulfate
		10	light protection; ambient temperature
		11	light protection; +7 °C; addition of sodium sulfate
		12	light protection; -20 °C

The molecular peaks of “compound 1” (**5.22**, $[M + H]^+$, *m/z* = 557) and “compound 2” (**5.22**, $\{[M + H]^+ + 16\}$, *m/z* = 573) were detected as ion counts from mass spectral analysis. To observe the process of oxidation for the ensemble of 12 experiments, ionic counts were plotted versus measurements. The results are displayed in Figure 5.22.

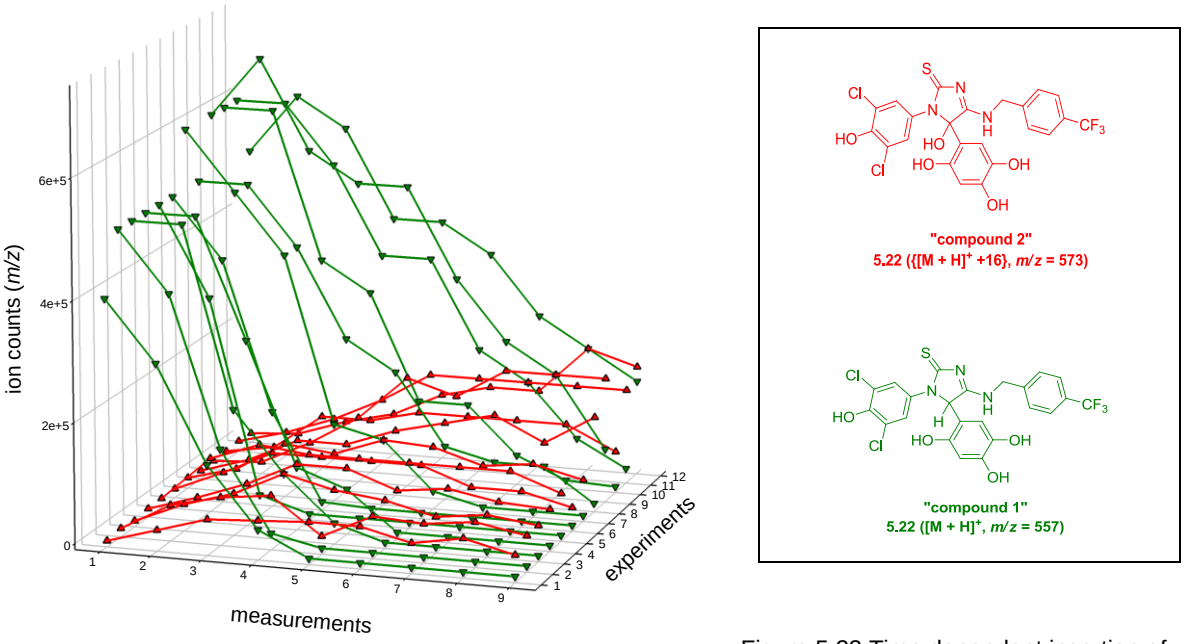


Figure 5.22 Time dependent insertion of oxygen into **5.22** (qualitative experiment).

As a result, temperature (-20 °C, +7 °C, +25 °C), light (λ = 250 nm, daylight) or condensed water in organic solvents did not retard oxidation and the formation of the $\{[M + H]^+ + 16\}$ derivative of **5.22** (“compound 2”). Besides, further decomposition of the “$\{[M + H]^+ + 16\}$ substance” became obvious from the mass spectrum 340 hours after completion of the synthesis.

The air-sensitivity of **5.22** was examined in more detail by means of a quantitative experiment, monitored by HPLC-MS analysis. Mass spectra were recorded from 0 to 336 hours after the completion of the synthesis. The investigations were performed with the crude products which were stored in glass vessels in solution (solvent: anhydrous methanol). Substance **5.62** was used as internal standard to analyze the time dependent conversion of $[M + H]^+$ (*m/z* = 557) to the oxidized **5.22** corresponding to a mass of *m/z* = 573 $\{[M + H]^+ + 16\}$. On column, the investigated compounds showed a sufficient separation of molecular peaks (cf. Figure 5.23). Detailed HPLC traces and molecular peaks under the specified conditions are given in section B.11 (appendix II).

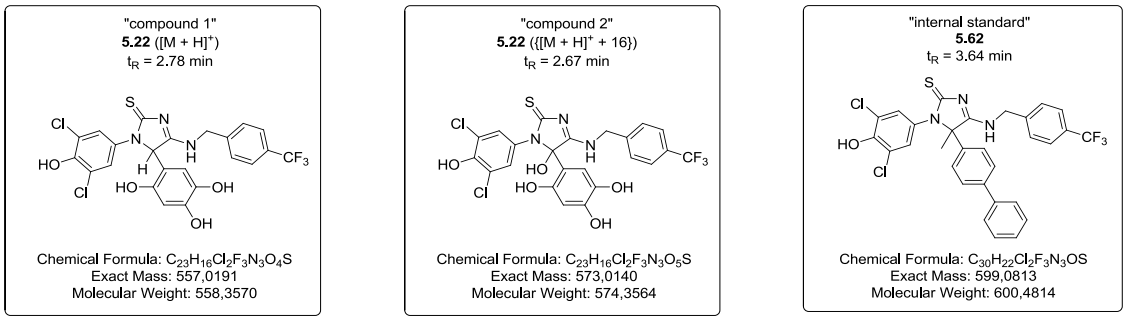


Figure 5.23 Structures, formulas and molecular masses of "compound 1" (**5.22**, $[M + H]^+$), "compound 2" (**5.22**, $\{[M + H]^+ +16\}$) and **5.62**.

To quantify the results, the peak area recorded for mass signal $[M + H]^+$ (*m/z* = 599) of **5.62** served as reference. The mean peak area derived from triplicate measurements of the internal standard was set to 100 %. Mean peak areas (triplicates) of "compound 1" the $[M + H]^+$ species of **5.22** was correlated to the reference and expressed as percentual value. The same procedure was applied to the $\{[M + H]^+ + 16\}$ species ("compound 2") of **5.22**. The time dependent process is plotted in Figure 5.24.

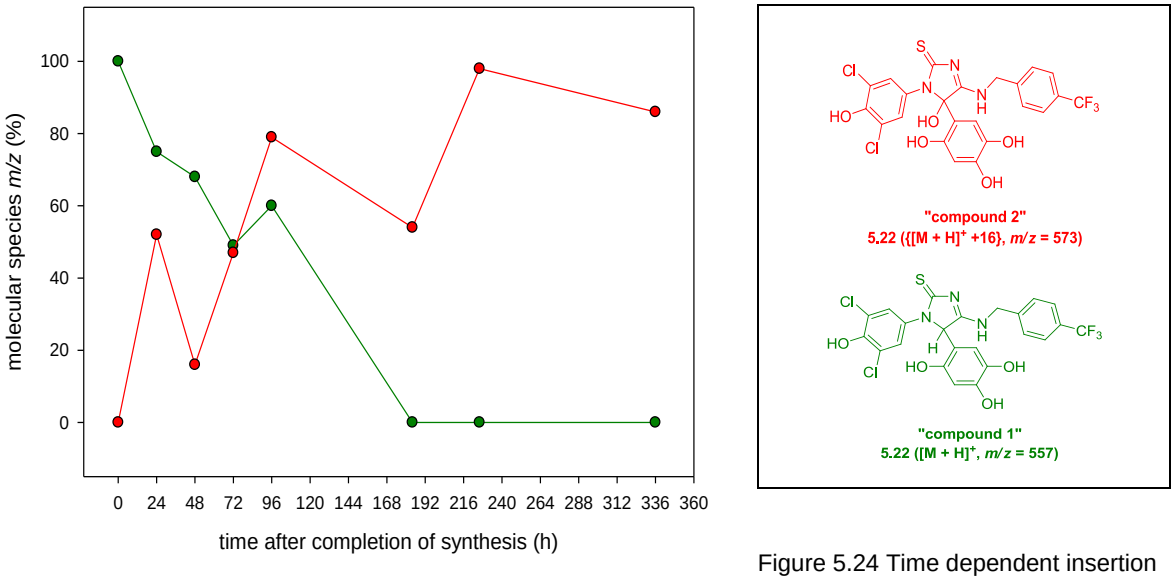


Figure 5.24 Time dependent insertion of oxygen to **5.22** (quantitative experiment)

Evaluation of data clearly showed a depression of the $[M + H]^+$ (*m/z* = 557) molecular peak of "compound 1" over 7 days. Within this time span, the *m/z* percentage value dropped from 100 % to 0 %. The opposite effect was observed for $\{[M + H]^+ + 16\}$ ("compound 2", *m/z* = 573), representing the predominant molecular species between 184 and 336 hours after completion of the experiment. As a consequence,

pharmacological tests of purified compounds **5.16**-**5.25** were performed 8 days after synthesis. As mentioned above, additional molecular fragmentation was observed after 340 hours. Hence, due to lack of chemical stability, these compounds were inappropriate for the determination of IC_{50} values, as side products, which might falsify the pharmacological data, could not be excluded.

Only very few information from the literature is available that might help to explain the observed insertion of oxygen. In fact, there was no preceding article on the molecules of interest, the 4-amino-1*H*-imidazole-2(5*H*)-thiones. However, Morel and coworkers, who performed a detailed study on the three-component cyclocondensation of 4-amino-2-(methylthio)imidazolidinium salts, observed autoxidation among several imidazole derivatives, which are structurally related to the compounds presented in this thesis. In particular, they found that 1-methyl-2-(methylthio)-5-phenyl-3-isopropyl-4-(isopropylamino) imidazolium chlorides (**16**) can be degraded to thioxoimidazolidines (**17**) via methyl chloride elimination (Figure 5.25). This was accompanied by an autoxidation in the presence of atmospheric oxygen (**18**, **19**).[58] Structure **19** was confirmed by mass and NMR spectral data. Besides, the structure was suggested due to the presence of a strong O-H stretching band in the infrared spectra of the isolated molecules. The authors reported that there were no details available regarding the oxidation process. In the same paper they postulate the intermediate hydroperoxide structure (**18**), which was reported in literature for related cyclic and acyclic systems.[64-66] Interestingly, Morel and coworkers were able to confirm the structural assignment of a 5-hydroxy derivative (**20**) by X-ray diffraction analysis.

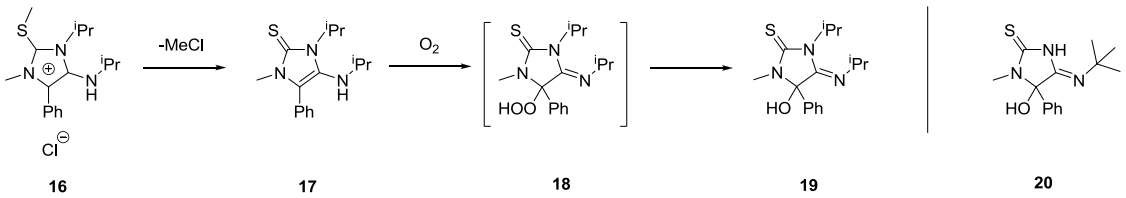


Figure 5.25 Autoxidation of 1-methyl-2-(methylthio)-5-phenyl-3-isopropyl-4-(isopropylamino)imidazolium chloride (**12**) and confirmed molecular structure of derivative **20** (according to Morel[58]).

In 2002, Törnqvist et al. elucidated the formation and degradation of some phenylthiohydantoins. In their study, the authors refer to an autoxidation process observed for 3-phenyl-1-methylthiohydantoin (**21**) under basic conditions (Figure 5.26).[67] They recommend the addition of oxygen to the carbanion moiety α to the carbonyl group.[68] According to the suggested mechanism, the hydrogen peroxide anion is eliminated to form a resonance-stabilized immonium ion that finally accepts a nucleophile (cf. **22**-

24a/b).[67] In addition, they employed $^{18}O_2$ for the oxidation of **23** and received molecules of structure **24b**, incorporating [^{18}O] in 50 % yield.

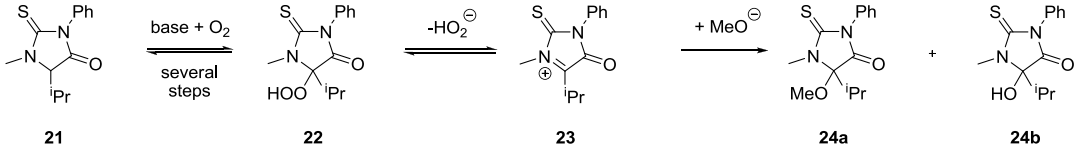


Figure 5.26 Autoxidation of 3-phenyl-1-methylthiohydantoin (**21**) (according to Törnqvist[67]).

Very recently, Angelova et al. described the chemoselective autoxidation of structural closely related 4-imino-1,5-dimethyl-3-(4-nitrophenyl)imidazolidin-2-one (**25**) to the 5-hydroxy derivative **28** (Figure 5.27).[61] In their paper, they present a mechanism for the conversion to the hydroxyl product. Here, they discuss a mechanism of enamine autoxidation involving single electron transfer from the tautomeric enamine to molecular oxygen (**26**). Concerning the hydroperoxide moiety they propose the formation of the hydroxyl product from a homolytic cleavage of the peroxide species (cf. **27**).[61]

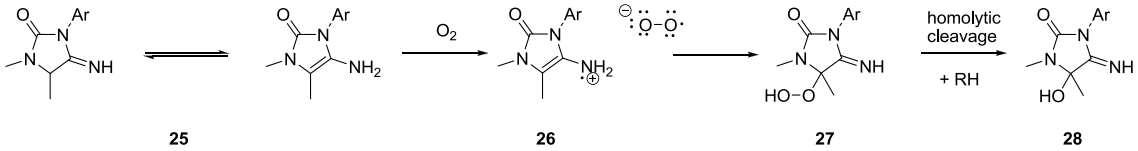
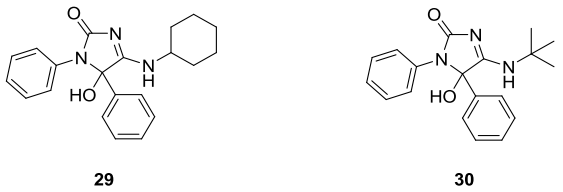


Figure 5.27 Autoxidation of 4-imino-1,5-dimethyl-3-(4-nitrophenyl)imidazolidin-2-one (**25**) (according to Angelova[61]).

Some hints to X-ray structural analysis of 4-amino-1*H*-imidazole-2(5*H*)-ones were found in molecules that were investigated by Fehlhammer and coworkers in two papers.[69, 70] Initially aiming at organo-metallic four-component condensations and the investigation of crystal packing modes of imidazole derivatives, some compounds were synthesized by multicomponent reaction synthesis. The authors obtained crystal structures of two oxidized entities (4-(cyclohexylamino)-5-hydroxy-1,5-diphenyl-1*H*-imidazol-2(5*H*)-one **29**, 4-(*tert*-butylamino)-5-hydroxy-1,5-diphenyl-1H-imidazol-2(5*H*)-one **30**). As indicated, both of them showed a hydroxyl group in position C-5 (Figure 5.28). However, Fehlhammer and coworkers suspected oxidation during the synthesis to give the observed molecules, but did not further evaluate or analyze this phenomenon.

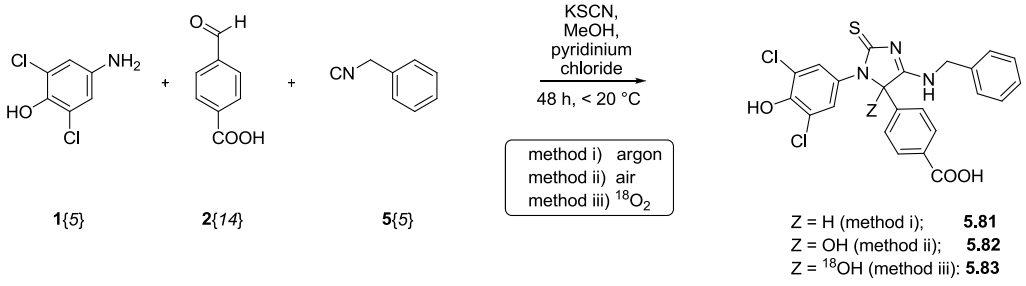


29 **30**

Figure 5.28 Structures of crystallized 4-amino-1*H*-imidazole-2(5*H*)-ones **29** and **30** (adopted from Andre[70]).

As stated above, according to the literature, there is only little knowledge about the autoxidation and insertion of oxygen. The cited studies might explain the occurrence of {$[M + H]^+ + 16$} molecular structures due to the presence of atmospheric oxygen. Besides, it remained unclear, how fast the process of oxidation takes place and whether the insertion of oxygen conditions might be prevented under certain conditions.

As a consequence, the process of oxidation observed for compounds **5.26**-**5.35**, was studied in detail by means of [^{18}O] labeling and HPLC-MS analysis. In particular, the focus was set to the recommended insertion of oxygen in position C-5 to yield 4-amino-1*H*-imidazole-2(5*H*)-thiones. Three experiments were performed in parallel to elucidate the behavior of the products when exposed to an atmosphere of argon, air and $^{18}O_2$, respectively. For the first case, under an atmosphere of argon, the generation of non-hydroxylated molecules was expected. For the latter two cases, exposure to air or a concentrated atmosphere of $^{18}O_2$, respectively, the incorporation of oxygen to position C-5 was expected. For the formation of the corresponding target molecules **5.81**-**5.83**, 4-amino-2,6-dichlorophenol (**1**{*5*}) was treated with 4-formylbenzoic acid (**2**{*14*}). Subsequently, (isocyanomethyl)benzene (**5**{*5*}) was added, combined with pyridinium chloride in anhydrous methanol (Figure 5.29).



1{*5*} **2**{*14*} **5**{*5*}

Z = H (method i); **5.81**
Z = OH (method ii); **5.82**
Z = ^{18}OH (method iii): **5.83**

Figure 5.29 Multicomponent synthesis of 4-(4-(benzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl)benzoic acid derivatives **5.81**-**5.83**. Reagents and conditions: (i) argon atmosphere, KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C, (ii) KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C, (iii) $^{18}O_2$-atmosphere, KSCN, anhydrous MeOH, pyridinium chloride, 48 h, < 20 °C.

Compounds **5.81**-**5.83** were synthesized at the same time under identical general conditions. After 2 hours and 72 hours after completion of the experiment, the crude reaction mixtures were analyzed by HPLC-MS. A detailed description, including the generation of $^{18}O_2$, and data of MS analysis is given in the experimental section.

In summary, under an atmosphere of argon, LC-MS control of the crude reaction mixture revealed $[M + H]^+$ (*m/z* = 485) as the predominant molecular peak. The corresponding experiment under atmospheric oxygen showed primarily the $\{[M + H]^+ +16\}$ (*m/z* = 501) species. Moreover, when $^{18}O_2$ was employed for the oxidation, ^{18}O was incorporated to C-5 and, as shown by LC-MS, formed the $\{[M + H]^+ +18\}$ (*m/z* = 503) molecule (Figure 5.30).

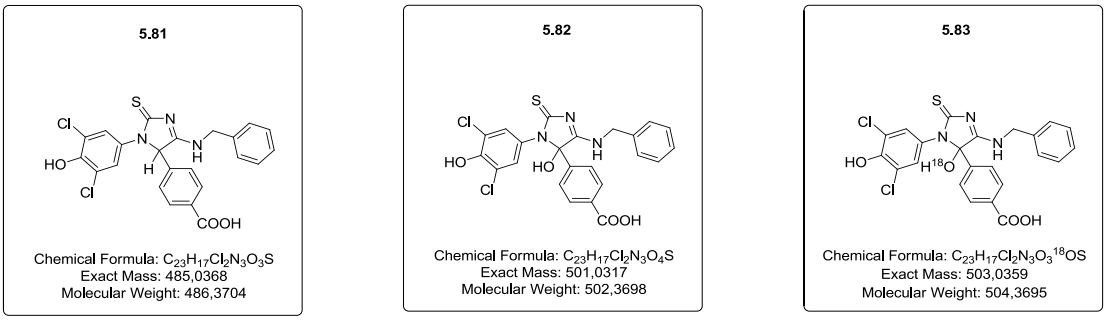


Figure 5.30 Molecular structures of 4-(4-(benzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl)benzoic acid derivatives **5.81**-**5.83**.

As a consequence, the data indicate autoxidation of 4-amino-1*H*-imidazole-2(5*H*)-thiones via the insertion of molecular oxygen from air. This process appears to be irreversible and is affected immediately by contact with air. Under inert gas conditions (argon), this process can be prevented, but the first contact with oxygen-containing atmosphere leads to the formation of oxidized compounds.

5.4.6 X-ray structure of 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones

To confirm the structures of the synthesized 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones, examples of compounds were crystallized for investigations by X-ray diffraction analysis. Whereas crystallization of **5.28** from methanol did not yield suitable crystals, compound **5.45** gave crystals, which were appropriate for X-ray analysis. The X-ray structure of **5.45** is shown in Figure 5.31.

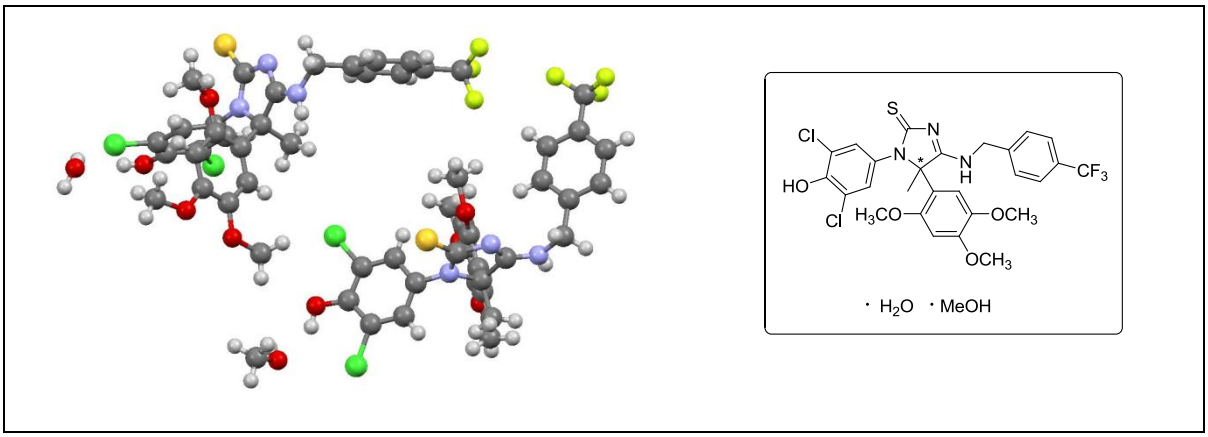


Figure 5.31 X-ray crystal structure of rac-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)-benzylamino]-5-(2,4,5-trimethoxyphenyl)-1*H*-imidazole-2(5*H*)-thione **5.45**.

The substance formed a single crystalline phase in which both enantiomers (*R* and *S*) were present in an ordered 1:1 ratio in the elementary cell. To describe the three-dimensional structure in more detail, an ORTEP-style plot and labeling scheme of (5*S*)-**5.45** is shown in Figure 5.32 and the experimental details (crystal data and structure refinement) including atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, torsion angles and hydrogen bonds are given in section A.3.1 (appendix I).

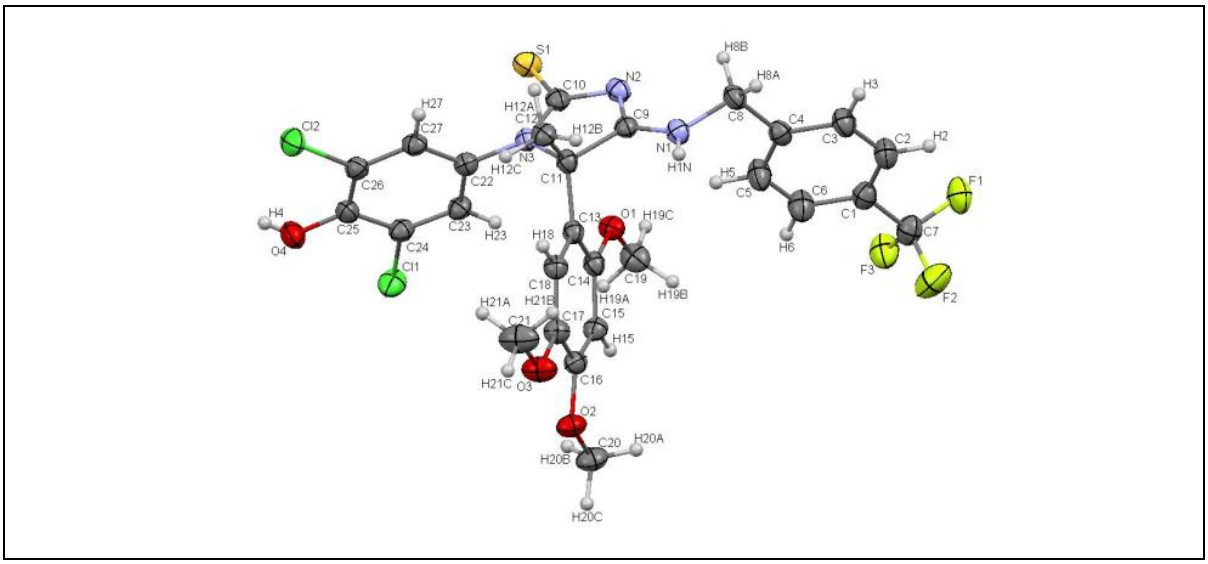


Figure 5.32 Molecular structure (ORTEP-style) of (5*S*)-**5.45** including atomic numbering scheme.

The molecular structure of (5*S*)-**5.45** shows a five-membered, non-aromatic heterocycle with $S_{(1)}$, $N_{(1)}$ and $C_{(8)}$ lying in plane. The phenyl rings are inclined to the imidazole-thione ring at various angles. The torsion angles $C_{(10)}$-$N_{(3)}$-$C_{(22)}$-$C_{(23)}$ and $N_{(3)}$-$C_{(11)}$-$C_{(13)}$-$C_{(14)}$ were at 71.9 ° and 55.3 °, respectively. The third torsion angle of (5*S*)-**5.45**, namely $C_{(8)}$-$N_{(1)}$-

$C_{(9)}$-$N_{(2)}$ is 9.9 °. Methoxy substituents were in plane with the phenyl ring fused to $C_{(11)}$. The amine hydrogen atom $H_{(1N)}$ is *trans* with respect to $N_{(2)}$. The corresponding *cis* conformation was not achieved, most likely due to steric hindrance of the bulky residues on $N_{(1)}$ and the neighboring 2,4,5-trimethoxyphenyl residue. It should be noted, that hydrogen was not found at the $N_{(2)}$ atom, in spite of reliable localization of the hydrogen atoms, even at the periphery of the molecule. According to the X-ray structural investigations, there is no hint to additional tautomeric forms of **5.45**. To substantiate these findings with respect to possible tautomeric isomers, NMR (^{1}H, ^{13}C) were performed.

As shown in Figure 5.33, two isomers were conceivable for **5.45** by a proton shift along the $N_{(2)}$=$C_{(9)}$-$N_{(1)}H_{(1N)}$ bond, resulting in the formation of tautomeric "imine" (structure **a**) and "amine" (structure **b**) structures. The latter "amine" form shares a resonance structure (structure **c**).

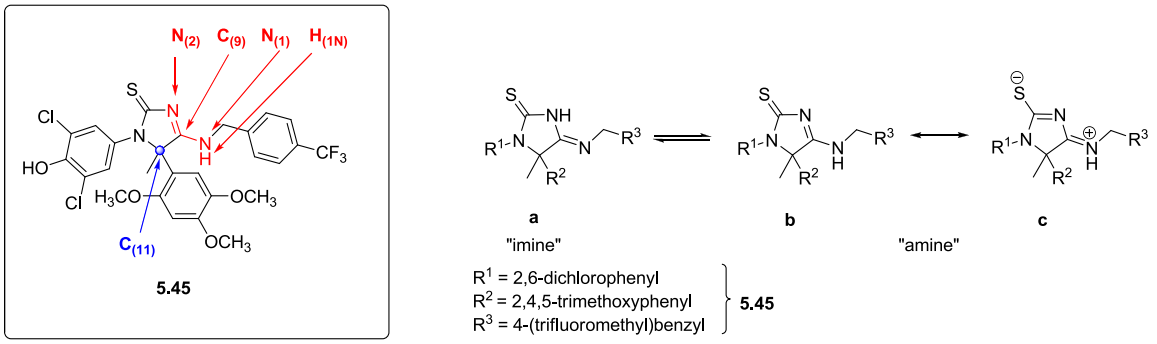


Figure 5.33 Tautomers and resonance structures of 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones; compound **5.45** as an example.

Medvedeva et al. reported that structurally related thioiminohydantoins prefer the amine form (see Figure 5.33, structure **b**) in the case of benzyl-substituents for R^3. In the same paper, they cite a shift of the tautomeric equilibrium in favor of the imine form when there is a decrease in the basicity of the exocyclic nitrogen atom.[56] The NMR spectra of some 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones are provided in Table 5.9.

Table 5.9 ^{1}H-NMR chemical shifts (600 MHz, DMSO-d_6, 298 K) of selected 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones.

Compound	**δ (ppm)[a]:** N***H***CH_2-*p*-C_6H_4-CF_3	**δ (ppm)[a]:** NHC***H*****$_2$**-*p*-C_6H_4-CF_3	**δ (ppm)[a]:** C_{quat}, ***C***S	**δ (ppm)[a]:** C_{quat}, ***C***-4
5.44	9.22 (t, *J* = 6.0 Hz, 1H)	4.74 – 4.61 (m, 2H)	195.52	180.70
5.45	8.88 (t, *J* = 5.9 Hz, 1H)	4.71 (dd, *J* = 15.5, 6.3 Hz, 1H), 4.57 (dd, *J* = 15.5, 6.3 Hz, 1H	195.03	181.57

5.51	8.97 (t, *J* = 6.0 Hz, 1H)	4.75 (dd, *J* = 15.4, 6.5 Hz, 1H), 4.54 (dd, *J* = 15.4, 5.5 Hz, 1H)	195.34	181.40
5.54	9.05 (t, *J* = 5.7 Hz, 1H)	4.74 (dd, *J* = 15.3, 6.1 Hz, 1H), 4.62 (dd, *J* = 15.3, 5.7 Hz, 1H)	195.35	181.66
5.55	9.22 (t, *J* = 6.1 Hz, 1H)	4.77 (dd, *J* = 15.2, 6.4 Hz, 1H), 4.61 (dd, *J* = 15.2, 5.7 Hz, 1H)	195.23	180.29
5.56	9.24 (t, *J* = 5.9 Hz, 1H)	4.68 (d, *J* = 5.8 Hz, 2H)	195.51	180.49
5.57	9.23 (t, *J* = 6.0 Hz, 1H)	4.67 (d, *J* = 5.9 Hz, 2H)	195.66	180.34
5.58	9.22 (t, *J* = 5.7 Hz, 1H)	4.67 (d, *J* = 5.4 Hz, 2H,	195.69	180.29
5.59	9.27 (t, *J* = 5.9 Hz, 1H)	4.75 – 4.61 (m, 2H)	195.85	180.10
5.60	9.32 (t, *J* = 5.9 Hz, 1H)	4.73 – 4.62 (m, 2H)	196.03	179.84
5.61	9.19 (t, *J* = 6.0 Hz, 1H)	4.71 – 4.61 (m, 2H)	195.57	180.71
5.64	9.20 (t, *J* = 6.0 Hz, 1H)	4.67 (qd, *J* = 15.5, 6.0 Hz, 2H)	195.79	180.63
5.65	9.26 (t, *J* = 5.9 Hz, 1H)	4.71 – 4.62 (m, 2H)	195.57	180.40

[a] relative to TMS

The vicinal $^{3}J_{\text{H-C-N-H}}$ coupling of the CH_2 group with the neigbouring NH (NHC***H***$_2$-*p*-C_6H_4-CF_3) became visible (Figure 5.34). The values of the ^{3}J coupling constant covered a range from 5.4 to 6.5 Hz, the geminal $^{2}J_{\text{H-C-H}}$ coupling from 15.2 to 15.5 Hz. For the investigated substances **5.45**, **5.51**, **5.54** and **5.55** the signals the CH_2 protons were separated, resulting in dubletts of dubletts (cf. Table 5.9).

NHC***H***$_2$-*p*-C_6H_4-CF_3:
compounds **5.45**, **5.51**, **5.54**, **5.55**

NHC***H***$_2$-*p*-C_6H_4-CF_3:
compound **5.64**

NHC***H***$_2$-*p*-C_6H_4-CF_3:
compounds **5.44**, **5.56**-**5.61**, **5.65**

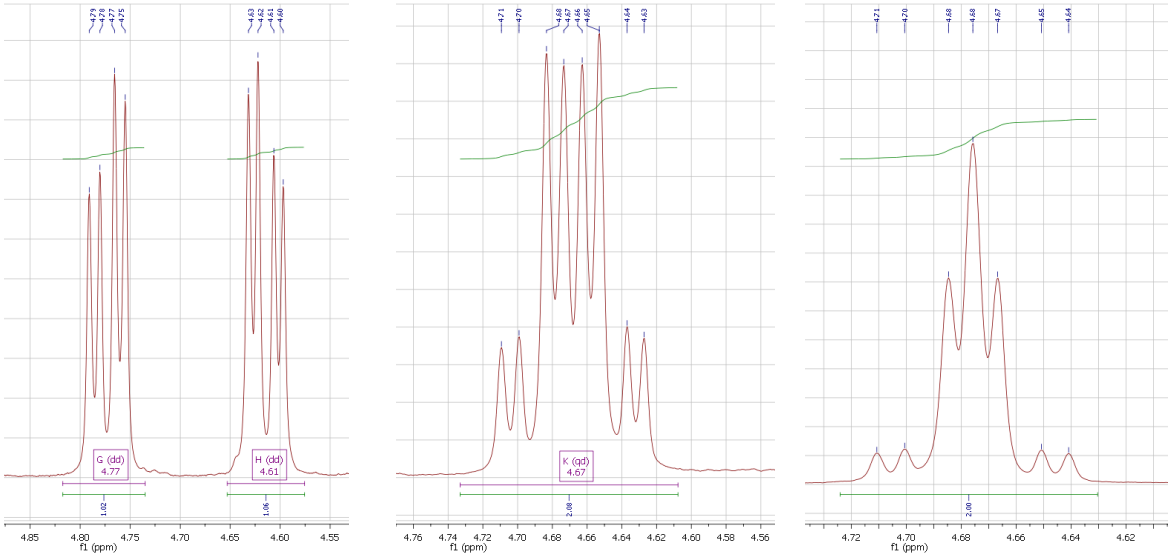


Figure 5.34 Coupling of the CH_2 protons (NHC***H***$_2$-p-C_6H_4-CF_3) with the neigbouring NH of selected 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones.

As a result, the ^{1}H-NMR spectra confirm that these compounds prefer the “amine” tautomeric form in deuterated DMSO at ambient temperature (298 K). The suggested structure is also in agreement with the ^{13}C-NMR data. It is important to mention, that a double set of signals, e.g. for the C=S group in the range of 175 – 180 ppm, suggesting the “imine” structure were not observed under these conditions. Likewise, only one signal was visible for the quaternary carbon atom $C_{(9)}$ at approximately 180 ppm.

It should be stressed that the synthesized 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones as well as the analogous amino-5-hydroxy-1*H*-imidazole-2(5*H*)-ones were synthesized and characterized as racemates. It may be speculated about a discrimination of the stereoisomers by hyaluronidases. Enantioseparations have not been performed so far, as the inhibitory activities of the racemic mixtures were still moderate.

In summary, it is concluded from X-ray analysis and NMR data that the synthesized cyclic amidines exist as tautomers with exocyclic NH group, namely as 4-amino-1*H*-imidazole-2(5*H*)-thiones.

5.5 Pharmacological results and discussion of second generation inhibitors

5.5.1 General conditions and screening mode

Cf. section 5.3.1

The IC_{50} values of the compounds were calculated based on 8-point concentration-response curves (experiment performed in duplicate).

5.5.2 Inhibitory activities of screening compounds on *Sag*Hyal$_{4755}$

Among the tested 352 generated 4-amino-1*H*-imidazole-2(5*H*)-thiones, 25 compounds were identified as screening hits representing a hit rate of 7.1 %. These hits were located on plate **ori.hya.44** (11 hits), **ori.hya.45** (7 hits), **ori.hya.46** (4 hits) and **ori.hya.47** (3 hits). A detailed on-screen assay validation and representation of screening data is given in appendix II (sections B.8.3, B.9.4). HPLC-MS analysis revealed the oxidized molecular species {$[M + H]^+$ +16} as the predominant structures. The percentual enzyme inhibition determined for the 4-amino-1*H*-imidazole-2(5*H*)-thione screening hits are summarized in Table 5.10.

Table 5.10 Inhibition of *Sag*Hyal$_{4755}$ by the screening hits from plates **ori.hya.44**-**47**.

Compound	Location (plate_well)	*Sag*Hyal$_{4755}$ % inhibition[a]
	ori.hya.44_A2	58 ± 2
	ori.hya.44_E2	53 ± 6
5.16	**ori.hya.44_A7**	64 ± 4
5.17	**ori.hya.44_D2**	62 ± 3
	ori.hya.44_D3	51 ± 5
	ori.hya.44_D5	62 ± 3
	ori.hya.44_D6	59 ± 3
	ori.hya.44_D7	61 ± 3
	ori.hya.44_A9	58 ± 3
	ori.hya.44_D9	60 ± 3
	ori.hya.44_D12	50 ± 4
5.18	**ori.hya.45_D2**	62 ± 2
	ori.hya.45_D5	58 ± 2
	ori.hya.45_D6	50 ± 2
	ori.hya.45_D7	57 ± 3
	ori.hya.45_D9	53 ± 4

5.21	**ori.hya.45_D10**	68 ± 4
	ori.hya.45_E10	53 ± 5
	ori.hya.46_D2	53 ± 1
	ori.hya.46_D3	57 ± 2
	ori.hya.46_D7	53 ± 2
	ori.hya.46_D9	61 ± 1
	ori.hya.47_D5	58 ± 1
	ori.hya.47_D7	62 ± 2
	ori.hya.47_E7	51 ± 5

[a] mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were set to an assumed final assay concentration of 200 µM; percent inhibition determined at pH 5.0 in the automated 96-well turbidimetric assay.

5.5.3 Inhibitory activities of purified compounds on *Sag*Hyal$_{4755}$

5.5.3.1 4-Amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thiones

Among the 25 identified hits, substances **5.16**-**5.18**, **5.21** (highlighted in Table 5.10) were selected, equaling the most potent compounds of this study. Together with the distinguished compounds **5.19**, **5.20** and **5.22-5.25** a small library of 10 purified 4-amino-1*H*-imidazole-2(5*H*)-thiones was subjected for preparative synthesis and retested on *Sag*Hyal$_{4755}$. The IC_{50} values determined for the 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thione derivatives **5.16**-**5.25** are summarized in Table 5.11.

Table 5.11 Inhibitory activity[a] and calculated $logD_{5.0}$ values[b] of purified 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thione derivatives **5.26**-**5.35**.

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (µM)[a]**	**BTH IC$_{50}$ (µM)[a]**	**$logD_{5.0}$[b]**
5.16	850 ± 35	inactive	3.2
5.17	230 ± 15	inactive	3.5
5.18	335 ± 35	inactive	4.3
5.19	270 ± 25	inactive	3.1
5.20	310 ± 20	inactive	2.5
5.21	380 ± 10	inactive	3.4
5.22	80 ± 4.3	inactive	3.7
5.23	250 ± 20	inactive	3.4
5.24	230 ± 15	inactive	3.7
5.25	1200 ± 55	inactive	2.9

[a] mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 in the 96-well turbidimetric assay; [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

The investigation of the purified compounds **5.17**, **5.18** and **5.21** confirmed the inhibition of the target enzyme *Sag*Hyal$_{4755}$ with IC_{50} values of 230 µM (**5.17**), 335 µM (**5.18**) and 380 µM (**5.21**), respectively. An exception was **5.16**, showing a considerable decrease in inhibitory activity (Figure 5.35).

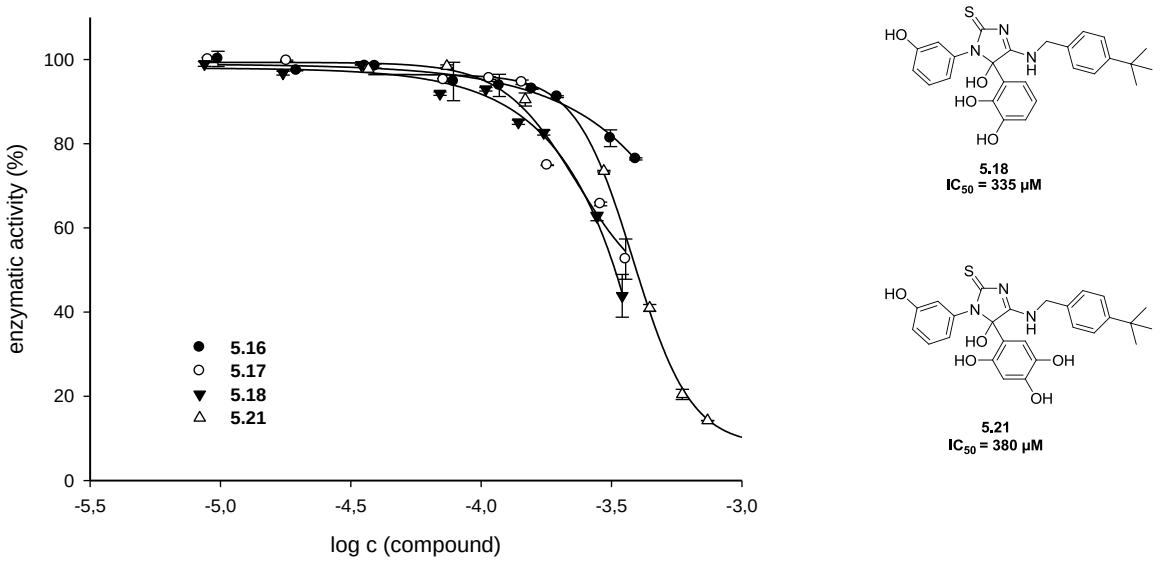


Figure 5.35 Concentration dependent inhibition of *Sag*Hyal$_{4755}$ by **5.16**-**5.18** and **5.21**.

Substances **5.19** (IC_{50} = 270 µM), **5.20** (IC_{50} = 310 µM), **5.23** (IC_{50} = 250 µM) and **5.24** (IC_{50} = 230 µM) showed moderate inhibition, compound **5.25** (1200 µM) was inactive. With an IC_{50} value of 80 µM, **5.22** represents a 3-fold more potent inhibitor compared to the next best substance of this library (Figure 5.36).

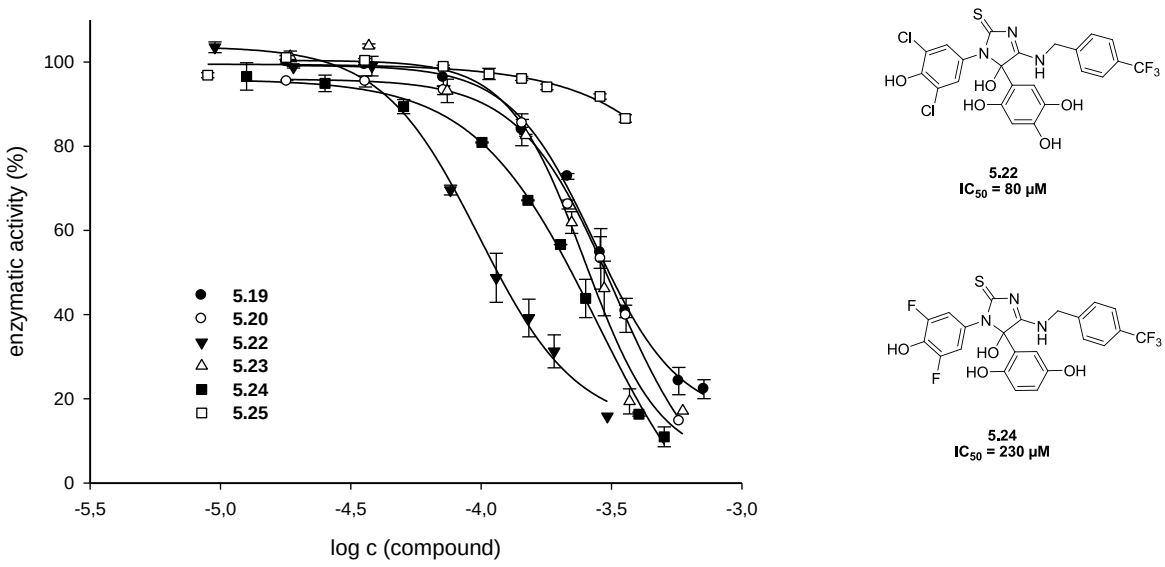


Figure 5.36 Concentration dependent inhibition of *Sag*Hyal$_{4755}$ in the presence of **5.19**-**5.20** and **5.22**-**25**.

Hence, **5.22** represented the most potent inhibitor among the 4-amino-1*H*-imidazole-2(5*H*)-thione series at this stage of the screening campaign. In view of the (calculated) $logD_{5.0}$ values, all inhibitors are expected to possess drug-like properties. However, chemical instability must be taken into account: the oxidized compounds with a mass of $\{[M + H]^+ + 16\}$ were stable molecules over a period of 14 days; thus, supposed that the hydroxy compounds represent the predominant molecular species, the measured inhibition of enzymatic activity can most probably be attributed to the product of autoxidation, although, other active molecules cannot be ruled out, e. g. $[M + H]^+$ or $[M + H]^+$ mixed with $\{[M + H]^+ + 16\}$. Additional molecular fragments might also contribute to the inhibitory activities of the investigated mixtures. In conclusion, the discussed compounds were inappropriate for a further hit to lead development.

The IC_{50} values determined for the 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thione derivatives **5.26**-**5.35** are summarized in Table 5.12.

Table 5.12 Inhibition of hyaluronidase activities[a] and calculated $logD_{5.0}$ values[b] of 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thione derivatives **5.26**-**5.35**.

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (µM)[a]**	**BTH IC$_{50}$ (µM)[a]**	**logD$_{5.0}$[b]**
5.26	70.4 ± 3.5	inactive	5.3
5.27	146 ± 1.5	inactive	5.2
5.28	7.9 ± 0.9	inactive	4.1
5.29	31.5 ± 1.3	60 % (c = 200 µM)	5.7
5.30	49.0 ± 2.4	inactive	6.1
5.31	180 ± 7.7	inactive	4.2
5.32	57.6 ± 1.5	inactive	4.9
5.33	133 ± 0.5	inactive	4.4
5.34	117 ± 5.4	inactive	4.9
5.35	55.7 ± 3.1	inactive	2.2

[a] mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were set to an assumed final assay concentration of 200 µM; IC_{50} values determined at pH 5.0 in the automated 96-well turbidimetric assay; [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Substances **5.26** and **5.27** were designed as stable, C-5 hydroxylated analogs of **5.22**. For **5.26** and **5.27** IC_{50} values of 70 µM and 146 µM, respectively, were calculated. As ring-hydroxylated products were unstable, a bioisosteric approach was applied. In a first attempt, the commercially available heterocyclic aldehydes, 1*H*-indole-carbaldehydes

(cf. **5.29**, **5.30**), 1*H*-benzo[*d*]imidazole-5-carbaldehyde (cf. **5.31**) and 1*H*-indazole-5-carbaldehyde (cf. **5.32**) were used as building blocks. The resulting products **5.29** (IC_{50} = 32 µM), **5.30** (IC_{50} = 49 µM) and **5.32** (IC_{50} = 58 µM) proved to be superior to the corresponding phenyl-substituted parent compounds. A 3-fold decrease in potency was investigated for **5.31**. In a second series, the phenyl group in the southern part of the "minimal inhibitor substrate" **5.26** was expanded in *para*-position by the addition of five- and six-membered ring heterocycles. Thereby substituents bearing imidazole (**5.33**), methylthiazole (**5.34**) and methylpiperazine moieties (**5.35**) were prepared to investigate the contribution of basic moieties to enzyme inhibition. Inhibitory activities at micromolar concentrations were retained (**5.33**: 133 µM, **5.34**: 117 µM). Interestingly, the investigation of the most basic compound **5.35** revealed an IC_{50} value of 56 µM. Considering the $logD_{5.0}$ value of 2.2, the molecule might offer beneficial properties to overcome the problem of extremely high plasma protein binding.

The desired chemical properties were furnished in **5.28**. Strikingly, with **5.28** the most potent inhibitor among 4-amino-1*H*-imidazole-2(5*H*)-thiones was found, revealing an IC_{50} value of 8 µM. Moreover the compound presumably incorporates drug-like properties with a calculated $logD_{5.0}$ of 4.1. The concentration dependent inhibition of *Sag*$Hyal_{4755}$ in presence of **5.28**-**5.30**, **5.35** is depicted in Figure 5.37.

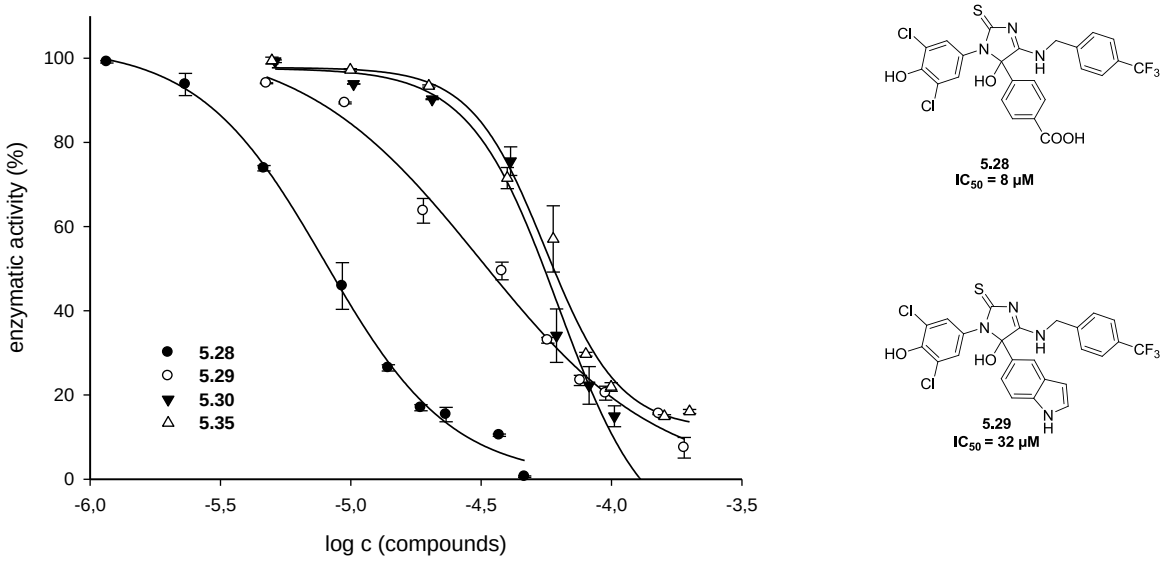


Figure 5.37 Concentration dependent inhibition of *Sag*$Hyal_{4755}$ in the presence of **5.28**-**5.30** and **5.35**.

5.5.3.2 4-Amino-5-methyl-1*H*-imidazole-2(5*H*)-thione derivatives

The IC_{50}-values determined for the 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thione derivatives **5.40**-**5.70** are summarized in Table 5.13.

Table 5.13 Inhibitory activities[a] and calculated $logD_{5.0}$ values[b] of 4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thione derivatives **5.40**-**5.70**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)[a]	$logD_{5.0}$[b]
5.40	inactive	inactive	3.9
5.41	350 ± 13.5	inactive	3.7
5.42	240 ± 14.8	inactive	2.4
5.43	810 ± 12.0	inactive	3.7
5.44	90.0 ± 4.7	inactive	6.4
5.45	50.4 ± 3.2	inactive	6.3
5.46	80.2 ± 2.8	inactive	5.3
5.47	47.9 ± 1.9	inactive	6.1
5.48	162 ± 3.0	inactive	4.4
5.49	125 ± 4.9	inactive	6.8
5.50	97.7 ± 2.1	inactive	6.0
5.51	90.3 ± 5.3	inactive	6.7
5.52	160 ± 7.2	inactive	6.5
5.53	154 ± 5.9	inactive	6.0
5.54	145 ± 7.8	inactive	5.9
5.55	130 ± 2.9	inactive	5.6
5.56	115 ± 1.5	inactive	6.7
5.57	120 ± 1.6	inactive	7.2
5.58	130 ± 3.0	inactive	7.4
5.59	95.1 ± 5.0	inactive	7.3
5.60	85.1 ± 4.4	inactive	6.2
5.61	70.2 ± 1.0	inactive	5.5
5.62	> 500	inactive	8.3
5.63	150 ± 8.8	inactive	5.8
5.64	100 ± 5.6	inactive	7.6
5.65	30 % (c = 120 µM)	inactive	6.8
5.66	59.0 ± 1.0	inactive	6.1
5.67	140 ± 5.6	inactive	3.4
5.68	136 ± 2.5	inactive	3.4

5.69	240 ± 10.8	50 % (c = 200 µM)	4.5
5.70	160 ± 7.8	inactive	5.4

[a] mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 in the auto 96-well turbidimetric assay; [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Compounds **5.44**, **5.45** and **5.49**-**66** were designed as 5-methyl derivatives of **5.22**. Substance **5.44** (IC_{50} = 90 µM), bearing a phenyl moiety without additional substituents, represents the prototypical molecule within this series. The introduction of methoxy residues yielding mono- (**5.51**, **5.52**), di- (**5.53**) and tri-methoxy derivatives (**5.54**, **5.55**), in general, revealed no major benefit regarding inhibitory activity. An exception was found with **5.45**, showing an IC_{50} value of 50 µM. Additional *para*-substituted moieties were introduced in compounds **5.46** and **5.56**-**5.62**. Halogen substituents in 1-(3,5-dichloro-4-hydroxyphenyl)- 5-methyl-4-(4-(trifluoromethyl)benzylamino)-1*H*-imidazole-2(5*H*)-thiones did not significantly influence the inhibitory activity, despite increased lipophilicity of molecules **5.56** to **5.58**. By contrast, when lipophilicity was reduced stepwise by *para*-CF_3 (**5.59**), *para*-NO_2 (**5.60**) and *para*-$B(OH)_2$ moieties (**5.61**), inhibition was slightly improved. Surprisingly, the 5-methyl analogue **5.46** of the potent inhibitor 5-hydroxy substance **5.28**, bearing a carboxylic group, showed a 10-fold lower inhibitory potency, although acidic groups were identified as activity-enhancing structural features in previous work. At first sight C-5 hydroxylation seemed to be superior to C-5 methylation. However, investigation of C-5 methylated compounds **5.49** (C-5 hydroxy analogue: **5.29**), **5.50** (C-5 hydroxy analogue: **5.32**) and **5.66** revealed only marginal differences in activity between the 5-methyl- and the 5-hydroxy-derivatives. Additional chain branching as in compounds **5.63** and **5.65** resulted in moderate to low enzyme inhibition. Substances **5.62** and **5.64** were designed to increase the lipophilicity by the incorporation of a bulky biphenylyl or naphthyl moiety. As a result, the biphenylyl substituted compound **5.62**, the analogue with highest (calculated) $logD_{5.0}$ value (8.3) within this series, showed only weak inhibition of *Sag*$Hyal_{4755}$. For naphthyl analogue **5.64** ($logD_{5.0}$ = 7.6) an IC_{50} value of 100 µM was calculated.

To substantiate the inhibitory activities and to elaborate structure-activity relationships of 4-imino-5-methyl-1*H*-imidazole-2(5*H*)-thiones, analogs of compound **5.46**, bearing a negatively charged benzoic acid moiety in position 5, were tested on *Sag*$Hyal_{4755}$. In order to find the right balance between lipophilicity (by experience a prerequisite for inhibitory activity) and drug-like properties, a matrix of virtually designed molecules was set up. An ensemble of 40 compounds (Figure 5.38) was generated by combination of 8 amines (x-axis) and 5 isocyanides (y-axis) (cf. Figure 5.39). The corresponding $logD_{5.0}$ values

(displayed on z-axis) were calculated for each molecule by the aid of ACDLabs software version 12.00 (Advanced Chemistry Development Inc., Toronto, Canada). From these data a 3-D mesh plot was drawn (Figure 5.39). The selected amine and isocyanide residues span a range from $logD_{5.0}$ 2.3 to 6.1.

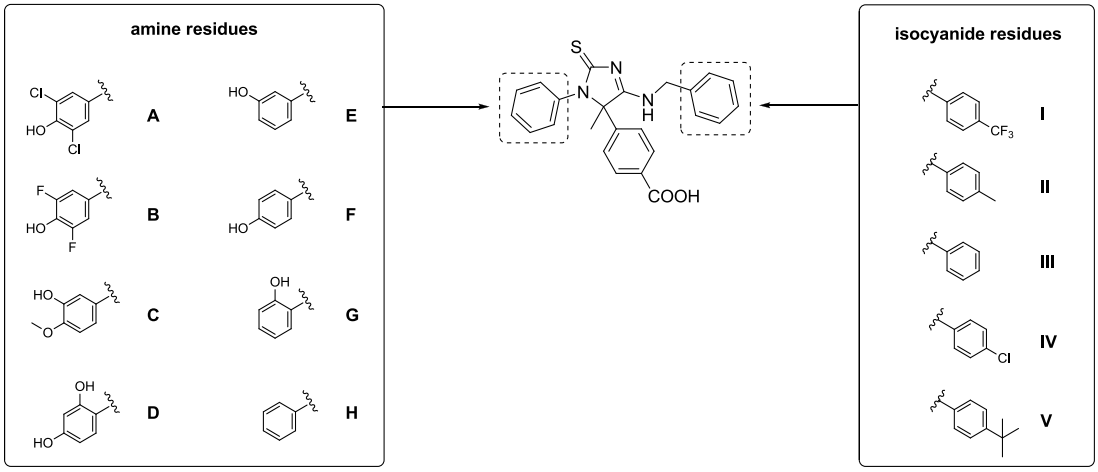


Figure 5.38 Amine residues (A-H) and isocyanide residues (I-V) selected as building blocks to virtually synthesize and to predict the properties of a small library of 4-(5-methyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl)benzoic acid derivatives.

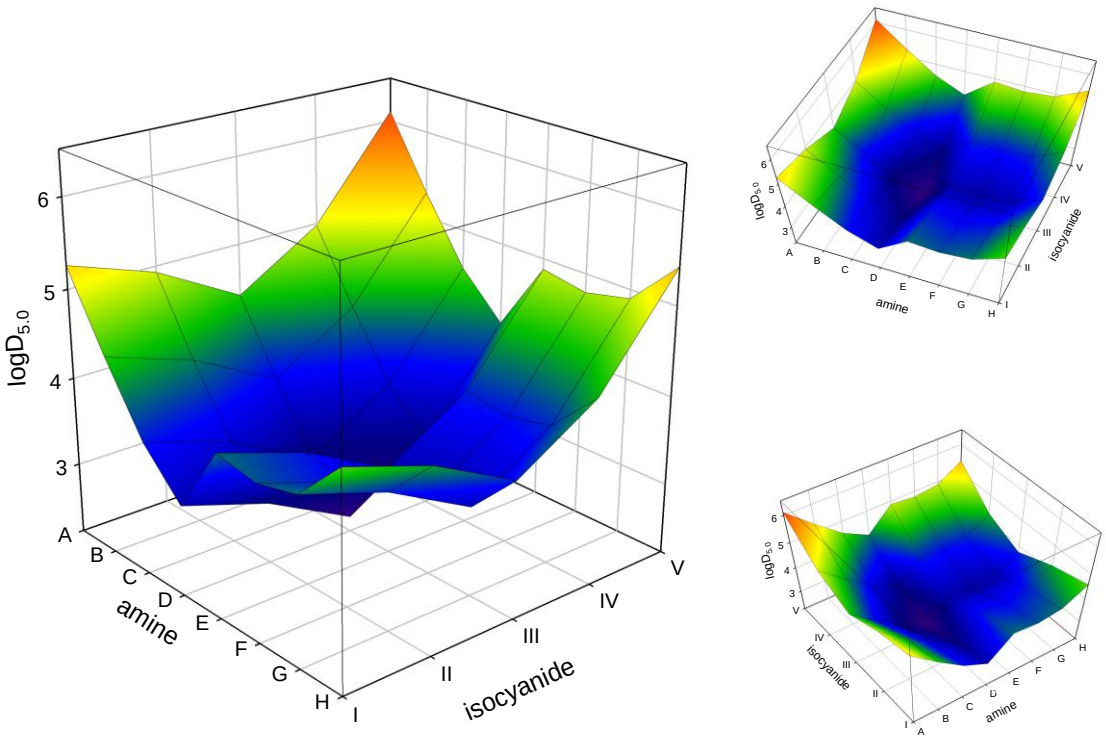


Figure 5.39 3-D mesh plot displaying calculated $logD_{5.0}$ values for a molecular matrix consisting of 8 amines and 5 isocyanides virtually used as building blocks as shown in Figure 5.38.

Out of 40 possible combinations, based on the predicted properties (Figure 5.39), a set of 10 representative molecules (**5.40**-**5.43**, **5.46**-**5.48** and **5.67**-**5.70**) was synthesized and tested for inhibition of SagHyal$_{4755}$. As expected, lipophilic inhibitors were most potent. In case of **5.47** (combination A-V) the (calculated) log$D_{5.0}$ of 6.1 corresponds to an IC_{50} value of 48 µM. When 4-amino-2,6-dichlorophenol was replaced by aniline as a building block (cf. **5.70**, combination H-V), the decrease in lipophilicity (log$D_{5.0}$ value = 5.4) was accompanied with a reduction of inhibitory potency to 160 µM. Substances of significantly lower log$D_{5.0}$ values, such as **5.40** (combination F-I, log$D_{5.0}$ = 3.9) and **5.43** (combination E-II, log$D_{5.0}$ = 3.7) turned out to be inactive or very weak inhibitors, respectively. However, moderate inhibitory activity was regained when the CF_3 group in **5.40** was replaced by a chlorine substituent (**5.41**, combination F-IV), despite a rather low log$D_{5.0}$ of 3.7. Interestingly, for **5.42**, synthesized from a combination of building blocks D-III, the calculated log$D_{5.0}$ was as low as 2.4 but the IC_{50} value amounted to 240 µM. Compared to the prototypical inhibitor **5.46** (combination A-I, log$D_{5.0}$ = 5.3), there was not increase in potency found for the remaining compounds **5.48** and **5.67**-**5.69**, which cover a log$D_{5.0}$ range from 3.4 to 4.5. Hence, no major trends in terms of structure-activity relationships (SAR) could be identified among the investigated molecules. The concentration dependent effects of **5.40**, **5.46** and **5.69** on the enzymatic activity of *Sag*Hyal$_{4755}$ are depicted in Figure 5.40.

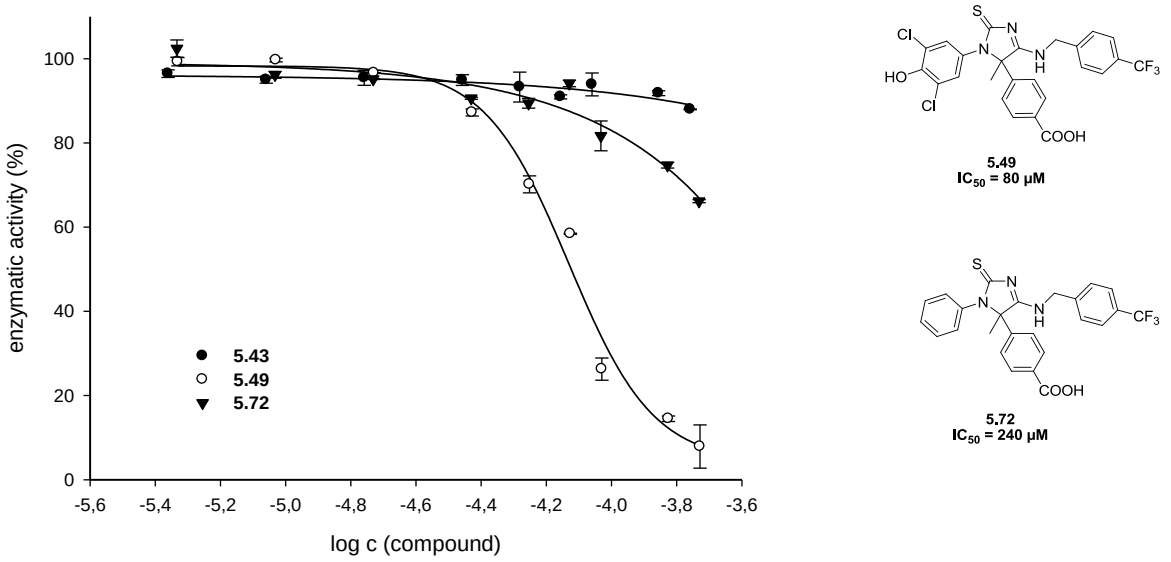


Figure 5.40 Concentration dependent effects of **5.43**, **5.49** and **5.72** on the activity of *Sag*Hyal$_{4755}$.

Hyaluronidase inhibitory activity was observed for benzoic acid derivatives covering a calculated $logD_{5.0}$ range from 2.4 to 6.1. With respect to 5-hydroxylated 4-amino-1*H*-imidazole-2(5*H*)-thiones, active compounds covered $logD_{5.0}$ values from 2.2 - 6.1 (Table 5.12). Replacement of 5-methyl by a 5-hydroxy group lowers the calculated $logD_{5.0}$ value by approximately 1.2. Modifications in 5-position might be key to adequate drug-like properties. Additionally, one might speculate about the contribution of fluorinated substituents replacing the 2,6-dichlorophenyl residue in the western part of the 4-amino-1*H*-imidazole-2(5*H*)-thione scaffold.

5.5.4 Inhibitory activities of purified compounds on BTH

The substances were routinely tested on BTH as a representative example of mammalian hyaluronidases. Only compounds **5.29** (60 % inhibition at a final concentration of 200 µM) and **5.69** (50 % inhibition at a final concentration of 200 µM) displayed some inhibitory activity. IC_{50} values were not calculated due to the occurrence of colored compounds at higher concentrations, indicating the presence of decomposition products and/or impurities.

5.5.5 Inhibitory activities of selected compounds on *Spn*Hyl

All compounds discussed in this chapter were designed as inhibitors of the hyaluronate lyase from *S. agalactiae* (*Sag*$Hyal_{4755}$). To study biological activity on the related hyaluronidase from *S. pneumonia* (*Spn*Hyl), an ensemble of 5 potent inhibitors was selected and tested by Dr. J. Hamberger from our workgroup. Under identical assay conditions including the same pH value, these substances were inactive on *Spn*Hyl (Table 5.14). A preference for negatively charged groups (cf. **5.28**, **5.46**), basic residues (cf. **5.35**, **5.66**) or space filling substituents (cf. **5.45**) was not detectable. Thus, the investigated 4-amino-1*H*-imidazole-2(5*H*)-thiones are selective for the enzyme *Sag*$Hyal_{4755}$, which was used as the biological target in this work.

Table 5.14 Inhibitory activity[a] and calculated $logD_{5.0}$ values[b] of 4-imino-1*H*-imidazole-2(5*H*)-thione derivatives **5.28**, **5.35**, **5.48**-**5.49** and **5.69**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	*Spn*Hyl IC_{50} (µM)[a]
5.28	7.9 ± 0.9	inactive
5.35	55.7 ± 3.1	inactive
5.45	50.4 ± 3.2	inactive
5.46	80.2 ± 2.8	inactive
5.66	59.0 ± 1.0	inactive

[a] mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 in the automated 96-well turbidimetric assay.

5.6 Outlook

Chemically stabilized 4-amino-1*H*-imidazole-2(5*H*)-thiones display inhibitory activity on the target enzyme *Sag*Hyal$_{4755}$. The evaluation of structurally closely related compounds corroborated the biological data for this class of hyaluronate lyase inhibitors. It should be stressed that the 4-amino-1*H*-imidazole-2(5*H*)-thiones were suggested by virtual chemistry, following a ligand-based drug-design approach. These substances are new chemical entities and represent a new class of (selective) inhibitors on the target enzyme.

Common structural features previously investigated for inhibitors of streptococcal hyaluronidase included hydrophilic groups combined with hydrophobic alkyl chains. These substances were proven to address the active site of hyaluronidases. Examples of potent inhibitors were crystallized in complex with *S. pneumonia* hyaluronidase (*Spn*Hyl) and the binding mode was determined by X-ray crystallography. Structural analysis disclosed the molecular interactions of these compounds with amino acids at the catalytic site of *Spn*Hyl.[8, 18] The most potent inhibitors were characterized as molecules combining a hydrophilic anchor group which binds in the center of the active site, and rather lipophilic moieties that eventually mimic sugar residues of the substrate hyaluronan. Strikingly, derivatives of ascorbic acid and indolylalkanoic acid were also identified as potent inhibitors of mammalian enzymes.[10, 11] Hence, the structural feature, that is hydrophilic anchor plus hydrophobic carbon chain, led to almost equipotent inhibition of both bacterial and mammalian hyaluronidases. A major drawback of these inhibitors is their very high plasma protein binding. In addition, some compounds showed surfactant-like properties *in vitro*.[11]

To illustrate a possible correlation between lipophilicity and enzyme inhibition among ascorbic acid derivatives and indolylalkanoic acids, the calculated $logD_{5.0}$ was plotted versus the IC_{50} value. The results are displayed in Figure 5.41.

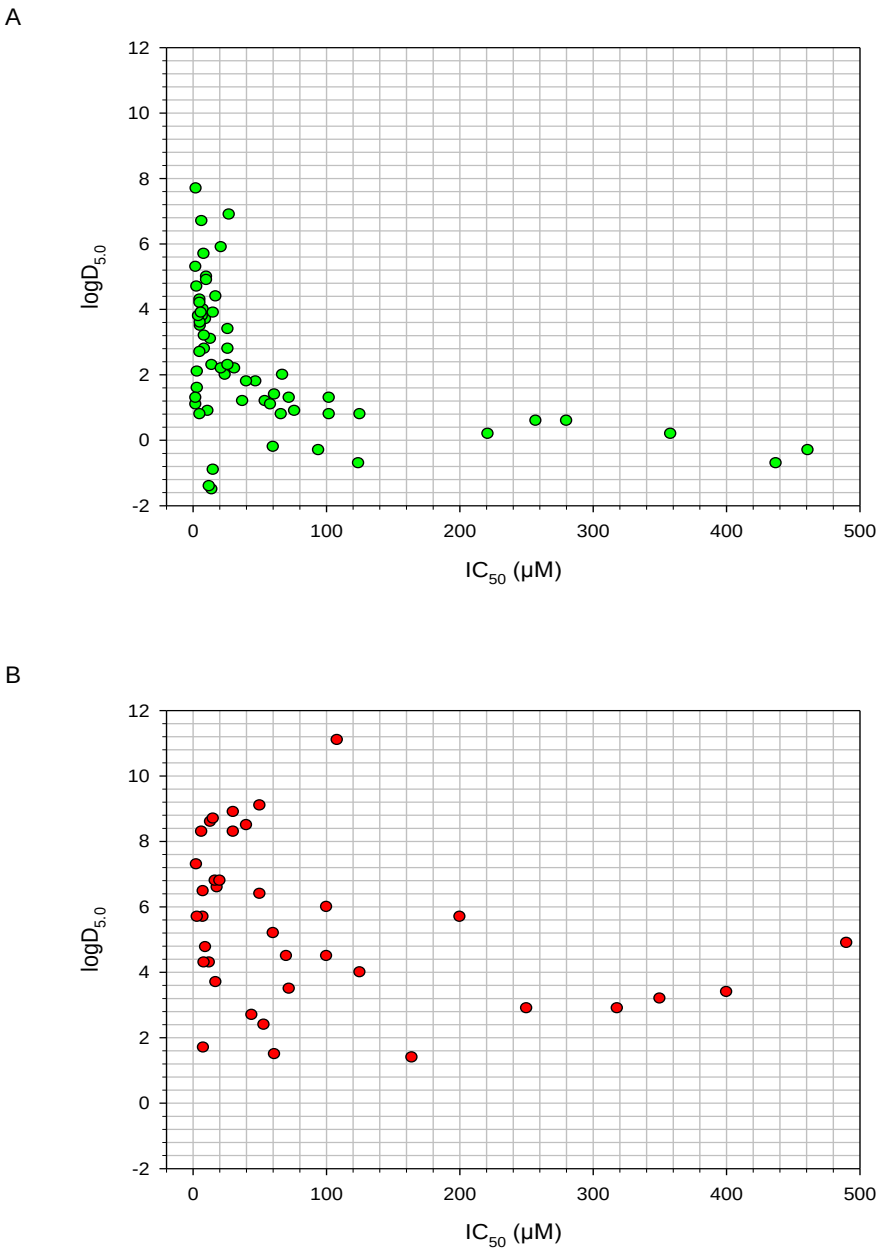


Figure 5.41 IC_{50} values (µM) plottet versus calculated $logD_{5.0}$ values of ascorbic acid derivatives (A) and indolylalkanoic acid derivatives (B) (data from Spickenreither[11]).

Surprisingly, in case of ascorbic acid derivatives, the most active compounds covered a wide range of $logD_{5.0}$ values, from approximately 0.8 to 6.0. According to Lipinski's rule of five, at first sight, this might allow for the identification of drug-like inhibitors. On the other side, it is hard to define a pharmacophore for these structures, as there seems to be no specific preference for hydrophilic or hydrophobic moieties. A similar behavior was

observed for indolalkylic acid derivatives. Again, a specific correlation between lipophilicity and inhibitory activity was difficult to analyze.

With regard to the structural requirements of 4-amino-1*H*-imidazole-2(5*H*)-thione-type hyaluronidase inhibitors, the five-membered “thiohydantoin” heterocycle might be regarded as a “center” or “hinge-region” to arrange lipophilic aromatic substituents at a short distance. These molecules share lipophilic residues which were fused directly to the aryl moieties. Another aspect is found in the anti-planar conformation, as the chemical scaffold allows a certain rotation and flexibility of substituents relative to the heterocyclic core. Possibly, additional insights into the stereochemical requirements can be gained from the investigation of pure enantiomers.

To illustrate a possible correlation between lipophilicity and enzyme inhibition among 4-amino-1*H*-imidazole-2(5*H*)-thiones, the calculated $logD_{5.0}$ was plotted versus the IC_{50} values (Figure 5.42).

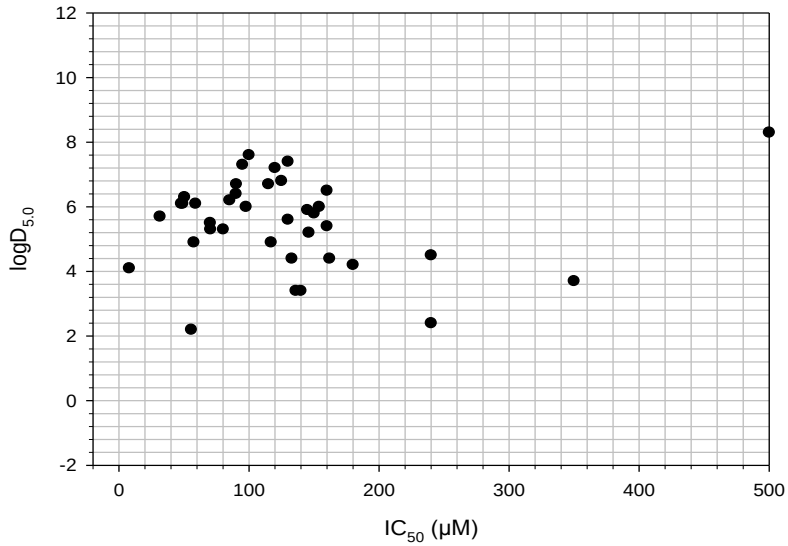


Figure 5.42 IC_{50} values (µM) plottet versus calculated $logD_{5.0}$ values of 4-amino-1*H*-imidazole-2(5*H*)-thiones.

By trend, there seems to be a correlation between lipophilicity and inhibition, suggesting potent 4-amino-1*H*-imidazole-2(5*H*)-thione hyaluronate lyase inhibitors to possess $logD_{5.0}$ in the range from approximately 3.0 to 8.0. However, only one compound (**5.28**) showed one-digit micromolar inhibitory activity. These considerations should not be overemphasized, as various substituent patterns and different chemical classes are compared. At least, the results suggest different binding modes in the active site for 4-amino-1*H*-imidazole-2(5*H*)-thione on the one hand and ascorbic acid and indole

derivatives, sharing lipophilic alkyl chains, on the other hand. Certainly, this concept must be restricted to the interaction with *Sag*Hyal$_{4755}$, as inhibition of the prototypical mammalian enzyme, BTH, was not observed.

To substantiate the electronic properties of these “topology-driven” hyaluronidase inhibitors, the surface polarities of inhibitors **5.28** (IC_{50} = 8 µM) and **5.46** (IC_{50} = 80 µM) were computed by a COMSO*sim*-based model.[71] Thermodynamic methods based on conductor-like screening models for realistic solvation (COSMO-RS) originated from the use of solvation thermodynamics and computational quantum mechanics. These methods rely on sigma profiles specific to each molecule. A sigma profile is a molecular-specific probability distribution of the surface polarization charge density (σ), which enables the application of solvation-thermodynamic models, e.g. to predict vapor-liquid equilibria.[72] To display surface polarity of (5*R*)-**5.28**, the COSMO*sim* surface model, color coded by σ, is given in Figure 5.43.

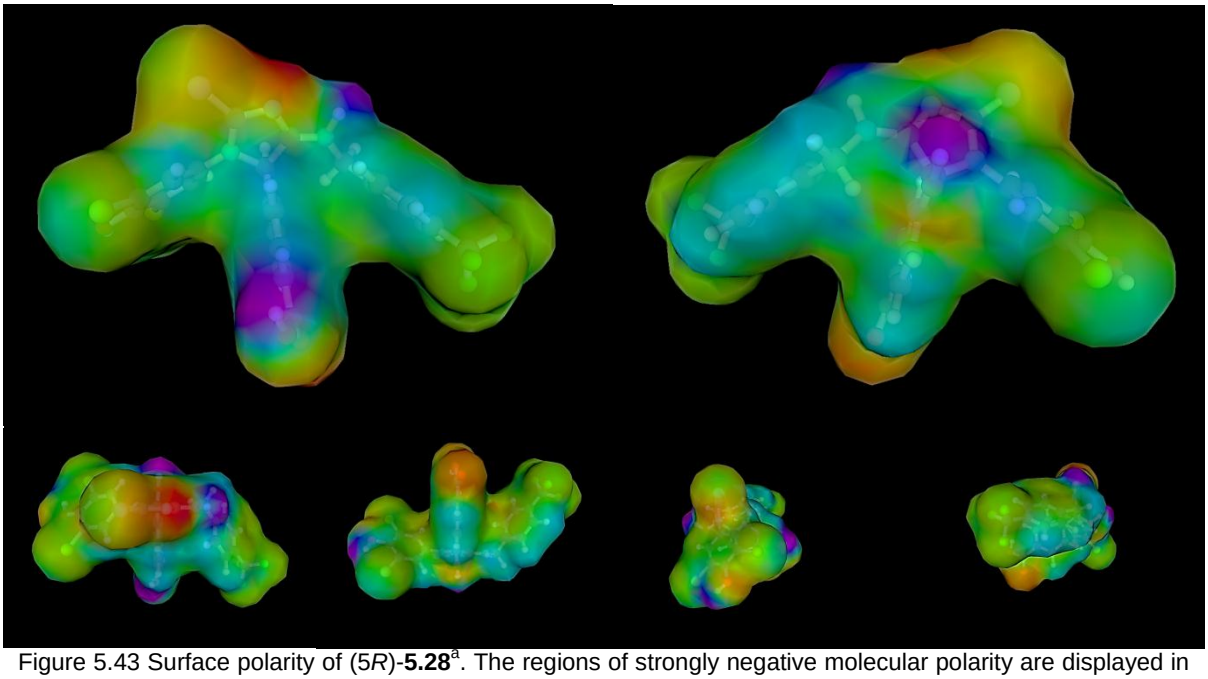
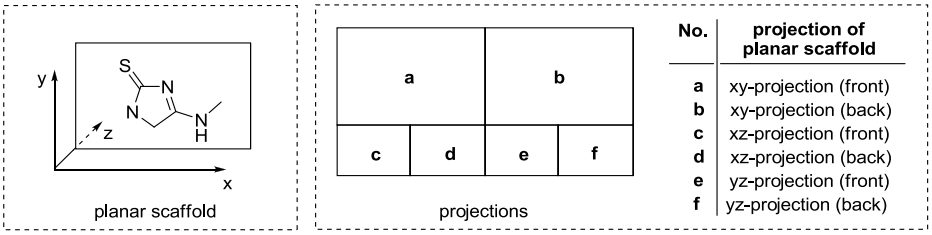


Figure 5.43 Surface polarity of (5*R*)-**5.28**[a]. The regions of strongly negative molecular polarity are displayed in red. The strongly positive molecular regions carrying negative σ are colored blue, while the neutral parts of the molecules with σ close to zero appear green (the negative molecular regions carry a positive polarization charge density, σ, because σ is just compensating the molecular electrostatic field and therefore has the opposite sign).[71]

[a] The planar scaffold of (5*R*)-**5.28** is fused to a plane level. The scaffold is shown from three spatial directions (xy-, xz, yz-projection).



The COSMO*sim* surface model of (5*S*)-**5.28** is given in Figure 5.44.

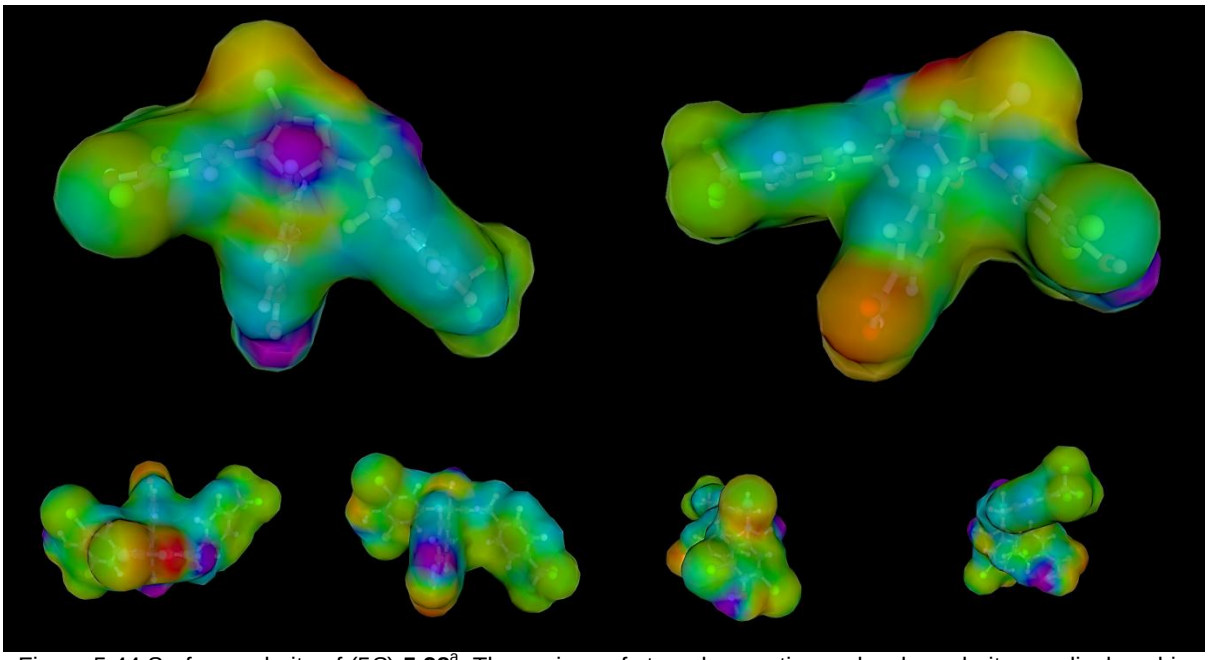


Figure 5.44 Surface polarity of (5*S*)-**5.28**[a]. The regions of strongly negative molecular polarity are displayed in red. The strongly positive molecular regions carrying negative σ are colored blue, while the neutral parts of the molecules with σ close to zero appear green; [a] see Figure 5.43.

As shown, the molecular surface of both enantiomers remained almost identical. The electronic surface at the center of the molecule is mainly imposed by the position of the hydroxyl group in position 5. Hence, the *R*- and *S*-stereoisomers can be described literally as mirror images. The COSMO*sim* surface model of (5*R*)-**5.46** is given in Figure 5.45.

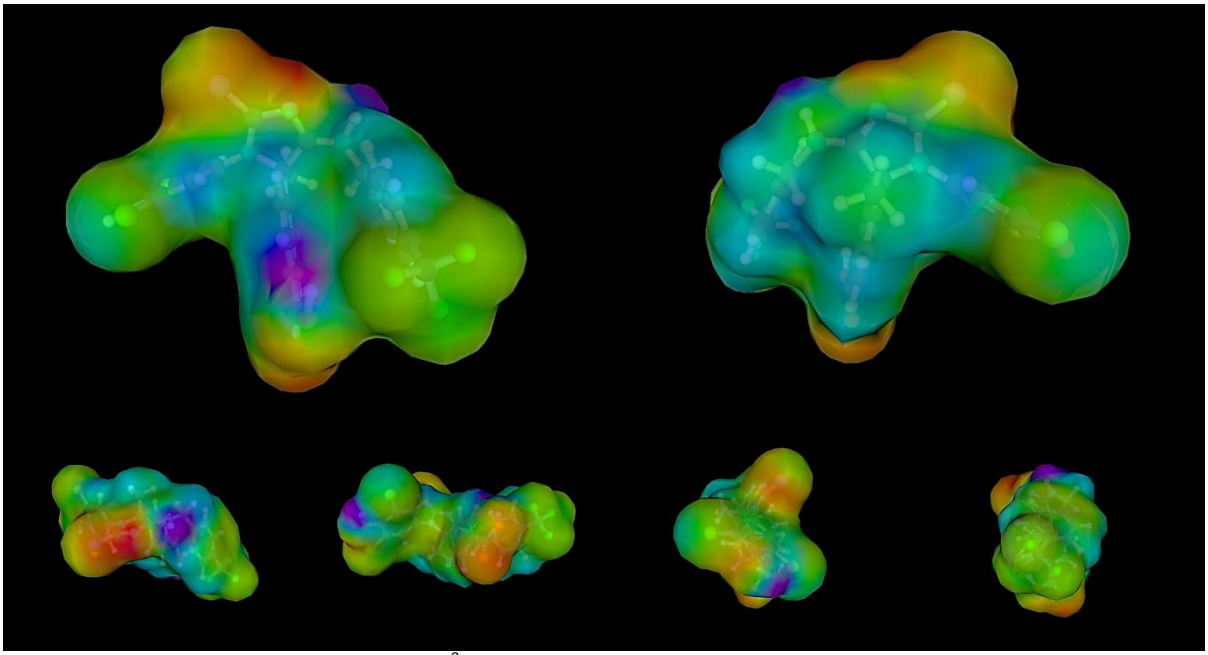


Figure 5.45 Surface polarity of (5*R*)-**5.46**[a]. The regions of strongly negative molecular polarity are displayed in red. The strongly positive molecular regions carrying negative σ are colored blue, while the neutral parts of the molecules with σ close to zero appear green; [a] see Figure 5.43.

The molecular surface, calculated for (5*R*)-**5.46** (Figure 5.45), indicates an aggregation of the 4-benzoic acid substituent and the 4-(trifluoromethyl)benzylamino residue. This results in a condensed, rather bulky lipophilic moiety. The situation is different for the computed surface of (5*R*)-**5.28** (Figure 5.43), where the aryl residues build up three separate and distinct zones. An aggregation or overlapping is not observed. When comparing the hydroxylated compound **5.28** and the corresponding methylated compound **5.46**, this might give an explanation for the 10-fold higher inbitory potency of the 4-amino-5-hydroxy-1*H*-imidazole-2(5*H*)-thione **5.28**.

To highlight promising structural modifications of hyaluronidase inhibitors with respect to future work, a sample for a computed protein-ligand interaction is given in Figure 5.46.

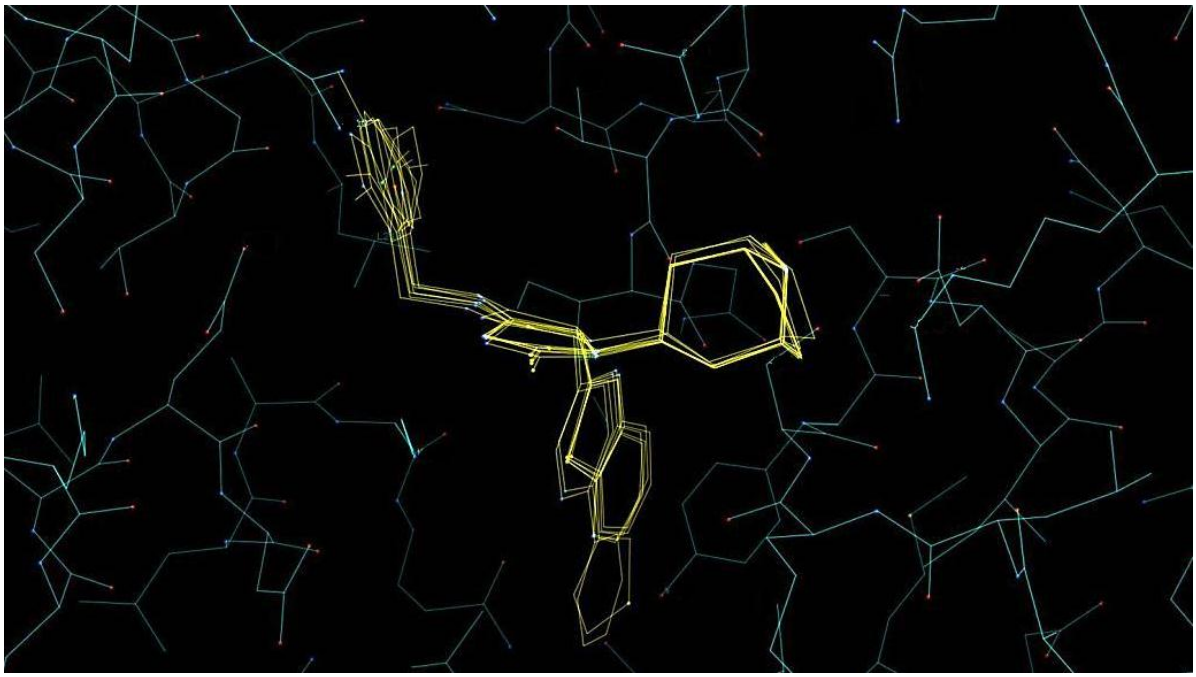


Figure 5.46 Crystal structure of *Sag*Hyal (PDB code 1LXM) with docked 4-amino-1*H*-imidazole-2(5*H*)-thiones.

The screenshot displays the hyaluronan binding site of the target enzyme *Sag*$Hyal_{4755}$ (pdb code 1lxm)[26] in complex with 4-amino-1*H*-imidazole-2(5*H*)-thione-type inhibitors. Note that enantiomeres of 5-hydroxylated or 5-methylated substances were not considered. Interestingly, the docking experiment proposes a highly conserved structural conformity for the computed molecules. Besides, the substances share a unitary orientation at the active site.

To identify common structural features, the molecular shape of the compounds was superimposed. A condensed topology, incorporating intermolecular similarity patterns, is given in Figure 5.47.

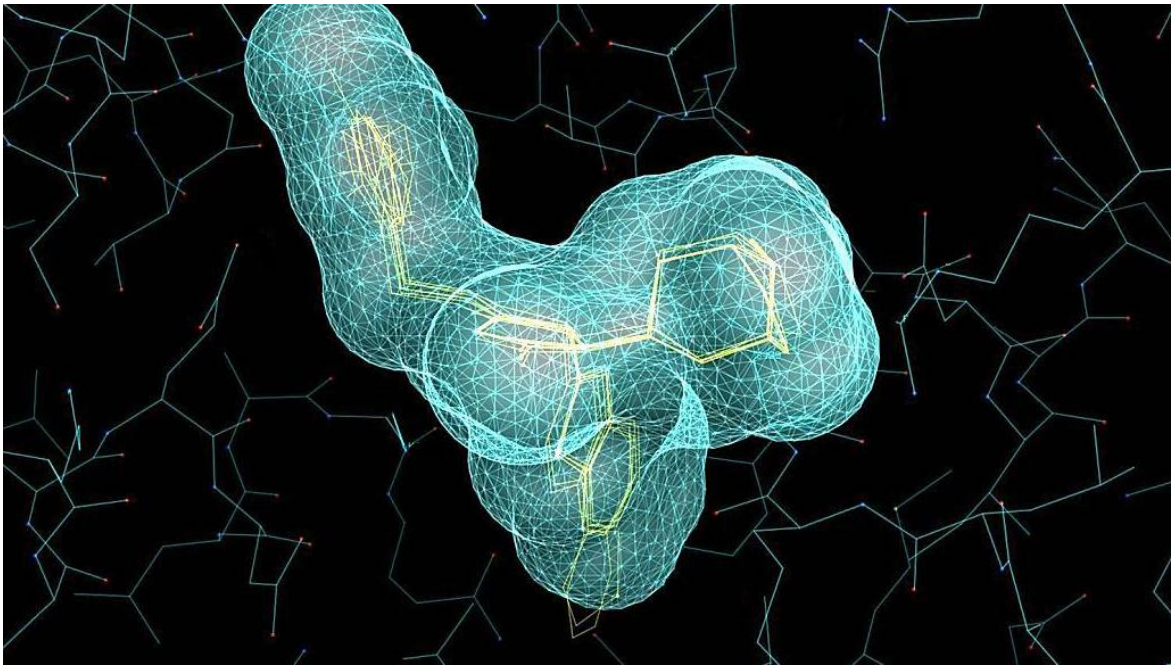


Figure 5.47 Superimposed electronic surface of 4-amino-1*H*-imidazole-2(5*H*)-thiones at the hyaluronan binding site of *Sag*Hyal (pdb code 1LXM).

Based on the molecular topology, modifications of the 4-amino-1*H*-imidazole-2(5*H*)-thiones core structure can be suggested for position 1 and 5. For example, alkyl substituents in position 1, e.g. space-filling cycloalkyl residues, might provide additional protein-inhibitor interactions. In this context, the alkylated substances **5.12** and **5.15** (see 5.3.3), might be regarded as prototypic structures. Moreover, aryl-substituents containing fused or connected (hetero)aromatic rings attached to position 5 might be valuable activity-enhancing residues.

To conclude, protein-inhibitor interactions of 4-amino-1*H*-imidazole-2(5*H*)-thiones are most likely driven by the topology of the inhibitors. Furthermore, these structures harbor a high degree of freedom for additional modifications.

5.7 Summary

In collaboration with Origenis GmbH a rational approach to identify novel lead inhibitors of the streptococcal lyase *Sag*Hyal$_{4755}$ was persued. *In silico* generated molecules were synthesized on demand via parallel synthesis, analyzed and tested for biological activity using medium-throughput techniques. Right from the start, the project was focused on the prediction of small molecules with drug-like properties and accessible by one-pot multicomponent synthesis.

In a first screening campaign three substances (“hits”) out of 2288 compounds were identified to possess moderate *in vitro* inhibitory activity in the micromolar range on the target enzyme. Re-testing of these screening hits as purified compounds, followed by rational modifications of similar molecules resulted in substances with retained biological activity in the micromolar range. The 4-amino-1*H*-imidazole-2(5*H*)-thione scaffold was selected as a promising novel structural motif to synthesize additional hyaluronidase inhibitors.

A second screening approach, consisting of 352 4-amino-1*H*-imidazole-2(5*H*)-thione derivatives revealed a total of 25 hits. Preparative synthesis and purification of selected substances confirmed moderate to good inhibition in the micromolar range. However, chemical stability of some derivatives was unsatisfactory, due to autoxidation and decomposition. To cope with this problem, the process of autoxidation was studied in detail by means of [^{18}O] labeling and HPLC-MS analysis.

With compounds **5.26**-**5.35**, stabilized C-5 hydroxylated molecules were obtained. All compounds were characterized as inhibitors following a dose-dependent manner on the target enzyme *Sag*Hyal$_{4755}$. Substance **5.28** (IC_{50} = 8 µM) was identified as one of the most potent inhibitor known so far.

Besides, modification of starting materials (aldehydes replaced by ketones) generated stable C-5 methylated compounds which were not prone to oxidation (substances **5.40**-**5.70**). Also, the molecular topology was highly conserved for these substances. The three-dimensional structure of corresponding molecules was determined by means of 2D-NMR, HRMS and X-ray diffraction analysis. A hit to lead development, under the guideline to meet the requirements in terms of drug-like properties, was pursued via rational variation of substituents on the core scaffold.

In summary, the applied innovative methods and modus operandi of the current approach were suitable to obtain novel lead inhibitors of the bacterial hyaluronidase *Sag*Hyal$_{4755}$.

5.8 Experimental section

5.8.1 General conditions

Chemicals and solvents were purchased from ACB Blocks Ltd (Moscow, Russia), Acros Organics (Geel, Belgium), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Bachem AG (Bubendorf, Switzerland), Biosynth AG (Staad, Switzerland), Chembridge Corporation (San Diego, USA), ChemPacific Corporation (Baltimore, USA), Fluka AG (Buchs, Switzerland), Frontier Scientific Inc. (Logan, USA), Maybridge (Trevillett, UK), Merck KGaA (Darmstadt, Germany), Priaton GmbH (Munich, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany) and Zerenex Molecular Ltd (Greater Manchester, UK) and used without further purification. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen); DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany). Millipore water was used throughout for the preparation of buffers and HPLC eluents.

Nuclear Magnetic Resonance spectra were recorded with Avance 300 (^{1}H: 300 MHz, ^{13}C: 75.5 MHz), Avance 400 (^{1}H: 400 MHz, ^{13}C: 100.6 MHz) and Avance III 600 Kryo spectrometer (^{1}H: 600 MHz, ^{13}C: 150.9 MHz) from Bruker BioSpin GmbH (Rheinstetten, Germany). Coupling constants (*J*) are reported in Hz throughout. Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), s br (for broad singulet), as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 90 and DEPT 135 (distortionless enhancement by polarization transfer): “+” primary and tertiary carbon atom (positive DEPT 135 signal), “-“ secondary carbon atom (negative DEPT 135 signal), “quat” quaternary carbon atom. For substances measured with the Avance 600 Kryo NMR spectrometer, 2D-NMR techniques (HSQC, HMBC, COSY) were used to assign ^{1}H and ^{13}C chemical shifts.

Flash chromatography was performed in glass columns on silica gel (Merck silica gel 60, particle size 0.040 – 0.063 mm). Automated flash chromatography was performed on a Varian IntelliFlash 310 using pre-packed Varian Superflash columns (Varian, Darmstadt, Germany). Reactions were routinely monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminum sheets and spots were visualized with UV light at 254 nm, and/or iodine vapor or ammonium molybdate/cerium(IV) sulphate solution. Microwave assisted reactions were performed on an Initiator 2.0 synthesizer (Biotage, Uppsala, Sweden). Lyophilisation was done on a Christ alpha 2-4 LD equipped with a vacuubrand

RZ 6 rotary vane vacuum pump. Melting points (mp) were measured on a BÜCHI 530 electrically heated copper block apparatus (Büchi AG, Flawil, Switzerland) using open capillaries and are uncorrected. Compounds were dried under reduced pressure (0.1 - 1 Torr) at room temperature.

Analytical RP-HPLC was performed on a system consisting of the following components (Thermo Separation Products, Switzerland): pump model was P4000 pump, adjusted to a SN400 controller and an AS3000 autosampler. For UV/VIS-detection served a Spectra Focus detector (Table 5.15).

Table 5.15 Analytical RP-HPLC system components and parameters.

stationary phase	column	Eurospher-100 C18, 4.0 x 250 mm, 5 µm (Knauer, Berlin, Germany)
	temperature	30 °C
mobile phase	solvent	A: acetonitrile (gradient grade) + 0.025 % TFA (v/v) B: water (millipore) + 0.025 % TFA (v/v)
	flow	0.7 mL/min
	gradient	00 to 30 min (A/B): 10/90 to 90/10 (v/v)
detector	wavelength λ	210 nm

HPLC-MS analysis was conducted on an Agilent Technologies 1100 Series (autosampler, pump, controller, UV detector) with a Hewlett-Packard 1100 Series detector (ESI-MSD) spectrometer (Table 5.16).

Table 5.16 Analytical HPLC-MS system components and parameters.

stationary phase	column	SunFire™ C18, 2.1 x 50 mm, 2.5 µm (Waters, Dublin, Ireland)
	temperature	40 °C
mobile phase	solvent	A: acetonitrile (gradient grade) B: water (LiChrosolv®) C (modifier): acetonitrile (gradient grade) + 2 % formic acid (for analysis) (v/v)
	flow	0.6 mL/min
	gradient	00 to 05 min (A/B): 5/95 to 95/0 (v/v)
detector	wavelength λ	220 nm, 250 nm
	MS system	Hewlett-Packard Series 1100 MSD (Hewlett-Packard, USA)

HPLC-MS analysis of compounds **5.36**-**5.38** was performed with a system including the following components: RP-HPLC and mass spectrometry analysis (MS) was conducted on

an Agilent Technologies 1290 Series (autosampler, pump, controller, UV detector) with an Agilent Technologies 6540 Series UHD Accurate-Mass (Q-TOF/LC MS) detector. For optimal signal detection, 2 different methods depending on the column-type were used: method 1 (Zorbax column, Table 5.17), method 2 (Accucore column, Table 5.18).

Table 5.17 Analytical HPLC-MS system components and parameters (method 1, Zorbax column).

stationary phase	column	Zorbax Eclipse Plus C18 Rapid Solution HD, 2.1 x 50 mm, 1.8 µm (Agilent Technologies, Santa Clara, USA)
	temperature	40 °C
mobile phase	solvent	A: acetonitrile (gradient grade) + 0.1 % formic acid (v/v)
		B: water (LiChrosolv®) + 0.1 % formic acid (v/v)
	flow	0.6 mL/min
	gradient	00 to 04 min (A/B): 5/95 to 90/10 (v/v)
detector	wavelength λ	220 nm
	MS system	Agilent Technologies 6540 Series UHD Accurate-Mass (Agilent Technologies, Santa Clara, USA)

Table 5.18 Analytical HPLC-MS system components and parameters (method 2, Accucore column).

stationary phase	column	Accucore aQ, 2.1 x 50 mm, 2.6 µm (Thermo Scientific, Santa Clara, USA)
	temperature	40 °C
mobile phase	solvent	A: acetonitrile (gradient grade) + 0.1 % formic acid (v/v)
		B: water (LiChrosolv®) + 0.1 % formic acid (v/v)
	flow	0.4 mL/min
	gradient	00 to 04 min (A/B): 0/100 to 90/10 (v/v)
detector	wavelength λ	220 nm
	MS system	Agilent Technologies 6540 Series UHD Accurate-Mass (Agilent Technologies, Santa Clara, USA)

Prior to preparative RP-HPLC, a standardized samples preparation was performed: crude products were precipitated (solvent indicated) and dried (rotary evaporator). Subsequently, the residues were dissolved in 1600 µL acetonitrile, 100 µL acetic acid and 800 µL H_2O (millipore). After filtration through Nylon-filters (13 mm, 0.45 µm, Roth GmbH, Karlsruhe, Germany) the samples were subjected to preparative RP-HPLC. After RP-HPLC purification, the solvent of the combined product fractions was evaporated (rotary evaporator). The residues were dissolved in 1600 µL *tert*-BuOH with 800 µL H_2O

(millipore) and subjected to lyophilisation (Alpha 2-4, Christ GmbH, Osterode, Germany). Purity of compounds tested for hyaluronidase inhibition was ≥ 95 %.

Preparative RP-HPLC was conducted on a system consisting of the following components: pump models were a Varian PrepStar Model SD-1 (main pump, operating solvents A, B), Dionex Ultimate 3000 pump (modifier pump, operating solvent C) and a Dionex P580 pump (analytical pump). The system was equipped with a Dionex Ultimate 3000 Variable Wavelength Detector. For mass detection an assembly of a Rheodyne SplitRatio 4000 MRA-switcher, Dionex UVD 340U and Dionex Model Surveyer MSQ mass spectrometer system was used. Samples were collected on a Gilson 215 Liquid Handler (injector module and sample collector). The Interface was a Dionex UCI-100 (Universal Chromatography Interface). Chromeleon Client Software (Version 6.80, Dionex Corp.) and Surveyor MSQ Tune (MSQ Standard 2.0 SP1, Thermo Electron Corp.) were embedded (Table 5.19).

Table 5.19 Preparative RP-HPLC system components and parameters.

stationary phase	column	XBridge™ Prep C18 5 µm OBD™, 19 x 50 mm, 5 µm (Waters, Dublin, Ireland)
	temperature	room temperature
mobile phase	solvent	A: acetonitrile (gradient grade) B: water (LiChrosolv®) C (modifier): 1:1 acetonitrile (gradient grade), water (millipore) + 10 % acetic acid (for analysis) (v/v/v)
	flow	40 mL/min (A,B), 2.4 mL/min (C; permanent modifier flow)
	gradient	method 1: 00 to 04 min (A/B): 5/95 to 95/5 (v/v) method 2: 00 to 05 min (A/B): 5/95 to 80/20 (v/v)
detector	wavelength λ	220 nm
	MS system	Dionex, Model Surveyer MSQ Mass Spectrometer System (Dionex, Sunnyvale, USA)

Alternatively, preparative RP-HPLC was performed on a system consisting of the following components: pump model was K-1800 (Knauer GmbH, Germany). For UV-detection served a K-2000 (Knauer GmbH, Germany) (cf. Table 5.20).

Table 5.20 Preparative RP-HPLC system components and parameters.

stationary phase	column	Nucleodur VP 250/21 100-5 C18ec, 21 x 250 mm, 5 µm (Macherey-Nagel, Düren, Germany)
	temperature	room temperature
mobile phase	solvent	A: acetonitrile (HPLC grade)
		B: water (millipore) + 0.1 % TFA + 5 % acetonitrile (v/v/v)
	flow	20.0 mL/min
	gradient	00 to 30 min (A/B): 20/80 to 90/10 (v/v)
detector	wavelength λ	220 nm

Mass spectrometry analysis (MS) was conducted on an Agilent Technologies 1290 Series (autosampler, pump, controller, UV detector) with an Agilent Technologies 6540 Series UHD Accurate-Mass (Q-TOF/LC MS) detector. Mass spectra (MS) were additionally recorded on a Finnigan MAT 95 (EI-MS 70 ev, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ES-MS) spectrometer.

5.8.2 Chemistry

5.8.2.1 Parallel synthesis

5.8.2.1.1 General conditions

Reaction conditions were adjusted to the microliter scale on 96 deep well plates (total volume: 1200 µL) and to the needs of an automated synthesis. Using a Tecan Genesis RSP 200/8 parallel synthesizer (Tecan Deutschland GmbH, Crailsheim, Germany) 80 or 88 reactions were performed simultaneously. After completion of the experiment, each compound was assessed on a PerSeptive BioSystems *Mariner*^{TM} ESI-TOF mass spectrometer. The success of the individual syntheses was analyzed and indicated automatically by an absolute threshold on signal intensity. The absolute threshold specified a threshold value relative to the total intensity per scan. For compounds displaying a target mass with an absolute threshold < 30 and < 100, the formation of products was regarded as failed or insufficient, respectively (category “red” and “yellow”). With an absolute threshold ≥ 100, the formation of products was regarded as successful (category “green”). The observed molecular ions for successfully synthesized compounds are listed in sections B.3 – B.6 (appendix II). After HPLC-MS analysis, the solvent was evaporated with a Genevac HT-4X centrifugal vacuum evaporator (Genevac Inc.,

Gardiner, USA). Finally, 500 µL of DMSO were added to each well to adjust to a final concentration of 0.02 M (assumption: formation of product 100 %). All compounds were immediately tested for inhibition of the activity of bacterial hyaluronidase *Sag*Hyal$_{4755}$. Final assay concentration of the respective inhibitors was adjusted to 200 µM (assuming 100 % conversion to product). After the testing procedures the well plates were sealed and stored at -20 °C.

5.8.2.1.2 Preparation of 1H-pyrrol-2(5H)-ones (ori.hya.10-19)

For the parallel synthesis of 1*H*-pyrrol-2(5*H*)-ones, solutions of the pertinent amines (0.2 M, 50 µL, 1 eq) in anhydrous EtOH were dispensed on 96 deep well plates. Aldehydes (0.2 M, 50 µL, 1 eq) in anhydrous EtOH were added, and the plates were stacked for 30 min at ambient temperature. Subsequently, isocyanides (0.2 M, 50 µL, 1 eq) in anhydrous EtOH were added. The well plates were sealed and stacked for 24 h at ambient temperature. After completion of the experiment, the compounds were analyzed (HPLC-MS), and the solvent was evaporated. Subsequently, the residues were dissolved in DMSO and the samples were stored as described previously.

5.8.2.1.3 Preparation of 4-amino-1H-imidazole-2(5H)-ones (ori.hya.20-27)

For the parallel synthesis of 4-amino-1*H*-imidazole-2(5*H*)-ones, amines (0.2 M, 50 µL, 1 eq) in MeOH (dry) were dispensed on 96 deep well plates. Aldehydes (0.2 M, 50 µL, 1 eq) in MeOH (dry) were added, and the plates were stacked for 30 min at ambient temperature. Subsequently a mixture of KOCN (0.8 M, 4 eq) and pyridine hydrochloride (0.8 M, 4 eq) in 50 µL MeOH (dry) and isocyanides (0.2 M, 50 µL, 1 eq) in MeOH (dry) were added. The well plates were sealed and stacked for 48 h at temperature below 20 °C. After completion of the experiment, the compounds were analyzed (HPLC-MS), and the solvent was evaporated. Subsequently, the residues were dissolved in DMSO and the samples were stored as described previously.

5.8.2.1.4 Preparation of 4-amino-1H-imidazole-2(5H)-thiones (ori.hya.28-35)

For the parallel synthesis of 4-amino-1*H*-imidazole-2(5*H*)-thiones, amines (0.2 M, 50 µL, 1 eq) in MeOH (dry) were dispensed on 96 deep well plates. Aldehydes (0.2 M, 50 µL, 1 eq) in MeOH (dry) were added, and the plates were stacked for 30 min at ambient temperature. Subsequently a mixture of KSCN (0.8 M, 4 eq) and pyridine hydrochloride (0.8 M, 4 eq) in 50 µL MeOH (dry) and isocyanides (0.2 M, 50 µL, 1 eq) in MeOH (dry)

were added. The well plates were sealed and stacked for 48 h at temperature below 20 °C. After completion of the experiment, the compounds were analyzed (HPLC-MS), and the solvent was evaporated. Subsequently, the residues were dissolved in DMSO and the samples were stored as described previously.

5.8.2.2 Preparation of the compounds 5.2, 5.3, 5.6, 5.10, 5.12-5.15

5-(3,5-Dihydroxyphenyl)-1-(3-hydroxyphenyl)-4-(pentan-2-ylamino)-1*H*-imidazol-2(5*H*)-one (5.2)[52]

The title compound was prepared from 3,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg) and 3-aminophenol (0.3 mmol, 33 mg) in anhydrous MeOH (1 mL per mmol). The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After addition of KOCN (4 eq), pyridinium chloride (4 eq) and 2-isocyanopentane (0.3 mmol, 29 mg) the mixture was stirred at room temperature overnight. Removal of the solvent gave the crude substance, which was purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 17.9 mg (16.2 %, pale yellow solid). MS (CI-MS) *m/z* (rel. int. in %) = 370.2 ($[M + H]^+$, 100). $C_{20}H_{23}N_3O_4$ (M_r = 369.41 g/mol).

4-(4-Chlorobenzylamino)-5-(3,5-dihydroxyphenyl)-1-(3-hydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.3)[52]

The title compound was prepared from 3,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg) and 3-aminophenol (0.3 mmol, 33 mg) in anhydrous MeOH (1 mL per mmol). The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After addition of KSCN (4 eq), pyridinium chloride (4 eq) and 1-chloro-4-(isocyanomethyl)benzene (0.3 mmol, 45 mg) the mixture was stirred at room temperature overnight. Removal of the solvent gave the crude substance, which was purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 6.2 mg (4.7 %, yellow solid). MS (CI-MS) *m/z* (rel. int. in %) = 440.1 ($[M + H]^+$, 100). $C_{22}H_{18}ClN_3O_3S$ (M_r = 439.91 g/mol).

1-(Cyclohexylmethyl)-5-(3,5-dihydroxyphenyl)-3-hydroxy-1*H*-pyrrol-2(5*H*)-one (5.6)[40]

To a mixture of cyclohexylmethanamine (0.6 mmol, 68 mg) and 3,5-dihydroxybenzaldehyde (0.6 mmol, 83 mg) in 5 mL anhydrous EtOH was added a solution of methyl 2-oxopropanoate (0.6 mmol, 61 mg) in 2.5 mL anhydrous EtOH. The reaction mixture was allowed to stand for 20 h at ambient temperature. The precipitated crude

product was filtered off, dried *in vacuo* and purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 1.5 mg (1.6 %, colorless solid). MS (CI-MS) *m/z* (rel. int. in %) = 304.1 ($[M + H]^+$, 100). $C_{17}H_{21}NO_4$ (M_r = 303.35 g/mol).

2-(3,5-Dihydroxyphenyl)-1-(2,3-dihydroxypropyl)-4-hydroxy-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carbonitrile (5.10)[40]

To a mixture of 3-aminopropane-1,2-diol (0.6 mmol, 46 mg) and 3,5-dihydroxybenzaldehyde (0.6 mmol, 83 mg) in 5 mL anhydrous EtOH was added a solution of ethyl 3-cyano-2-oxopropanoate (0.6 mmol, 85 mg) in 2.5 mL anhydrous EtOH. The reaction mixture was allowed to stand for 20 h at ambient temperature. The precipitated crude product was filtered off, dried *in vacuo* and purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 2.0 mg (1.1 %, colorless solid). MS (CI-MS) *m/z* (rel. int. in %) = 307.1 ($[M + H]^+$, 100). $C_{14}H_{14}N_2O_6$ (M_r = 306.27 g/mol).

5-(3,5-Dihydroxyphenyl)-1-(3-hydroxyphenyl)-4-(pentan-2-ylamino)-1*H*-imidazole-2(5*H*)-thione (5.12)[52]

The title compound was prepared from 3,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg) and 3-aminophenol (0.3 mmol, 33 mg) in anhydrous MeOH (1 mL per mmol). The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq), pyridinium chloride (4 eq) and 2-isocyanopentane (0.3 mmol, 29 mg), the mixture was stirred at room temperature overnight. Removal of the solvent gave the crude substance, which was purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 6.9 mg (5.7 %, gray solid). MS (CI-MS) *m/z* (rel. int. in %) = 386.1 ($[M + H]^+$, 100). $C_{20}H_{23}N_3O_3S$ (M_r = 385.48 g/mol).

4-(4-Chlorobenzylamino)-5-(3,5-dihydroxyphenyl)-1-(3-hydroxyphenyl)-1*H*-imidazol-2(5*H*)-one (5.13)[52]

The title compound was prepared from 3,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg) and 3-aminophenol (0.3 mmol, 33 mg) in anhydrous MeOH (1 mL per mmol). The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KOCN (4 eq), pyridinium chloride (4 eq) and 1-chloro-4-(isocyanomethyl)benzene (0.3 mmol, 45 mg) the mixture was stirred at room temperature overnight. Removal of the solvent gave the crude substance, which was purified by RP-

HPLC (Table 5.19, method 2). Yield (preparative HPLC): 46.4 mg (36.6 %, orange solid). MS (CI-MS) *m/z* (rel. int. in %) = 424.1 ($[M + H]^+$, 100). $C_{22}H_{18}ClN_3O_4$ (M_r = 423.85 g/mol).

4-(*sec*-Butylamino)-5-(3,5-dihydroxyphenyl)-1-(3-hydroxyphenyl)-1*H*-imidazol-2(5*H*)-one (5.14)[52]

The title compound was prepared from 3,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg) and 3-aminophenol (0.3 mmol, 33 mg) in anhydrous MeOH (1 mL per mmol). The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KOCN (4 eq), pyridinium chloride (4 eq) and 2-isocyanobutane (0.3 mmol, 25 mg) the mixture was stirred at room temperature overnight. Removal of the solvent gave the crude substance which was purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 17.1 mg (16.0 %, pale yellow solid). MS (CI-MS) *m/z* (rel. int. in %) = 356.2 ($[M + H]^+$, 100). $C_{19}H_{21}N_3O_4$ (M_r = 355.39 g/mol).

4-(*sec*-Butylamino)-5-(3,5-dihydroxyphenyl)-1-(3-hydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.15)[52]

The title compound was prepared from 3,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg) and 3-aminophenol (0.3 mmol, 33 mg) in anhydrous MeOH (1 mL per mmol). The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq), pyridinium chloride (4 eq) and 2-isocyanobutane (0.3 mmol, 25 mg) the mixture was stirred at room temperature overnight. Removal of the solvent gave the crude substance, which was purified by RP-HPLC (Table 5.19, method 2). Yield (preparative HPLC): 17.1 mg (16.0 %, yellow solid). MS (CI-MS) *m/z* (rel. int. in %) = 372.1 ($[M + H]^+$, 100). $C_{19}H_{21}N_3O_3S$ (M_r = 371.45 g/mol).

5.8.2.3 Preparation of the compounds 5.16-5.25

General procedure[52]

A solution of the pertinent aldehyde (1 eq) and amine (1 eq) in anhydrous MeOH (1 mL per mmol) was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq), pyridinium chloride (4 eq) and the corresponding isocyanide (1 eq), the mixture was stirred overnight below 20 °C. Insoluble material was removed by filtration, and the solvent (MeOH) was removed under reduced pressure. After addition of ethyl acetate as solvent, precipitated material was removed by filtration

and the crude product was obtained as residue after evaporation. The crude substance was submitted to preparative HPLC (method indicated) yielding the corresponding {[M + H]$^+$ + 16} product.[a]

[a] molecular [M + H]$^+$ species of compounds **5.26**-**5.35** showed complete autoxidation to the corresponding {[M + H]$^+$ + 16} derivatives within the first 48 h. HPLC isolation of "{[M + H]$^+$ + 16} substances" was performed not before 72 h after completion of the experiment.

5-(2,5-Dihydroxyphenyl)-5-hydroxy-1-(4-hydroxyphenyl)-4-[4-(trifluoromethyl)benzyl amino]-1*H*-imidazole-2(5*H*)-thione (5.16)

The title compound was prepared from 2,3-dihydroxybenzaldehyde (0.3 mmol, 41 mg), 4-aminophenol (0.3 mmol, 33 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 65 mg, 44 %; yield (preparative HPLC): 6.9 mg, 4.7 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 496.1 (10), 474.1 ([M + H]$^+$, 100), 385.1 (5), 218.1 (5). MS (CI-MS)[d] *m/z* (rel. int. in %) = 512.0 (5), 496.1 (20), 490.1 ({[M + H]$^+$ + 16}, 10), 474.1 ([M + H]$^+$, 100). $C_{23}H_{18}F_3N_3O_4S$ (M_r* = 489.47 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 473.47 g/mol) and {[M + H]$^+$ + 16} (M_r* = 489.47 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (473.47 g/mol) = 50 %; relative abundance (TIC): M_r* (489.47 g/mol) = 4 %; [d] 48 h after completion of the experiment; relative abundance (TIC): M_r (473.47 g/mol) = 50 %; relative abundance (TIC): M_r* (489.47 g/mol) = 10 %.

5-(2,3-Dihydroxyphenyl)-5-hydroxy-1-(3-hydroxyphenyl)-4-[4-(trifluoromethyl)benzyl amino]-1*H*-imidazole-2(5*H*)-thione (5.17)

The title compound was prepared from 2,3-dihydroxybenzaldehyde (0.3 mmol, 41 mg), 3-aminophenol (0.3 mmol, 33 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 95 mg, 65 %; yield (preparative HPLC): 4.9 mg, 3.3 %. MS (CI-MS)[b] *m/z* (rel. int. in %) =

496.1 (5), 474.1 ([M + H]$^+$, 100), 385.1 (5). MS (CI-MS)[d] *m/z* (rel. int. in %) = 496.1 (15), 490.1 ({[M + H]$^+$ + 16}, 10), 474.1 ([M + H]$^+$, 100). $C_{23}H_{18}F_3N_3O_4S$ (M_r* = 489.47 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 473.47 g/mol) and {[M + H]$^+$ + 16} (M_r* = 489.47 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (473.47 g/mol) = 65 %; relative abundance (TIC): M_r* (489.47 g/mol) = 10 %; [d] 48 h after completion of the experiment; relative abundance (TIC): M_r (473.47 g/mol) = 50 %; relative abundance (TIC): M_r* (489.47 g/mol) = 12 %.

4-(4-*tert*-Butylbenzylamino)-5-(2,3-dihydroxyphenyl)-5-hydroxy-1-(3-hydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.18)

The title compound was prepared from 2,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg), 3-aminophenol (0.3 mmol, 33 mg) and 1-*tert*-butyl-4-(isocyanomethyl)benzene (0.3 mmol, 52 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 95 mg, 66 %; yield (preparative HPLC): 4.2 mg, 2.9 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 484.1 (5), 462.2 ([M + H]$^+$, 100). MS (CI-MS)[d] *m/z* (rel. int. in %) = 484.1 (5), 476.2 ({[M + H]$^+$ + 16}, 10), 462.2 ([M + H]$^+$, 100). $C_{26}H_{27}N_3O_4S$ (M_r* = 477.58 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 461.58 g/mol) and {[M + H]$^+$ + 16} (M_r* = 477.58 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (461.58 g/mol) = 60 %; relative abundance (TIC): M_r* (477.58 g/mol) = 6 %; [d] 48 h after completion of the experiment; relative abundance (TIC): M_r (461.58 g/mol) = 60 %; relative abundance (TIC): M_r* (477.58 g/mol) = 8 %.

4-(4-Chlorobenzylamino)-5-(3,5-dihydroxyphenyl)-5-hydroxy-1-(3-hydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.19)

The title compound was prepared from 2,4,5-trihydroxybenzaldehyde (0.3 mmol, 46 mg), 3-aminophenol (0.3 mmol, 33 mg) and 1-*tert*-butyl-4-(isocyanomethyl)benzene (0.3 mmol, 52 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 70 mg, 47 %; yield (preparative HPLC): 6.1 mg, 4.1 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 547.1 (5), 494.0 (5), 478.0 (20), 456.1 ({[M + H]$^+$ + 16}, 95) 440.1 ([M + H]$^+$, 100). MS (CI-MS)[d]

m/z (rel. int. in %) = 478.0 (15), 476.2 ({[M + H]$^+$ + 16}, 100), 440.1 ([M + H]$^+$, 90). $C_{22}H_{18}ClN_3O_4S$ (M_r* = 455.91 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 439.91 g/mol) and {[M + H]$^+$ + 16} (M_r* = 455.91 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (439.91 g/mol) = 35 %; relative abundance (TIC): M_r* (455.91 g/mol) = 25 %; [d] 48 h after completion of the experiment; relative abundance (TIC): M_r (439.91 g/mol) = 35 %; relative abundance (TIC): M_r* (455.91 g/mol) = 35 %.

5-Hydroxy-1-(3-hydroxyphenyl)-4-(4-(trifluoromethyl)benzylamino)-5-(2,4,5-trihydroxy phenyl)-1H-imidazole-2(5H)-thione (5.20)

The title compound was prepared from 2,4,5-trihydroxybenzaldehyde (0.3 mmol, 46 mg), 3-aminophenol (0.3 mmol, 33 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave brown oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 80 mg, 53 %; yield (preparative HPLC): 1.6 mg, 1.1 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 565.1 (15), 512.1 (10), 491.1 ([M + H]$^+$, 100). MS (CI-MS)[c] *m/z* (rel. int. in %) = 508.4 (20), 506.4 ({[M + H]$^+$ + 16}, 100). $C_{23}H_{18}F_3N_3O_5S$ (M_r* = 505.47 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 489.47 g/mol) and {[M + H]$^+$ + 16} (M_r* = 505.47 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (489.47 g/mol) = 50 %; relative abundance (TIC): M_r* (505.47 g/mol) = 1.5 %; [c] 7 d after completion of the experiment; relative abundance (TIC): M_r (489.47 g/mol) = 0 %; relative abundance (TIC): M_r* (505.47 g/mol) = 40 %.

4-(4-*tert*-Butylbenzylamino)-5-hydroxy-1-(3-hydroxyphenyl)-5-(2,4,5-trihydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.21)

The title compound was prepared from 2,4,5-trihydroxybenzaldehyde (0.3 mmol, 46 mg), 3-aminophenol (0.3 mmol, 33 mg) and 1-*tert*-butyl-4-(isocyanomethyl)benzene (0.3 mmol, 52 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave brown oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a yellow solid. Yield (precipitation): 70 mg, 47 %; yield (preparative HPLC): 4.6 mg, 3.1 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 500.2 (15),

479.2 ([M + H]$^+$, 100). MS (CI-MS)[c] *m/z* (rel. int. in %) = 495.9 (30), 494.9 ({[M + H]$^+$ + 16}, 100). $C_{26}H_{27}N_3O_5S$ (M_r* = 493.57 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 477.57 g/mol) and {[M + H]$^+$ + 16} (M_r* = 493.57 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (477.57 g/mol) = 65 %; relative abundance (TIC): M_r* (493.57 g/mol) = 4.5 %; [c] 7 d after completion of the experiment; relative abundance (TIC): M_r (477.57 g/mol) = 0 %; relative abundance (TIC): M_r* (493.57 g/mol) = 40 %.

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-4-[4-(trifluoromethyl)benzylamino]-5-(2,4,5-trihydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.22)

The title compound was prepared from 2,4,5-trihydroxybenzaldehyde (0.3 mmol, 46 mg), 3,5-dichloro-4-(trifluoromethyl)aniline (0.3 mmol, 53 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave brown oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a gray solid. Yield (precipitation): 115 mg, 67 %; yield (preparative HPLC): 6.1 mg, 3.7 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.24 (s, 1H, O***H***-4'), 9.32 – 9.11 (m, 2H, Ph-O***H***), 9.03 – 8.88 (m, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.15 (s, 1H, O***H***-5), 7.75 – 7.64 (m, 2H, Ph-***H***), 7.60 – 7.49 (m, 2H, Ph-***H***), 7.06 (s, 2H, Ph-***H***), 6.90 – 6.79 (m, 1H, Ph-***H***), 6.25 (s, 1H, Ph-***H***), 4.75 – 4.53 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). MS (CI-MS)[b] *m/z* (rel. int. in %) = 580.0 (10), 558.0 ([M + H]$^+$, 100), 485.2 (15), 423.0 (5), 385 (10). MS (CI-MS)[c] *m/z* (rel. int. in %) = 614.9 ([M + H + MeCN]$^+$ + 16, 25), 575.9 ({[M + H]$^+$ + 16}, 60), 573.9 (100). $C_{23}H_{16}Cl_2F_3N_3O_4S$ (M_r* = 574.36 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 558.36 g/mol) and {[M + H]$^+$ + 16} (M_r* = 574.36 g/mol). NMR spectral data (^{1}H-NMR, ^{13}C-NMR) refer to M_r*. [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (558.36 g/mol) = 35 %; relative abundance (TIC): M_r* (574.36 g/mol) = 3.5 %; [c] 7 d after completion of the experiment; relative abundance (TIC): M_r (558.36 g/mol) = 0 %; relative abundance (TIC): M_r* (574.36 g/mol) = 75 %.

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-4-(4-methylbenzylamino)-5-(2,4,5-trihydroxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.23)

The title compound was prepared from 2,4,5-trihydroxybenzaldehyde (0.3 mmol, 46 mg), 3,5-dichloro-4-(trifluoromethyl)aniline (0.3 mmol, 53 mg) and 1-(isocyanomethyl)-4-methylbenzene (0.3 mmol, 40 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave orange oil, which

was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 80 mg, 51 %; yield (preparative HPLC): 4.6 mg, 3.0 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 649.0 (35), 576.1 (10), 526.0 (10), 504.9 ([M + H]$^+$, 100), 428.2 (60), 415.2 (20), 373.2 (25), 335.1 (90), 253.2 (25). MS (CI-MS)[c] *m/z* (rel. int. in %) = 522.9 (70), 520.9 ({[M + H]$^+$ + 16}, 100). $C_{26}H_{19}Cl_2N_3O_5S$ (M_r* = 520.39 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 504.39 g/mol) and {[M + H]$^+$ + 16} (M_r* = 520.39 g/mol). [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (504.39 g/mol) = 25 %; relative abundance (TIC): M_r* (520.39 g/mol) = 1.5 %; [c] 7 d after completion of the experiment; relative abundance (TIC): M_r (504.39 g/mol) = 10 %; relative abundance (TIC): M_r* (520.39 g/mol) = 25 %.

1-(3,5-Difluoro-4-hydroxyphenyl)-5-(2,5-dihydroxyphenyl)-5-hydroxy-4-[4-(trifluoro methyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.24)

The title compound was prepared from 2,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg), 3,5-difluoro-4-(trifluoromethyl)aniline (0.3 mmol, 44 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave orange oil, which was stored at -20 °C for 7 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as yellow solid. Yield (precipitation): 150 mg, 95 %; yield (preparative HPLC): 7.3 mg, 4.6 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.17 (s, 1H, O***H***-4'), 9.30 – 9.14 (m, 1H, Ph-O***H***), 9.01 – 8.91 (m, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.73 – 8.63 (m, 1H, Ph-O***H***), 7.81 (s, 1H, O***H***-5), 7.74 – 7.49 (m, 4H, Ph-***H***), 7.05 – 6.88 (m, 1H, Ph-***H***), 6.82 – 6.71 (m, 2H, Ph-***H***), 6.61 – 6.49 (m, 2H, Ph-***H***), 4.76 – 4.51 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). MS (CI-MS)[b] *m/z* (rel. int. in %) = 532.1 (22), 510.1 ([M + H]$^+$, 100), 485.2 (19). MS (CI-MS)[c] *m/z* (rel. int. in %) = 567.9 ([[M + H + MeCN]$^+$ + 16}, 30), 526.9 (35), 525.9 ({[M + H]$^+$ + 16}, 100). $C_{23}H_{16}F_5N_3O_4S$ (M_r* = 525.45 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 509.45 g/mol) and {[M + H]$^+$ + 16} (M_r* = 525.45 g/mol). NMR spectral data (^{1}H-NMR, ^{13}C-NMR) refer to M_r*. [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (509.45 g/mol) = 50 %; relative abundance (TIC): M_r* (525.45 g/mol) = 0.5 %; [c] 7 d after completion of the experiment; relative abundance (TIC): M_r (509.45 g/mol) = 0 %; relative abundance (TIC): M_r* (525.45 g/mol) = 90 %.

5-(2,5-Dihydroxyphenyl)-5-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(4-(trifluoro-methyl)benzylamino)-1*H*-imidazole-2(5*H*)-thione (5.25)

The title compound was prepared from 2,5-dihydroxybenzaldehyde (0.3 mmol, 41 mg), 5-amino-2-methoxyphenol (0.3 mmol, 42 mg) and 1-(isocyanomethyl)-4-(trifluoro-methyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil, which was stored at -20 °C for 2 days. A full conversion to the corresponding {$[M + H]^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {$[M + H]^+$ + 16] as a pale yellow solid. Yield (precipitation): 110 mg, 71 %; yield (preparative HPLC): 5.6 mg, 3.6 %. MS (CI-MS)[b] *m/z* (rel. int. in %) = 542.0 (5), 526.1 (15), 503.9 ($[M + H]^+$, 100), 385.1 (10), 218.1 (5). MS (CI-MS)[d] *m/z* (rel. int. in %) = 542.0 (15), 526.1 (25), 519.9 ({$[M + H]^+$ + 16}, 10), 503.9 ($[M + H]^+$, 100), 385.1 (20), 361.1 (5), 240.1 (5), 218.1 (10). $C_{24}H_{20}F_3N_3O_5S$ (M_r* = 519.49 g/mol).

Note: mass spectral data refer to $[M + H]^+$ (M_r = 503.49 g/mol) and {$[M + H]^+$ + 16} (M_r* = 519.49 g/mol). NMR spectral data (^{1}H-NMR, ^{13}C-NMR) refer to M_r*. [b] 4 h after completion of the experiment; relative abundance (TIC): M_r (503.49 g/mol) = 40 %; relative abundance (TIC): M_r* (519.49 g/mol) = 7 %; [d] 48 h after completion of the experiment; relative abundance (TIC): M_r (503.49 g/mol) = 10 %; relative abundance (TIC): M_r* (519.49 g/mol) = 25 %.

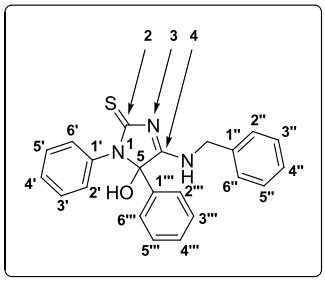
5.8.2.4 Preparation of the compounds 5.26-5.35

General procedure[52]

A solution of the pertinent aldehyde (1 eq) and 4-amino-2,6-dichlorophenol (1 eq) in anhydrous MeOH (1 mL per mmol) was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq), pyridinium chloride (4 eq) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (1 eq), the mixture was stirred overnight below 20 °C. Insoluble material was removed by filtration, and the solvent (MeOH) was removed under reduced pressure. After addition of ethyl acetate as solvent, precipitated material was removed by filtration and the crude product was obtained as residue after evaporation. The crude substance was subjected to preparative HPLC (method indicated) yielding the corresponding {$[M + H]^+$ + 16] product.[a]

[a] molecular $[M + H]^+$ species of compounds **5.26**-**5.35** showed complete autoxidation to the corresponding {$[M + H]^+$ + 16} derivatives within the first 48 h. HPLC isolation of {$[M + H]^+$ + 16} substances was performed no earlier than 72 h after completion of the experiment.

Labeling of atoms (4-amino-1*H*-imidazole-2(5*H*)-ones derivatives)



1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-phenyl-4-[4-(trifluoromethyl)benzyl amino]-1*H*-imidazole-2(5*H*)-thione (5.26)

The title compound was prepared from benzaldehyde (0.4 mmol, 58 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave red oil, which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as an orange solid. Yield (precipitation): 170 mg, 39 %; yield (preparative HPLC): 18.6 mg, 4.2 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.30 (s, 1H, O***H***-4'), 9.31 (t, *J* = 6.2 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.19 (s, 1H, O***H***-5), 7.68 (t, *J* = 10.4 Hz, 2H, Ph-***H***-3'',5''), 7.44 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 7.41 – 7.33 (m, 3H, Ph-***H***-3''',4''',5'''), 7.29 – 7.26 (m, 2H, Ph-***H***-2''',6'''), 6.99 (s, 2H, Ph-***H***-2',6'), 4.68 – 4.59 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.49 (C_{quat}, ***C***S), 177.45 (C_{quat}, ***C***-4), 148.39 (C_{quat}, Ph-***C***-4'), 142.47 (C_{quat}, Ph-***C***-1''), 136.17 (C_{quat}, Ph-***C***-1'''), 129.17 (+, Ph-***C***-2',6'), 129.04 (C_{quat}, Ph-***C***-3',5'), 128.59 (+, Ph-***C***-3''',4''',5'''), 128.01 (+, Ph-***C***-2'',6''), 125.54 (+, Ph-***C***-2''',6'''), 125.17 (+, Ph-***C***-3'',5''), 123.34 (C_{quat}, Ph-***C***-4''), 121.20 (C_{quat}, Ph-***C***-1'), 93.94 (C_{quat}, 5-***C***), 45.02 (-, NH***C***H_2-*p*-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 568.9 (35), 566.9 ([M* + H + MeCN]$^+$, 55), 528.9 (15), 527.9 (75), 525.8 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 526.0365, found [M* + H]$^+$ 526.03696. $C_{23}H_{16}Cl_2F_3N_3O_2S$ (M_r* = 526.36 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 526.36 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-4-[4-(trifluoromethyl)benzylamino]-5-(2,4,5-trimethoxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.27)

The title compound was prepared from 2,4,5-trimethoxybenzaldehyde (0.4 mmol, 59 mg) according to the general procedure. The crude product was obtained as residue after

evaporation (ethyl acetate), and gave a dark solid, which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a yellow solid. Yield (precipitation): 175 mg, 71 %; yield (preparative HPLC): 14.8 mg, 6.0 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 10.10 (s br, 1H, O***H***-4'), 9.00 (t, *J* = 6.2 Hz, 1H N***H***CH$_2$-*p*-C$_6$H$_4$-CF$_3$), 8.06 (s, 1H, O***H***-5), 7.72 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.49 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 7.11 (s, 1H, Ph-***H***-6'''), 7.05 (s, 2H, Ph-***H***-2',6'), 6.65 (s, *J* = 6.3 Hz, 1H, Ph-***H***-3'''), 4.62 (ddd, *J* = 97.8, 15.4, 6.1 Hz, 2H, NHC***H***$_2$-*p*-C$_6$H$_4$-CF$_3$), 3.76 (s, 3H, OC***H***$_3$-2'''), 3.65 (s, 3H, OC***H***$_3$-4'''), 3.59 (s, 3H, OC***H***$_3$-5'''). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.45 (C$_{quat}$, ***C***S), 177.87 (C$_{quat}$, ***C***-4), 150.67 (C$_{quat}$, Ph-***C***-2'''), 150.42 (C$_{quat}$, Ph-***C***-4'''), 142.80 (C$_{quat}$, Ph-***C***-1''), 142.55 (C$_{quat}$, Ph-***C***-4'), 141.72 (C$_{quat}$, Ph-***C***-5'''), 128.13 (+, Ph-***C***-2',6'), 127.95 (+, Ph-***C***-2'',6''), 125.14 (+, Ph-***C***-3'',5''), 125.10 (C$_{quat}$, Ph-***C***-3',5'), 123.40 (C$_{quat}$, Ph-***C***-4''), 121.04 (C$_{quat}$, Ph-***C***-1'), 114.34 (C$_{quat}$, Ph-***C***-1'''), 112.71 (+, Ph-***C***-6'''), 97.58 (+, Ph-***C***-3'''), 66.91 (C$_{quat}$, ***C***-5), 56.22 (OC***H***$_3$-4'''), 56.12 (+, OC***H***$_3$-5'''), 55.75 (+, OC***H***$_3$-2'''), 44.89 (-, NH***C***H$_2$-*p*-C$_6$H$_4$-CF$_3$). MS (CI-MS) *m/z* (rel. int. in %) = 659.1 (20), 657.0 ([M* + H + MeCN]$^+$, 30), 620.0 (15), 619.0 (20), 617.9 (80), 615.9 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 616.0682, found [M* + H]$^+$ 616.0699. $C_{26}H_{22}Cl_2F_3N_3O_5S$ (M_r* = 616.44 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 616.44 g/mol).

4-(1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-2-thioxo-4-[4-(trifluoromethyl) benzylamino]-2,5-dihydro-1*H*-imidazol-5-yl)benzoic acid (5.28)

The title compound was prepared from 4-formylbenzoic acid (0.4 mmol, 60 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a brown solid, which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 215 mg, 94 %; yield (preparative HPLC): 39.8 mg, 17.4 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 9.40 (s, 1H, N***H***CH$_2$-*p*-C$_6$H$_4$-CF$_3$), 8.37 (s, 1H, O***H***-5), 7.93 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.70 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.44 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.41 (d, *J* = 8.4 Hz, 2H, Ph-***H***-2''',6'''), 7.03 (s, *J* = 8.5 Hz, 2H, Ph-***H***-2',6'), 4.64 (s, 2H, NHC***H***$_2$-*p*-C$_6$H$_4$-CF$_3$). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.65 (C$_{quat}$, ***C***S), 177.09 (C$_{quat}$, ***C***-4), 166.80 (C$_{quat}$, Ph-***C***OOH), 148.62 (C$_{quat}$, Ph-***C***-4'), 142.35 (C$_{quat}$, Ph-***C***-1''), 140.53 (C$_{quat}$, Ph-***C***-4'''), 131.53 (C$_{quat}$, Ph-***C***-1'''), 129.59 (+, Ph-***C***-3''',5'''), 129.32 (C$_{quat}$, Ph-***C***-

3',5'), 129.21 (+, Ph-***C***-2',6'), 128.02 (+, Ph-***C***-2''',6'''), 125.98 (+, Ph-***C***-2'',6''), 125.23 (+, Ph-***C***-3'',5''), 123.46 (C_{quat}, Ph-***C***-4''), 121.28 (C_{quat}, Ph-***C***-1'), 93.63 (C_{quat}, ***C***-5), 45.11 (-, NH***C***H_2-*p*-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 614.9 (10), 614.0 (15), 612.9 (40), 610.9 ([M* + H + MeCN]$^+$, 70), 574.1 (15), 573.0 (20), 571.9 (70), 569.9 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 570.0263, found [M* + H]$^+$ 570.0263. $C_{24}H_{16}Cl_2F_3N_3O_4S$ (M_r* = 570.37 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 570.37 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-(1*H*-indol-5-yl)-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.29)

The title compound was prepared from 1*H*-indole-5-carbaldehyde (0.4 mmol, 44 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a dark brown solid, which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a yellow solid. Yield (precipitation): 165 mg, 73 %; yield (preparative HPLC): 11.6 mg, 5.1 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 11.18 (s, 1H, indole-N***H***), 10.21 (s br, 1H, O***H***-4'), 9.12 (t, *J* = 6.3 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.04 (s, 1H, O***H***-5), 7.66 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.60 (s, 1H, Ar-***H***-4'''), 7.42 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 7.39 (d, *J* = 8.5 Hz, 1H, Ar-***H***-7'''), 7.35 (d, *J* = 2.7 Hz, 1H, Ar-***H***-2'''), 7.04 (s, 2H, Ph-***H***-2',6'), 6.88 (dd, *J* = 8.5, 1.6 Hz, 1H, Ar-***H***-6'''), 6.44 – 6.42 (m, 1H, Ar-***H***-3'''), 4.60 (tt, *J* = 15.4, 7.7 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.25 (C_{quat}, ***C***S), 178.18 (C_{quat}, ***C***-4), 148.13 (C_{quat}, Ph-***C***-4'), 142.71 (C_{quat}, Ph-***C***-1''), 135.78 (C_{quat}, indole-***C***), 129.75 (C_{quat}, Ph-***C***-3',5'), 129.47 (C_{quat}, indole-***C***), 129.00 (+, Ph-***C***-2',6'), 127.95 (+, Ph-***C***-2'',6''), 127.14 (C_{quat}, indole-***C***), 126.23 (+, Ar-***C***-2'''), 126.18 (C_{quat}, Ph-***C***-4''), 125.11 (+, Ph-***C***-3'',5''), 121.03 (C_{quat}, Ph-***C***-1'), 118.15 (+, Ar-***C***-6'''), 117.80 (+, Ar-***C***-4'''), 111.52 (+, Ar-***C***-7'''), 101.68 (+, Ar-***C***-3'''), 94.71 (C_{quat}, ***C***-5), 45.02 (-, NH***C***H_2-p-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 606.2 ([M* + H + MeCN]$^+$, 10), 568.9 (20), 566.9 (70), 564.9 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 564.0401, found [M* + H]$^+$ 564.05001. $C_{25}H_{17}Cl_2F_3N_4O_2S$ (M_r* = 565.39 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 565.39 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-(1*H*-indol-6-yl)-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.30)

The title compound was prepared from 1*H*-indole-6-carbaldehyde (0.4 mmol, 44 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a dark brown solid which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a yellow solid. Yield (precipitation): 190 mg, 84 %; yield (preparative HPLC): 13.7 mg, 6.1 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 11.17 (s, 1H, indole-N***H***), 10.23 (s br, 1H, O***H***-4'), 9.15 (t, *J* = 6.2 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.09 (s, 1H, O***H***-5), 7.66 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.55 (d, *J* = 8.4 Hz, 1H, Ar-***H***-4'''), 7.49 (s, 1H, Ar-***H***-7'''), 7.41 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.40 – 7.38 (m, 1H, Ar-***H***-2'''), 7.05 (s, 2H, Ph-***H***-2',6'), 6.81 – 6.77 (m, 1H, Ar-***H***-5'''), 6.44 – 6.40 (m, 1H, Ar-***H***-3'''), 4.61 (qd, *J* = 15.3, 6.1 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.39 (C_{quat}, ***C***S), 177.99 (C_{quat}, ***C***-4), 148.18 (C_{quat}, Ph-***C***-4'), 142.57 (C_{quat}, Ph-***C***-1''), 135.35 (C_{quat}, indole-***C***), 129.63 (C_{quat}, Ph-***C***-3',5'), 129.42 (C_{quat}, indole-***C***), 129.07 (+, Ph-***C***-2',6'), 128.48 (C_{quat}, indole-***C***), 127.88 (+, Ph-***C***-2'',6''), 126.61 (+, Ar-***C***-2'''), 125.12 (+, Ph-***C***-3'',5''), 123.34 (C_{quat}, Ph-***C***-4''), 121.05 (C_{quat}, Ph-***C***-1'), 120.15 (+, Ar-***C***-4'''), 115.91 (+, Ar-***C***-5'''), 109.35 (+, Ar-***C***-7'''), 101.07 (+, Ar-***C***-3'''), 94.61 (C_{quat}, ***C***-5), 44.92 (-, NH***C***H_2-*p*-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 607.9 (20), 605.9 ([M* + H + MeCN]$^+$, 30), 568.8 (15), 567.9 (20), 566.9 (80), 564.9 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 565.0474, found [M* + H]$^+$ 565.0476. $C_{25}H_{17}Cl_2F_3N_4O_2S$ (M_r* = 565.39 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 565.39 g/mol).

5-(1*H*-Benzo[*d*]imidazol-5-yl)-1-(3,5-dichloro-4-hydroxyphenyl)-5-hydroxy-4-[4-(trifluoro methyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.31)

The title compound was prepared from 1*H*-benzo[*d*]imidazole-5-carbaldehyde (0.4 mmol, 44 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave beige solid, which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a yellow solid. Yield (precipitation): 170 mg, 85 %; yield (preparative HPLC): 13.7 mg, 6.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 12.50 (s, 1H, benzimidazole-N***H***), 9.22 (t, *J* = 6.1 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.26 – 8.23

(m, 1H, Ar-***H***-2'''), 8.19 (s, 1H, O***H***-5), 7.82 (d, *J* = 8.1 Hz, 1H, Ar-***H***-6'''), 7.66 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.42 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.11 (s, 1H, Ar-***H***-4'''), 7.04 (s, 2H, Ph-***H***-2',6'), 6.99 (d, *J* = 8.5 Hz, 1H, Ar-***H***-7'''), 4.67 – 4.57 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.44 (C_{quat}, ***C***S), 177.81 (C_{quat}, ***C***-4), 149.16 (C_{quat}, benzimidazole-***C***), 148.26 (C_{quat}, Ph-***C***-4'), 143.13 (+, Ar-***C***-2'''), 142.51 (C_{quat}, Ph-***C***-1''), 138.65 (C_{quat}, benzimidazole-***C***), 129.62 (C_{quat}, Ph-***C***-3',5'), 129.26 (+, Ph-***C***-2',6'), 129.17 (+, Ar-***C***-7'''), 128.98 (+, Ar-***C***-4'''), 127.93 (+, Ph-***C***-2'',6''), 127.84 (C_{quat}, benzimidazole-***C***), 125.49 (+, Ar-***C***-6'''), 125.15 (+, Ph-***C***-3'',5''), 123.34 (C_{quat}, Ph-***C***-4''), 121.11 (C_{quat}, Ph-***C***-1'), 94.32 (C_{quat}, ***C***-5), 44.97 (-, NH***C***H$_2$-*p*-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 647.9 (10), 610.9 (10), 609.9 (15), 608.9 (70), 606.9 ([M* + H + MeCN]$^+$, 100), 569.1 (10), 567.9 (40), 565.9 ([M* + H]$^+$, 55). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 566.0427, found [M* + H]$^+$ 566.0430. $C_{24}H_{16}Cl_2F_3N_5O_2S$ (M_r* = 566.38 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 566.38 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-(1*H*-indazol-5-yl)-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.32)

The title compound was prepared from 1*H*-indazole-5-carbaldehyde (0.4 mmol, 44 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a dark oil which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 200 mg, 90 %; yield (preparative HPLC): 11.9 mg, 5.3 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 13.15 (s, 1H, indazole-N***H***), 10.21 (s br, 1H, O***H***-4'), 9.23 (t, *J* = 6.2 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.21 (s, 1H, O***H***-5), 8.09 (s, 1H, Ar-***H***-3'''), 7.84 (s, 1H, Ar-***H***-4'''), 7.67 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.55 (d, *J* = 8.8 Hz, 1H, Ar-***H***-7'''), 7.43 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.09 (d, *J* = 8.0 Hz, 1H, Ar-***H***-6'''), 7.04 (s, 2H, Ph-***H***-2',6'), 4.68 – 4.57 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.47 (C_{quat}, ***C***S), 177.68 (C_{quat}, ***C***-4), 148.29 (C_{quat}, Ph-***C***-4'), 142.48 (C_{quat}, Ph-***C***-1''), 139.71 (C_{quat}, indazole-***C***), 134.19 (+, 3'''-ArC), 129.62 (C_{quat}, Ph-***C***-3',5'), 129.20 (C_{quat}, indazole-***C***), 129.08 (+, Ph-***C***-2',6'), 127.93 (+, Ph-***C***-2'',6''), 125.16 (+, Ph-***C***-3'',5''), 123.33 (C_{quat}, Ph-***C***-4''), 123.11 (+, Ar-***C***-6'''), 122.44 (C_{quat}, indazole-***C***), 121.14 (C_{quat}, Ph-***C***-1'), 118.58 (+, Ar-***C***-4'''), 110.49 (+, Ar-***C***-7'''), 94.19 (C_{quat}, ***C***-5), 44.99 (-, NH***C***H$_2$-*p*-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 610.1 (10), 608.9 (35), 606.9 ([M* + H + MeCN]$^+$, 60), 569.9 (15), 568.9 (20), 567.9

(80), 565.9 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 566.0427, found [M* + H]$^+$ 566.0427. $C_{24}H_{16}Cl_2F_3N_5O_2S$ (M_r* = 566.38 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 566.38 g/mol).

5-[4-(1*H*-Imidazol-1-yl)phenyl]-1-(3,5-dichloro-4-hydroxyphenyl)-5-hydroxy-4-[4-(trifluoromethyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.33)

The title compound was prepared from 4-(1*H*-imidazol-1-yl)benzaldehyde (0.4 mmol, 69 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a pale brown solid, which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a colorless solid. Yield (precipitation): 125 mg, 53 %; yield (preparative HPLC): 12.1 mg, 5.1 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.33 (s, 1H, O***H***-4'), 9.40 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.41 (s, 1H, imidazole-***H***), 8.36 (s, 1H, O***H***-5), 7.81 (s, 1H imidazole-***H***), 7.71 (t, *J* = 7.7 Hz, 4H, Ph-***H***-3'',5'', Ph-***H***-3''',5'''), 7.46 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.42 (d, *J* = 8.6 Hz, 2H, Ph-***H***-2''',6'''), 7.17 (s, 1H, imidazole-***H***), 7.10 (s, 2H, Ph-***H***-2',6'), 4.70 – 4.60 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.56 (C_{quat}, ***C***S), 177.16 (C_{quat}, ***C***-4), 148.42 (C_{quat}, Ph-***C***-4'), 142.39 (C_{quat}, Ph-***C***-1''), 137.06 (C_{quat}, Ph-***C***-1'''), 135.42 (+, imidazole-***C***), 134.72 (C_{quat}, Ph-***C***-4''), 129.26 (+, Ph-***C***-2',6'), 129.12 (+, imidazole-***C***), 129.00 (C_{quat}, Ph-***C***-3',5'), 128.00 (+, Ph-***C***-2'',6''), 127.33 (+, Ph-***C***-2''',6'''), 125.25 (+, Ph-***C***-3''',5'''), 123.34 (C_{quat}, Ph-***C***-4''), 121.29 (C_{quat}, Ph-***C***-1'), 120.26 (+, Ph-***C***-2''',6'''), 118.07 (+, imidazole-***C***), 93.54 (C_{quat}, ***C***-5), 45.08 (-, NH***C***H_2-*p*-C_6H_4-CF_3). MS (CI-MS) *m/z* (rel. int. in %) = 637.1 (10), 636.0 (20), 635.0 (55), 632.9 ([M* + H + MeCN]$^+$, 75), 596.0 (15), 595.0 (20), 593.9 (60), 591.9 ([M* + H]$^+$, 100), 358.9 (30), 337.4 (20). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 592.0583, found [M* + H]$^+$ 592.0585. $C_{26}H_{18}Cl_2F_3N_5O_2S$ (M_r* = 592.42 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 592.42 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-[4-(2-methylthiazol-4-yl)phenyl]-4-(4-(trifluoromethyl)benzylamino)-1*H*-imidazole-2(5*H*)-thione (5.34)

The title compound was prepared from 4-(2-methylthiazol-4-yl)benzaldehyde (0.4 mmol, 81 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a dark yellow solid which was stored at -20 °C

for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a yellow solid. Yield (precipitation): 220 mg, 88 %; yield (preparative HPLC): 45.3 mg, 18.2 %. *Note:* NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass data refer to the stable MH$^+$ + 16 species (abbreviation: M*). ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 10.31 (s, 1H, O***H***-4'), 9.39 (t, *J* = 6.2 Hz, 1H, N***H***CH$_2$-*p*-C$_6$H$_4$-CF$_3$), 8.29 (s, 1H, O***H***-5), 7.96 (s, 1H, thiazole-***H***), 7.93 (d, *J* = 8.6 Hz, 2H, Ph-***H***-3''',5'''), 7.69 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.47 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 7.33 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 7.05 (s, 2H, Ph-***H***-2',6'), 4.68 – 4.61 (m, 2H, NHC***H***$_2$-*p*-C$_6$H$_4$-CF$_3$), 2.70 – 2.67 (m, 3H, thiazole-C***H***$_3$). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.50 (C$_{quat}$, ***C***S), 177.38 (C$_{quat}$, ***C***-4), 165.75 (C$_{quat}$, thiazole-***C***), 152.95 (C$_{quat}$, thiazole-***C***), 148.39 (C$_{quat}$, Ph-***C***-4'), 142.47 (C$_{quat}$, Ph-***C***-1''), 135.51 (C$_{quat}$, Ph-***C***-4'''), 134.78 (C$_{quat}$, Ph-***C***-1'''), 129.17 (+, Ph-***C***-2',6'), 129.10 (C$_{quat}$, Ph-***C***-3',5'), 128.00 (C$_{quat}$, Ph-***C***-4''), 126.10 (+, Ph-***C***-2''',6'''), 126.04 (+, Ph-***C***-3''',5'''), 125.22 (+, Ph-***C***-3'',5''), 125.18 (+, Ph-***C***-2'',6''), 121.22 (C$_{quat}$, Ph-***C***-1'), 114.72 (+, thiazole-***C***), 93.87 (C$_{quat}$, ***C***-5), 45.06 (-, NH***C***H$_2$-*p*-C$_6$H$_4$-CF$_3$), 18.89 (+, thiazole-***C***H$_3$). MS (CI-MS) *m/z* (rel. int. in %) = 665.9 (20), 663.9 ([M* + H + MeCN]$^+$, 25), 625.9 (15), 625.0 (65), 622.9 ([M* + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 623.0351, found [M* + H]$^+$ 623.0355. $C_{27}H_{19}Cl_2F_3N_4O_2S_2$ (M_r* = 623.50 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass data spectral refer to the stable {[M + H]$^+$ + 16} species (M_r* = 623.50 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-[4-(4-methylpiperazin-1-yl)phenyl]-4-(4-(trifluoromethyl)benzylamino)-1*H*-imidazole-2(5*H*)-thione (5.35)

The title compound was prepared from 4-(4-methylpiperazin-1-yl)benzaldehyde (0.4 mmol, 82 mg) according to the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a yellow solid which was stored at -20 °C for 19 days. A full conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. Separation by RP-HPLC (Table 5.19, method 1) yielded the autoxidized species {[M + H]$^+$ + 16} as a pale yellow solid. Yield (precipitation): 225 mg, 90 %; yield (preparative HPLC): 37.7 mg, 15.1 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 9.19 (t, *J* = 6.2 Hz, 1H, N***H***CH$_2$-*p*-C$_6$H$_4$-CF$_3$), 8.06 (s, 1H, O***H***-5), 7.69 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.44 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.13 (d, *J* = 8.9 Hz, 2H, Ph-***H***-2''',6'''), 7.04 (s, 2H, Ph-***H***-2',6'), 6.95 (d, *J* = 9.0 Hz, 2H, Ph-***H***-3''',5'''), 4.62 (d, *J* = 6.1 Hz, 2H, NHC***H***$_2$-*p*-C$_6$H$_4$-CF$_3$), 3.26 (s br, 4H, piperazine-***H***), 2.88 (s br, 4H, piperazine-***H***), 2.53 (s, 3H, piperazine-C***H***$_3$). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.23 (C$_{quat}$, ***C***S), 177.63 (C$_{quat}$, ***C***-4), 150.47 (C$_{quat}$, Ph-***C***-4'''), 148.27 (C$_{quat}$, Ph-***C***-4'), 142.56 (C$_{quat}$, Ph-***C***-

1''), 129.63 (C_{quat}, Ph-***C***-1'''), 129.52 (C_{quat}, Ph-***C***-3',5'), 129.05 (+, Ph-***C***-2',6'), 127.99 (+, Ph-***C***-2'',6''), 126.46 (+, Ph-***C***-2''',6'''), 125.18 (+, Ph-***C***-3'',5''), 123.36 (C_{quat}, Ph-***C***-4''), 121.15 (C_{quat}, Ph-***C***-1'), 114.97 (+, Ph-***C***-3''',5'''), 94.08 (C_{quat}, ***C***-5), 53.30 (-, piperazine-***C***H_2), 46.20 (-, piperazine-***C***H_2), 44.98 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 43.81 (+, piperazine-***C***H_3). MS (CI-MS) *m/z* (rel. int. in %) = 669.0 (20), 668.0 (25), 667.0 (80), 665.0 ([M* + H + MeCN]$^+$, 100), 627.0 (15), 626.0 (65), 624.0 ([M* + H]$^+$, 50), 413.3 (10), 412.5 (30), 411.5 (25), 373.9 (40), 354.3 (85), 353.4 (90), 332.9 (10). HRMS (ESI-MS) *m/z* calcd. [M* + H]$^+$ 624.1209, found [M* + H]$^+$ 624.1212. $C_{28}H_{26}Cl_2F_3N_5O_2S$ (M_r* = 624.50 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass spectral data refer to the stable {[M + H]$^+$ + 16} species (M_r* = 624.50 g/mol).

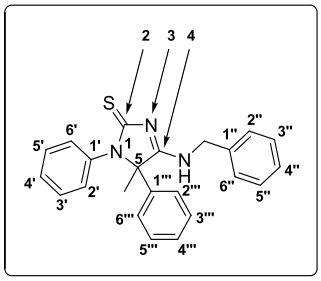
5.8.2.5 Preparation of the compounds 5.36-5.70

General procedure

Method A: A solution of the pertinent methyl ketone (1 eq) and 4-amino-2,6-dichlorophenol (1 eq) in anhydrous MeOH (1 mL per mmol) was stirred for 2.5 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq), pyridinium chloride (4 eq) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (1 eq), the mixture was stirred overnight below 20 °C. Insoluble material was removed by filtration, and the solvent (MeOH) was removed under reduced pressure. After addition of ethyl acetate as solvent, precipitated material was removed by filtration and the crude product was obtained as residue after evaporation. The crude substance was subjected to RP-HPLC (method indicated) yielding the target molecules.

Method B: A solution of the pertinent methyl ketone (1 eq) and amine (1 eq) in anhydrous MeOH (1 mL per mmol) was stirred for 3.0 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq), pyridinium chloride (4 eq) and the corresponding isocyanide (1 eq), the mixture was stirred overnight below 20 °C. After stirring at 0 °C overnight, solids were removed by filtration, the crude product was precipitated as described and subjected to RP-HPLC. Insoluble material was removed by filtration, and the solvent (MeOH) was removed under reduced pressure. After addition of ethyl acetate as solvent, precipitated material was removed by filtration and the crude product was obtained as residue after evaporation. The crude substance was subjected to preparative HPLC (method indicated) yielding the target molecules.

Labeling of atoms (4-amino-5-methyl-1*H*-imidazole-2(5*H*)-thiones derivatives)



1-(3,5-Dichloro-4-hydroxyphenyl)-5-(4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.36)

The title compound was prepared from 1-(4-hydroxyphenyl)ethanone (0.3 mmol, 41 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 115 mg, 71 %; yield (preparative HPLC): beige solid, 2.6 mg, 1.6 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.38 (s, 1H, O***H***-4'), 9.74 (s, 1H, Ph-O***H***-4'''), 9.13 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.71 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.46 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 6.99 (d, *J* = 8.7 Hz, 2H, Ph-H-2''',6'''), 6.82 – 6.78 (m, 2H, Ph-H-3''',5'''), 6.70 (s, 2H, Ph-H-2',6'), 4.66 (qd, *J* = 15.6, 6.0 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.73 (s, *J* = 9.5 Hz, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.67 (C_{quat}, ***C***S), 180.38 (C_{quat}, ***C***-4), 158.32 (C_{quat}, Ph-***C***-4'''), 146.40 (C_{quat}, Ph-***C***-4'), 143.14 (C_{quat}, Ph-***C***-1''), 133.88 (C_{quat}, Ph-***C***-1'''), 129.69 (+, Ph-***C***-2',6'), 127.92 (+, Ph-***C***-2'',6''), 127.62 (+, Ph-***C***-2''',6'''), 127.60 (C_{quat}, Ph-***C***-3',5'), 125.00 (+, Ph-***C***-3'',5''), 124.48 (C_{quat}, Ph-***C***-4''), 121.43 (C_{quat}, Ph-***C***-1'), 115.11 (+, Ph-***C***-3''',5'''), 73.05 (C_{quat}, ***C***-5), 44.88 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 17.13 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 541.0 (20), 539.0 ([$M^{+\bullet}$], 35), 389.9 (35), 202.0 (30), 176.2 (40), 159.0 (100), 146.0 (70), 121.0 (40), 109.1 (35), 72.1 (40), 59.1 (50). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 539.0449, found $[M + H]^+$ 539.0436. $C_{24}H_{18}Cl_2F_3N_3O_2S$ (M_r = 540.38 g/mol).

(*E*)-*N*'-(3,5-Dichloro-4-hydroxyphenyl)-*N*-[4-(trifluoromethyl)benzyl]formimidamide (5.37a)

1-(2,5-Dihydroxyphenyl)ethanone (0.6 mmol, 91 mg) was treated with 4-amino-2,6-dichlorophenol according to method A of the general procedure. Under these conditions the target molecule 1-(3,5-dichloro-4-hydroxyphenyl)-5-(2,5-dihydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione **5.40** was not obtained after the addition of the corresponding isocyanide. However, a full conversion to (*E*)-*N*'-(3,5-

dichloro-4-hydroxyphenyl)-*N*-[4-(trifluoromethyl)benzyl]formimidamide occurred. The crude product was obtained as residue after evaporation (ethyl acetate), and gave orange oil which was subjected to RP-HPLC (Table 5.19, method 2) to yield a yellow solid. Yield (precipitation): 100 mg, 92 %; yield (preparative HPLC): 9.6 mg, 8.8 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 7.90 (s, 1H, Ph-O***H***), 7.83 (d, *J* = 8.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.71 (d, *J* = 8.2 Hz, 2H, Ph-***H***), 7.60 (s, 1H, N=C***H***$NHCH_2$-*p*-C_6H_4-CF_3), 7.56 (d, 2H, Ph-***H***), 6.94 (s, 2H, Ph-***H***), 4.54 (s, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 151.01 (+, N=***C***$HNHCH_2$-*p*-C_6H_4-CF_3), 142.69 (C_{quat}, Ph-***C***), 136.96 (C_{quat}, Ph-***C***), 134.61 (C_{quat}, Ph-***C***), 129.44 (C_{quat}, Ph-***C***), 127.99 (+, Ph-***C***), 124.93 (+, Ph-***C***), 122.45 (+, Ph-***C***), 118.98 (+, Ph-***C***), 56.40 (-, NH***C***H_2-*p*-C_6H_4-CF_3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 364.0 (52), 363.1 (20), 362.0 ($[M]^{+\bullet}$, 90), 334.0 (64), 207.0 (40), 204.9 (66), 179.0 (52), 177.0 (85), 175.1 (45), 174.0 (100), 159.0 (80), 127.0 (30), 115.0 (25), 113.0 (69), 109.0 (46), 106.1 (33), 78.1 (53), 52.1 (23). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 362.0201, found $[M + H]^+$ 362.0195. $C_{15}H_{11}Cl_2F_3N_2O$ (M_r* = 363.16 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass data refer to the isolated (*E*)-*N*'-(3,5-dichloro-4-hydroxyphenyl)-*N*-(4-(trifluoromethyl)benzyl)formimidamide species (M_r* = 363.16 g/mol).

(*E*)-*N*'-(3,5-Dichloro-4-hydroxyphenyl)-*N*-[4-(trifluoromethyl)benzyl]formimidamide (5.38a)

1-(2,6-Dihydroxyphenyl)ethanone (0.6 mmol, 91 mg) was treated with 4-amino-2,6-dichlorophenol according to method A of the general procedure. Under these conditions the target molecule 1-(3,5-dichloro-4-hydroxyphenyl)-5-(2,6-dihydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione **5.42** was not obtained after addition of the corresponding isocyanide. Instead, a full conversion to (*E*)-*N*'-(3,5-dichloro-4-hydroxyphenyl)-*N*-[4-(trifluoromethyl)benzyl]formimidamide occurred. The crude product was obtained as residue after evaporation (ethyl acetate), and gave orange oil. Yield (precipitation): 95 mg, 87 %. MS (CI-MS) *m/z* (rel. int. in %) = 363.1 ($[M + H]^+$, 100), 156.1 (5). $C_{15}H_{11}Cl_2F_3N_2O$ (M_r = 363.16 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass data refer to the isolated (*E*)-*N*'-(3,5-dichloro-4-hydroxyphenyl)-*N*-(4-(trifluoromethyl)benzyl)formimidamide species (M_r* = 363.16 g/mol).

(*E*)-*N*'-(3,5-Dichloro-4-hydroxyphenyl)-*N*-[4-(trifluoromethyl)benzyl]formimidamide (5.39a)

1-(2,4,5-Trihydroxyphenyl)ethanone (0.6 mmol, 101 mg) was treated with 4-amino-2,6-dichlorophenol according to method A of the general procedure. Under these conditions

the target molecule 1-(3,5-dichloro-4-hydroxyphenyl)-5-(2,4,5-trihydroxyphenyl)-5-methyl-4-(4-(trifluoromethyl)benzylamino)-1*H*-imidazole-2(5*H*)-thione **5.41** was not obtained after the addition of the corresponding isocyanide. Instead, a full conversion to (*E*)-*N*'-(3,5-dichloro-4-hydroxyphenyl)-*N*-(4-(trifluoromethyl)benzyl)formimidamide occurred. The crude product was obtained as residue after evaporation (ethyl acetate), and gave orange oil. Yield (precipitation): 75 mg, 69 %. MS (CI-MS) *m/z* (rel. int. in %) = 363.1 ($[M + H]^+$, 100), 156.1 (10). $C_{15}H_{11}Cl_2F_3N_2O$ (M_r* = 363.16 g/mol).

Note: NMR spectral data (^{1}H-NMR, ^{13}C-NMR) and mass data refer to the isolated (*E*)-*N*'-(3,5-dichloro-4-hydroxyphenyl)-*N*-(4-(trifluoromethyl)benzyl)formimidamide species (M_r* = 363.16 g/mol).

4-(1-(4-Hydroxyphenyl)-5-methyl-2-thioxo-4-[4-(trifluoromethyl)benzylamino]-2,5-dihydro-1*H*-imidazol-5-yl)benzoic acid (5.40)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 4-aminophenol (0.8 mmol, 87 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.8 mmol, 148 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave beige solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 195 mg, 49 %; yield (preparative HPLC): dark oil, 13.2 mg, 3.3 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.52 (s, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.24 (s, 1H, O***H***-5), 7.98 – 7.96 (m, 2H, Ph-***H***-3''',5'''), 7.72 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.46 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.33 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 6.61 (d, *J* = 8.9 Hz, 2H, Ph-***H***-3',5'), 6.55 – 6.53 (m, 2H, Ph-***H***-2',6'), 4.75 – 4.64 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.84 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.24 (C_{quat}, ***C***S), 175.69 (C_{quat}, ***C***-4), 166.75 (C_{quat}, Ph-***C***OOH), 157.12 (C_{quat}, Ph-***C***-4'), 142.21 (C_{quat}, Ph-***C***-1''), 141.33 (C_{quat}, Ph-***C***-4'''), 131.24 (C_{quat}, Ph-***C***-1'''), 130.67 (C_{quat}, Ph-***C***-1'), 130.59 (+, Ph-***C***-2',6'), 129.78 (+, Ph-***C***-3''',5'''), 128.04 (+, Ph-***C***-2'',6''), 127.37 (C_{quat}, Ph-***C***-4''), 127.06 (+, Ph-***C***-2''',6'''), 125.39 (+, Ph-***C***-3'',5''), 115.32 (+, Ph-***C***-3',5'), 66.91 (C_{quat}, ***C***-5), 45.71 (-, NH***C***H_2-p-C_6H_4-CF_3), 20.00 (+, C***H***$_3$-5). MS (CI-MS) *m/z* (rel. int. in %) = 575.2 (5), 500.1 ($[M + H]^+$, 100). HRMS (ESI scan) *m/z* calcd. $[M + H]^+$ 500.1177, found $[M + H]^+$ 500.1189. $C_{25}H_{20}F_3N_3O_3S$ (M_r = 499.50 g/mol).

4-[4-(4-Chlorobenzylamino)-1-(4-hydroxyphenyl)-5-methyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.41)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 4-aminophenol (0.8 mmol, 87 mg) and 1-chloro-4-(isocyanomethyl)benzene (0.8 mmol, 121 mg) according to method B of the general procedure. The crude product was

obtained as residue after evaporation (ethyl acetate), and gave brown oil, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 290 mg, 78 %; yield (preparative HPLC): pale red solid, 4.6 mg, 1.2 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.54 (s, 1H, O***H***-4'), 9.03 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-Cl), 8.08 – 8.00 (m, 1H, Ph-COO***H***), 7.95 (dd, *J* = 10.4, 3.6 Hz, 2H, Ph-***H***-3''',5'''), 7.41 – 7.38 (m, 2H, Ph-***H***-2''',6'''), 7.27 (dd, *J* = 12.3, 8.5 Hz, 4H, Ph-***H***-2'',6'', Ph-***H***-3'',5''), 6.60 – 6.58 (m, 2H, Ph-***H***-3',5'), 6.54 – 6.51 (m, 2H, Ph-***H***-2',6'), 4.56 (qd, *J* = 15.2, 5.9 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-Cl), 1.77 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.72 (C_{quat}, ***C***S), 180.11 (C_{quat}, ***C***-4), 166.72 (C_{quat}, Ph-***C***OOH), 156.90 (C_{quat}, Ph-***C***-4'), 141.83 (C_{quat}, Ph-***C***-4'''), 136.90 (C_{quat}, Ph-***C***-1'''), 131.83 (C_{quat}, Ph-***C***-1''), 131.05 (C_{quat}, Ph-***C***-1'), 130.62 (+, Ph-***C***-2',6'), 129.70 (+, Ph-***C***-3''',5'''), 129.53 (C_{quat}, Ph-***C***-4''), 129.16 (+, Ph-***C***-2'',6''), 128.39 (+, Ph-***C***-2''',6'''), 126.95 (+, Ph-***C***-3'',5''), 115.21 (+, Ph-***C***-3',5'), 73.55 (C_{quat}, ***C***-5), 44.72 (-, NH***C***H_2-*p*-C_6H_4-Cl), 19.95 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 509.1 (10), 507.1 ([M + H + MeCN]$^+$, 20), 467.8 (40), 465.9 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 565.0474, found [M + H]$^+$ 565.0476. $C_{24}H_{20}ClN_3O_3S$ (M_r = 465.95 g/mol).

4-[4-(Benzylamino)-1-(3-hydroxy-4-methoxyphenyl)-5-methyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.42)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 5-amino-2-methoxyphenol (0.8 mmol, 111 mg) and (isocyanomethyl)benzene (0.8 mmol, 94 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave pale brown solid which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 210 mg, 57 %; yield (preparative HPLC): pale brown solid, 11.9 mg, 3.2 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.03 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-Ph), 7.96 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.36 – 7.24 (m, 5H, Ph-***H***-2'',3'',4'',5'',6''), 7.22 (d, *J* = 7.3 Hz, 2H, Ph-***H***-2''',6'''), 6.75 (d, *J* = 8.7 Hz, 1H, Ph-***H***-5'), 6.19 (d, *J* = 2.4 Hz, 1H, Ph-***H***-2'), 6.12 (dd, *J* = 8.6, 2.4 Hz, 1H, Ph-***H***-6'), 4.57 (ddd, *J* = 53.0, 15.1, 5.9 Hz, 2H, NHC***H***$_2$-*p*-Ph), 3.69 (s, 3H, OC***H***$_3$-4'''), 1.78 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.58 (C_{quat}, ***C***S), 180.09 (C_{quat}, ***C***-4), 166.82 (C_{quat}, Ph-***C***OOH), 147.27 (C_{quat}, Ph-***C***-4'), 146.07 (C_{quat}, Ph-***C***-3'), 141.96 (C_{quat}, Ph-***C***-4'''), 137.77 (C_{quat}, Ph-***C***-1''), 131.02 (C_{quat}, Ph-***C***-1'''), 129.64 (+, Ph-***C***-3''',5'''), 129.61 (C_{quat}, Ph-***C***-1'), 128.41 (+, Ph-***C***-3'',4'',5''), 127.23 (+, Ph-***C***-2''',6'''), 126.93 (+, Ph-***C***-2'',6''), 120.18 (+, Ph-***C***-6'), 116.94 (+, Ph-***C***-2'), 111.45 (+, Ph-***C***-5'), 73.55 (C_{quat}, ***C***-5), 55.45 (+, O***C***H_3-4'), 45.48 (-, NH***C***H_2-*p*-Ph), 19.88 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 504.1 ([M + H + MeCN]$^+$, 10), 464.0 (10), 462.9 (30), 462.0 ([M + H]$^+$, 100). HRMS

(ESI-MS) *m/z* calcd. $[M + H]^+$ 462.1482, found $[M + H]^+$ 462.1485. $C_{25}H_{23}N_3O_4S$ (M_r = 461.53 g/mol).

4-[1-(3-Hydroxyphenyl)-5-methyl-4-(4-methylbenzylamino)-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.43)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 3-aminophenol (0.8 mmol, 88 mg) and 1-(isocyanomethyl)-4-methylbenzene (0.8 mmol, 105 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a pale yellow solid which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 320 mg, 89 %; yield (preparative HPLC): colorless solid, 7.4 mg, 2.1 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.45 (s br, 1H, O***H***-3'), 9.00 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CH_3), 7.96 (d, *J* = 8.4 Hz, 2H, Ph-***H***-3''',5'''), 7.29 (d, *J* = 8.4 Hz, 2H, Ph-***H***-2''',6'''), 7.11 (q, *J* = 8.1 Hz, 4H, Ph-***H***-2'',6'', Ph-***H***-3'',5''), 7.02 (dd, *J* = 13.1, 5.1 Hz, 1H, Ph-***H***-5'), 6.63 (dd, *J* = 8.1, 1.7 Hz, 1H, Ph-***H***-4'), 6.21 – 6.15 (m, 2H, Ph-***H***-2', Ph-***H***-6'), 4.62 – 4.43 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CH_3), 2.26 (s, 3H, $NHCH_2$-*p*-C_6H_4-C***H***$_3$), 1.77 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.34 (C_{quat}, ***C***S), 180.05 (C_{quat}, ***C***-4), 166.88 (C_{quat}, Ph-***C***OOH), 157.33 (C_{quat}, Ph-***C***-3'), 141.73 (C_{quat}, Ph-***C***-4'''), 138.01 (C_{quat}, Ph-***C***-1'), 136.40 (C_{quat}, Ph-***C***-4''), 134.68 (C_{quat}, Ph-***C***-1''), 129.68 (+, Ph-***C***-3''',5'''), 129.13 (+, Ph-***C***-2'',6''), 128.94 (+, Ph-***C***-5'), 128.58 (C_{quat}, Ph-***C***-1'''), 127.22 (+, Ph-***C***-3'',5''), 126.83 (+, Ph-***C***-2''',6'''), 119.89 (+, Ph-***C***-6'), 116.52 (+, Ph-***C***-2'), 114.85 (+, Ph-***C***-4'), 73.62 (C_{quat}, ***C***-5), 45.25 (-, NH***C***H_2-*p*-C_6H_4-CH_3), 20.64 (+, $NHCH_2$-*p*-C_6H_4-***C***H_3), 19.89 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 488.1 ($[M + H + MeCN]^+$, 10), 448.0 (10), 446.9 (25), 445.9 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 446.1533, found $[M + H]^+$ 446.1532. $C_{25}H_{23}N_3O_3S$ (M_r = 445.53 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-5-phenyl-4-[4-(trifluoromethyl)benzyl amino]-1*H*-imidazole-2(5*H*)-thione (5.44)

The title compound was prepared from acetophenone (0.6 mmol, 72 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave pale red oil, which was subjected to preparative HPLC (Table 5.19, method 1). Yield (crude product): 175 mg, 33 %; yield (preparative HPLC): colorless solid, 2.9 mg, 0.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.39 (s, 1H, O***H***-4'), 9.22 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.71 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.49 – 7.41 (m, 5H, Ph-***H***), 7.20 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 6.69 (s, 2H, Ph-***H***-

2',6'), 4.74 – 4.61 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.81 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.52 (C_{quat}, ***C***S), 180.70 (C_{quat}, ***C***-4), 148.93 (C_{quat}, Ph-***C***-4'), 142.65 (C_{quat}, Ph-***C***-1''), 136.96 (C_{quat}, Ph-***C***-1'''), 129.80 (+, Ph-***C***-2',6'), 129.17 (C_{quat}, Ph-***C***-3',5'), 129.00 (+, Ph-***C***), 127.92 (+, 4C, Ph-***C***), 126.54 (+, Ph-***C***-3'',5''), 125.32 (+, Ph-***C***-2'',6''), 123.33 (C_{quat}, Ph-***C***-4''), 121.51 (C_{quat}, Ph-***C***-1'), 73.98 (C_{quat}, ***C***-5), 44.96 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.65 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 525.0 ([$M^{+\bullet}$], 31), 524.1 ([$M^{+\bullet}$], 21), 523.0 ([$M^{+\bullet}$], 53), 159.0 (100), 130.1 (44), 103.1 (33), 69.2 (31), 57.2 (49), 55.1 (46). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 524.0572, found $[M + H]^+$ 524.0581. $C_{24}H_{18}Cl_2F_3N_3OS$ (M_r = 524.39 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-5-(2,4,5-trimethoxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.45)

The title compound was prepared from 1-(2,4,5-trimethoxyphenyl)ethanone (0.6 mmol, 126 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a pale yellow solid, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 230 mg, 62 %; yield (preparative HPLC): pale red solid, 15.9 mg, 4.3 %. Single crystals of **5.48** were grown from a solution of 10 mg HPLC-purified product in anhydrous methanol (1.5 mL) in a 2 mL plastic vial. The solvent was slowly evaporated at room temperature over 14 days. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 10.30 (s, 1H, O***H***-4'), 8.88 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.73 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.49 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 6.84 – 6.65 (m, 4H, Ph-***H***-2',6', Ph-***H***-3''',6'''), 4.71 (dd, *J* = 15.5, 6.3 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 4.57 (dd, *J* = 15.5, 6.3 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 3.81 (d, *J* = 6.6 Hz, 3H, OC***H***$_3$-2'''), 3.69 (d, *J* = 10.1 Hz, 3H, OC***H***$_3$-4'''), 3.63 (s, 3H, OC***H***$_3$-5'''), 1.74 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.03 (C_{quat}, ***C***S), 181.57 (C_{quat}, ***C***-4), 152.31 (C_{quat}, Ph-***C***-2'''), 150.99 (C_{quat}, Ph-***C***-4'''), 148.52 (C_{quat}, Ph-***C***-4'), 143.06 (C_{quat}, Ph-***C***-1''), 142.04 (C_{quat}, Ph-***C***-5'''), 129.88 (+, Ph-***C***-2',6'), 129.53 (C_{quat}, Ph-***C***-3',5'), 127.91 (+, Ph-***C***-3'',5''), 125.23 (+, Ph-***C***-2'',6''), 123.5 (C_{quat}, Ph-***C***-4''), 121.36 (C_{quat}, Ph-***C***-1'), 114.86 (+, Ph-***C***-6'''), 114.47 (C_{quat}, Ph-***C***-1'''), 98.25 (+, Ph-***C***-3'''), 71.53 (C_{quat}, ***C***-5), 56.84 (+, O***C***H_3-4'''), 56.40 (+, O***C***H_3-5'''), 55.93 (+, O***C***H_3-2'''), 44.82 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 23.07 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 613.0 ([$M^{+\bullet}$], 16), 447.0 (28), 445.0 (49), 220.1 (30), 193.1 (22), 159.1 (43), 123.0 (38), 83.2 (56), 69.1 (87), 55.1 (100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 614.0889, found $[M + H]^+$ 614.0894. $C_{27}H_{24}Cl_2F_3N_3O_4S$ (M_r = 614.46 g/mol).

4-{1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-2-thioxo-4-[4-(trifluoromethyl) benzylamino]-2,5-dihydro-1*H*-imidazol-5-yl}benzoic acid (5.46)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 320 mg, 70 %; yield (preparative HPLC): gray solid, 14.1 mg, 3.1 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.43 (s, 1H, O***H***-4'), 9.26 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.00 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.71 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.45 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.34 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 6.74 (s, 2H, Ph-***H***-2',6'), 4.67 (d, *J* = 5.9 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.85 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.77 (C_{quat}, ***C***S), 180.30 (C_{quat}, ***C***-4), 166.72 (C_{quat}, Ph-***C***OOH), 149.06 (C_{quat}, Ph-***C***-4'), 142.52 (C_{quat}, Ph-***C***-1''), 141.39 (C_{quat}, Ph-***C***-4'''), 131.30 (C_{quat}, Ph-***C***-1'''), 129.91 (+, Ph-***C***-3''',5'''), 129.89 (+, Ph-***C***-2',6'), 129.06 (C_{quat}, Ph-***C***-3',5'), 128.05 (+, Ph-***C***-2'',6''), 126.99 (+, Ph-***C***-2''',6'''), 125.36 (+, Ph-***C***-3'',5''), 123.32 (C_{quat}, Ph-***C***-4''), 121.60 (C_{quat}, Ph-***C***-1'), 73.73 (C_{quat}, ***C***-5), 45.06 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.77 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 611.9 (10), 610.9 (40), 608.9 ($[M + H + MeCN]^+$, 30), 571.9 (10), 570.9 (20), 569.9 (70), 567.9 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 568.0471, found $[M + H]^+$ 568.0468. $C_{25}H_{18}Cl_2F_3N_3O_3S$ (M_r = 568.39 g/mol).

4-[4-(4-*tert*-Butylbenzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.47)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 4-amino-2,6-dichlorophenol (0.8 mmol, 142 mg) and 1-*tert*-butyl-4-(isocyanomethyl)benzene (0.8 mmol, 139 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a green solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 200 mg, 45 %; yield (preparative HPLC): pale lilac solid, 12.0 mg, 2.7 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 13.07 (s, 1H, Ph-COO***H***), 10.41 (s, 1H, O***H***-4'), 9.13 (t, *J* = 5.8 Hz, 1H, N***H***CH_2-*p*-C_6H_4-$C(CH_3)_3$), 7.98 (d, *J* = 8.6 Hz, 2H, Ph-***H***-3''',5'''), 7.36 – 7.31 (m, 4H, Ph-***H***-3'',5'', Ph-***H***-2''',6'''), 7.15 (d, *J* = 8.3 Hz, 2H, Ph-***H***-2'',6''), 6.74 (s, 2H, Ph-***H***-2',6'), 4.62 – 4.46 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-$C(CH_3)_3$), 1.83 (s, *J* = 5.8 Hz, 3H, C***H***$_3$-5), 1.25 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.80 (C_{quat}, ***C***S), 180.04 (C_{quat}, ***C***-4), 166.72 (C_{quat}, Ph-***C***OOH), 149.80 (C_{quat}, Ph-***C***-4''), 148.99 (C_{quat}, Ph-***C***-4'), 141.63 (C_{quat}, Ph-***C***-4'''), 134.42 (C_{quat}, Ph-***C***-1''), 131.18 (C_{quat}, Ph-***C***-1'''), 129.92 (+, Ph-***C***-3''',5'''), 129.68 (+, Ph-***C***-2',6'), 129.16 (C_{quat}, Ph-***C***-3',5'), 127.15 (+, Ph-***C***-2'',6''), 126.99 (+, Ph-***C***-3'',5''),

125.17 (+, Ph-***C***-2''',6'''), 121.56 (C_{quat}, Ph-***C***-1'), 73.61 (C_{quat}, ***C***-5), 45.38 (-, NH***C***H_2-*p*-C_6H_4-$C(CH_3)_3$), 34.19 (C_{quat}, ***C***$(CH_3)_3$), 31.19 (+, $C(\mathbf{C}H_3)_3$), 19.73 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 597.0 ($[M + H + MeCN]^+$, 5), 560.0 (10), 559.0 (20), 557.9 (70), 556.0 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 556.1223, found $[M + H]^+$ 556.1223. $C_{28}H_{27}Cl_2N_3O_3S$ (M_r = 556.50 g/mol).

4-[4-(Benzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.48)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 4-amino-2,6-dichlorophenol (0.8 mmol, 142 mg) and (isocyanomethyl)benzene (0.8 mmol, 94 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a pale brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 280 mg, 70 %; yield (preparative HPLC): pale yellow solid, 23.2 mg, 5.8 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.20 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-Ph), 7.98 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.33 (t, *J* = 7.8 Hz, 4H, Ph-***H***-3'',5'', Ph-***H***-2''',6'''), 7.27 (t, *J* = 7.3 Hz, 1H, Ph-***H***-4''), 7.23 (d, *J* = 7.2 Hz, 2H, Ph-***H***-2'',6''), 6.74 (s, 2H, Ph-***H***-2',6'), 4.59 (ddd, *J* = 42.0, 15.0, 5.9 Hz, 2H, NHC***H***$_2$-*p*-Ph), 1.84 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.83 (C_{quat}, ***C***S), 180.16 (C_{quat}, ***C***-4), 166.74 (C_{quat}, Ph-***C***OOH), 149.16 (C_{quat}, Ph-***C***-4'), 141.53 (C_{quat}, Ph-***C***-4'''), 137.58 (C_{quat}, Ph-***C***-1''), 131.34 (C_{quat}, Ph-***C***-1'''), 129.90 (+, Ph-***C***-3''',5'''), 129.82 (+, Ph-***C***-2',6'), 129.60 (C_{quat}, Ph-***C***-3',5'), 128.98 (+, Ph-***C***-4''), 128.44 (+, Ph-***C***-2''',6'''), 127.31 (+, Ph-***C***-2'',6''), 126.95 (+, Ph-***C***-3'',5''), 121.59 (C_{quat}, Ph-***C***-1'), 73.63 (C_{quat}, ***C***-5), 45.56 (-, NH***C***H_2-*p*-Ph), 19.75 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 545.1 (10), 543.0 (20), 540.9 ($[M + H + MeCN]^+$, 35), 502.0 (75), 499.9 ($[M + H]^+$, 100). HRMS (ESI scan) *m/z* calcd. $[M + H]^+$ 500.0597, found $[M + H]^+$ 500.0598. $C_{24}H_{19}Cl_2N_3O_3S$ (M_r = 500.40 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(1*H*-indol-5-yl)-5-methyl-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.49)

The title compound was prepared from 1-(1*H*-indol-5-yl)ethanone (0.8 mmol, 127 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave dark oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 280 mg, 62 %; yield (preparative HPLC): pale gray solid, 10.5 mg, 1.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 11.25 (s, 1H, indole-N***H***), 10.32 (s, 1H, O***H***-4'), 9.08 (t, *J* = 5.7 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.67

(d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.50 – 7.45 (m, 2H, Ph-***H***-6''',7'''), 7.43 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 7.41 – 7.39 (m, 1H, Ph-***H***-2'''), 7.09 (s, 1H, Ph-***H***-4'''), 6.71 (s, 2H, Ph-***H***-2',6'), 6.47 – 6.43 (m, 1H, Ph-***H***-3'''), 4.75 – 4.54 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.86 (s, 3H, CH_3-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.22 (C_{quat}, ***C***S), 181.44 (C_{quat}, ***C***-4), 148.72 (C_{quat}, Ph-***C***-4'), 142.80 (C_{quat}, Ph-***C***-1''), 140.79 (C_{quat}, indole-***C***), 135.54 (C_{quat}, indole-***C***), 129.59 (+, Ph-***C***-2',6'), 129.50 (C_{quat}, Ph-***C***-3',5'), 127.86 (+, Ph-***C***-2'',6''), 127.39 (C_{quat}, indole-***C***), 126.95 (C_{quat}, Ph-***C***-4''), 126.47 (+, Ar-***C***-2'''), 125.23 (+, Ph-***C***-3'',5''), 121.37 (C_{quat}, Ph-***C***-1'), 120.87 (+, Ar-***C***-4'''), 112.01 (+, Ar-***C***-6'''), 111.85 (+, Ar-***C***- 7'''), 101.69 (+, Ar-***C***-3'''), 74.54 (C_{quat}, ***C***-5), 44.87 (-, NH***C***H$_2$-*p*-C_6H_4-CF_3), 20.20 (+, ***C***H$_3$-5). MS (CI-MS) *m/z* (rel. int. in %) = 606.0 ([M + H + MeCN]$^+$, 10), 566.0 (20), 564.9 (75), 562.9 ([M + H]$^+$, 100). HRMS (ESI scan) *m/z* calcd. [M + H]$^+$ 563.0681, found [M + H]$^+$ 563.0681. $C_{26}H_{19}Cl_2F_3N_4OS$ (M_r = 563.42 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(1*H*-indazol-5-yl)-5-methyl-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.50)

The title compound was prepared from 1-(1*H*-indazol-5-yl)ethanone (0.8 mmol, 128 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave dark oil which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 240 mg, 53 %; yield (preparative HPLC): pale yellow solid, 7.3 mg, 1.6 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 13.22 (s, 1H, indazole-N***H***), 10.36 (s, 1H, O***H***-4'), 9.15 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.11 (d, *J* = 14.0 Hz, 1H, Ar-***H***-3'''), 7.71 (d, *J* = 1.1 Hz, 1H, Ar-***H***-4'''), 7.69 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.66 (d, *J* = 8.8 Hz, 1H, Ar-***H***-7'''), 7.44 (d, *J* = 6.6 Hz, 2H, Ph-***H***-2'',6''), 7.05 (dd, *J* = 8.8, 1.4 Hz, 1H, Ar-***H***-6'''), 6.72 (s, 2H, Ph-***H***-2',6'), 4.71 – 4.60 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.87 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.46 (C_{quat}, ***C***S), 180.98 (C_{quat}, ***C***-4), 148.87 (C_{quat}, Ph-***C***-4'), 142.67 (C_{quat}, Ph-***C***-1''), 139.42 (C_{quat}, indazole-***C***), 134.16 (+, Ar-***C***-3'''), 129.73 (+, Ph-***C***-2',6'), 129.32 (C_{quat}, Ph-***C***-3',5'), 128.59 (C_{quat}, indazole-***C***), 127.87 (+, Ph-***C***-2'',6''), 125.29 (+, Ph-***C***-3'',5''), 125.11 (C_{quat}, Ph-***C***-4''), 124.37 (+, Ar-***C***-6'''), 122.53 (C_{quat}, indazole-***C***), 121.48 (C_{quat}, Ph-***C***-1'), 119.31 (+, Ar-***C***-4'''), 111.18 (+, Ar-***C***-7'''), 74.18 (C_{quat}, ***C***-5), 44.94 (-, NH***C***H$_2$-*p*-C_6H_4-CF_3), 20.10 (+, ***C***H$_3$-5). MS (CI-MS) *m/z* (rel. int. in %) = 606.9 (25), 605.0 ([M + H + MeCN]$^+$, 40), 566.0 (65), 564.0 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 564.0634, found [M + H]$^+$ 564.0639. $C_{25}H_{18}Cl_2F_3N_5OS$ (M_r = 564.41 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(2-methoxyphenyl)-5-methyl-4-[4-(trifluoromethyl) benzylamino])-1*H*-imidazole-2(5*H*)-thione (5.51)

The title compound was prepared from 1-(2-methoxyphenyl)ethanone (0.6 mmol, 90 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow oil which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 145 mg, 39 %; yield (preparative HPLC): pale gray solid, 7.7 mg, 2.3 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.28 (s, 1H, O***H***-4'), 8.97 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.72 (t, *J* = 9.2 Hz, 2H, Ph-***H***-3'',5''), 7.48 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.42 (t, *J* = 8.5 Hz, 1H, Ph-***H***-4'''), 7.26 (d, *J* = 8.3 Hz, 1H, Ph-***H***-6'''), 7.10 (d, *J* = 8.3 Hz, 1H, Ph-***H***-3'''), 6.93 (d, *J* = 7.7 Hz, 1H, Ph-***H***-5'''), 6.70 (s, 2H, Ph-***H***-2',6'), 4.75 (dd, *J* = 15.4, 6.5 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 4.54 (dd, *J* = 15.4, 5.5 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 3.69 (s, 3H, OC***H***$_3$-2'''), 1.76 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.34 (C_{quat}, ***C***S), 181.40 (C_{quat}, ***C***-4), 157.33 (C_{quat}, Ph-***C***-2'''), 148.65 (C_{quat}, Ph-***C***-4'), 143.03 (C_{quat}, Ph-***C***-1''), 131.10 (+, Ph-***C***-4'''), 129.66 (+, Ph-***C***-2',6'), 129.18 (+, Ph-***C***-6'''), 127.92 (+, Ph-***C***-2'',6''), 127.70 (C_{quat}, Ph-***C***-3',5'), 125.21 (+, Ph-***C***-3'',5''), 123.72 (C_{quat}, Ph-***C***-1'''), 123.37 (C_{quat}, Ph-***C***-4''), 121.41 (C_{quat}, Ph-***C***-1'), 120.44 (+, Ph-***C***-5'''), 111.55 (+, Ph-***C***-3'''), 71.56 (C_{quat}, ***C***-5), 55.74 (+, O***C***H_3-2'''), 44.86 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 22.91 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 555.1 (29), 553.0 ([$M^{+\bullet}$], 61), 318.1 (57), 160.1 (54), 159.0 (100), 133.1 (51), 105.1 (54), 57.1 (27), 55.1 (27). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 553.0605, found $[M + H]^+$ 553.0592. $C_{25}H_{20}Cl_2F_3N_3O_2S$ (M_r = 554.41 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(4-methoxyphenyl)-5-methyl-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.52)

The title compound was prepared from 1-(4-methoxyphenyl)ethanone (0.6 mmol, 90 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave dark yellow oil which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 170 mg, 31 %; yield (preparative HPLC): colorless solid, 12.8 mg, 2.3 %; mp 137-138 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.31 (s, 1H, O***H***-4'), 8.98 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.73 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.48 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 7.42 (t, *J* = 7.7 Hz, 1H, Ph-***H***-3'''), 7.26 (d, *J* = 7.5 Hz, 1H, Ph-***H***-2'''), 7.10 (d, *J* = 8.1 Hz, 1H, Ph-***H***-6'''), 6.93 (t, *J* = 7.7 Hz, 1H, Ph-***H***-5'''), 6.70 (s, 2H, Ph-***H***-2',6'), 4.75 (dd, *J* = 15.4, 6.4 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 4.54 (dd, *J* = 15.4, 5.4 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 3.69 (s, 3H, OC***H***$_3$-4'''), 1.76 (s, 3H, CH_3-***5***). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.33 (C_{quat}, ***C***S), 181.40 (C_{quat}, ***C***-4), 157.34 (C_{quat}, Ph-***C***-4'''), 148.64 (C_{quat}, Ph-***C***-4'), 143.03 (C_{quat}, Ph-***C***-1''), 131.12 (+, Ph-

C-3'''), 129.68 (+, Ph-***C***-2',6'), 129.19 (+, Ph-***C***-2'''), 127.93 (+, Ph-***C***-2'',6''), 127.72 (C_{quat}, Ph-***C***-4''), 125.22 (+, Ph-***C***-3'',5''), 123.73 (C_{quat}, Ph-***C***-1'''), 123.37 (C_{quat}, Ph-***C***-3',5'), 121.42 (C_{quat}, Ph-***C***-1'), 120.45 (+, Ph-***C***-5'''), 111.56 (+, Ph-***C***-6'''), 71.58 (C_{quat}, ***C***-5), 55.75 (+, O***C***H_3-4'''), 44.87 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 22.92 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 555.3 (20), 553.0 ([$M^{+\bullet}$], 44), 447.1 (24), 445.1 (20), 319.1 (28), 310.1 (22), 207.1 (37), 148.9 (22), 111.1 (27), 105.0 (22), 95.1 (35), 85.1 (35), 71.1 (54), 69.1 (67), 57.1 (100), 55.1 (85). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 553.0605, found $[M + H]^+$ 553.0599. $C_{25}H_{20}Cl_2F_3N_3O_2S$ (M_r = 554.41 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(3,4-dimethoxyphenyl)-5-methyl-4-[4-(trifluoro methyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.53)

The title compound was prepared from 1-(3,4-dimethoxyphenyl)ethanone (0.6 mmol, 108 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow oil which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 110 mg, 31 %; yield (preparative HPLC): pale yellow solid, 4.9 mg, 1.4 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.39 (s, 1H, O***H***-4'), 9.19 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.71 (d, *J* = 8.0 Hz, 2H, Ph-***H***-3'',5''), 7.53 (d, *J* = 7.9 Hz, 2H, Ph-***H***-2'',6''), 6.90 (d, *J* = 8.9 Hz, 1H, Ph-***H***-5'''), 6.74 (s, 2H, Ph-***H***-2',6'), 6.32 (s, 2H, Ph-***H***-2''',6'''), 4.74 (dd, *J* = 15.2, 6.4 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 4.62 (dd, *J* = 15.2, 5.7 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 3.78 – 3.71 (m, 6H, OC***H***$_3$-3''',4'''), 1.70 (s, *J* = 7.8 Hz, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.32 (C_{quat}, ***C***S), 181.09 (C_{quat}, ***C***-4), 151.77 (C_{quat}, Ph-***C***-3'''), 148.89 (C_{quat}, Ph-***C***-4'), 148.21 (C_{quat}, Ph-***C***-4'''), 142.73 (C_{quat}, Ph-***C***-1''), 129.79 (+, Ph-***C***-2',6'), 129.44 (C_{quat}, Ph-***C***-1'''), 128.25 (+, Ph-***C***-2'',6''), 127.91 (C_{quat}, Ph-***C***-3',5') 125.27 (+, Ph-***C***-3'',5''), 123.61 (+, Ph-***C***-5'''), 123.30 (C_{quat}, Ph-***C***-4''), 121.46 (C_{quat}, Ph-***C***-1'), 104.24 (+, Ph-***C***-2''',6'''), 74.14 (C_{quat}, ***C***-5), 60.17 (+, O***C***H_3-4'''), 56.15 (+, O***C***H_3-3'''), 44.99 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 20.89 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 759.2 (20), 662.0 (5), 606.0 (15), 584.1 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 584.0711, found $[M + H]^+$ 584.0910. $C_{26}H_{22}Cl_2F_3N_3O_3S$ (M_r = 584.44 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-5-(2,3,4-trimethoxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.54)

The title compound was prepared from 1-(2,3,4-trimethoxyphenyl)ethanone (0.6 mmol, 126 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave red oil which was

subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 145 mg, 43 %; yield (preparative HPLC): beige solid, 8.4 mg, 2.3 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.33 (s, 1H, O***H***-4'), 9.04 (t, *J* = 5.7 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.72 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.55 (d, *J* = 8.1 Hz, 2H, Ph-***H***-2'',6''), 6.93 (d, *J* = 8.9 Hz, 1H, Ph-***H***-5'''), 6.77 (s, 2H, Ph-***H***-2',6'), 6.74 (d, *J* = 8.9 Hz, 1H, Ph-***H***-6'''), 4.74 (dd, *J* = 15.3, 6.1 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 4.62 (dd, *J* = 15.3, 5.7 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 3.78 (s, 3H, OC***H***$_3$-4'''), 3.72 (s, 3H, OC***H***$_3$-3'''), 3.70 (s, 3H, OC***H***$_3$-2'''), 1.70 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.35 (C_{quat}, ***C***S), 181.66 (C_{quat}, ***C***-4), 154.64 (C_{quat}, Ph-***C***-4'''), 151.77 (C_{quat}, Ph-***C***-3'''), 148.79 (C_{quat}, Ph-***C***-4'), 142.79 (C_{quat}, Ph-***C***-1''), 141.61 (C_{quat}, Ph-***C***-2'''), 129.67 (+, Ph-***C***-2',6'), 128.25 (+, Ph-***C***-2'',6''), 127.89 (C_{quat}, Ph-***C***-3',5'), 125.21 (+, Ph-***C***-3'',5''), 123.62 (+, Ph-***C***-5'''), 123.27 (C_{quat}, Ph-***C***-4''), 121.44 (C_{quat}, Ph-***C***-1'''), 120.91 (C_{quat}, Ph-***C***-1'), 106.84 (+, Ph-***C***-6'''), 71.50 (C_{quat}, ***C***-5), 60.44 (+, O***C***H_3-2'''), 60.15 (+, O***C***H_3-3'''), 55.93 (+, O***C***H_3-4'''), 44.97 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 22.89 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 613.2 ([$M^{+\bullet}$], 8), 447.0 (47), 445.0 (70), 378.2 (26), 363.1 (33), 220.1 (24), 174.1 (37), 159.1 (80), 109.1 (38), 85.1 (48), 71.2 (60), 69.1 (62), 57.1 (100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 614.0889, found $[M + H]^+$ 614.0889. $C_{27}H_{24}Cl_2F_3N_3O_4S$ (M_r = 614.46 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-5-(3,4,5-trimethoxyphenyl)-1*H*-imidazole-2(5*H*)-thione (5.55)

The title compound was prepared from 1-(3,4,5-trimethoxyphenyl)ethanone (0.3 mmol, 63 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave pale yellow solid which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 90 mg, 49 %; yield (preparative HPLC): colorless solid, 5.0 mg, 2.7 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.41 (s, 1H, O***H***-4'), 9.22 (t, *J* = 6.1 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.72 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.51 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 6.74 (s, 2H, Ph-***H***-2',6'), 6.34 (s, 2H, Ph-***H***-2''',6'''), 4.77 (dd, *J* = 15.2, 6.4 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 4.61 (dd, *J* = 15.2, 5.7 Hz, 1H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 3.67 – 3.61 (m, 9H, OC***H***$_3$-3''',4''',5'''), 1.78 (s, *J* = 7.8 Hz, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.23 (C_{quat}, ***C***S), 180.29 (C_{quat}, ***C***-4), 153.00 (C_{quat}, Ph-***C***-3''',5'''), 148.90 (C_{quat}, Ph-***C***-4'), 142.72 (C_{quat}, Ph-***C***-1''), 138.22 (C_{quat}, Ph-***C***-4'''), 132.45 (C_{quat}, Ph-***C***-1'''), 129.84 (+, Ph-***C***-2',6'), 129.19 (C_{quat}, Ph-***C***-3',5'), 128.25 (+, Ph-***C***-2'',6''), 125.35 (+, Ph-***C***-3'',5''), 123.30 (C_{quat}, Ph-***C***-4''), 121.46 (C_{quat}, Ph-***C***-1'), 104.34 (+, Ph-***C***-2''',6'''), 74.04 (C_{quat}, ***C***-5), 60.17 (+, O***C***H_3-4'''), 56.09 (+, O***C***H_3-3''',5'''), 45.01 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 20.19 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 613.1 ([$M^{+\bullet}$], 10), 444.9 (35), 159.0 (32), 97.1 (34), 83.1 (38), 74.0 (63),

73.1 (35), 71.1 (58), 69.1 (59), 60.1 (74), 57.1 (100), 55.1 (75). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 614.0889, found $[M + H]^+$ 614.0891. $C_{27}H_{24}Cl_2F_3N_3O_4S$ (M_r = 614.46 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(4-fluorophenyl)-5-methyl-4-[(4-(trifluoromethyl)-benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.56)

The title compound was prepared from 1-(4-fluorophenyl)ethanone (0.6 mmol, 83 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave pale red oil which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 240 mg, 74 %; yield (preparative HPLC): colorless solid, 9.2 mg, 1.7 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.27 (s, 1H, O***H***-4'), 9.24 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.72 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.45 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.32 – 7.23 (m, 4H, *p*-C_6***H***$_4$-F), 6.71 (s, 2H, Ph-***H***-2',6'), 4.68 (d, *J* = 5.8 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.81 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.51 (C_{quat}, ***C***S), 180.49 (C_{quat}, ***C***-4), 161.35 (C_{quat}, Ph-***C***-4'''), 149.81 (C_{quat}, Ph-***C***-4'), 142.60 (C_{quat}, Ph-***C***-1''), 133.27 (C_{quat}, Ph-***C***-1'''), 129.83 (+, Ph-***C***-2',6'), 129.10 (C_{quat}, Ph-***C***-3',5'), 129.01 (+, Ph-***C***-2''',6'''), 127.86 (+, Ph-***C***-3'',5''), 125.35 (+, Ph-***C***-2'',6''), 123.33 (C_{quat}, Ph-***C***-4''), 121.61 (C_{quat}, Ph-***C***-1'), 115.81 (+, Ph-***C***-3''',5'''), 73.45 (C_{quat}, ***C***-5), 44.98 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.99 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 541.0 ([$M^{+\bullet}$], 5), 309.0 (46), 306.9 (73), 221.0 (63), 218.9 (100), 159.1 (30), 120.0 (92), 55.2 (78). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 542.0478, found $[M + H]^+$ 542.0486. $C_{24}H_{17}Cl_2F_4N_3OS$ (M_r = 542.38 g/mol).

5-(4-Chlorophenyl)-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.57)

The title compound was prepared from 1-(4-chlorophenyl)ethanone (0.6 mmol, 93 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 80 mg, 24 %; yield (preparative HPLC): colorless solid, 3.2 mg, 0.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.43 (s, 1H, O***H***-4'), 9.23 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.72 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.54 – 7.50 (m, 2H, Ph-***H***-3''',5'''), 7.45 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 7.24 – 7.21 (m, 2H, Ph-***H***-2''',6'''), 6.76 (s, 2H, Ph-***H***-2',6'), 4.67 (d, *J* = 5.9 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.81 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 195.66 (C_{quat}, ***C***S), 180.34 (C_{quat}, ***C***-4), 149.04 (C_{quat}, Ph-***C***-4'), 142.54 (C_{quat}, Ph-***C***-1''), 135.90 (C_{quat}, Ph-***C***-1'''),

133.74 (C_{quat}, Ph-***C***-4'''), 129.90 (+, Ph-***C***-2',6'), 129.07 (C_{quat}, Ph-***C***-3',5'), 128.95 (+, Ph-***C***-3''',5'''), 128.67 (+, Ph-***C***-2''',6'''), 127.92 (+, Ph-***C***-3'',5''), 125.36 (+, Ph-***C***-2'',6''), 123.34 (C_{quat}, Ph-***C***-4''), 121.61 (C_{quat}, Ph-***C***-1'), 73.48 (C_{quat}, ***C***-5), 45.02 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.83 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 559.0 ([$M^{+\bullet}$], 30), 557.2 ([$M]^{+\bullet}$, 15), 314.0 (18), 164.1 (31), 159.1 (100), 97.2 (40), 71.2 (51), 57.2 (82), 55.1 (56). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 558.0183, found $[M + H]^+$ 558.0184. $C_{24}H_{17}Cl_3F_3N_3OS$ (M_r = 558.83 g/mol).

5-(4-Bromophenyl)-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl) benzylamino])-1*H*-imidazole-2(5*H*)-thione (5.58)

The title compound was prepared from 1-(4-bromophenyl)ethanone (0.6 mmol, 119 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 135 mg, 37 %; yield (preparative HPLC): colorless solid, 6.4 mg, 1.8 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.22 (t, *J* = 5.7 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.72 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.65 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.45 (d, *J* = 7.9 Hz, 2H, Ph-***H***-2'',6''), 7.16 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 6.75 (s, 2H, Ph-***H***-2',6'), 4.67 (d, *J* = 5.4 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.80 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.69 (C_{quat}, ***C***S), 180.29 (C_{quat}, ***C***-4), 149,39 (C_{quat}, Ph-***C***-4'), 142.54 (C_{quat}, Ph-***C***-1''), 136.32 (C_{quat}, Ph-***C***-4'''-ArC), 131.89 (+, Ph-***C***-3''',5'''), 129.86 (+, Ph-***C***-2',6'), 129.07 (C_{quat}, Ph-***C***-3',5'), 128.94 (+, Ph-***C***-2''',6'''), 127.91 (+, Ph-***C***-2'',6''), 125.36 (+, Ph-***C***-3'',5''), 123.46 (C_{quat}, Ph-***C***-4''), 122.37 (C_{quat}, Ph-***C***-1'''), 121.62 (C_{quat}, Ph-***C***-1'), 73.54 (C_{quat}, ***C***-5), 45.02 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.78 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 603.0 ([$M^{+\bullet}$], 40), 601.0 ([$M^{+\bullet}$], 24), 358.9 (20), 343.8 (28), 210.0 (26), 159.0 (100), 109.1 (32), 60.1 (48). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 602.9708, found $[M + H]^+$ 602.9716. $C_{24}H_{17}BrCl_2F_3N_3OS$ (M_r = 603.28 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-5-(4-(trifluoromethyl)phenyl)-1*H*-imidazole-2(5*H*)-thione (5.59)

The title compound was prepared from 1-(4-(trifluoromethyl)phenyl)ethanone (0.6 mmol, 113 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 190 mg, 53 %; yield (preparative HPLC): beige solid, 7.6 mg, 2.1 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] =

10.45 (s, 1H, O***H***-4'), 9.27 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.83 (d, *J* = 8.4 Hz, 2H, Ph-***H***-3''',5'''), 7.71 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.45 (d, *J* = 7.5 Hz, 4H, Ph-***H***-2'',6'', Ph-***H***-2''',6'''), 6.74 (s, 2H, Ph-***H***-2',6'), 4.75 – 4.61 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.87 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.85 (C_{quat}, ***C***S), 180.10 (C_{quat}, ***C***-4), 149.11 (C_{quat}, Ph-***C***-4'), 142.47 (C_{quat}, Ph-***C***-1''), 141.39 (C_{quat}, Ph-***C***-1'''), 129.93 (+, Ph-***C***-2',6'), 128.96 (C_{quat}, Ph-***C***-3',5'), 127.94 (+, Ph-***C***-2'',6''), 127.76 (+, Ph-***C***-2''',6'''), 125.93 (+, Ph-***C***-3''',5'''), 125.35 (+, Ph-***C***-3'',5''), 123.31 (C_{quat}, Ph-***C***-4''), 123.00 (C_{quat}, Ph-***C***-4'''), 121.65 (C_{quat}, Ph-***C***-1'), 73.59 (C_{quat}, ***C***-5), 45.07 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.71 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 593.0 ([$M^{+\bullet}$], 19), 592.0 ([$M^{+\bullet}$], 12), 591.1 ([$M^{+\bullet}$], 25), 348.0 (14), 332.0 (14), 204.0 (13), 159.0 (100), 109.1 (16). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 592.0446, found $[M + H]^+$ 592.0450. $C_{25}H_{17}Cl_2F_6N_3OS$ (M_r = 592.38 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-5-(4-nitrophenyl)-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.60)

The title compound was prepared from 1-(4-nitrophenyl)ethanone (0.6 mmol, 99 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave orange oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 215 mg, 63 %; yield (preparative HPLC): pale yellow solid, 6.7 mg, 1.4 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 10.46 (s, 1H, O***H***-4'), 9.32 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.33 – 8.26 (m, 2H, Ph-***H***-3''',5'''), 7.72 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.53 – 7.49 (m, 2H, Ph-***H***-2''',6'''), 7.44 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 6.82 (s, 2H, Ph-***H***-2',6'), 4.73 – 4.62 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.90 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 196.03 (C_{quat}, ***C***S), 179.84 (C_{quat}, ***C***-4), 149.21 (C_{quat}, Ph-***C***-4'), 147.66 (C_{quat}, Ph-***C***-1'''), 143.80 (C_{quat}, Ph-***C***-4'''), 142.39 (C_{quat}, Ph-***C***-1''), 129.99 (+, Ph-***C***-2',6'), 128.89 (C_{quat}, Ph-***C***-3',5'), 128.40 (+, Ph-***C***-2''',6'''), 127.85 (+, Ph-***C***-2'',6''), 125.41 (+, Ph-***C***-3'',5''), 124.07 (+, Ph-***C***-3''',5'''), 123.32 (C_{quat}, Ph-***C***-4''), 121.71 (C_{quat}, Ph-***C***-1'), 73.45 (C_{quat}, ***C***-5), 45.10 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.84 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 568.0 ([$M^{+\bullet}$], 27), 159.0 (94), 131.1 (20), 109.1 (36), 91.1 (45), 83.2 (49), 71.2 (48), 69.1 (70), 57.2 (99), 55.2 (100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 569.0423, found $[M + H]^+$ 569.0432. $C_{24}H_{17}Cl_2F_3N_4O_3S$ (M_r = 569.38 g/mol).

4-{1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-2-thioxo-4-[4-(trifluoromethyl) benzylamino]-2,5-dihydro-1*H*-imidazol-5-yl}phenylboronic acid (5.61)

The title compound was prepared from 4-acetylphenylboronic acid (0.6 mmol, 98 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave pale yellow oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 140 mg, 40 %; yield (preparative HPLC): pale yellow solid, 3.1 mg, 0.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.39 (s, 1H, O***H***-4'), 9.19 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.16 (s, 2H, B(O***H***)$_2$), 7.84 (d, *J* = 8.2 Hz, 2H, Ph-***H***-2''',6'''), 7.70 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.44 (d, *J* = 7.9 Hz, 2H, Ph-***H***-2'',6''), 7.17 (d, *J* = 8.3 Hz, 2H, Ph-***H***-3''',5'''), 6.71 (s, 2H, Ph-***H***-2',6'), 4.71 – 4.61 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.81 (s, 3H, 5-CH_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 195.57 (C_{quat}, ***C***S), 180.71 (C_{quat}, ***C***-4), 148.89 (C_{quat}, Ph-***C***-4'), 146.76 (C_{quat}, Ph-***C***-1'''), 142.65 (C_{quat}, Ph-***C***-1''), 138.51 (C_{quat}, Ph-***C***-4'''), 134.68 (+, Ph-***C***-2''',6'''), 129.76 (+, Ph-***C***-3',5'), 129.26 (+, Ph-***C***-2',6'), 127.91 (+, Ph-***C***-2'',6''), 125.49 (+, Ph-***C***-3''',5'''), 125.30 (+, Ph-***C***-3'',5''), 123.32 (C_{quat}, Ph-***C***-4''), 121.49 (C_{quat}, Ph-***C***-1'), 74.00 (C_{quat}, ***C***-5), 44.96 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.66 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 590.0 (5), 568.1 ([M + H]$^+$, 100), 180.1 (3), 158.2 (4). HRMS (ESI scan) m/z calcd. [M + H]$^+$ 568.0642, found [M + H]$^+$ 568.0652. $C_{24}H_{19}BCl_2F_3N_3O_3S$ (M_r = 568.20 g/mol).

5-(Biphenyl-4-yl)-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)-benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.62)

The title compound was prepared from 1-(biphenyl-4-yl)ethanone (0.8 mmol, 157 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 260 mg, 54 %; yield (preparative HPLC): pale yellow solid, 4.8 mg, 1.0 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.41 (s, 1H, O***H***-4'), 9.26 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.04 – 8.02 (m, 2H, Ph-***H***-2'',6''), 7.83 – 7.81 (m, 2H, Ph-***H***-3'',5''), 7.77 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.75 – 7.73 (m, 2H, Ph-***H***-8''',12'''), 7.71 (s, 1H, Ph-***H***-10'''), 7.69 (dd, *J* = 8.2, 1.1 Hz, 2H, Ph-***H***-9''',11'''), 7.29 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 6.76 (s, 2H, Ph-***H***-2',6'), 4.70 (d, *J* = 5.9 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.85 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.57 (C_{quat}, ***C***S), 180.66 (C_{quat}, ***C***-4), 148.97 (C_{quat}, Ph-***C***-4'), 144.52 (C_{quat}, Ph-***C***-1''), 142.64 (C_{quat}, Ph-***C***-7''), 140.74 (C_{quat}, Ph-***C***-1'''), 138.70 (C_{quat}, Ph-***C***-4'''), 129.87 (+, Ph-***C***-2',6'), 129.22 (C_{quat}, Ph-***C***-3',5'), 128.89 (+, Ph-***C***-9''',11'''), 127.90 (+, Ph-***C***-2'',6''), 127.23 (+, Ph-***C***-8''',12'''), 126.98 (+, Ph-***C***-2''',6'''), 126.84 (+, Ph-***C***-3''',5'''), 126.70 (+, Ph-***C***-3'',5''), 125.35 (+, Ph-***C***-10'''), 123.35 (C_{quat}, Ph-***C***-4''), 121.56 (C_{quat}, Ph-***C***-1'), 73.82 (C_{quat},

C-5), 45.01 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.78 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 643.1 (10), 641.1 ([M + H + MeCN]$^+$, 20), 602.0 (60), 600.0 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 600.0885, found [M + H]$^+$ 600.0892. $C_{30}H_{22}Cl_2F_3N_3OS$ (M_r = 600.48 g/mol).

3-{1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-2-thioxo-4-[4-(trifluoromethyl) benzylamino]-2,5-dihydro-1*H*-imidazol-5-yl}benzonitrile (5.63)

The title compound was prepared from 3-acetylbenzonitrile (0.8 mmol, 116 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave gray solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 280 mg, 64 %; yield (preparative HPLC): colorless solid, 19.3 mg, 4.4 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.53 (s, 1H, O***H***-4'), 9.26 (t, *J* = 5.6 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.92 (d, *J* = 7.8 Hz, 1H, Ph-***H***-2'''), 7.71 (d, 2H, Ph-***H***-3'',5''), 7.68 (m, 2H, Ph-***H***-4''',6'''), 7.49 (dd, *J* = 19.6, 7.5 Hz, 1H, Ph-***H***-5'''), 7.44 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 6.77 (s, 2H, Ph-***H***-2',6'), 4.67 (d, *J* = 5.2 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 2.07 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.86 (C_{quat}, ***C***S), 179.93 (C_{quat}, ***C***-4), 149.17 (C_{quat}, Ph-***C***-4'), 142.49 (C_{quat}, Ph-***C***-1''), 133.05 (+, Ph-***C***-2'''), 131.72 (+, Ph-***C***-5'''), 130.50 (+, Ph-***C***-4''',6'''), 130.36 (C_{quat}, Ph-***C***-1'''), 130.07 (+, Ph-***C***-2',6'), 128.89 (C_{quat}, Ph-***C***-3',5'), 128.00 (+, Ph-***C***-2'',6''), 125.37 (+, Ph-***C***-3'',5''), 123.32 (C_{quat}, Ph-***C***-4''), 121.68 (C_{quat}, Ph-***C***-1'), 118.26 (***C***N-3'''), 111.93 (C_{quat}, Ph-***C***-3''), 73.46 (C_{quat}, ***C***-5), 44.98 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.77 (+, ***C***H_3-5). MS (ESI-MS) m/z (rel. int. in %) = 593.0 (10), 592.0 (65), 589.9 ([M + H + MeCN]$^+$, 100), 548.9 ([M + H]$^+$, 60). HRMS (ESI scan) m/z calcd. [M + H]$^+$ 549.0525, found [M + H]$^+$ 549.0526. $C_{25}H_{17}Cl_2F_3N_4OS$ (M_r = 549.39 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-5-(naphthalen-2-yl)-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.64)

The title compound was prepared from 1-(naphthalen-2-yl)ethanone (0.6 mmol, 102 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow oil, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 205 mg, 60 %; yield (preparative HPLC): colorless solid, 4.8 mg, 1.4 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 10.37 (s, 1H, O***H***-4'), 9.20 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.02 (t, *J* = 9.5 Hz, 1H, Ar-***H***-4'''), 7.96 (t, *J* = 8.3 Hz, 2H, Ar-***H***-5''',8'''), 7.86 (s, 1H, Ar-***H***-2'''), 7.68 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.57 (pd, *J* = 6.9, 1.5 Hz, 2H, Ar-***H***-6''',7'''), 7.44 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''),

7.20 (d, *J* = 8.6 Hz, 1H, Ar-***H***-3'''), 6.76 (s, 2H, Ph-***H***-2',6'), 4.67 (qd, *J* = 15.5, 6.0 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.94 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.79 (C_{quat}, ***C***S), 180.63 (C_{quat}, ***C***-4), 148.94 (C_{quat}, Ph-***C***-4'), 142.61 (C_{quat}, Ph-***C***-1''), 133.94 (C_{quat}, Ar-***C***-2'''), 132.68 (C_{quat}, naphthalene-***C***), 132.52 (C_{quat}, naphthalene-***C***), 129.79 (+, Ph-***C***-2',6'), 129.26 (+, Ph-***C***-3',5'), 128.86 (+, Ar-***C***-4'''), 128.30 (+, Ar-***C***-5'''), 127.91 (+, Ph-***C***-2'',6''), 127.57 (+, Ar-***C***-8'''), 127.13 (+, Ar-***C***-7'''), 126.82 (+, Ar-***C***-6'''), 126.24 (+, Ar-***C***-1'''), 125.29 (+, Ph-***C***-3'',5''), 123.74 (+, Ar-***C***-3'''), 123.31 (C_{quat}, Ph-***C***-4''), 121.53 (C_{quat}, Ph-***C***-1'), 74.12 (C_{quat}, ***C***-5), 45.00 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.91 (+, ***C***H_3-5). RP-HPLC (220 nm, gradient 1): 97.5 % (t_R = 26.7 min, k = 2.5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 576.1 ([$M^{+\bullet}$], 5), 573.0 ([$M^{+\bullet}$], 28), 445.0 (6), 330.1 (16), 285.0 (18), 218.9 (20), 180.1 (48), 159.0 (100), 155.1 (42), 127.1 (40), 57.2 (28). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 574.0729, found $[M + H]^+$ 574.0736. $C_{28}H_{20}Cl_2F_3N_3OS$ (M_r = 577.44 g/mol).

5-(Benzo[*d*][1,3]dioxol-5-yl)-1-(3,5-dichloro-4-hydroxyphenyl)-5-methyl-4-[4-(trifluoromethyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.65)

The title compound was prepared from 1-(benzo[*d*][1,3]dioxol-5-yl)ethanone (0.6 mmol, 98 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave orange oil, which was subjected to RP-HPLC (Table 5.19, method 1). Yield (crude product): 170 mg, 50 %; yield (preparative HPLC): pale yellow solid, 5.4 mg, 1.6 %. ^{1}H-NMR (600 MHz, DMSO-*d*$_6$): δ [ppm] = 10.41 (s, 1H, O***H***-4'), 9.26 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.71 (d, *J* = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.46 (d, *J* = 8.0 Hz, 2H, Ph-***H***-2'',6''), 6.99 (d, *J* = 8.0 Hz, 1H, Ar-***H***-4'''), 6.77 (s, 2H, Ph-***H***-2',6'), 6.71 – 6.67 (m, 2H, Ar-***H***-6''',7'''), 6.06 (d, *J* = 8.4 Hz, 2H, Ar-***H***-2'''), 4.71 – 4.62 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.77 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-*d*$_6$): δ [ppm] = 195.57 (C_{quat}, ***C***S), 180.40 (C_{quat}, ***C***-4), 148.93 (C_{quat}, Ph-***C***-4'), 147.69 (C_{quat}, dioxole-***C***), 147.58 (C_{quat}, dioxole-***C***), 142.59 (C_{quat}, Ph-***C***-1''), 130.73 (C_{quat}, Ph-***C***-5''), 129.74 (+, Ph-***C***-2',6'), 129.19 (+, Ph-***C***-3',5'), 127.93 (+, Ph-***C***-2'',6''), 125.30 (+, Ph-***C***-3'',5''), 123.27 (C_{quat}, Ph-***C***-4''), 121.54 (C_{quat}, Ph-***C***-1'), 120.57 (+, Ar-***C***-6'''), 108.50 (+, Ar-***C***-4'''), 106.92 (+, Ar-***C***-7'''), 101.57 (+, Ar-***C***-2'''), 73.79 (C_{quat}, ***C***-5), 45.89 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 20.10 (+, ***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 569.1 ([$M^{+\bullet}$], 22), 567.0 ([$M^{+\bullet}$], 29), 444.9 (14), 334.1 (51), 308.0 (17), 279.0 (27), 174.0 (100), 159.0 (82), 149.1 (29), 109.0 (25), 55.2 (36). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 568.0471, found $[M + H]^+$ 568.0482. $C_{25}H_{18}Cl_2F_3N_3O_3S$ (M_r = 568.39 g/mol).

1-(3,5-Dichloro-4-hydroxyphenyl)-5-(1*H*-indazol-6-yl)-5-methyl-4-[(4-(trifluoromethyl)benzylamino]-1*H*-imidazole-2(5*H*)-thione (5.66)

The title compound was prepared from 1-(1*H*-indol-6-yl)ethanone (0.8 mmol, 128 mg) according to method A of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave dark oil, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 360 mg, 80 %; yield (preparative HPLC): pale gray solid, 11.0 mg, 1.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 13.18 (s, 1H, indazole-N***H***), 10.38 (s, 1H, O***H***-4'), 9.17 (t, *J* = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 8.13 (s, 1H, Ar-***H***-3'''), 7.88 (d, *J* = 8.5 Hz, 1H, Ar-***H***-4'''), 7.68 (d, *J* = 8.1 Hz, 2H, Ph-***H***-3'',5''), 7.44 (s, 1H, Ar-***H***-7'''), 7.42 (d, *J* = 6.7 Hz, 2H, Ph-***H***-2'',6''), 6.85 (d, *J* = 8.6 Hz, 1H, Ar-***H***-5'''), 6.75 (s, 2H, Ph-***H***-2',6'), 4.72 – 4.60 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.88 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.68 (C_{quat}, ***C***S), 180.74 (C_{quat}, ***C***-4), 148.93 (C_{quat}, Ph-***C***-4'), 142.62 (C_{quat}, Ph-***C***-1''), 134.53 (C_{quat}, indazole-***C***), 133.54 (+, Ar-***C***-3'''), 129.68 (+, Ph-***C***-2',6'), 129.28 (C_{quat}, Ph-***C***-3',5'), 127.99 (C_{quat}, indazole-***C***), 127.80 (+, Ph-***C***-2'',6''), 125.30 (+, Ph-***C***-3'',5''), 125.12 (C_{quat}, indazole-***C***), 123.32 (C_{quat}, Ph-***C***-4''), 122.59 (C_{quat}, Ph-***C***-1'), 121.50 (+, Ar-***C***-4'''), 118.53 (+, Ar-***C***-5'''), 108.67 (+, Ar-***C***-7'''), 74.17 (C_{quat}, ***C***-5), 44.93 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 19.88 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 608.0 (10), 607.0 (30), 604.9 ([M + H + MeCN]$^+$, 40), 565.9 (75), 563.9 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 564.0634, found [M + H]$^+$ 564.0635. $C_{25}H_{18}Cl_2F_3N_5OS$ (M_r = 564.41 g/mol).

4-[1-(3-Hydroxy-4-methoxyphenyl)-5-methyl-4-(4-methylbenzylamino)-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.67)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 5-amino-2-methoxyphenol (0.8 mmol, 111 mg) and 1-(isocyanomethyl)-4-methylbenzene (0.8 mmol, 105 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 265 mg, 70 %; yield (preparative HPLC): yellow solid, 11.1 mg, 2.9 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.98 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CH_3), 7.95 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.28 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 7.11 (q, *J* = 8.3 Hz, 4H, Ph-***H***-2'',6'', Ph-***H***-3'',5''), 6.75 (d, *J* = 8.7 Hz, 1H, Ph-***H***-5'), 6.19 (d, *J* = 2.5 Hz, 1H, Ph-***H***-2'), 6.11 (dd, *J* = 8.6, 2.5 Hz, 1H, Ph-***H***-6'), 4.59 – 4.45 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CH_3), 3.69 (s, 3H, OC***H***$_3$-4'''), 2.26 (s, 3H, NHCH_2-*p*-C_6H_4-C***H***$_3$), 1.77 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.59 (C_{quat}, ***C***S), 180.01 (C_{quat}, ***C***-4), 166.85 (C_{quat}, Ph-***C***OOH), 147.26 (C_{quat}, Ph-***C***-4'), 146.06 (C_{quat}, Ph-***C***-3'), 141.94 (C_{quat}, Ph-***C***-4'''), 136.39 (C_{quat}, Ph-***C***-1''), 134.73 (C_{quat}, Ph-

C-4''), 131.08 (C_{quat}, Ph-***C***-1'''), 129.64 (+, Ph-***C***-3''',5'''), 128.83 (+, Ph-***C***-2'',6''), 128.65 (C_{quat}, Ph-***C***-1'), 127.23 (+, Ph-***C***-3'',5''), 126.91 (+, Ph-***C***-2''',6'''), 120.17 (+, Ph-***C***-6'), 116.95 (+, Ph-***C***-2'), 111.44 (+, Ph-***C***-5'), 73.50 (C_{quat}, ***C***-5), 55.44 (+, O***C***H_3-4'), 45.20 (-, NH***C***H_2-*p*-C_6H_4-CH_3), 20.64 (+, NHCH_2-*p*-C_6H_4-***C***H_3), 19.88 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 517.0 ($[M + H + MeCN]^+$, 10), 478.0 (10), 477.0 (30), 475.9 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 476.1639, found $[M + H]^+$ 476.1642. $C_{26}H_{25}N_3O_4S$ (M_r = 475.56 g/mol).

4-[4-(4-Chlorobenzylamino)-1-(3-hydroxy-4-methoxyphenyl)-5-methyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.68)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), 5-amino-2-methoxyphenol (0.8 mmol, 111 mg) and 1-chloro-4-(isocyanomethyl)benzene (0.8 mmol, 121 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave a brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 280 mg, 71 %; yield (preparative HPLC): gray solid, 11.3 mg, 2.8 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.02 (t, *J* = 5.9 Hz, 1H, N***H***CH_2-*p*-C_6H_4-Cl), 7.96 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.42 – 7.38 (m, 2H, Ph-***H***-3'',5''), 7.28 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 7.25 (d, *J* = 8.4 Hz, 2H, Ph-***H***-2'',6''), 6.75 (d, *J* = 8.7 Hz, 1H, Ph-***H***-5'), 6.18 (d, *J* = 2.5 Hz, 1H, Ph-***H***-2'), 6.11 (dd, *J* = 8.6, 2.4 Hz, 1H, Ph-***H***-6'), 4.54 (qt, *J* = 21.4, 10.8 Hz, 2H, NHC***H***$_2$-*p*-C_6H_4-Cl), 3.69 (s, 3H, OC***H***$_3$-4'''), 1.77 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.51 (C_{quat}, ***C***S), 180.12 (C_{quat}, ***C***-4), 166.89 (C_{quat}, Ph-***C***OOH), 147.29 (C_{quat}, Ph-***C***-4'), 146.07 (C_{quat}, Ph-***C***-3'), 141.66 (C_{quat}, Ph-***C***-4'''), 136.89 (C_{quat}, Ph-***C***-1''), 131.82 (C_{quat}, Ph-***C***-4''), 129.71 (+, Ph-***C***-3''',5'''), 129.53 (C_{quat}, Ph-***C***-1'), 129.14 (+, Ph-***C***-2'',6''), 128.39 (+, Ph-***C***-3'',5''), 127.92 (C_{quat}, Ph-***C***-1'''), 126.85 (+, Ph-***C***-2''',6'''), 120.16 (+, Ph-***C***-6'), 116.92 (+, Ph-***C***-2'), 111.46 (+, Ph-***C***-5'), 73.58 (C_{quat}, ***C***-5), 55.45 (+, O***C***H_3-4'), 44.71 (-, NH***C***H_2-*p*-C_6H_4-Cl), 19.88 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 536.9 ($[M + H + MeCN]^+$, 10), 497.9 (40), 496.0 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 496.1092, found $[M + H]^+$ 496.1093. $C_{25}H_{22}ClN_3O_4S$ (M_r = 495.98 g/mol).

4-{5-Methyl-1-phenyl-2-thioxo-4-[4-(trifluoromethyl)benzylamino]-2,5-dihydro-1H-imidazol-5-yl}benzoic acid (5.69)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 128 mg), aniline (0.8 mmol, 75 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.8 mmol, 148 mg) according to method B of the general procedure. The crude product was obtained as

residue after evaporation (ethyl acetate), and gave pale brown solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 290 mg, 70 %; yield (preparative HPLC): yellow solid, 8.7 mg, 2.3 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.16 (t, J = 6.0 Hz, 1H, N***H***CH_2-*p*-C_6H_4-CF_3), 7.98 (d, J = 8.5 Hz, 2H, Ph-***H***-3''',5'''), 7.71 (d, J = 8.2 Hz, 2H, Ph-***H***-3'',5''), 7.44 (t, J = 10.0 Hz, 2H, Ph-***H***-2'',6''), 7.33 (d, J = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 7.29 – 7.23 (m, 3H, Ph-***H***-2',4',6'), 6.80 – 6.75 (m, 2H, Ph-***H***-3',5'), 4.73 – 4.62 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-CF_3), 1.83 (s, 3H, C***H***$_3$-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.53 (C_{quat}, ***C***S), 180.30 (C_{quat}, ***C***-4), 166.77 (C_{quat}, Ph-***C***OOH), 142.65 (C_{quat}, Ph-***C***-4''), 141.70 (C_{quat}, Ph-***C***-4'''), 137.04 (C_{quat}, Ph-***C***-1'), 131.19 (C_{quat}, Ph-***C***-1'''), 129.83 (+, Ph-***C***-3''',5'''), 129.47 (+, Ph-***C***-3',5'), 128.72 (+, Ph-***C***-2',4',6'), 127.85 (+, Ph-***C***-2'',6''), 126.95 (+, Ph-***C***-2''',6'''), 125.35 (+, Ph-***C***-3'',5''), 123.34 (C_{quat}, Ph-***C***-1''), 73.81 (C_{quat}, ***C***-5), 45.01 (-, NH***C***H_2-*p*-C_6H_4-CF_3), 20.02 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 526.0 (10), 525.0 ([M + H + MeCN]$^+$, 50), 483.9 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 484.1301, found [M + H]$^+$ 484.1304. $C_{25}H_{20}F_3N_3O_2S$ (M_r = 483.51 g/mol).

4-[4-(4-*tert*-Butylbenzylamino)-5-methyl-1-phenyl-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.70)

The title compound was prepared from 4-acetylbenzoic acid (0.8 mmol, 131 mg), aniline (0.8 mmol, 75 mg) and 1-*tert*-butyl-4-(isocyanomethyl)benzene (0.8 mmol, 139 mg) according to method B of the general procedure. The crude product was obtained as residue after evaporation (ethyl acetate), and gave yellow solid, which was subjected to RP-HPLC (Table 5.19, method 2). Yield (crude product): 290 mg, 70 %; yield (preparative HPLC): pale yellow solid, 8.7 mg, 2.3 %. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 9.04 (t, J = 5.8 Hz, 1H, N***H***CH_2-*p*-C_6H_4-$C(CH_3)_3$), 7.97 (t, J = 6.4 Hz, 2H, Ph-***H***-3''',5'''), 7.36 – 7.33 (m, 2H, Ph-***H***-2'',6''), 7.31 (d, J = 8.5 Hz, 2H, Ph-***H***-2''',6'''), 7.26 – 7.23 (m, 3H, Ph-***H***-3',4',5'), 7.16 (t, J = 8.7 Hz, 2H, Ph-***H***-3'',5''), 6.79 – 6.76 (m, 2H, Ph-***H***-2',6'), 4.62 – 4.47 (m, 2H, NHC***H***$_2$-*p*-C_6H_4-$C(CH_3)_3$), 1.81 (s, 3H, C***H***$_3$-5), 1.25 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 195.58 (C_{quat}, ***C***S), 180.07 (C_{quat}, ***C***-4), 166.81 (C_{quat}, Ph-***C***OOH), 149.75 (C_{quat}, Ph-***C***-4''), 141.87 (C_{quat}, Ph-***C***-4'''), 137.12 (C_{quat}, Ph-***C***-1'), 134.55 (C_{quat}, Ph-***C***-1''), 131.22 (C_{quat}, Ph-***C***-1'''), 129.73 (+, Ph-***C***-3''',5'''), 129.50 (+, Ph-***C***-2',6'), 127.81 (+, Ph-***C***-3',4',5'), 127.12 (+, Ph-***C***-3'',5''), 126.94 (+, Ph-***C***-2''',6'''), 125.17 (+, Ph-***C***-2'',6''), 73.70 (C_{quat}, ***C***-5), 45.33 (-, NH***C***H_2-*p*-C_6H_4-$C(CH_3)_3$), 34.19 (C_{quat}, ***C***$(CH_3)_3$), 31.19 (+, $C(\mathbf{C}H_3)_3$), 19.99 (+, ***C***H_3-5). MS (CI-MS) *m/z* (rel. int. in %) = 513.0 ([M + H + MeCN]$^+$, 10), 474.1 (10), 473.0 (30), 472.0 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 472.2053, found [M + H]$^+$ 472.2055. $C_{28}H_{29}N_3O_2S$ (M_r = 471.61 g/mol).

5.8.2.6 Preparation of the compounds 5.81-5.83

4-[4-(Benzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.81)

The title compound was prepared from 4-formylbenzoic acid (0.8 mmol, 120 mg) and 4-amino-2,6-dichlorophenol (0.8 mmol, 142 mg) in 8 mL anhydrous MeOH under an atmosphere of nitrogen in a septum-equipped glass vessel. The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq) and pyridinium chloride (4 eq) the mixture was bubbled with argon and evacuated. (Isocyanomethyl)benzene (0.8 mmol, 94 mg) was added with a syringe, and the mixture was stirred at ambient temperature overnight. For mass spectral analysis a sample from the supernatant (10 µL diluted with 990 µL MeOH) was subjected to LC-MS. According to this procedure, the crude product was analyzed 2 h and 72 h, respectively, after the completion of the experiment.

LC-MS analysis (parameters cf. Table 5.17) 2 h after completion of the experiment is illustrated in Figure 5.48.

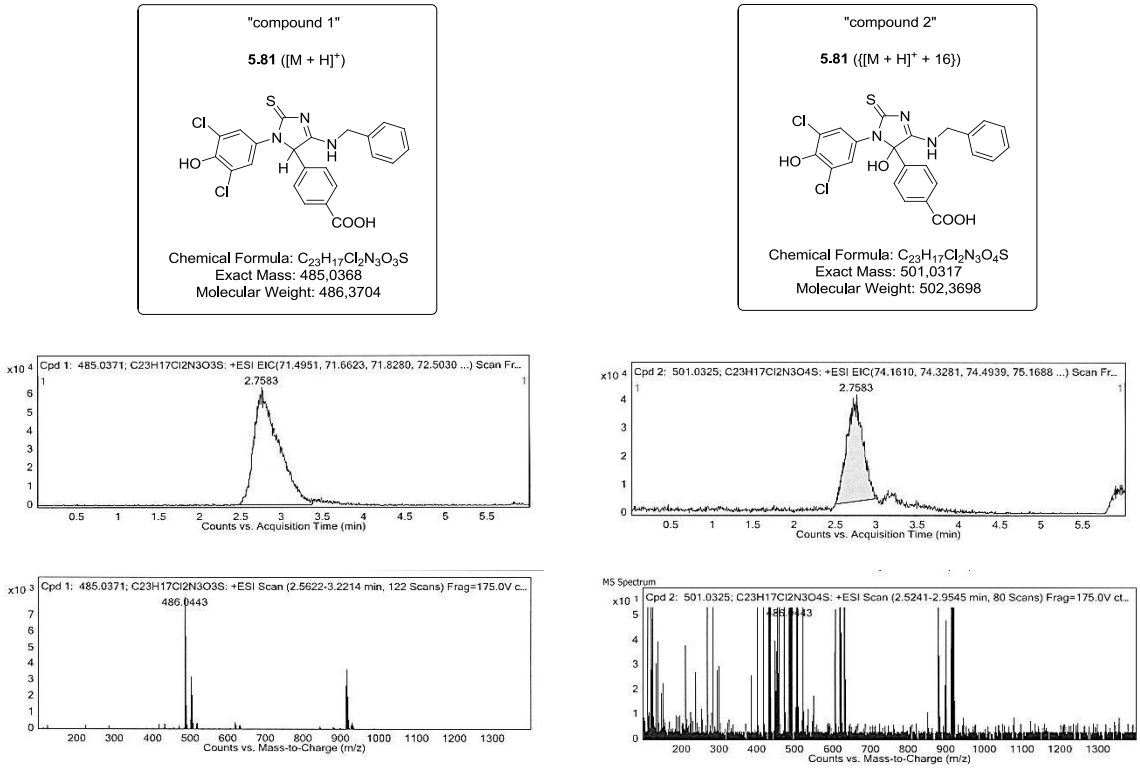


Figure 5.48 LC-MS analysis of **5.81** recorded 2 hours after completion of the experiment. Counts vs. acquisition time plot and counts vs. mass-to-charge plot of "compound 1" (**5.81** ($[M + H]^+$)) shown on the left; counts vs. acquisition time plot and counts vs. mass-to-charge plot of "compound 2" (**5.81**, {$[M + H]^+ + 16$}) indicated on the right; LC-MS column: Zorbax Eclipse Plus C18 Rapid Solution HD (Agilent Technologies, Santa Clara, USA).

The counts vs. acquisition time plot indicated a single peak (t_R = 2.7583 min). The corresponding counts vs. mass-to-charge (*m/z*) plot displayed a mass of 486.0443 g/mol ("compound 1", **5.81** ($[M + H]^+$)) as predominant species. The signal for the C-5 hydroxylated species ("compound 2", **5.81** ({$[M + H]^+$ +16})) was not detected.

LC-MS analysis (parameters cf. Table 5.18) 72 h after completion of the experiment is illustrated in Figure 5.49.

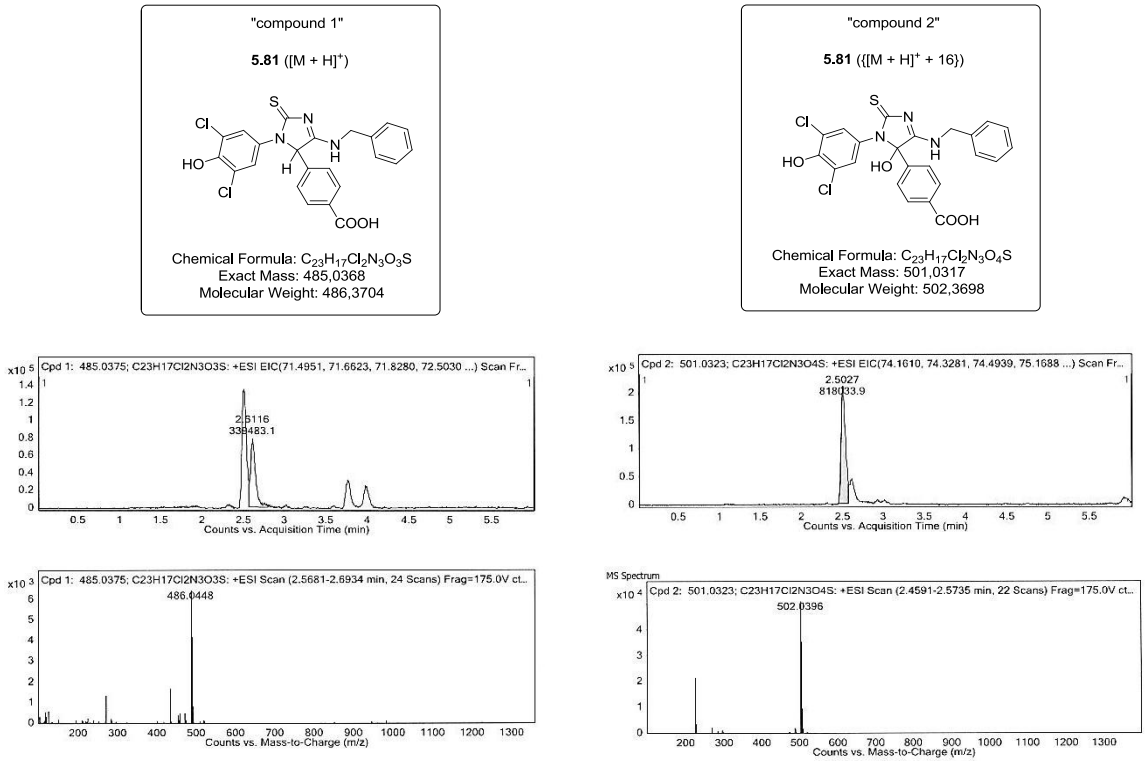


Figure 5.49 LC-MS analysis of **5.81** recorded 72 hours after completion of the experiment. Counts vs. acquisition time plot and counts vs. mass-to-charge plot of "compound 1" (**5.81**, ($[M + H]^+$)) shown on the left; counts vs. acquisition time plot and counts vs. mass-to-charge plot of "compound 2" (**5.81**, {$[M + H]^+$ + 16}) indicated on the right; LC-MS column: Accucore aQ (Thermo Scientific, Santa Clara, USA).

The counts vs. acquisition time plot indicated a double-peak (t_R = 2.5027 min, 2.6116 min). The corresponding counts vs. mass-to-charge (*m/z*) plot displayed a mass of 486.0443 g/mol ("compound 1", **5.81** ($[M + H]^+$)) and a mass of 502.0396 g/mol ("compound 2", **5.81** ({$[M + H]^+$ +16})).

4-[4-(Benzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-5-hydroxy-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.82)

The title compound was prepared from 4-formylbenzoic acid (0.8 mmol, 120 mg) and 4-amino-2,6-dichlorophenol (0.8 mmol, 142 mg) in 8 mL anhydrous MeOH under an

atmosphere of nitrogen in a septum-equipped glass vessel. The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KSCN (4 eq) and pyridinium chloride (4 eq) the mixture was bubbled with air. (Isocyanomethyl)benzene (0.8 mmol, 94 mg) was added and the mixture was stirred at ambient temperature overnight. For mass spectral analysis a sample from the supernatant (10 µL diluted with 990 µL MeOH) was subjected to LC-MS. Following this method, the crude product was analyzed 2 h and 72 h, respectively, after the completion of the experiment.

LC-MS analysis (parameters cf. Table 5.17) 2 h after completion of the experiment is illustrated in Figure 5.50.

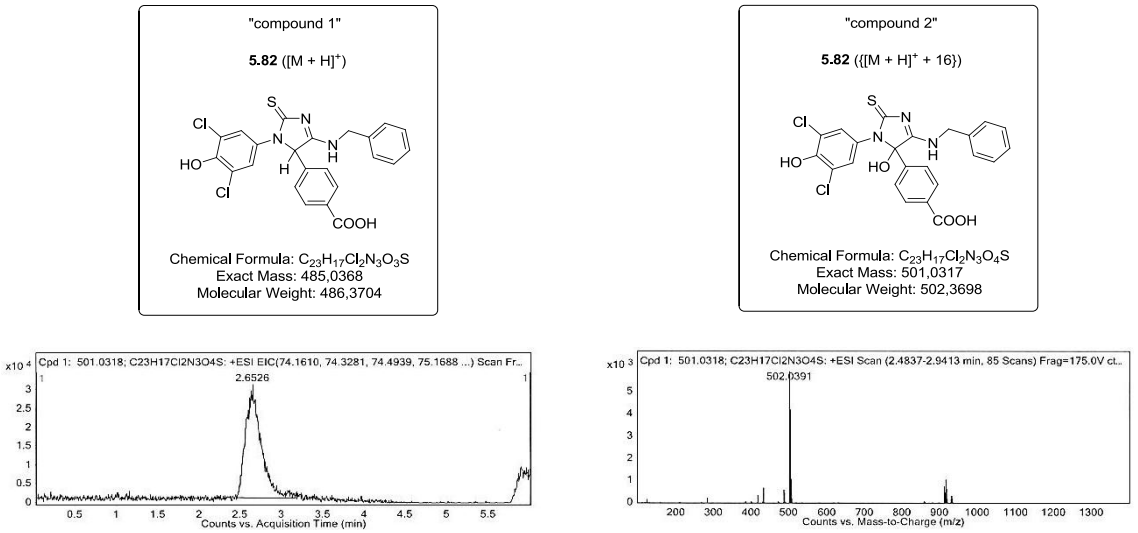


Figure 5.50 LC-MS analysis of **5.82** recorded 2 hours after completion of the experiment. “Compound 1” (**5.82**, ([M + H]$^+$)) non-existent; counts vs. acquisition time plot of “compound 2” (**5.82**, {[M + H]$^+$ + 16}) indicated on the left, counts vs. mass-to-charge plot of “compound 2” indicated on the right; LC-MS column: Zorbax Eclipse Plus C18 Rapid Solution HD (Agilent Technologies, Santa Clara, USA).

The counts vs. acquisition time plot indicated a single peak (t_R = 2.6526 min). The corresponding counts vs. mass-to-charge (*m/z*) plot displayed only a mass of 502.0396 g/mol (“compound 2”, **5.82** ({[M + H]$^+$ +16})).

LC-MS analysis (parameters cf. Table 5.18) 72 h after completion of the experiment is illustrated in Figure 5.51.

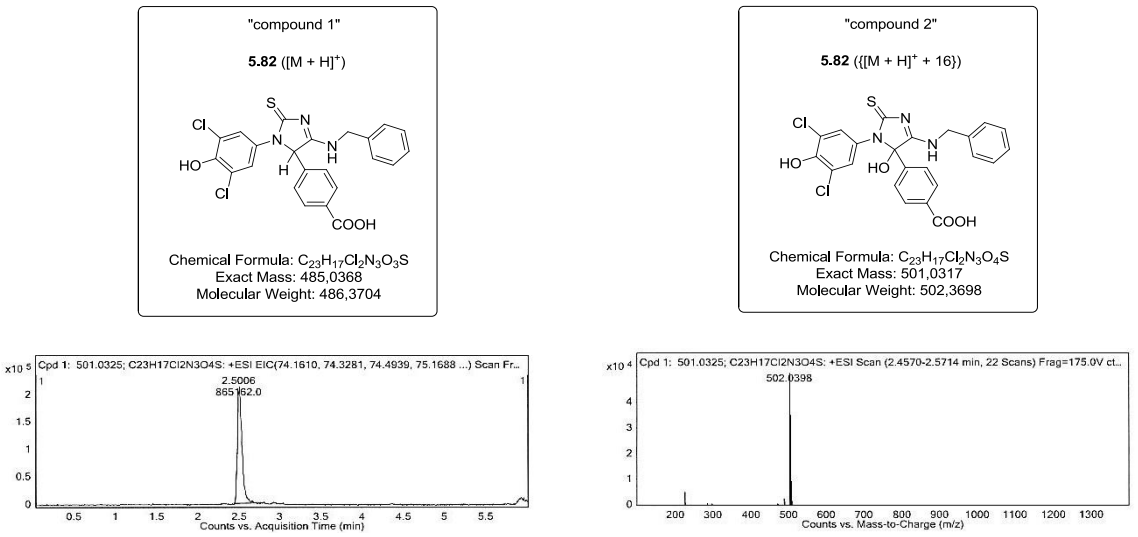


Figure 5.51 LC-MS analysis of **5.82** recorded 72 hours after completion of the experiment. "Compound 1" (**5.82**, ([M + H]$^+$)) non-existent; counts vs. acquisition time plot of "compound 2" (**5.82**, {[M + H]$^+$ + 16}) (M: non-existent); counts vs. mass-to-charge plot of "compound 2" indicated on the right; LC-MS column: Accucore aQ (Thermo Scientific, Santa Clara, USA).

The counts vs. acquisition time plot indicated a single peak (t_R = 2.5006 min). The corresponding counts vs. mass-to-charge (*m/z*) plot displayed a mass of 502.0396 g/mol ("compound 2", **5.82** ({[M + H]$^+$ +16})).

4-[4-(Benzylamino)-1-(3,5-dichloro-4-hydroxyphenyl)-5-[^{18}O]hydroxy-2-thioxo-2,5-dihydro-1*H*-imidazol-5-yl]benzoic acid (5.83)

$^{18}O_2$ oxygen gas was generated from $H_2{}^{18}O$ in an electrolysis apparatus (Figure 5.52).[e]

[e] $H_2{}^{18}O$ (20 mL) was a gift from G. Bernhardt, University of Regensburg

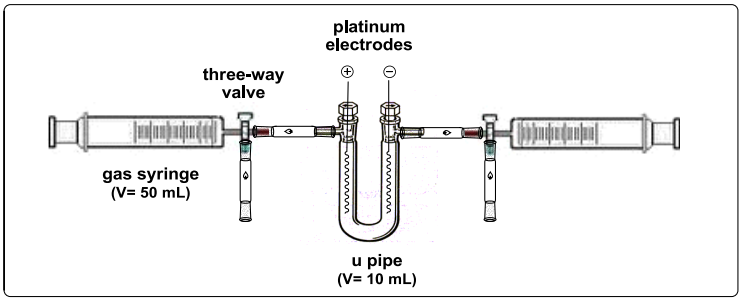


Figure 5.52 Electrolysis apparatus for the generation of $^{18}O_2$ oxygen gas

10 mL of $H_2{}^{18}O$ were filled in an "u pipe", which was connected (three-way valves) to gas syringes. Six drops of concentrated sulfuric acid were added to $H_2{}^{18}O$. Platinum electrodes were assembled to both sides of the u pipe and hermetically sealed. Subsequently, the apparatus was purged with argon. Platinum electrodes were connected

to direct-current (14 V). During electrolysis (3 hours), a total volume of 30 mL of $^{18}O_2$ oxygen gas was collected in the gas syringe next to positive electrode (anode). Hydrogen gas was collected in the opposite syringe. The $^{18}O_2$ oxygen gas fraction was transferred to an aerostat and instantly used for the experiment.

The title compound was prepared from 4-formylbenzoic acid (0.8 mmol, 120 mg) and 4-amino-2,6-dichlorophenol (0.8 mmol, 142 mg) in 8 mL anhydrous MeOH under an atmosphere of nitrogen in a septum-equipped glass vessel. The solution was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After addition of KSCN (4 eq) and pyridinium chloride (4 eq) the mixture was bubbled with argon and evacuated. A portion of $^{18}O_2$ (30 mL, 2.4 mmol) was added to the glass vessel. (Isocyanomethyl)benzene (0.8 mmol, 94 mg) was added with a syringe and the mixture was stirred at ambient temperature overnight. For mass spectral analysis a sample from the supernatant (10 µL diluted with 990 µL MeOH) was subjected to LC-MS. Following this method, the crude product was analyzed 2 h and 72 h, respectively, after the completion of the experiment.

LC-MS analysis (parameters cf. Table 5.17) 2 h after completion of the experiment is illustrated in Figure 5.53.

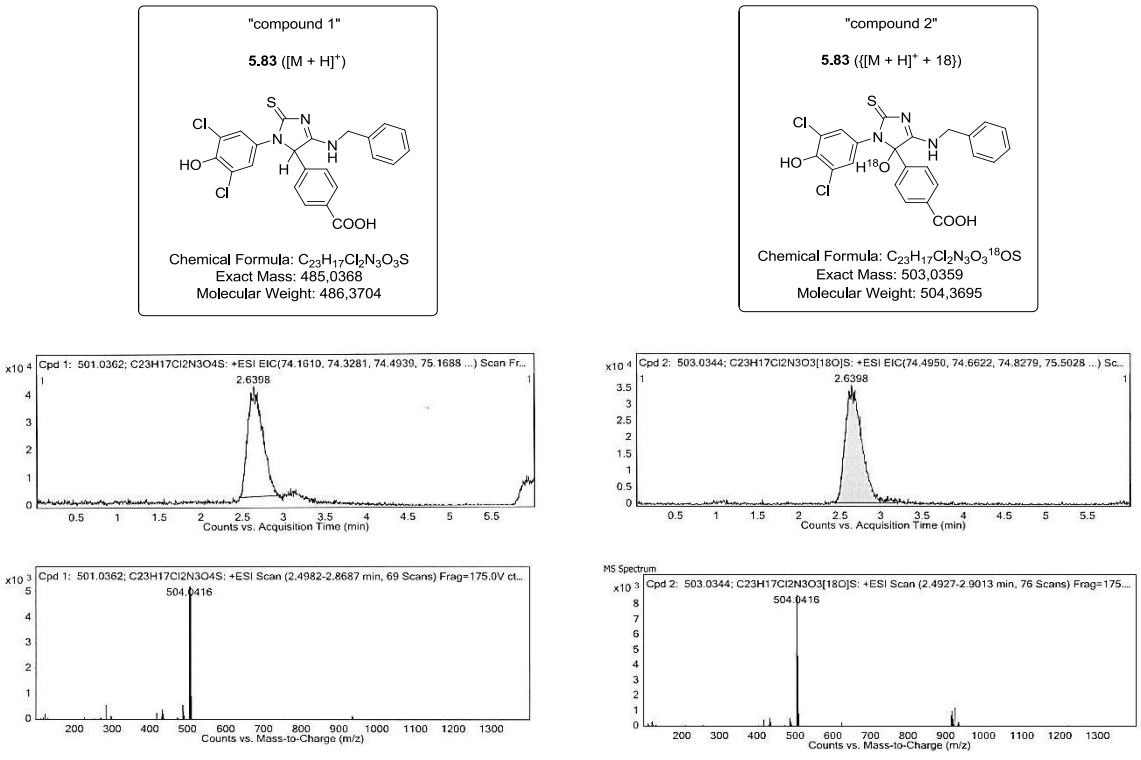


Figure 5.53 LC-MS analysis of **5.83** recorded 2 hours after completion of the experiment. Counts vs. acquisition time plot and counts vs. mass-to-charge plot of “compound 1” (**5.83**, ([M + H]$^+$) shown on the left (detection exclusively of “compound 2”); counts vs. acquisition plot and counts vs. mass-to-charge plot of “compound 2” (**5.83**, {[M + H]$^+$ + 18}) indicated on the right; LC-MS column: Zorbax Eclipse Plus C18 Rapid Solution HD (Agilent Technologies, Santa Clara, USA).

The counts vs. acquisition time plot indicated a single peak (t_R = 2.6398 min). The corresponding counts vs. mass-to-charge (*m/z*) plot displayed a mass of 504.0416 g/mol ("compound 2", **5.83** ({[M + H]$^+$ +18})).

LC-MS analysis (parameters cf. Table 5.18) 72 h after completion of the experiment is illustrated in Figure 5.54.

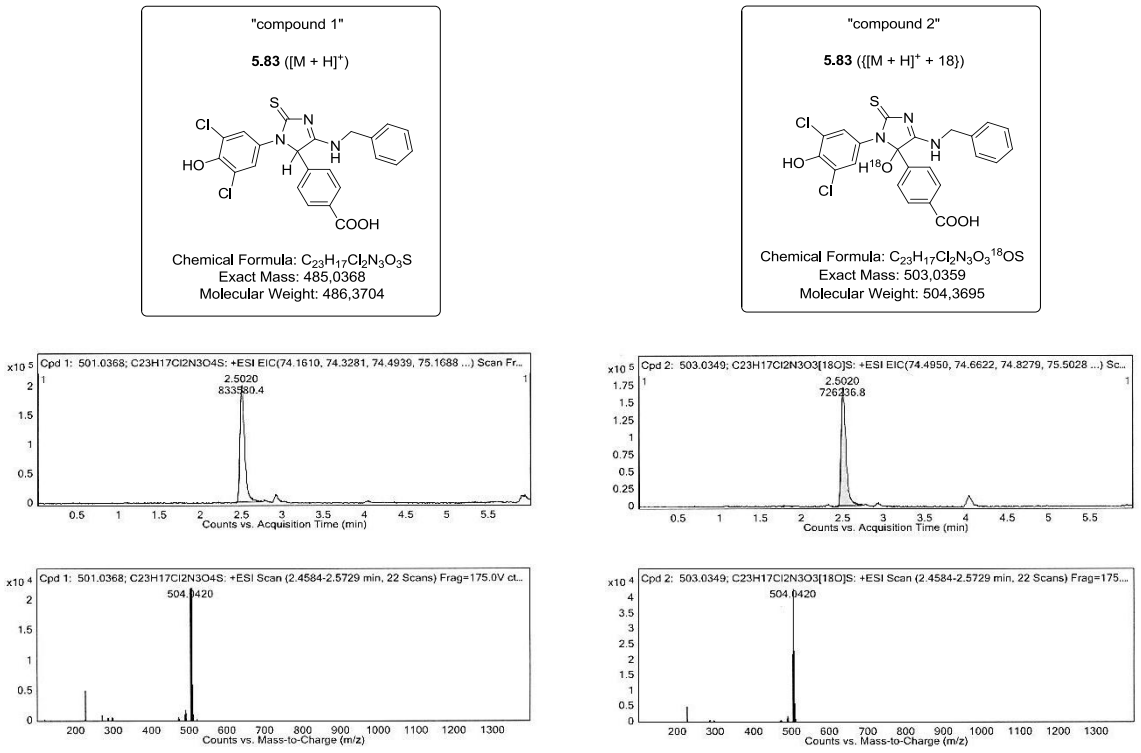


Figure 5.54 LC-MS analysis of **5.83** recorded 72 hours after completion of the experiment. Counts vs. acquisition time plot and counts vs. mass-to-charge plot of "compound 1" (**5.83**, ([M + H]$^+$) shown on the left (detection exclusively of "compound 2"); counts vs. acquisition plot and counts vs. mass-to-charge plot of "compound 2" (**5.83**, {[M + H]$^+$ + 18}) indicated on the right; LC-MS column: Accucore aQ (Thermo Scientific, Santa Clara, USA).

The counts vs. acquisition time plot indicated a single peak (t_R = 2.5020 min). The corresponding counts vs. mass-to-charge (*m/z*) displayed a mass of 504.0416 g/mol ("compound 2", **5.83** ({[M + H]$^+$ +18})).

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6 Screening of 3-amino substituted imidazo [1,2-*a*]pyridines for the inhibition of streptococcal hyaluronidases

6.1 Introduction

Previously, hydroxylated 2-phenylindoles have been identified as inhibitors of streptococcal hyaluronidases.[1] Besides, moderate inhibition was observed for some related benzimidazole derivatives bearing a similar substitution pattern.[2] In Figure 6.1 structures and *Sag*Hyal$_{4755}$ inhibitory activities of representative hydroxylated 2-phenylindoles (**1**, **2**, **4**, **5**) and 2-phenylbenzimidazoles (**3**, **6**) are summarized. Chloro substituents (cf. **2**, **5**) were found to enhance the biological activity of phenylindoles. Similarly, in the benzimidazole series, hyaluronidase inhibiton correlated with lipophilicity (cf. **3**, **6**).

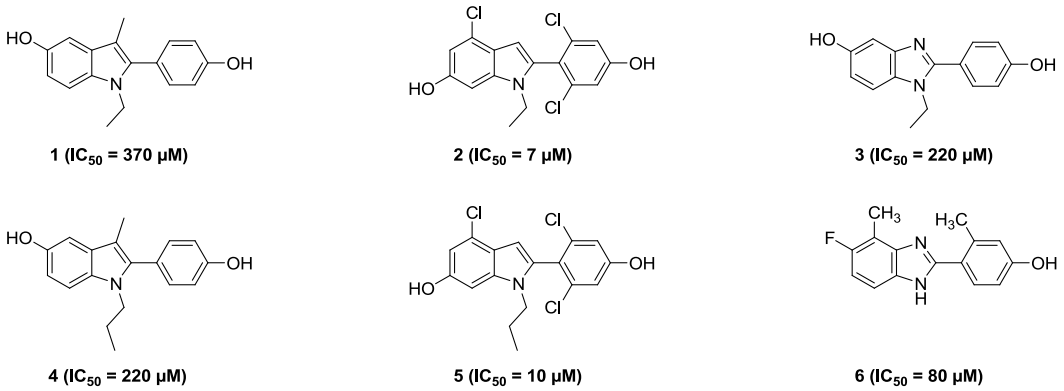


Figure 6.1 Structures of selected hydroxlated 2-phenylindoles (**1**, **2**, **4**, **5**) and 2-phenylbenzimidazoles (**3**, **6**) with inhibitory activity on *Sag*Hyal$_{4755}$.

These findings motivated us to explore novel heterocycles as hyaluronidase inhibitors. Only molecules accessible by multicomponent synthesis were taken into account. Moreover, the designed substances should possess drug-like properties. Under these premisses, imidazo[1,2-*a*]pyridines were selected as novel structural motifs.

The structural motif of imidazo[1,2-*a*]pyridine can be found in a broad variety of biologically active compounds, including drugs for the treatment of gastric disorders[3, 4], viral diseases[5-8] and bacterial infections.[9, 10] The imidazopyridine-type drug zolpidem is used as a medication for insomnia. In addition, the closely related heterocyclic substances alpidem, necopidem and saripidem demonstrate the importance of this structural moiety (Figure 6.2).

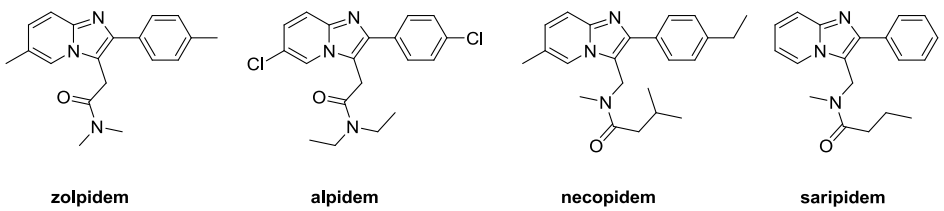
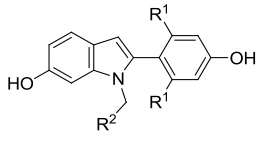


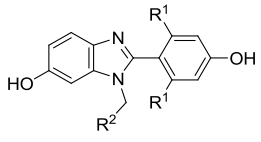
Figure 6.2 Structures of zolpidem, alpidem, necopidem and saripidem.

Aiming at lyase inhibitors with improved drug-like properties, two different strategies were pursued. For the first approach, the focus was set on computer-assisted drug-design accompanied with multicomponent reaction synthesis of compound libraries. The evaluation of these compounds was performed according to electronic similarity.

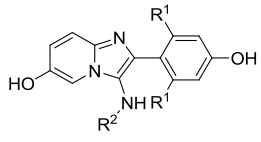
In a second approach, the imidazo[1,2-*a*]pyridine scaffold was maintained. In contrast to the previous strategy, the selection of starting materials was primarily based on experience and rational decision. To avoid the synthesis of unsuitable compounds, it became necessary to carefully analyze the chemical properties, for example lipophilicity. To estimate appropriate substituents, a series of structurally related heterocyles was considered. As shown Figure 6.3, correlating 2-phenylindoles (**7a-d**), 2-phenylbenzimidazoles (**8a-d**) and 3-amino substituted 2-phenylimidazo[1,2-*a*]pyridines (**9a-d**) were tabulated and compared based on $logD_{5.0}$ values. All compounds were virtually substituted with conserved residues (chlorine, pentyl) to increase $logD_{5.0}$ values (Figure 6.3.).



No.	R^1	R^2	$logD_{5.0}$
a	H	CH_3	3.55
b	H	$(CH_2)_4CH_3$	5.59
c	Cl	CH_3	4.39
d	Cl	$(CH_2)_4CH_3$	6.43



No.	R^1	R^2	$logD_{5.0}$
a	H	CH_3	1.22
b	H	$(CH_2)_4CH_3$	3.22
c	Cl	CH_3	2.49
d	Cl	$(CH_2)_4CH_3$	4.52



No.	R^1	R^2	$logD_{5.0}$
a	H	CH_3	-0.51
b	H	$(CH_2)_4CH_3$	1.67
c	Cl	CH_3	0.46
d	Cl	$(CH_2)_4CH_3$	2.72

Figure 6.3 Arrangement of *in silico* designed and analyzed heterocycles bearing indole, benzimidazole and imidazo[1,2*a*]pyridine scaffold.

6.2 Chemistry

For the first screening campaign, the reaction of aminopyridines **10**, aldehydes **11** and isocyanides **12** was utilized for the construction of a series of 3-amino substituted 2-phenylimidazo[1,2-*a*]pyridines of general structure **13** (c.f. Figure 6.4).

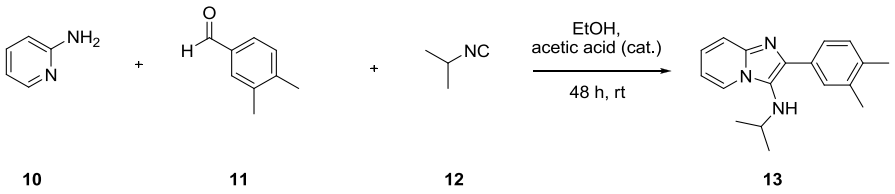


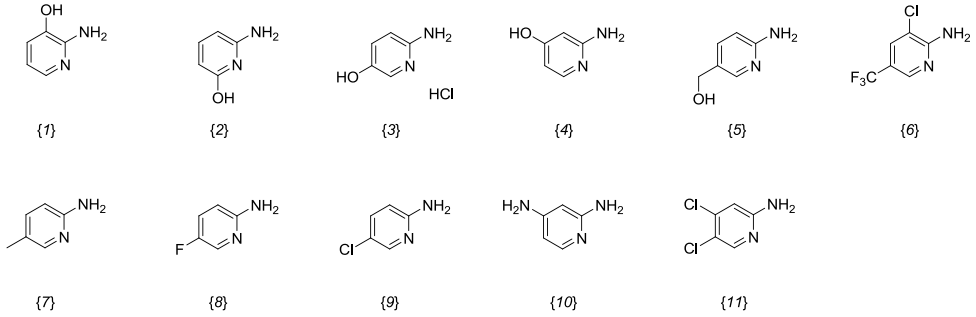
Figure 6.4 General synthesis (multicomponent synthesis) of 3-amino substituted imidazo[1,2-*a*]pyridines (**13**). Reagents and conditions: anhydrous EtOH, (cat.) acetic acid, 48 h, rt.

According to the method described by Groebke et al., target molecules were available from one-pot three component condensation.[11] In parallel synthesis, aminopyridines and aldehydes were dissolved in anhydrous methanol and transformed to 96-deep well plates with an automated liquid handling system. After the generation of the corresponding imine species, isocyanides and a catalytic amount of acetic acid were added. The mixture was allowed to react for 48 hours at ambient temperature to give the target molecules.

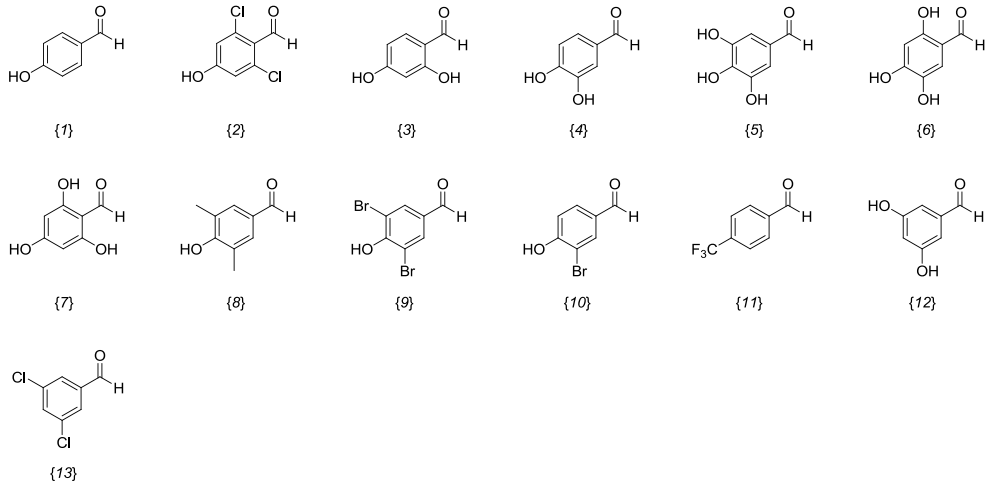
A selection of 26 aminopyridines, 27 aldehydes and 20 isocyanides was used to set up a library of 792 3-amino substituted imidazo[1,2-*a*]pyridines (**ori.hya.1**-**9**). All starting materials were commercially available chemicals. After HPLC mass spectral analysis (ESI-TOF), the solvent was evaporated and the crude substances were dispensed in DMSO, adjusting a concentration of 20 mM. Deep-well plates were sealed and stored at -20 °C. For biological tests, the compounds were diluted and transferred by an automated system to 96-well screening plates. Starting materials and mass spectral analysis for compounds of plates **ori.hya.1**-**9** are shown in section B.2 (appendix II).

By analogy with the protocol shown in Figure 6.4, 3-amino substituted 2-phenylimidazo[1,2-*a*]pyridines were synthesized in a second screening campaign. The corresponding starting materials are shown in Figure 6.5. A screening library of 560 substances was obtained by combinatorial variation of 11 aminopyridines, 13 aldehydes and 35 isocyanides (**ori.hya.48**-**54**). Details for mass spectral analysis of the corresponding compounds are given in section B.7 (appendix II).

aminopyridines 10{*1*}-{*11*}:



aldehydes 11{*1*}-{*13*}:



isocyanides 12 {*1*}-{*35*}:

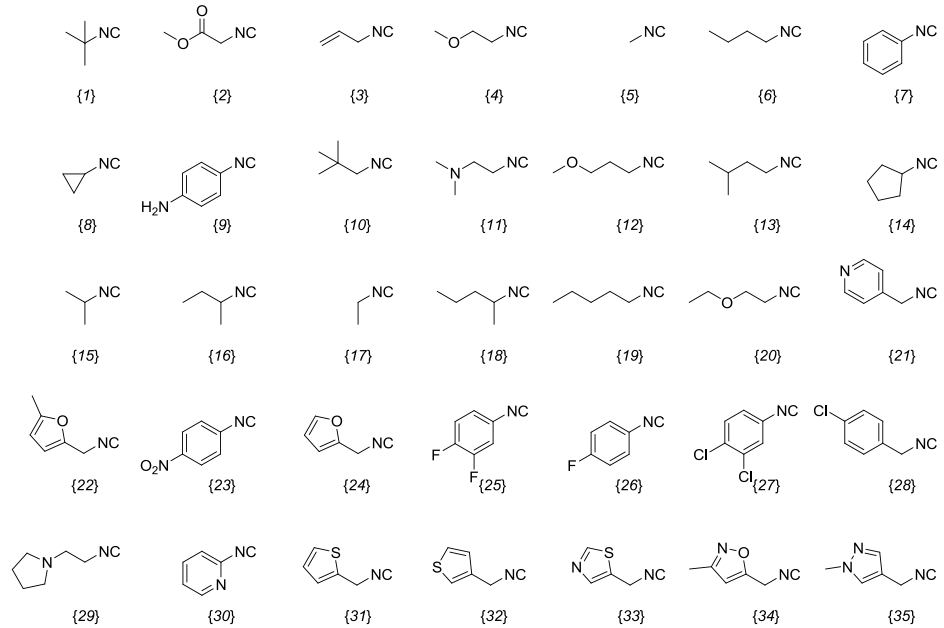


Figure 6.5 Aminopyridines **10**{*1*}-{*11*}, aldehydes **11**{*1*}-{*13*} and isocyanides **12**{*1*}-{*35*} used to construct a library of 560 potential hyaluronidase inhibitors.

160 compounds were synthesized on plates **ori.hya.48**, **49**. Aminopyridines **10**{*1*}-{*4*}, aldehydes **11**{*1*}-{*2*} and isocyanides **12**{*1*}-{*20*} served as starting materials. A combination of 6-aminopyridin-3-ol **10**{*1*} with aldehydes **11**{*1*}, {*2*} was superior to the comparable aminopyridines **10**{*1*}, {*2*}, {*4*}. The latter showed no conversion to the target molecules. No reaction was observed for isocyanides **12**{*9*}, {*11*}.

A matrix of 240 compounds was used for the miniaturized synthesis performed on plates **ori.hya.50**-**52**. Aminoyridines **10**{*5*}-{*10*}, hydroxylated aldehydes **11**{*2*}-{*10*} and isocyanides **12**{*1*}, {*2*}, {*4*}, {*6*}, {*15*} served as starting materials. The reaction worked well. Merely, when **10**{*10*} and **11**{*6*} were used as starting materials, a significant lower occurrence of the target molecules was observed.

The synthesis of an ensemble of 160 compounds was performed on screening plates **ori.hya.53**, **54** by variation of aminopyridines **10**{*5*}-{*10*}, aldehydes **11**{*2*}, {*11*}-{*13*} and isocyanides **12**{*1*}, {*2*}, {*4*}, {*11*}, {*15*}, {*21*}-{*35*}. The usage of **12**{*21*}, {*34*} and **12**{*28*}, {*29*}, {*32*}, {*33*} as starting material gave either low or no conversion to the products.

In total, 1352 3-amino substituted imidazo[1,2-*a*]pyridines were synthesized in both campaigns. The substances were synthesized by one-pot reactions on a miniaturized scale in 96 deep-well plates.

6.3 Pharmacological results and discussion

6.3.1 General conditions and screening mode

Cf. section 5.3.1

6.3.2 Inhibitory activities of screening compounds on *Sag*Hyal$_{4755}$

Among the tested 1352 3-amino substituted 2-phenylimidazo[1,2-*a*]pyridines, four compounds (**6.1**-**6.4**) were identified as screening hits. Referred to the size of the substance library, this equals a hit rate of 0.3 %. In appendix II, a detailed on-screen assay validation (cf. sections B.8.1, B.8.4) and representation of screening data (cf. sections B.9.2, B.9.5) is given. Chemical structures of identified hits (**6.1**-**6.4**) are shown in Figure 6.6.

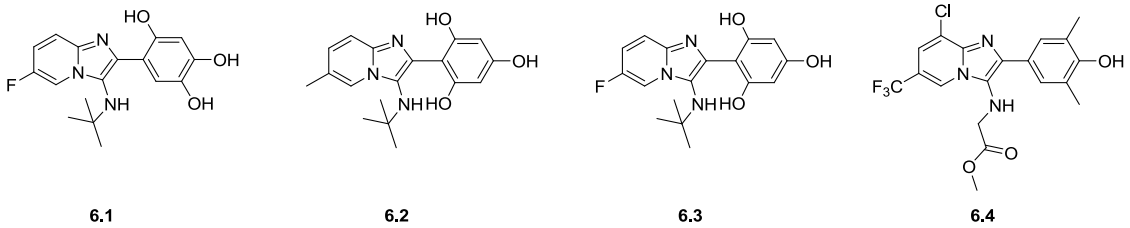


Figure 6.6 Structures of identified screening hits **6.1**-**6.4**.

At this stage, the criteria to denote a compound as hit (inhibitory effect > 50 %, weighted effect ≥ 1 (cf. section 4.3.2)) were loosened to some extent (inhibitory effect 20 % - 50 %, weighted effect ≥ 1). Accordingly, 4 additional substances (**6.5**-**6.8**, Figure 6.7) with certified mass were pharmacologically characterized. These molecules were investigated to gain new ideas for novel inhibitors.

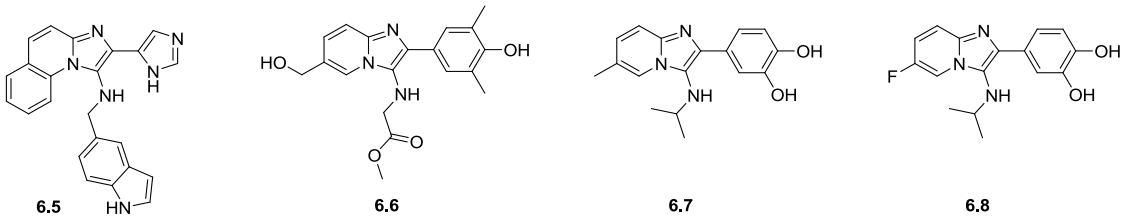


Figure 6.7 Structures of additional compounds of the screening campaign displaying inhibitory activity between 20 % and 50 % (**6.5**-**6.8**).

The inhibitory activities of compounds **6.1**-**6.8** are summarized in Table 5.1.

Table 6.1 Inhibitory activities of compounds **6.1**-**6.8** on *Sag*Hyal$_{4755}$, BTH and *Spn*Hyl.

Compound	***Sag*Hyal$_{4755}$ IC_{50} (µM)[a]**	**BTH % inhibition**	***Spn*Hyl IC_{50} (µM)[a]**	**Comment**
6.1	67 ± 5	inactive	90 ± 13	hit
6.2	59 ± 5	inactive	99 ± 11	hit
6.3	62 ± 4	inactive	95 ± 15	hit
6.4	99 ± 1	inactive	7 ± 1	hit
6.5	73 ± 7	inactive	21 ± 1	missed hit criteria
6.6	99 ± 2	inactive	8 ± 0.3	missed hit criteria
6.7	99 ± 6	inactive	inactive	missed hit criteria
6.8	97 ± 11	inactive	inactive	missed hit criteria

[a] mean values SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 in the 96-well turbidimetric assay.

The values determined for *Sag*Hyal$_{4755}$ were compared to screening data on *Spn*Hyl (data provided by Dr. J. Hamberger from our workgroup).[12] For the *Spn*Hyl screening campaign the assay parameters (ingredients, pH, microtiter plates, incubation time) were not

modified or changed. In addition, compounds **6.1**-**6.8** were tested on the mammalian hyaluronidase BTH.

Screening hits **6.1** and **6.8** were subjected to preparative synthesis and were retested as purified compounds on *Sag*Hyal$_{4755}$ using the turbidimetric assay. The compounds showed IC_{50} values of 630 µM (**6.1**) and 255 µM (**6.8**), respectively (Figure 6.8).

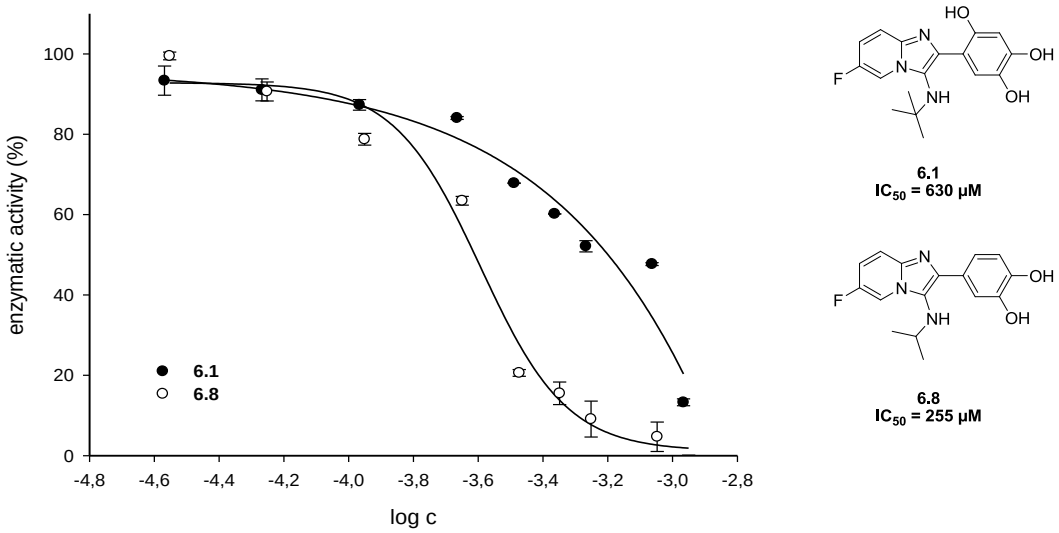


Figure 6.8 Concentration dependant inhibition of *Sag*Hyal$_{4755}$ by **6.1** and **6.8**.

The results on the purified compounds **6.1** and **6.8** did not support the screening results, which suggested higher inhibitory potency for both compounds at *Sag*Hyal$_{4755}$ and in particular at *Spn*Hyl (cf. Table 5.1). According to the screening data, the inhibitory activity on *Spn*Hyl appeared to be even higher. In the case of **6.1**, most likely, the aldehyde (2,4,5-trihydroxybenzaldehyde) was responsible for this result, as similar discrepencies were observed before for screening compounds, bearing trihydroxylated groups. Most likely, substances **6.2** and **6.3**, bearing an identical moiety, show the same phenomenon. In the case of **6.8**, at least a lower difference between the results of the screening data and the testing of the purified substance was observed. False positive results became not obvious in case of other dihydroxylated compounds. Substances **6.4** and **6.6** pretend strong inhibition of *Sag*Hyal$_{4755}$ but were found almost inactive on *Spn*Hyl. To corroborate these results, the molecules should be synthesized in pure form.. Compounds bearing aromatic or heterocyclic residues in position 3 were not among the screening hits, except for **6.5**, which showed moderate inhibition on *Sag*Hyal$_{4755}$ (IC_{50} = 73 µM) and *Spn*Hyl (IC_{50} = 21 µM).

6.4 Outlook

For multicomponent synthesis, additional structural modifications of imidazopyridines as potential hyaluronidase inhibitors could be suggested. The concepts (concept 1, concept 2) were drafted in Figure 6.9.

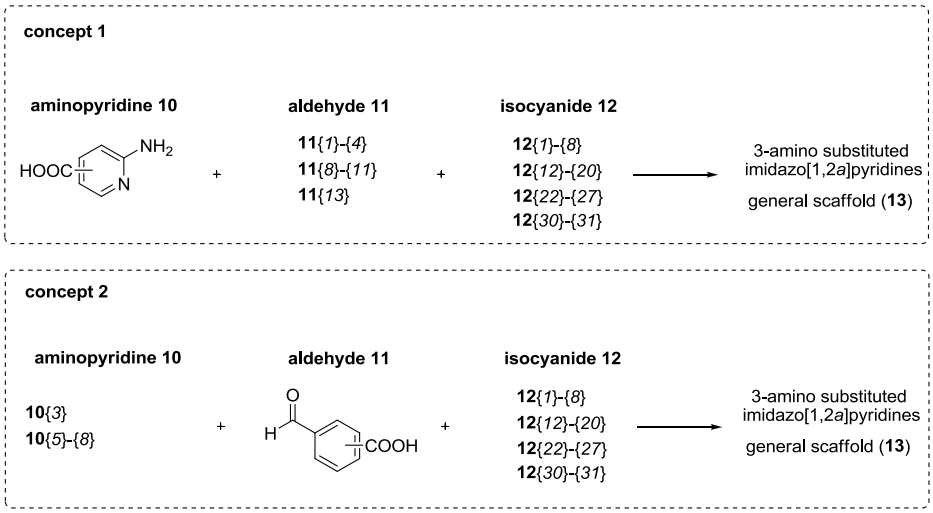


Figure 6.9 Proposed multicomponent synthesis of additional 3-amino substituted imidazo[1,2*a*]pyridines.

Here, the introduction of a carboxylic moiety is recommended, either attached to the aminopyridine (concept 1) or to the aldehyde building block (concept 2). Synthesis of structurally related compounds was recently reported in literature.[13-15] Eventually, this may promote the discovery of additional inhibitors of *Sag*Hyal$_{4755}$.

6.5 Summary

3-Amino substituted imidazo[1,2*a*]pyridines were designed, synthesized in parallel and tested for the first time for inhibition of the bacterial hyaluronidase *Sag*Hyal$_{4755}$. The achievement of drug-like properties was a key demand for the design and synthesis of imidazopyridines described in this chapter. Hence, the considered substances were predicted to be in accordance to criteria of druglikeness e.g. Lipinski's rule of five. As lipophilicity was taken into account, plasma protein binding, which was found to be very high for previously sythesized hyaluronidase, inhibitors might be reduced.

In two screening campaigns a total of 1352 3-amino substituted 2-phenylimidazo[1,2-*a*]pyridines was prepared via multicomponent reactions. Four molecules were identified as

screening hits, with highest inhibitory activities residing in screening compounds **6.1** (67 % inhibition) and **6.4** (99 % inhibition).

However, re-investigation of the purified compounds **6.1** and **6.8** did not confirm the inhibitory potency of the screening hits. In the case of **6.1** (IC_{50} = 630 µM) the discrepancy between screening of the mixture from the microtiter plates and the investigation of the pure compound appears to be associated with the presence of a trihydroxylated phenyl moiety. For **6.8**, the purified compound had an IC_{50} value of 255 µM corresponding to moderate inhibition of the target enzyme.

Taken together, the concept of molecular modeling, rational scaffold-orientated design and synthesis of hyaluronidase inhibitors led to new chemical entities, which were biologically active under assay conditions. This is supported by the results of investigations on *Spn*Hyl, where other imidazopyridines were recently identified as screening hits.[12] Further investigations with purified 3-amino-substituted imidazo[1,2*a*]pyridines, including studies on SpnHyl and plasma protein binding, have to be performed to substantiate the results and to prove the working hypothesis.

6.6 Experimental section

6.6.1 General conditions

Cf. section 5.8.1

6.6.2 Chemistry

6.6.2.1 Parallel synthesis

6.6.2.1.1 General conditions

Cf. section 5.8.2.1.1

6.6.2.1.2 Preparation of 3-aminoimidazo[1,2a]pyridines (ori.hya.1-9, 48-54)

Aminopyridines (0.2 M, 50 µL, 1 eq) in anhydrous MeOH) were dispensed on 96 deep-well plates, the pertinent aldehydes (0.2 M, 50 µL, 1 eq) in anhydrous MeOH and 10 µL of acetic acid were added. The plates were stacked for 30 min at ambient temperature, before the isocyanides (0.3 M, 50 µL, 1.5 eq) in anhydrous MeOH were added. The well plates were sealed and stored for 48 h at room temperature. The solvent was evaporated and 500 µL of DMSO were added to adjust to a theoretical concentration of 20 mM.

6.6.2.2 Preparation of the compounds 6.1, 6.8

5-[3-(*tert*-Butylamino)-6-fluoroimidazo[1,2-*a*]pyridin-2-yl]benzene-1,2,4-triol (6.1)

The title compound was prepared by analogy with the procedure described in 6.6.2.1.2 using 5-fluoropyridin-2-amine (1 mmol, 114 mg), 2,4,5-trihydroxybenzaldehyde (1 mmol, 154 mg), 100 µL of acetic acid in 6 mL of anhydrous MeOH. 2-Isocyano-2-methylpropane (1.5 mmol, 125 mg) was added subsequently. The crude product was subjected to automated flash-chromatography (column: SF10-4g, UV detection: 254 nm, DCM/MeOH 98/2 v/v) to yield an orange solid (90 mg, 27 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.52 (dd, *J* = 4.4, 2.2 Hz, 1H, Ar-***H***), 8.01 (d, *J* = 8.4 Hz, 1H, Ar-***H***), 7.30 (dd, *J* = 9.8, 2.4 Hz, 1H, Ar-***H***), 6.29 (dd, *J* = 8.7, 2.4 Hz, 1H, Ar-***H***), 6.21 (d, *J* = 2.4 Hz, 1H, Ar-***H***), 3.34 (s, 1H, N***H***$C(CH_3)_3$), 1.29 (s, 9H, NHC(C***H***$_3)_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 157.87 (C_{quat}, Ar-***C***), 157.70 (C_{quat}, Ar-***C***), 154.23 (C_{quat}, Ar-***C***), 151.10 (C_{quat}, Ar-***C***), 141.25 (C_{quat}, Ar-***C***), 136.69 (C_{quat}, Ar-***C***), 136.60 (+, Ar-***C***), 128.16 (C_{quat}, Ar-***C***), 124.36 (C_{quat}, Ar-***C***), 116.53 (+, Ar-***C***), 116.35 (+, Ar-***C***), 115.60 (+, Ar-***C***), 109.90 (+, Ar-***C***), 59.31 (C_{quat}, NH***C***$(CH_3)_3$), 29.49 (+, NHC(***C***$H_3)_3$). MS (ESI-MS, 70 eV) m/z (rel. int. in %) = 331.9 ([$M^{+\bullet}$], 100), 332.9 ([M + H]$^+$, 20). $C_{17}H_{18}FN_3O_3$ (M_r = 331.34 g/mol).

4-[6-Fluoro-3-(isopropylamino)imidazo[1,2-*a*]pyridin-2-yl]benzene-1,2-diol (6.8)

The title compound was prepared according to the procedure described in 6.6.2.1.2 using 5-fluoropyridin-2-amine (1 mmol, 114 mg), 3,4-dihydroxybenzaldehyde (1 mmol, 138 mg), 100 µL acetic acid in 6 mL MeOH (dry). 2-Isocyanopropane (1.5 mmol, 104 mg) was added subsequently. The crude product was subjected to automated flash-chromatography (column: SF10-4g, UV detection: 254 nm, DCM/MeOH 97/3 v/v) to yield a colorless oil (100 mg, 33 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 8.50 (dd, *J* = 4.4, 2.2 Hz, 1H, Ar-***H***), 8.05 (d, *J* = 8.6 Hz, 1H, Ar-***H***), 7.62 (dd, *J* = 9.9, 4.9 Hz, 1H, Ar-***H***), 7.33 (ddd, *J* = 9.8, 8.3, 2.4 Hz, 1H, Ar-***H***), 6.34 (dd, *J* = 8.6, 2.5 Hz, 1H, Ar-***H***), 6.28 (d, *J* =

2.4 Hz, 1H, Ar-***H***), 3.35 (s, 1H, N***H***CH$(CH_3)_2$), 3.26 (dq, *J* = 12.2, 6.2 Hz, 1H, NHC***H***$(CH_3)_2$), 1.04 (d, *J* = 6.3 Hz, 6H, NHCH(C***H***$_3)_2$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 158.27 (C_{quat}, Ar-***C***), 157.70 (C_{quat}, Ar-***C***), 154.23 (C_{quat}, Ar-***C***), 151.10 (C_{quat}, Ar-***C***), 136.69 (C_{quat}, Ar-***C***), 136.60 (+, Ar-***C***), 128.16 (C_{quat}, Ar-***C***), 124.36 (C_{quat}, Ar-***C***), 116.53 (+, Ar-***C***), 116.35 (+, Ar-***C***), 115.95 (+, Ar-***C***), 115.60 (+, Ar-***C***), 109.90 (+, Ar-***C***), 48.28 (+, NH***C***H$(CH_3)_2$), 22.59 (+, NHCH(***C***H$_3)_2$). MS (ESI-MS, 70 eV) m/z (rel. int. in %) = 301.9 ([$M^{+\bullet}$], 100), 302.9 ([M + H]$^+$, 25). $C_{16}H_{16}FN_3O_2$ (M_r = 301.32 g/mol).

6.7 References

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7 Screening of synthetic organic molecules, natural products and peptide mimetics of hyaluronan for hyaluronidase inhibition

7.1 Introduction

Recently (2009), Girish et al. published a comprehensive, but uncritical, review on the biological and therapeutic perspective of inhibitors of hyaluronidases.[1] According to this survey, inhibitors of bacterial and mammalian hyaluronidases were found among proteins, glycosaminoglycans, polysaccharides, fatty acids, lanostanoids, antibiotics, antinematodal, synthetic organic and plant derived bioactive compounds such as alkaloids, antioxidants, polyphenols, flavonoids, terpenoids and anti-inflammatory drugs.[1] It should be noted, that classifications as competitive or noncompetitive inhibitors as well as IC_{50} values were not given by Girish et al. for the various hyaluronidase inhibitors. Nevertheless, IC_{50} values strongly depend on the method of the assay and are often difficult to compare. It is documented that incubation conditions, different isoenzymes or enzyme concentrations, substrate concentrations or pH value can cause huge discrepancies between inhibitory activities determined in different laboratories.

In search for novel lead inhibitors of the bacterial hyaluronidase *Sag*$Hyal_{4755}$, some purported hyaluronidase inhibitors, included in the review by Girish et al., were analyzed in our laboratory under standardized conditions in the turbidimetric assay. In addition, heterocyclic compounds resembling reported inhibitors, but having a different substitution pattern, and newe chemical entities were investigated for their inhibitory activity on *Sag*$Hyal_{4755}$. The compounds were also analyzed on a mammalian hyaluronidase, bovine testicular hyaluronidase (BTH). In this context, hyaluronidases from prokaryotes and mammals must be clearly distinguished, as these enzymes cleave the substrate hyaluronan by a different catalytic mechanism. BTH is an ortholog of the human spam adhesion molecule (SPAM 1), also termed PH-20. Hence, the investigation on BTH was regarded as an approach to evaluate potential inhibitors of the human hyaluronidase PH-20.

The selected compounds were small molecules. The aim was to identify substances, which might serve as basis for the development of drug-like inhibitors. A number of compounds included in this screening were previously synthesized in our workgroup. In many cases, these agents were initially designed to target different biomolecules. Accordingly, activities at biological targets other than hyaluronidases must be kept in mind.

7.2 Inhibitory activity of screening compounds

7.2.1 Benzo[*b*]furane derivatives

Braun et al. identified a number of benzoxazole-2-thiones as potent and selective inhibitors of the streptococcal lyase *Sag*Hyal$_{4755}$. Within this series, 3-phenyl-1-(2-thioxobenzo[*d*]oxazol-3(2*H*)-yl)propan-1-one proved to be the most potent inhibitor with an IC_{50} value of 15 µM at a pH value of 5.0.[2, 3] Disadvantage of the 3-acylbenzoxazoles was their limited stability against hydrolysis, resulting in *N*-deacylated degradation products. In search for bioisosteric replacements of the heterocyclic core, a number of 2-methylbenzo[*b*]furanes were investigated by Braun for inhibition of *Sag*Hyal$_{4755}$ activity. These special compounds proved to be much less potent than expected,[2] however, based on these results, the scaffold should not yet be considered inappropriate. To elucidate the impact of structural modifications, a set of six benzo[*b*]furans (Figure 7.1) was screened for hyaluronidase inhibition.

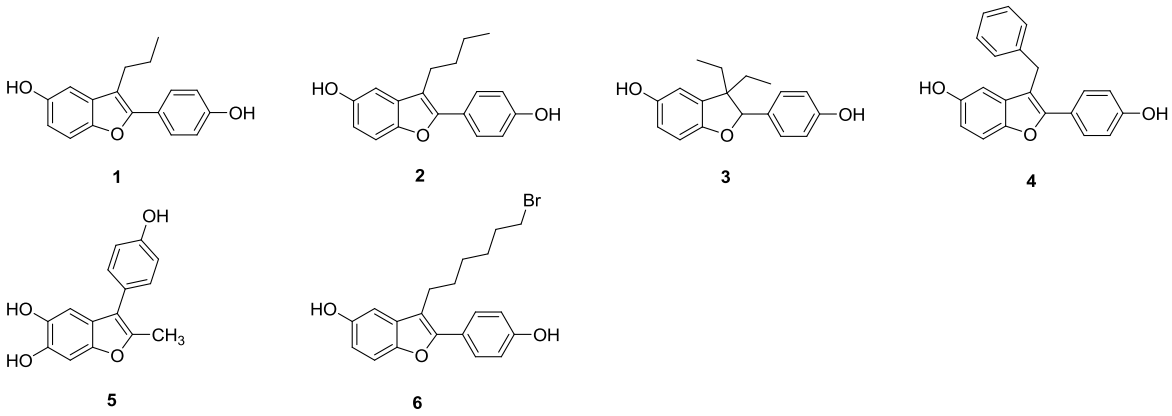


Figure 7.1 Structures of investigated benzo[*b*]furanes.

The hyaluronidase inhibitory activities of compounds **1**-**6** are summarized in Table 7.1.

Table 7.1 Inhibitory activity on hyaluronidases and log$D_{5.0}$ values of benzo[*b*]furanes **1**-**6**.

Compound	***Sag*Hyal$_{4755}$ IC_{50} (µM)[a]**	**BTH IC_{50} (µM)**	**log$D_{5.0}$[b]**
1	60 ± 8	inactive	4.9
2	73 ± 6	inactive	5.4
3	21 ± 4	inactive	3.9
4	120 ± 10	inactive	5.3
5	227 ± 3	inactive	3.4
6	9.2 ± 2	inactive	6.5

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Previously, compounds **1**-**6** were synthesized in our workgroup as antiestrogens and investigated for estrogen receptor binding. The following substances were published before: **1**[4, 5], **2**[4, 5], **4**[4], **6**[5]. When investigated on *Sag*Hyal$_{4755}$, surprisingly, the 3-alkyl-2-(4-hydroxyphenyl)benzofuranes **1**-**3** turned out to be rather potent hyaluronidase inhibitors. The 3-benzyl-substituted benzofurane **4** was a moderate inhibitor. Dislocation of the 4-hydroxyphenyl moiety from position 2 to 3 (cf. **5**) resulted in a decrease in inhibitory potency. The most potent derivative was found with **6** (IC_{50} = 9 µM), bearing a 6-bromohexyl moiety. All six substances were inactive on BTH.

In conclusion, small molecules incorporating the 2-(4-hydroxyphenyl)-benzofuran-5-ol motif might be regarded as promising scaffolds for the inhibition of streptococcal hyaluronidases. A potential binding of structurally modified inhibitors bearing a hydroxylated 2-phenylbenzo[*b*]furan motif to the estrogen receptor (ER) or cytotoxic effects should be kept in mind.

7.2.2 Benzo[*b*]thiophene derivatives

For the first time, derivatives of benzo[*b*]thiophenes (Figure 7.2), structural analogs of 2-phenylindoles and 2-phenylbenzo[*b*]furanes, were investigated as inhibitors of bacterial and mammalian hyaluronidases.

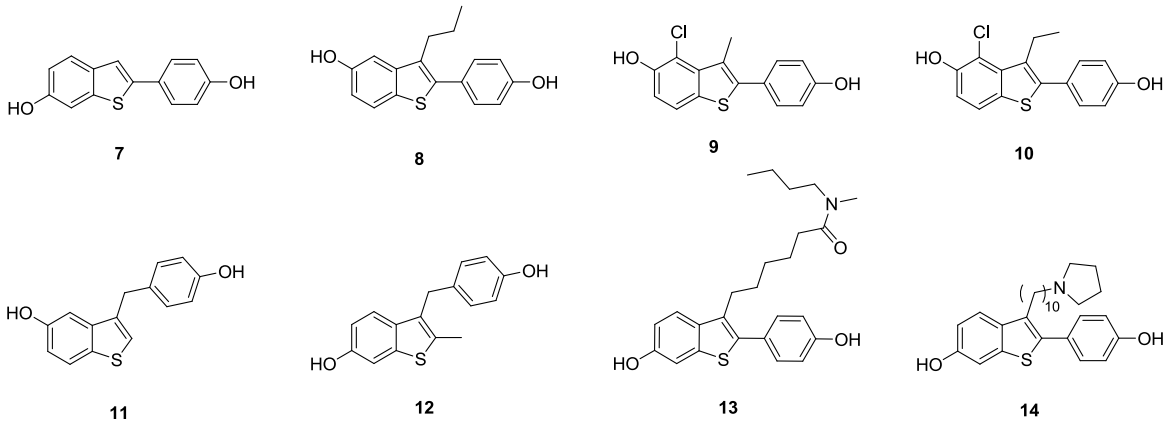


Figure 7.2 Structures of investigated benzo[*b*]thiophene-type derivatives.

The hyaluronidase inhibitory activities of compounds **7**-**14** are summarized in Table 7.2.

Table 7.2 Inhibitory activity on hyaluronidases and $logD_{5.0}$ values of benzo[*b*]thiophenes **7-14**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	$logD_{5.0}$[b]
7	950	inactive	3.0
8	38 ± 2	inactive	4.8
9	24 ± 2	inactive	4.4
10	16 ± 1	inactive	4.9
11	145 ± 13	inactive	4.0
12	20 ± 4	inactive	3.9
13	inactive	inactive	5.5
14	700	inactive	3.9

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Previously, the selected compounds were synthesized in our workgroup. The benzothiophenes **8**[6], **13**[7], **14**[7] are published and described as ligands of the estrogen receptor (ER).[7] By analogy with the related benzo[*b*]furanes, the 4-hydroxyphenyl moiety was identified as an effective motif to gain inhibitory activity on the bacterial hyaluronate lyase *Sag*Hyal$_{4755}$. Among the 2-(4-hydroxyphenyl)benzo[*b*]furanes, the lipophilic compounds **9** (IC_{50} = 24 µM, $logD_{5.0}$ = 4.4) and **10** (IC_{50} = 16 µM, $logD_{5.0}$ = 4.9) were most active. With a 4-hydroxyphenyl group attached to position 3, best results were obtained for substance **12** with an IC_{50} value of 20 µM ($logD_{5.0}$ = 3.9). Interestingly, the introduction of alkyl spacers (cf. **13**, **14**) resulted in a (total) loss of inhibition. None of these compounds inhibited the activity of the mammalian hyaluronidase BTH. Single crystals of **10**, suitable for X-ray diffraction studies, were grown by evaporation of the solvent (anhydrous methanol). The packing arrangement of a single crystalline phase contained symmetric dimers in which two molecules were present. To illustrate the configuration, an ORTEP-style plot and labeling scheme of **10** is given in Figure 7.3.

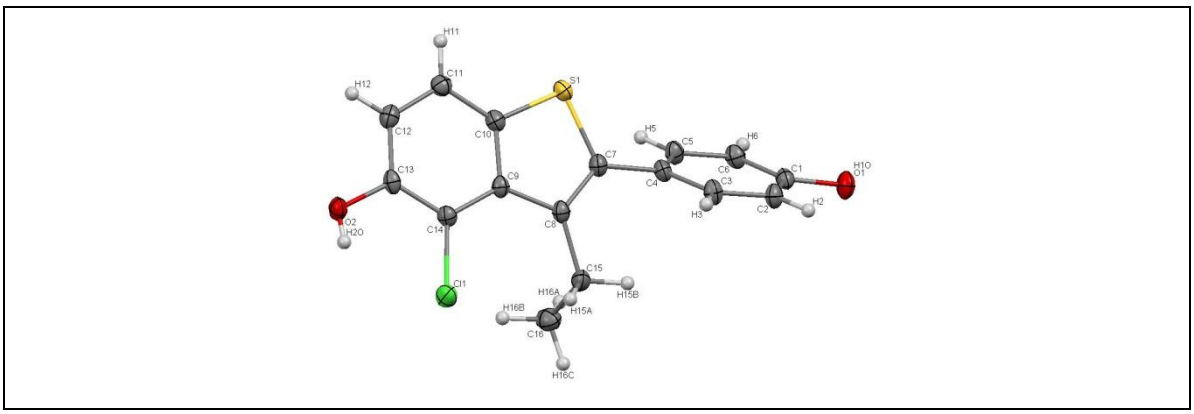


Figure 7.3 Molecular structure (ORTEP-style) of **10** including atomic numbering scheme.

Experimental details (crystal data and structure refinement) including atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, torsion angles and hydrogen bonds are given in section A.3.2 (appendix I). The molecular structure of **10** shows the planar geometry of the benzo[*b*]thiophen scaffold with $C_{(7)}$-$S_{(1)}$-$C_{(10)}$-$C_{(11)}$ lying in plane. The torsion angle between the phenyl residue and the thiophene moiety along $C_{(5)}$-$C_{(4)}$-$C_{(7)}$-$C_{(8)}$ is at 122.3 °.

It is noteworthy that small molecules incorporating the 2-(4-hydroxyphenyl)benzothiophen-5-ol motif are among the most promising scaffolds for the inhibition of streptococcal hyaluronidase identified so far. Potential binding of hydroxylated 2-phenyl-benzo[*b*]thiophenes to the estrogen receptor (ER) or cytotoxic effects should be kept in mind.

7.2.3 Benzimidazole derivatives

Benzimidazole-type substances were shown as inhibitors for the hyaluronate lyase from *S. agalactiae* strain 4755 (*Sag*$Hyal_{4755}$).[2, 3] The compounds are selective for the bacterial enzyme, whereas bovine testicular hyaluronidase was not affected.[2] Hence, for the current selection, an ensemble of four molecules bearing the 2-(4-hydroxyphenyl) motif was investigated. The structures of the analyzed molecules are shown in Figure 7.4.

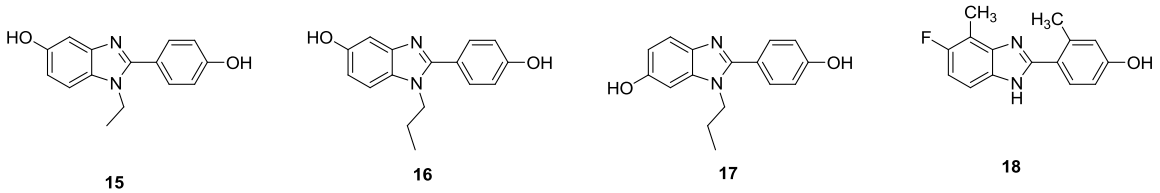


Figure 7.4 Structures of investigated benzimidazole-type substances.

The hyaluronidase inhibitory activities of compounds **15**-**18** are summarized in Table 7.3.

Table 7.3 Inhibitory activity of benzimidazole-type derivatives.

Compound	***Sag*$Hyal_{4755}$ IC_{50} (µM)[a]**	**BTH IC_{50} (µM)**	**log$D_{5.0}$[b]**
15	220 ± 70	inactive	1.2
16	inactive	inactive	1.7
17	inactive	inactive	1.7
18	80 ± 17	inactive	2.0

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

The following substances were published before: **16**[8], **17**[8]. Compound **18** was provided by P. Baumeister from our workgroup. Among the investigated benzimidazole derivatives, only compound **18** showed noteworthy inhibition of the bacterial hyaluronidase. Probably, the benzo[*b*]furane and the benzo[*b*]thiphene scaffold harbor a higher potential the development of novel lead inhibitors. Compared to the latter, the benzimidazole skeleton causes a strong decrease in lipophilicity (cf. $logD_{5.0}$ values of **15**-**18**), which is supposed to determine the binding affinity for hyaluronidases.

7.2.4 Alkanoic acid derivatives

Previously, Salmen identified derivatives of diphenylpropionic acid as inhibitors of *Sag*Hyal$_{4755}$ and BTH.[1, 9] Based on these observations, additional alkanoic acids (acetic acid, propanoic acid, butanoic acid and pentanoic acid derivatives) were tested for inhibition of hyaluronidases. The hyaluronidase inhibitory activities of compounds **19**-**36** (Figure 7.5) are summarized in Table 7.4.

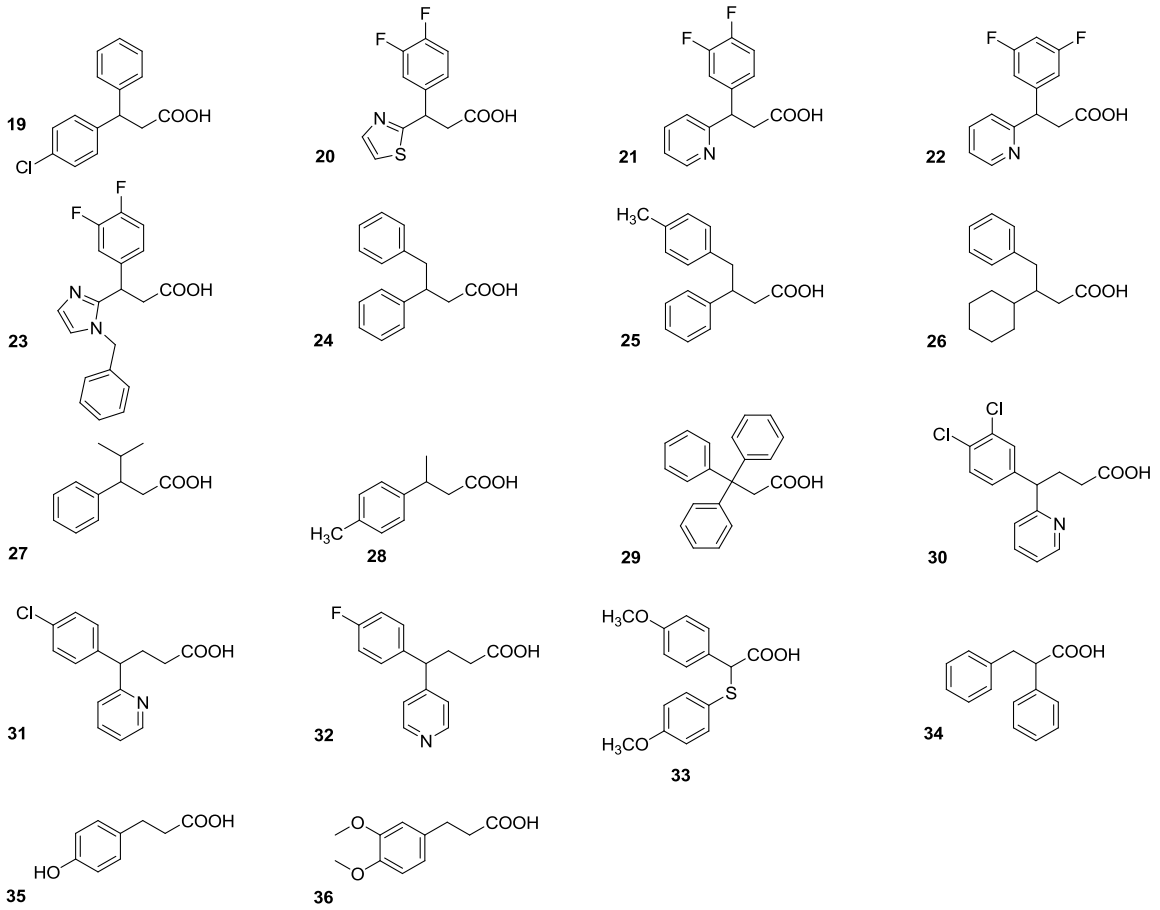


Figure 7.5 Structures of investigated (het)arylalkanoic acids.

Table 7.4 Inhibition of hyaluronidases by (het)arylalkanoic acids.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)
19	92 ± 4	inactive
20	>1000	inactive
21	>1000	inactive
22	>1000	inactive
23	>1000	inactive
24	240 ± 22	inactive
25	260 ± 40	inactive
26	345 ± 15	inactive
27	>1000	inactive
28	inactive	inactive
29	115 ± 9	inactive
30	290 ± 17	inactive
31	700	inactive
32	inactive	inactive
33	410	inactive
34	850	inactive
35	inactive	inactive
36	inactive	inactive

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes).

The following substances were published before: **19**[10], **20**[11], **23**[11], **26**[12], **27**[13], **30**[14], **31**[14], **32**[15], **33**[16], **34**. Substances **28**, **29**, **34**-**36** were commercially available. Among the carboxylic acids, compounds **19** (IC_{50} = 92 µM) and **29** (IC_{50} = 115 µM) were identified as the most potent inhibitors of *Sag*Hyal$_{4755}$. Inhibition of the mammalian enzyme BTH was not observed.

Taken together, several of the investigated (het)arylalkanoic acids revealed moderate to weak inhibitory activity on the bacterial hyaluronidase. Attempts to optimize the structures were not performed in this thesis. However, the results added knowledge to the project: Based on these observations, several commercially available non-steroidal anti-inflammatory drugs (NSAIDs) were predicted as potential inhibitors of *Sag*Hyal$_{4755}$. These studies are subject of ongoing projects in our workgroup.

7.2.5 Miscellaneous substances

To study additional small-molecules, compounds **37**-**53** were tested for hyaluronidase inhibitory activity. The structures are displayed in Figure 7.6.

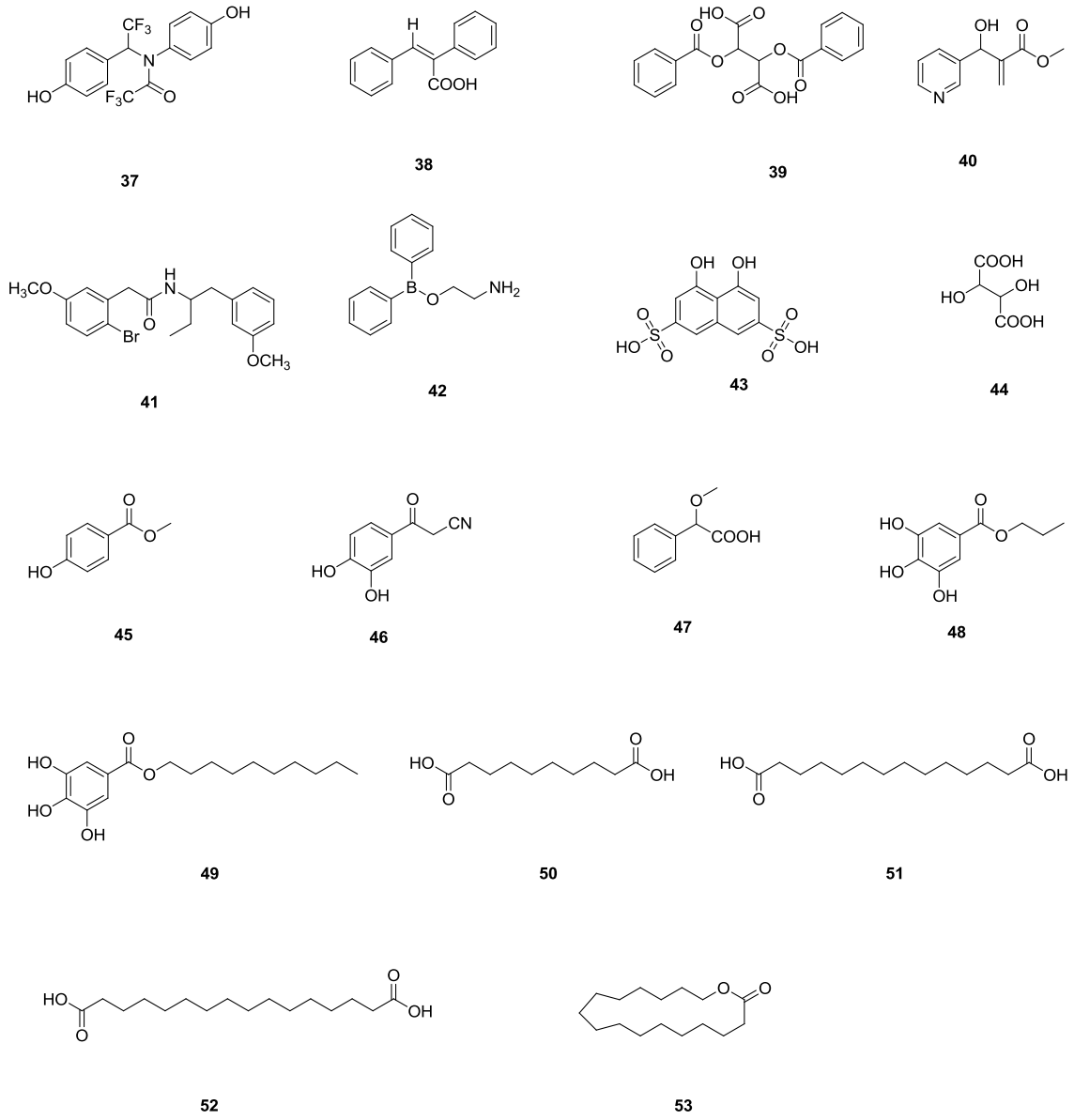


Figure 7.6 Structures of investigated miscellaneous substances.

The hyaluronidase inhibitory activities of compounds **19**-**29** are summarized in Table 7.5.

Table 7.5 Inhibitory activity of miscellaneous substances on hyaluronidases.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)[a]
37	120 ± 36	inactive
38	500	inactive
39	Inactive	inactive
40	Inactive	inactive
41	Inactive	inactive
42	480	inactive
43	>1000	inactive
44	>1000	inactive
45	Inactive	inactive
46	690	inactive
47	Inactive	inactive
48	>1000	inactive
49	10 ± 8	inactive
50	480	inactive
51	100 ± 8	>1000
52	17 ± 3	41 ± 5
53	Inactive	inactive

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes).

Compound **41** was published before[17], substances **38**-**40**, **42**-**53** were commercially available. Most of these compounds were inactive or showed weak inhibition of *Sag*Hyal$_{4755}$ and BTH. As expected, the inhibitory activity of the dicarboxylic acids **50**-**52** correlated with the length of the alkyl spacer. Accordingly, hexadecanedioic acid (**52**) was the most active inhibitor in this series. Besides, **52** showed relatively strong inhibition of the mammalian enzyme BTH. Remarkably, the loss of the carboxylic moiety led to inactive compounds, as, e.g., in case of **53**. However, these molecules are far from being drug-like, due to surfactant-like structural features.

7.2.6 Screening of bioactive natural products for hyaluronidase inhibition

Natural products (bioactive compounds) and medicinal plants represent a promising source of potential hyaluronidase inhibitors.[18] As an example, rosmarinic acid, was reported to possess various biological activities including inhibition of hyaluronidase.[1, 19] Very recently, novel phenylpropanoids from herbal plants *Lycopus lucidas*[19], *Cimicifuga dahurica* and *Cimicifuga heracleifolia*[20], *Meehania urticifolia*[21] and *Keiskea japonica*[22] were purported to have hyaluronidase inhibiting activities. The structures of the selected molecules are displayed in Figure 7.7.

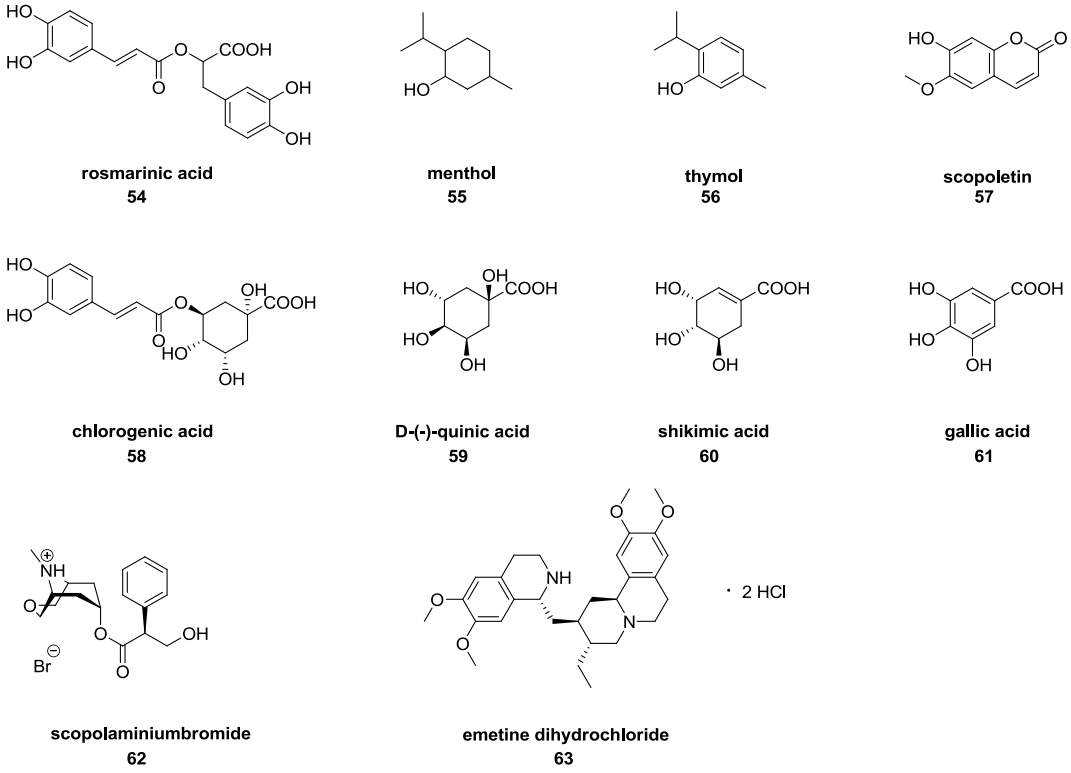


Figure 7.7 Structures of natural products investigated for hyaluronidase inhibition.

The substances **54**-**63** were commercially available and investigated with respect to hyaluronidase inhibition. In this series, rosmarinic acid (**54**) served as reference.

The hyaluronidase inhibitory activities of compounds **54**-**63** are summarized in Table 7.6.

Table 7.6 Inhibitory activity of investigated natural products.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	Notation
54	325 ± 55	inactive	rosmarinic acid
55	inactive	inactive	menthol
56	>1000	inactive	thymol
57	inactive	inactive	scopoletin
58	inactive	inactive	chlorogenic acid
59	inactive	inactive	D-(-)-quinic acid
60	inactive	inactive	shikimic acid
61	inactive	inactive	gallic acid
62	inactive	inactive	scopolaminiumbromide
63	inactive	inactive	emetine dihydrochloride

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes).

Except for rosmarinic acid, the investigated substances, proved to be inactive on both *Sag*Hyal$_{4755}$ and BTH, rosmarinic acid was inactive on BTH (turbidimetric acid, pH 5.0). As discussed before, the selection of compound was restricted to small molecules. Hence, a screening of more complex structures was not attempted.

7.2.7 Screening of (approved) drugs for hyaluronidase inhibition

In the review by Girish et al. , anti-inflammatory, anti-allergic and antibiotic drugs are listed as hyaluronidase inhibitors.[1] This was confirmed in our laboratory in case of indomethacin, which is used as a reference inhibitor in this thesis. Besides, the anti-inflammatory agents phenylbutazone and oxyphenbutazone were stated to be potent hyaluronidase inhibitors.[23] This hint and the discovery of active compounds incorporating a “thiohydantoin” scaffold (see chapter 5) inspired us to investigate a small library containing structurally related five-membered heterocycles (cf. Figure 7.8).

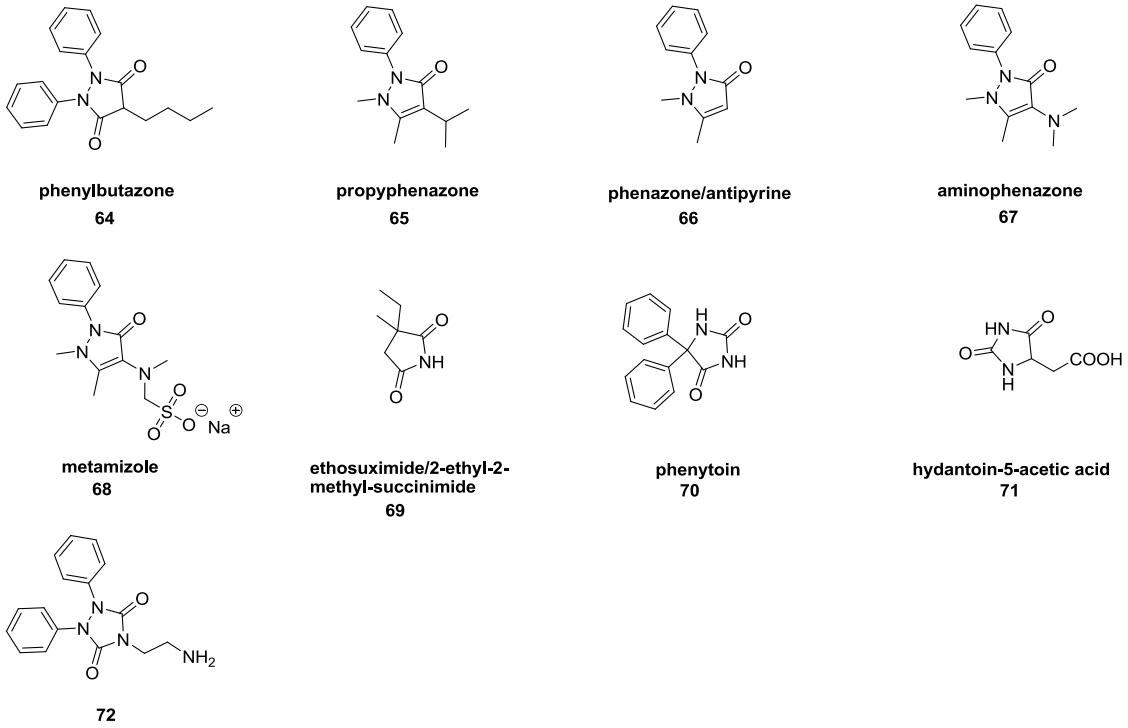


Figure 7.8 Structures of investigated drugs and related substances.

The hyaluronidase inhibitory activities of compounds **64**-**72** are summarized in Table 7.7.

Table 7.7 Inhibitory activity of investigated drugs and related substances.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	Notation
64	650	inactive	phenylbutazone
65	inactive	inactive	propyphenazone
66	inactive	inactive	phenazone (antipyrine)
67	inactive	inactive	aminophenazone
68	inactive	inactive	metamizole
69	inactive	inactive	ethosuximide
70	inactive	inactive	phenytoin
71	inactive	inactive	hydantoin-5-acetic acid
72	inactive	inactive	

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes).

The substances **64**-**71** were commercially available. Compound **72** was provided by N. Pluym from our workgroup. Phenylbutazone (**64**) served as reference inhibitor. Under the present screening conditions, except for phenylbutazone, the investigated substances, were inactive on *Sag*Hyal$_{4755}$ and BTH.

7.2.8 Peptide-based substances

Over the past decade, a number of peptide-based therapeutics that block the binding of CD44 or RHAMM-specific ligands have been developed and tested in experimental models of disease.[24] For example, peptide mimetics of HA with affinity to RHAMM were identified.[25] This demonstrated that non-HA ligands specific to a given HA binding protein can be engineered.[25] Hits were identified among HA-mimetic peptide libraries containing a number of negatively charged (*R*)- and (*S*)-amino acids. Strikingly, the most active peptides consisted of 8 to 12 amino acids.[25, 26] These peptides were predicted to bind closely to basic amino acids in RHAMM which were known to be involved in the interaction with HA.[27] As peptide mimetics offer a novel therapeutic strategy to block specific functions of CD44 and RHAMM, the idea arose to characterize octapeptides with regard to inhibition of hyaluronidases (*Sag*Hyal$_{4755}$ and BTH). The structures of the selected peptide-ligands are displayed in Figure 7.9.

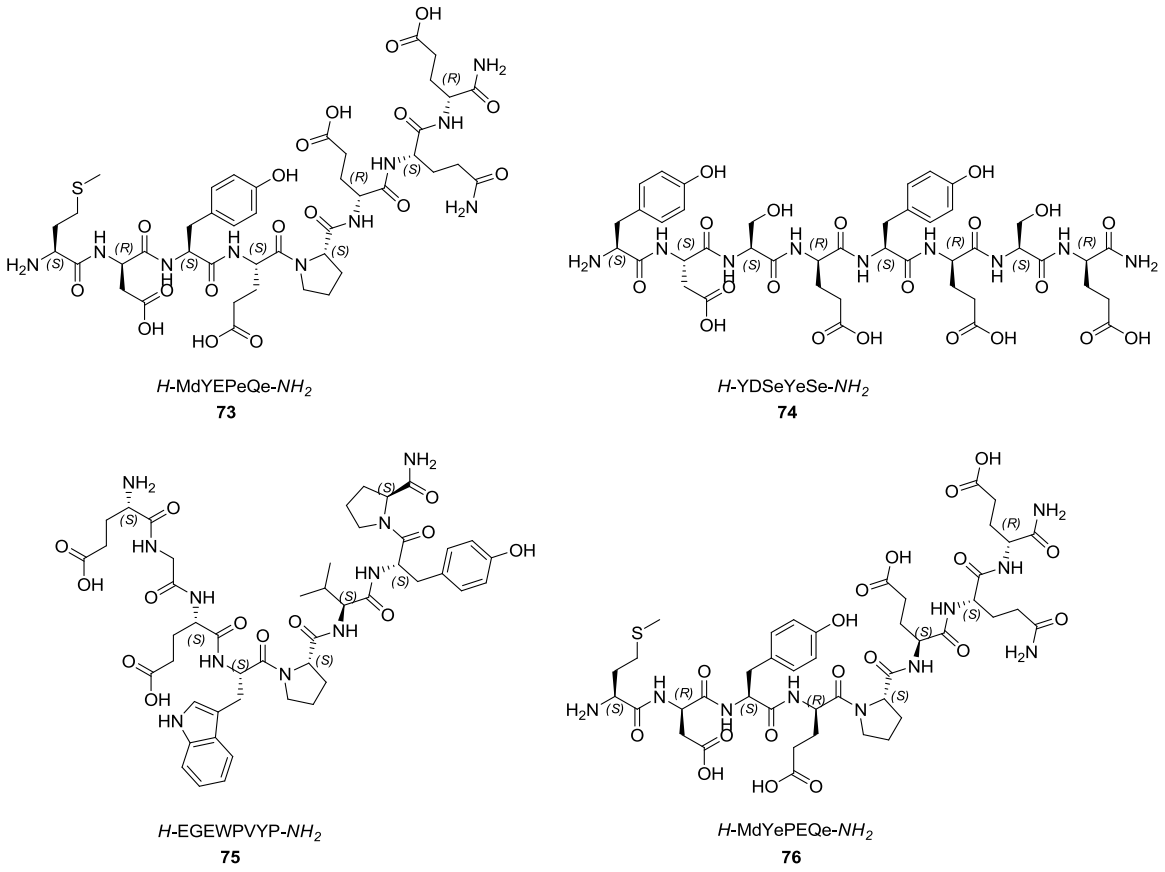


Figure 7.9 Structures of investigated peptides.

The hyaluronidase inhibitory activities of compounds **73**-**76** are summarized in Table 7.8.

Table 7.8 Inhibitory activity of peptides **73**-**76** on *Sag*Hyal$_{4755}$ and BTH.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	Notation
73	570 ± 20	inactive	*H*-MdYEPeQe-NH_2
74	640 ± 35	inactive	*H*-YDSeYeSe-NH_2
75	930 ± 10	inactive	*H*-EGEWPVYP-NH_2
76	610 ± 20	inactive	*H*-MdYePEQe-NH_2

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes).

The peptides were kindly provided by Prof. Dr. C. Cabrele (Institute of Organic Chemistry, University of Bochum). The peptide sequences were adopted from Ziebell et al. (**73**[27], **74**[27], **75**[25], **76**[25]). All peptides (**73-76**) were identified as weak inhibitors of *Sag*Hyal$_{4755}$. By contrast, inhibitory activity on the bovine testicular hyaluronidase was not observed. The inhibition of the bacterial enzyme is rather poor, probably due to major structural differences of the HA-binding site in *Sag*Hyal$_{4755}$ compared to RHAMM. Nevertheless, such peptides might offer a molecular approach to the mimicry of the natural ligand HA. Most likely, computer-assisted design of peptides containing 8 to 12 amino acids is key to the rational discovery of more active and selective protein-based inhibitors.

7.2.9 Conformationally restricted 2-phenylindole derivatives

To explore conformationally restricted 2-phenylindoles (Figure 7.10), 6-alkyl-5,6-dihydroindolo[2,1-*a*]isoquinolines (**77**-**84**), 11-alkyl-6,11-dihydro-5*H*-benzo[*a*]carbazoles (**85**, **86**) and 5,10-diethyl-5,10-dihydroindeno[1,2-*b*]indole-2,8-diol (**87**), were selected for investigation on *Sag*Hyal$_{4755}$ and BTH. Here, the indole and the 2-(4-hydroxyphenyl) substituent were bridged by a methylene or an ethylene group to constrain the rotation around the sigma bond between indole-C-2 and phenyl residue, resulting in a more planar shape of the core structure. Previously, the selected compounds were synthesized in our workgroup and investigated for their estrogen receptor affinity. The following substances were published before: **77**[17, 28], **78**[29], **84**[30], **85**[31].

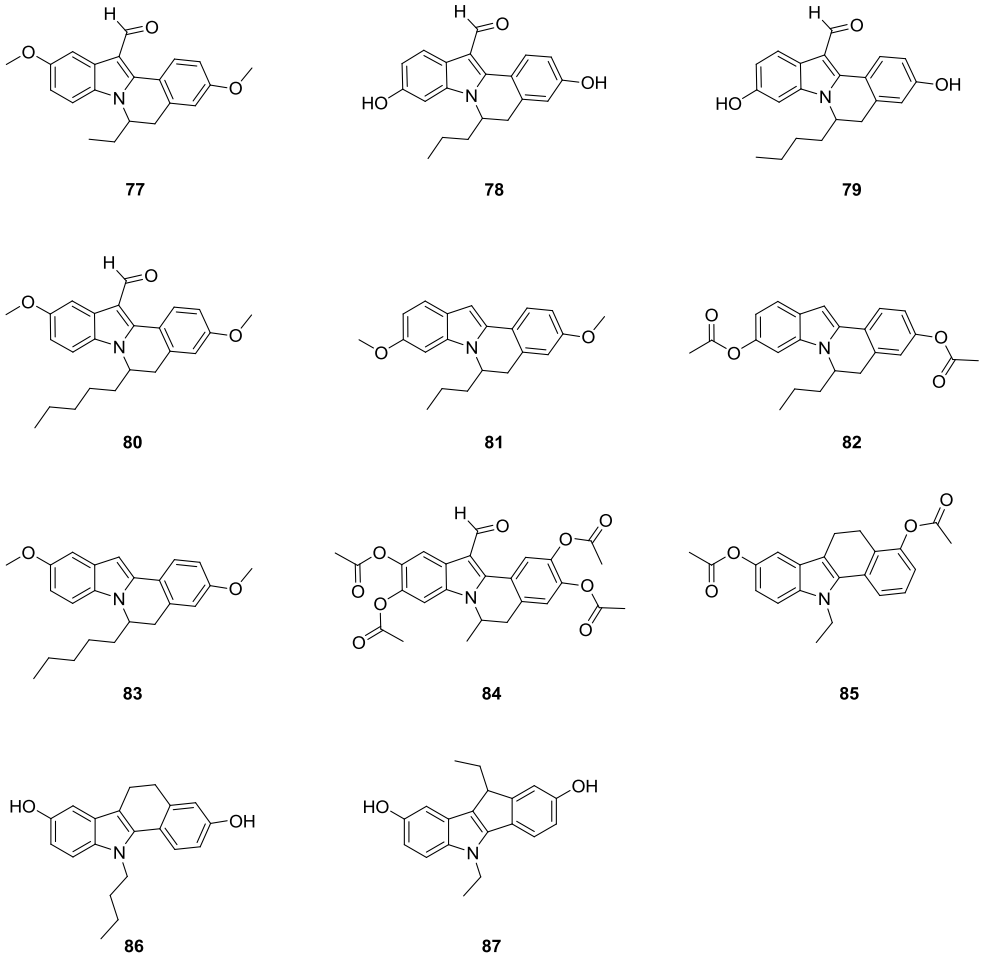


Figure 7.10 Structures of investigated conformationally restricted 2-phenylindoles.

The compounds **77**-**87** were investigated for inhibition of *Sag*Hyal$_{4755}$ and BTH (Table 7.9).

Table 7.9 Activity of conformationally restricted 2-phenylindoles **77**-**87** on *Sag*Hyal$_{4755}$ and BTH.

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (μM)[a]**	**BTH IC$_{50}$ (μM)**
77	inactive	inactive
78	inactive	inactive
79	inactive	inactive
80	inactive	inactive
81	inactive	inactive
82	inactive	inactive
83	inactive	inactive
84	inactive	inactive
85	inactive	inactive
86	inactive	inactive
87	inactive	inactive

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC$_{50}$ values determined at pH 5.0 in the turbidimetric assay (cuvettes).

None of the investigated compounds **77-87** showed an inhibitory effect on *Sag*Hyal$_{4755}$ and BTH. Obviously, the rigid three-dimensional structure is incompatible with binding to the enzymes.

7.2.10 2-Phenylindolecarbaldehyde derivatives

A series of hydrazones (**88**-**93**, **96**) and Knoevenagel condensation products of 2-phenylindole-3-carbaldehydes (**94**, **95**, **97**) was tested for inhibition of hyaluronidases. Previously, the selected compounds were synthesized in our workgroup and investigated for their estrogen receptor affinity. For substance **88** cf. ref.[32] The corresponding structures are shown in Figure 7.11.

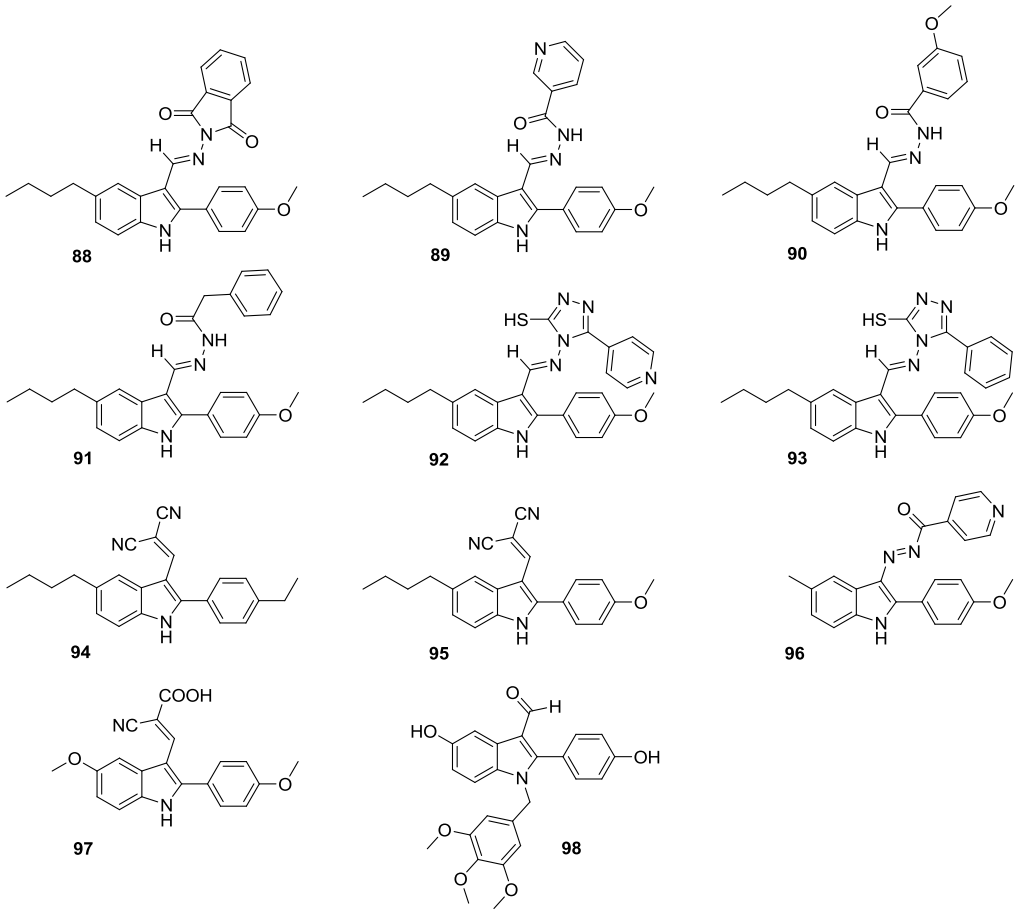


Figure 7.11 Structures of hydrazones and Knoevenagel condensation products of 2-phenylindole-3-carbaldehydes.

The results of the investigations of compounds **88**-**97** on *Sag*Hyal$_{4755}$ and BTH are summarized in Table 7.10.

Table 7.10 Activities of 2-phenylindole-3-carbaldehyde derivatives on *Sag*Hyal$_{4755}$ and BTH.

Compound	***Sag*Hyal$_{4755}$ IC_{50} (µM)[a]**	**BTH IC_{50} (µM)[a]**
88	inactive	inactive
89	inactive	inactive
90	inactive	inactive
91	inactive	inactive
92	inactive	inactive
93	inactive	inactive
94	inactive	inactive
95	inactive	inactive
96	inactive	inactive
97	380 ± 45	inactive
98	37% (200 µM)[b]	inactive

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] % inhibition of *Sag*Hyal$_{4755}$ at indicated inhibitor concentration.

As a result, most of the investigated compounds did not affect *Sag*Hyal$_{4755}$ and BTH. An exception was substance **97**. In this case, presumably, the carboxylic moiety plays a key role in enhancing the binding to the bacterial hyaluronate lyase. Moreover, cleavage of the methyl ether to the corresponding phenolic hydroxyl group as in **98** can increase binding affinity for hyaluronidase.

7.2.11 *N*-Alkylated 2-phenylindoles

A small library of *N*-alkylated 2-phenylindoles was tested for the inhibition of *Sag*Hyal$_{4755}$ and BTH. Previously, the selected compounds were synthesized in our workgroup and investigated for their estrogen receptor affinity. The following substances were published before: **99**-**102**[33], **103**[34], **104**[34], **105**[34], **106**[35], **107**[36], **109**[37]. The corresponding molecules are shown in Figure 7.12.

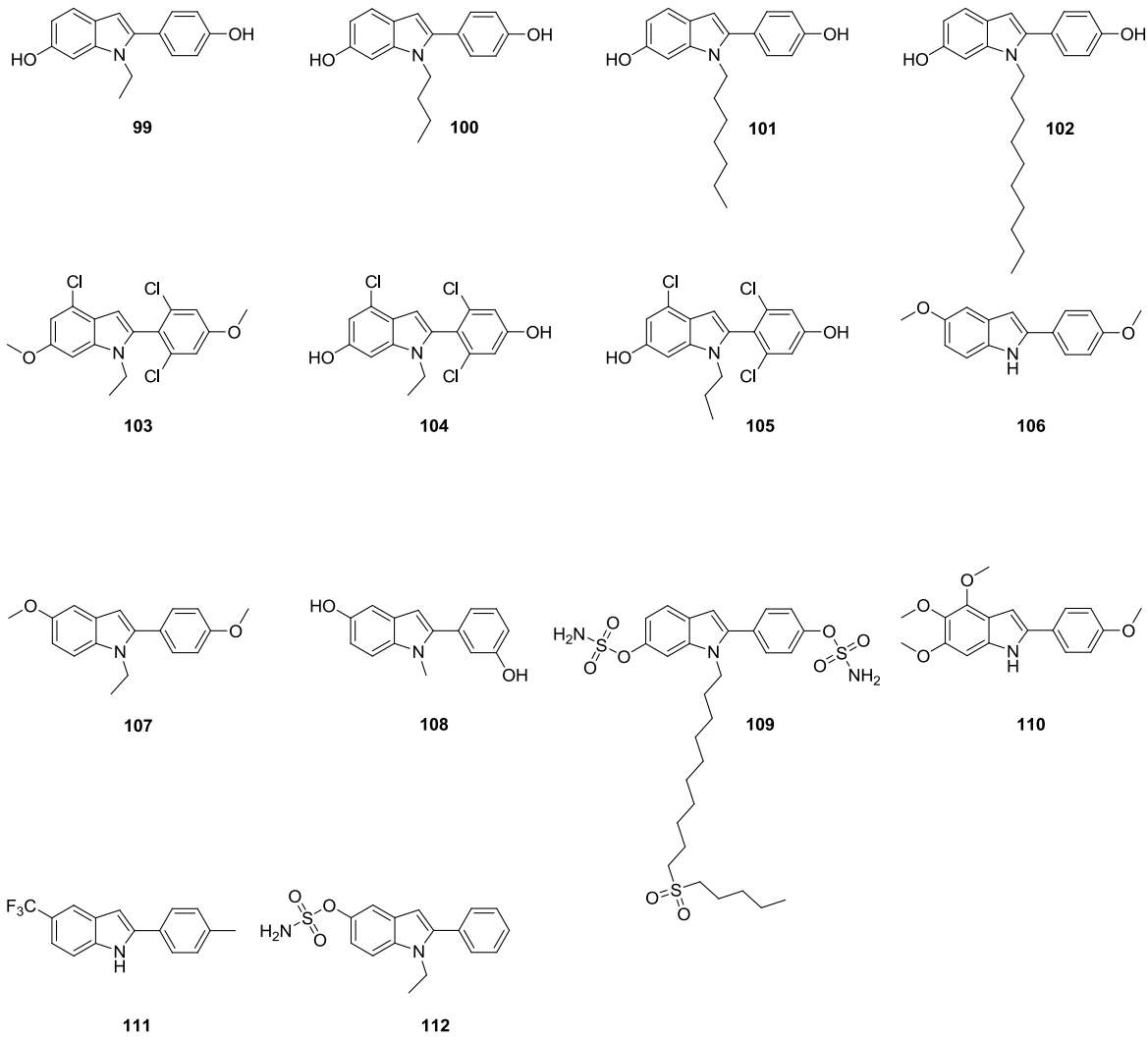


Figure 7.12 Structures of *N*-alkylated 2-phenylindoles **99**-**112**.

The hyaluronidase inhibitory activities of compounds **98**-**111** are summarized in Table 7.11.

Table 7.11 Inhibitory activity on *Sag*Hyal$_{4755}$ and BTH and log$D_{5.0}$ values of *N*-alkylated 2-phenylindoles.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	log$D_{5.0}$[b]
99	320 ± 25	inactive	3.6
100	66 ± 17	inactive	4.6
101	18.2 ± 9.6	inactive	6.1
102	15 ± 5.8	inactive	7.6
103	inactive	inactive	5.1
104	8.5 ± 1.3	inactive	5.2
105	10 ± 3.6	inactive	5.7
106	inactive	inactive	2.2
107	inactive	inactive	3.7
108	460 ± 44	inactive	2.9
109	inactive	inactive	5.6
110	inactive	inactive	not calculated
111	inactive	inactive	5.2
112	620 ± 26	inactive	3.3

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes). [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

The inhibitory potency of **99**-**102** for *Sag*Hyal$_{4755}$ increased with elongation of the carbon chain. Interestingly, compound **101** (7 carbon atoms) and compound **102** (10 carbon atoms) were almost equipotent. In agreement with previous investigations,[9, 38, 39] the results suggest a positive correlation between lipophilicity and enzyme inhibition. However, compound **109**, containing a 10-(pentylsulfonyl)decyl substituent at the indole-nitrogen, did not show inhibitory activities.

Aiming at drug-like properties, the weak inhibitor **99**, containing a relatively short aliphatic chain, was considered a structural basis for the search of structurally derived analogs. Accordingly, compounds **103**-**108** and **110**-**112** were selected for biological tests. By trend, only hydroxylated compounds were capable of inhibiting hyaluronidase *Sag*Hyal$_{4755}$. Strikingly, compounds **104** and **105** were identified as one-digit micromolar inhibitors of *Sag*Hyal$_{4755}$. Remarkably, these small molecules turned out to be even more potent than 2-phenylindole bearing lipophilic *N*-alkyl chains (see compounds **101**, **102**). Replacement of the hydroxyl (cf. **104**) by a methyl ether group (cf. **103**) led to a total loss of inhibition.

Single crystals of **104**, suitable for X-ray diffraction studies were grown by evaporation of the solvent (anhydrous methanol). The packing arrangement of a single crystalline phase contained monomers. To illustrate the configuration, an ORTEP-style plot and labeling scheme of **104** is given in Figure 7.13.

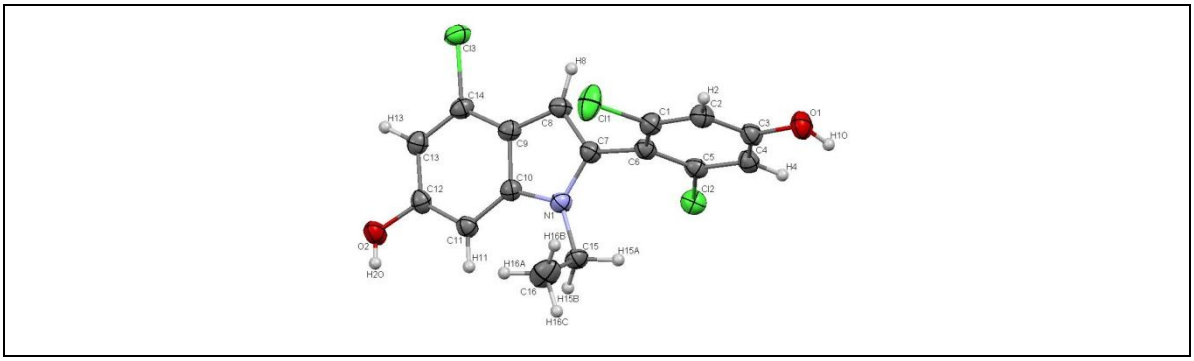


Figure 7.13 Molecular structure (ORTEP-style) of **104** including atomic numbering scheme.

Experimental details (crystal data and structure refinement) including atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, torsion angles and hydrogen bonds are given in section A.3.3 (appendix I). The molecular structure of **104** shows the indole scaffold with $C_{(7)}$-$N_{(1)}$-$C_{(10)}$-$C_{(11)}$ lying in plane. The torsion angle between the phenyl ring and indole ring along $C_{(1)}$-$C_{(6)}$-$C_{(7)}$-$C_{(8)}$ is 68.8 °.

In summary, the discovery of the potent inhibitors **104**, **105** became a valuable source for the design and development of novel, drug-like inhibitors of streptococcal enzymes.

7.2.12 *N*-Alkylated 3-methyl-2-phenylindole derivatives

A small library of *N*-alkylated 3-methyl-2-phenylindoles was tested for the inhibition of *Sag*Hyal$_{4755}$ and BTH. Previously, the selected compounds were synthesized in our workgroup and investigated for their estrogen receptor affinity. The following substances were published before: **113**[40], **115**[35], **116**[35], **117**[41], **118**[41], **119**-**121**[35], **122**[42], **123**[43], **124**[43], **125**[44], **127**[45], **128**[36], **130**[36], **133**[36]. The corresponding molecules are shown in Figure 7.14.

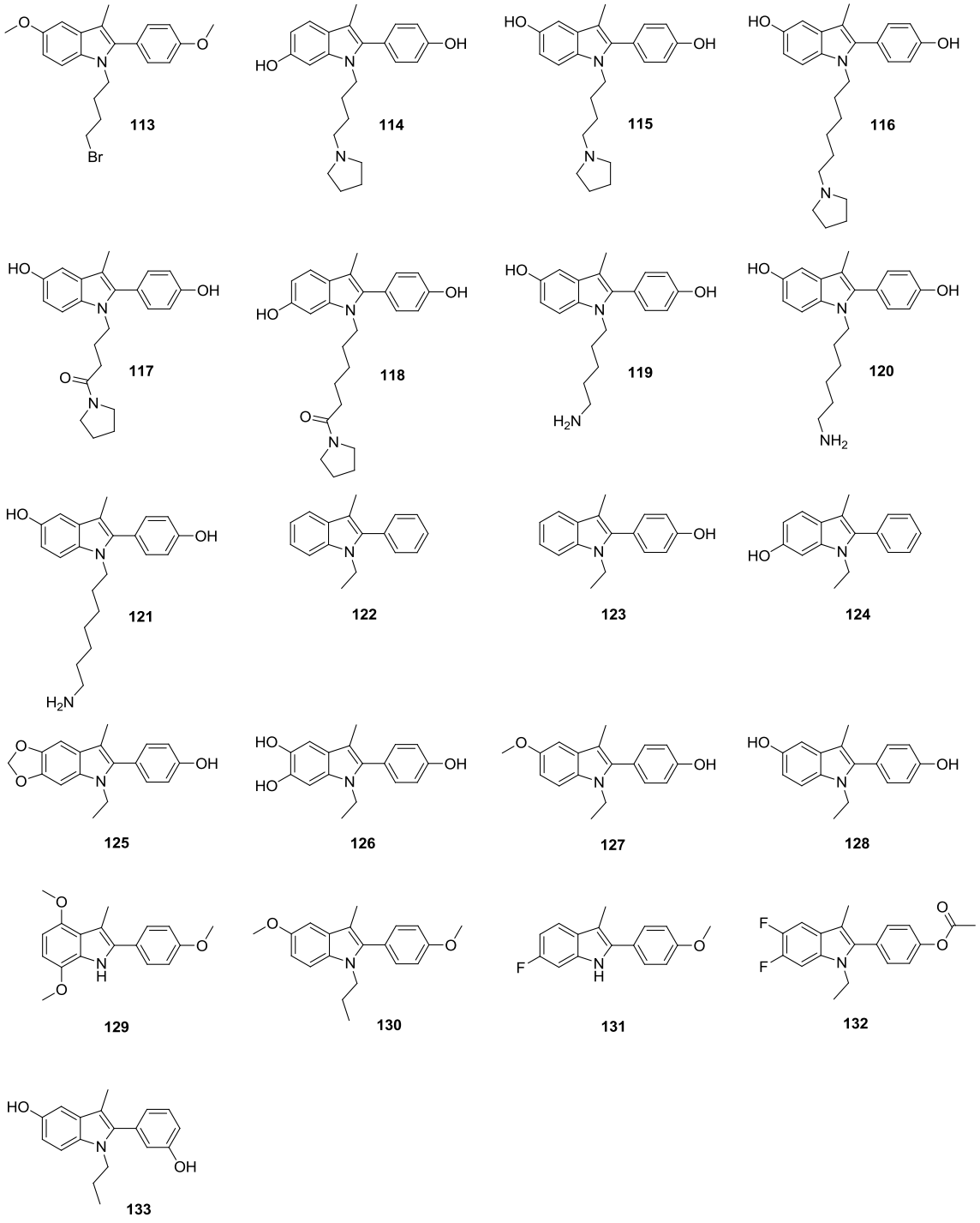


Figure 7.14 Structures of *N*-alkylated 3-methyl-2-phenylindoles **113**-**133**.

The hyaluronidase inhibitory activities of compounds **113**-**133** are summarized in Table 7.12.

Table 7.12 Inhibitory activities on *Sag*Hyal$_{4755}$ and BTH and log$D_{5.0}$ values of *N*-alkyl-3-methyl-2-phenyl-indoles.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (μM)[a]	BTH IC_{50} (μM)	log$D_{5.0}$[c]
113	inactive	inactive	5.3
114	140 ± 11	inactive	1.2
115	318 ± 10	inactive	1.4
116	300 ± 32	inactive	2.1
117	15% (200 μM)[b]	inactive	3.3
118	20% (200 μM)[b]	inactive	3.8
119	60% (200 μM)[b]	inactive	0.4
120	70% (200 μM)[b]	inactive	0.9
121	30% (200 μM)[b]	inactive	1.5
122	inactive	inactive	4.8
123	570 ± 14	inactive	4.2
124	750 ± 39	inactive	4.2
125	95 ± 5	inactive	3.4
126	16.4 ± 7	inactive	3.0
127	79% (200 μM)[b]	inactive	3.5
128	370 ± 48	inactive	3.7
129	inactive	inactive	3.1
130	inactive	inactive	4.5
131	inactive	inactive	4.0
132	inactive	inactive	4.3
133	103 ± 21	inactive	4.2

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] % inhibition of *Sag*Hyal$_{4755}$ at indicated inhibitor concentration; [c] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

N-Alkylated 3-methyl-2-phenylindoles comprising chains of 5 to 10 carbon atoms were identified as potent inhibitors of *Sag*Hyal$_{4755}$.[9] The attachment of bromo- (**113**), pyrrolidino- (**114**-**116**), pyrrolidinocarbonyl- (**117**, **118**) and amino groups (**119**-**121**) at the aliphatic chain resulted in weak hyaluronidase inhibitors.

Single crystals of **128**, suitable for X-ray diffraction studies were grown by evaporation of the solvent (methanol). The packing arrangement of a single crystalline phase contained symmetric dimers in which two molecules were present. To illustrate the configuration, an ORTEP-style plot and labeling scheme of **128** is given in Figure 7.15.

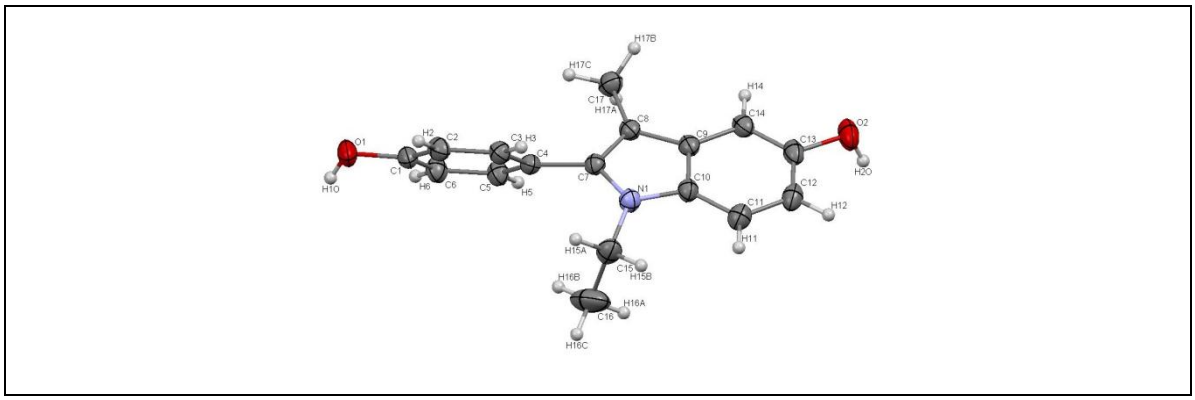


Figure 7.15 Molecular structure (ORTEP-style) of 128 including atomic numbering scheme.

Experimental details (crystal data and structure refinement) including atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, torsion angles and hydrogen bonds are given in section A.3.4 (appendix I). The molecular structure of **128** shows the planar indole scaffold with $C_{(7)}$-$N_{(1)}$-$C_{(10)}$-$C_{(9)}$ lying in plane. The torsion angle between the phenyl ring and indole ring along $C_{(5)}$-$C_{(4)}$-$C_{(7)}$-$C_{(8)}$ is at 55.0°.

To investigate the influence on potency by different positioning of the hydroxyl groups on the 3-methyl-2-phenylindole skeleton, compounds **122**-**128** were compared under standardized conditions. The core structure (**122**) and mono-hydroxylated 3-methyl-2-phenylindoles (**123**, **124**) were inactive or weak inhibitors, respectively. Compared to **128**, two hydroxyl groups significantly increased inhibitory activity. In addition, vicinal hydroxyl residues in position 5 and 6 of the indole skeleton (cf. **126**) were tolerated and led to a potent inhibitor. By contrast, replacement of hydroxyl groups by methoxy- (**129**, **130**) and fluoro-substituents (**131**, **132**) resulted in inactive compounds. As suggested by **133**, the position of the hydroxy group in the phenyl residue of 3-methyl-2-phenylindoles is of minor importance.

7.2.13 *N*-Benzyl-3-methyl-2-phenylindole derivatives

To get additional hints to promising structural modifications of inhibitors bearing a 2-phenylindoles scaffold, *N*-benzylated 3-methyl-2-phenylindoles compounds **134**-**142** were tested for hyaluronidase inhibition. The molecular structures are shown Figure 7.16.

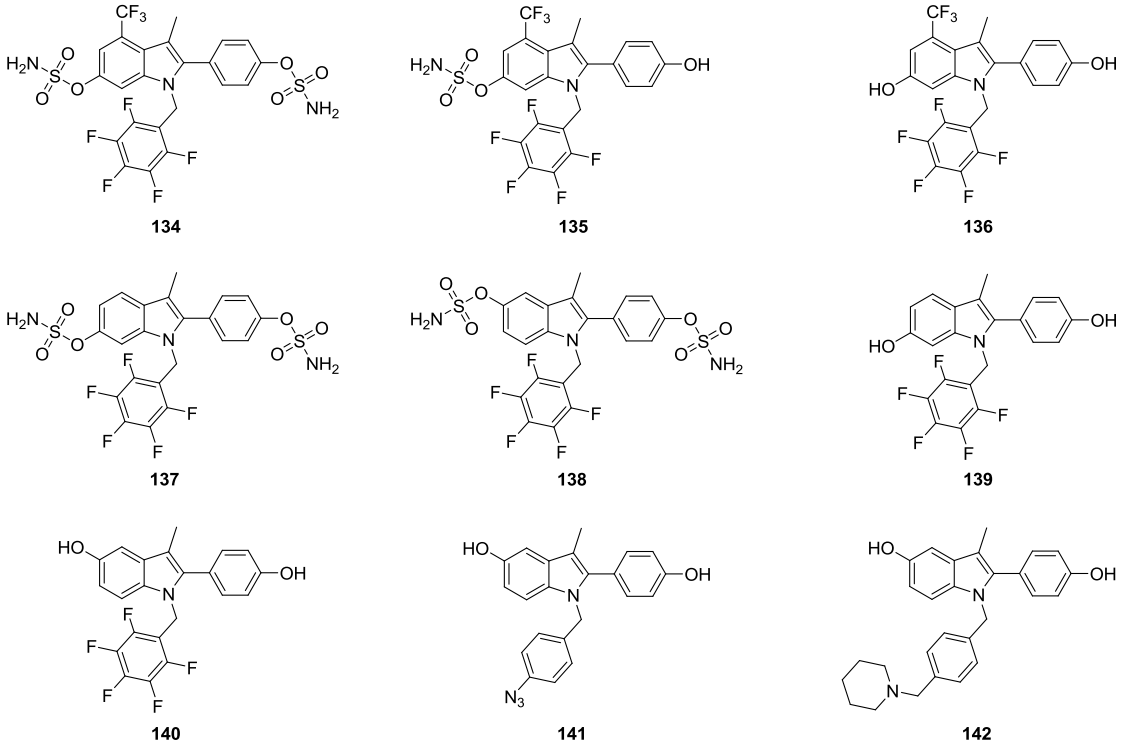


Figure 7.16 Structures of *N*-benzylated 3-methyl-2-phenylindoles **134**-**142**.

The hyaluronidase inhibitory activities of compounds **134**-**142** are summarized in Table 7.13.

Table 7.13 Activities of *N*-benzyl-3-methyl-2-phenylindoles on hyaluronidases and calculated $logD_{5.0}$ values.

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (µM)[a]**	**BTH IC$_{50}$ (µM)**	**log$D_{5.0}$[b]**
134	9 ± 2	inactive	4.9
135	5.2 ± 1.2	inactive	5.7
136	5.7 ± 0.8	inactive	6.9
137	27 ± 5	inactive	3.8
138	27 ± 7	inactive	3.8
139	20.1 ± 2	inactive	5.7
140	18.5 ± 3	inactive	5.7
141	22 ± 7	inactive	5.9
142	120 ± 19	inactive	3.2

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Previously, the selected compounds were synthesized in our workgroup and investigated for their estrogen receptor affinity and as inhibitors of the steroid sulfatase (EC 3.1.6.2.). For substance **135** (cf. ref.[44]).

Comparing **134**-**136** on *Sag*Hyal$_{4755}$, sequential cleavage of the sulfamic ester groups resulted in equipotent inhibitors. This also holds for the indoles **137** and **138** compared to **139** and **140**, respectively. Calculation of the calculated log$D_{5.0}$ values revealed significant lower lipophilicity of the sulfamates relative to the corresponding phenols. There is no correlation between the observed IC_{50} values and log$D_{5.0}$ values for compounds **134**-**141**. In this context, the chemical and enzymatic stability of the sulfamic ester group has to be considered. Golob and Walter reported that sulfatase inhibitors were at least partly converted to the free hydroxyl derivatives.[33, 37] In particular, they found that the sulfamate moitey was hydrolyzed to the free hydroxyl derivative within a few days when kept under tissue culture conditions. The authors speculate that this conversion might occur especially in vivo, i. e. the sulfamates are prodrug-like compounds.[33] Taking into consideration these observations, it was conceivable that the inhibition of hyaluronate lyase had to be mainly attributed to hydroxylated 2-phenylindole derivatives.

A qualitative HPLC-MS experiment was performed to analyze the chemical stability of substances **134**-**136** under assay conditions. The compounds (c = 100 µM) were incubated in buffer medium (c(BSA) = 0.2 mg/mL, c(HA) = 2.0 mg/mL, pH 5.0) at 37 °C for 30 minutes. Compound **136** served as reference compound. Subsequently, the assay incubation mixture was subjected to HPLC and analyzed by means of mass spectroscopy (Table 7.14).

Table 7.14 HPLC-MS spectral data of **134**-**136** (concentration: 100 µM) after incubation under assay conditions at 37 °C for 30 minutes.

Compound	MS-scan for 134	MS-scan for 135	MS-scan for 136
134 (M_r = 645.5)	646.1 ($[M + H]^+$, 100 %)	n.d.	n.d.
135 (M_r = 566.4)		567.1 ($[M + H]^+$, 100 %)	n.d.
136 (M_r = 487.3)			488.1 ($[M + H]^+$, 100 %)

As a result, for the incubation of the substances under *in vitro* assay conditions in the buffer medium, according to HPLC-MS, hydrolysis of the sulfamated residues was not observed. This data suggests that the indole derivatives bearing one or two sulfamate groups are actually equipotent to the corresponding hydroxy compounds. As discussed above, the situation might be different under *in vivo* conditions.

7.2.14 *N*-Alkyl-3-ethyl-2-phenylindole derivatives

A set of three *N*-alkylated 3-ethyl-2-phenylindoles was tested for inhibition of *Sag*Hyal$_{4755}$ and BTH. The corresponding molecules are shown in Figure 7.17.

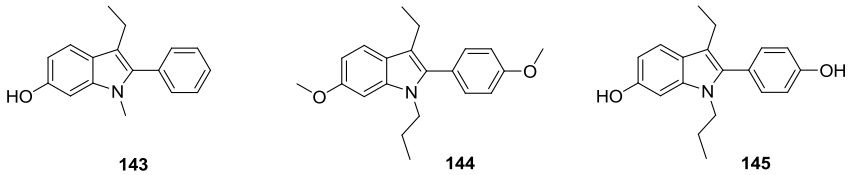


Figure 7.17 Structures of *N*-alkylated 3-ethyl-2-phenylindoles **143**-**145**.

Previously, the compounds **143-145** were synthesized in our workgroup and investigated for estrogen receptor affinity. For compounds **144** and **145** (cf. ref.[36]).

The hyaluronidase inhibitory activities of compounds **143**-**145** are summarized in Table 7.15.

Table 7.15 Hyaluronidase inhibitory activity and log$D_{5.0}$ values of *N*-alkyl-3-ethyl-2-phenylindoles **143**-**145**.

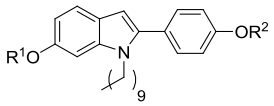
Compound	***Sag*Hyal$_{4755}$ IC_{50} (µM)[a]**	**BTH IC_{50} (µM)**	**log$D_{5.0}$[b]**
143	inactive	inactive	4.2
144	inactive	inactive	4.7
145	67 ± 13	inactive	4.6

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

The hydroxylated 3-ethyl-2-phenylindole **145** displayed inhibitory activity on *Sag*Hyal$_{4755}$. Nevertheless, the focus for structural modifications was set to less lipophilic 3-methyl-2-phenylindole derivatives.

7.2.15 Sulfamoyloxy-substituted 3-methyl-2-phenylindoles

Previously, the indole-type inhibitor **146** (Figure 7.18) from our workgroup was crystallized in complex with *S. pneumonia* hyaluronidase (*Spn*Hyl) and the binding mode was determined by X-ray crystallography.[38] As the indole moiety binds to the catalytic site of the enzyme, this phenylindole was tested for inhibition of the related hyaluronate lyase from *S. agalactiae* (*Sag*Hyal$_{4755}$) resulting in an IC_{50} value of 11 µM.[38]



Compound	R^1	R^2
146	SO_2NH_2	SO_2NH_2
147	SO_2NH_2	H
148	H	H

Figure 7.18 Structures of the sulfamoyloxy-substituted *N*-alkylated 3-methyl-2-phenylindoles **146**, **147** and the partent compound **148**.

The hyaluronidase inhibitory activities of compounds **146**-**148** are summarized in Table 7.16.

Table 7.16 Inhibitory activity of *N*-alkylated 3-methyl-2-phenylindoles **146**-**148** on hyaluronidases

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (µM)[a]**	**BTH IC$_{50}$ (µM)**	**logD$_{5.0}$[b]**
146	11 ± 5	inactive	4.9
147	8 ± 3	inactive	5.8
148	13 ± 6	inactive	6.9

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

To optimize 2-phenylindole of general structure **146** with respect to drug-likeness, the contribution of the sulfamate moieties, the effect of the length of the alkyl chains and the influence of novel substitution patterns (*N*-benzyl substituent, methyl group in position 3 of the 2-phenylindole scaffold) were investigated systematically. At first, to analyze the effect of sequential replacement of the sulfamoyl with hydroxyl groups, compounds **146**-**148** were tested towards inhibition of *Sag*Hyal$_{4755}$. In accordance to Salmen, the hydroxylated analogs (**147**, **148**) were about as active as the parent molecule **146**. In agreement with previous studies, inhibition of the mammalian hyaluronidase BTH was not observed.

In continuation of these studies, a small library of *N*-alkylated 2-phenylindoles (**149-154**) related to **146-148** was tested for inhibition of *Sag*Hyal$_{4755}$ and BTH. The following substances were published before: **149**[37], **150**[37], **152**-**154**[37]. The corresponding molecules are shown in Figure 7.19.

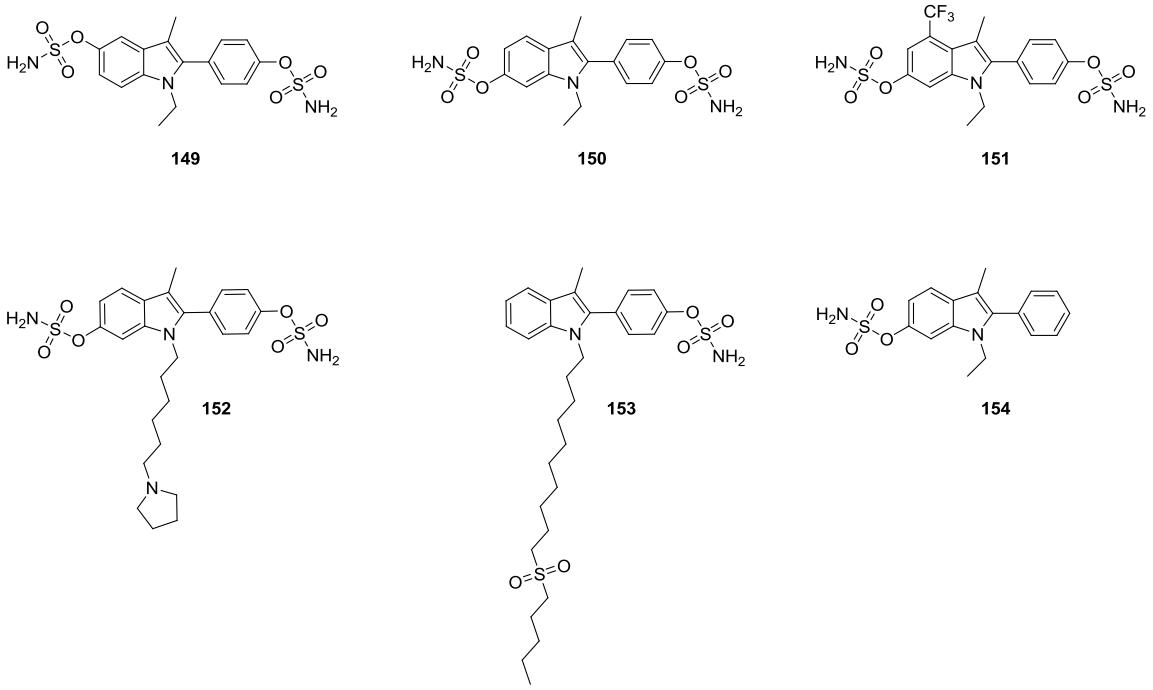


Figure 7.19 Structures of sulfamoyloxy-substituted 3-methyl-2-phenylindoles **149**-**154**.

The hyaluronidase inhibitory activities of compounds **149**-**154** are summarized in Table 7.17.

Table 7.17 Inhibitory activity of sulfamoyloxy-substituted 3-methyl-2-phenylindoles **149**-**154**.

Compound	***Sag*Hyal$_{4755}$ IC$_{50}$ (µM)[a]**	**BTH IC$_{50}$ (µM)**	**logD$_{5.0}$[b]**
149	124 ± 26	inactive	1.8
150	109 ± 10	inactive	1.8
151	20.9 ± 6	inactive	2.9
152	219 ± 41	inactive	0.2
153	inactive	inactive	7.2
154	550 ± 52	inactive	3.3

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Compounds **149**, **150**, **152** and **154** displayed moderate inhibition of the bacterial hyaluronate lyase, whereas substance **153** was inactive. Highest activity resided in **151**. Again, a 2-phenylindole, bearing a short aliphatic chain on the indole-nitrogen, was identified as potent inhibitor.

7.2.16 6,7-Dichloro-1*H*-indoles

In search for novel phenylindoles and structurally related indole-based heterocycles, 6,7-dichloro-1*H*-indoles derivatives **155**-**165** were tested for inhibitory activity on *Sag*Hyal$_{4755}$ and BTH. The structures are illustrated in Figure 7.20. The molecules were structurally derived from the β-carboline alkaloid bauerine C, having a unique 7,8-dichloro substitution pattern.[46] Bauerine C was first isolated from the blue-green alga *Dichothrix baueriana* and has been reported to possess antiproliferative as well as antiviral properties.[47] Very recently, such scaffolds were characterized as versatile chemotypes for the development of potent and selective kinase inhibitors.[48, 49] However, the selected compounds were not reported to possess kinase inhibitory activity.

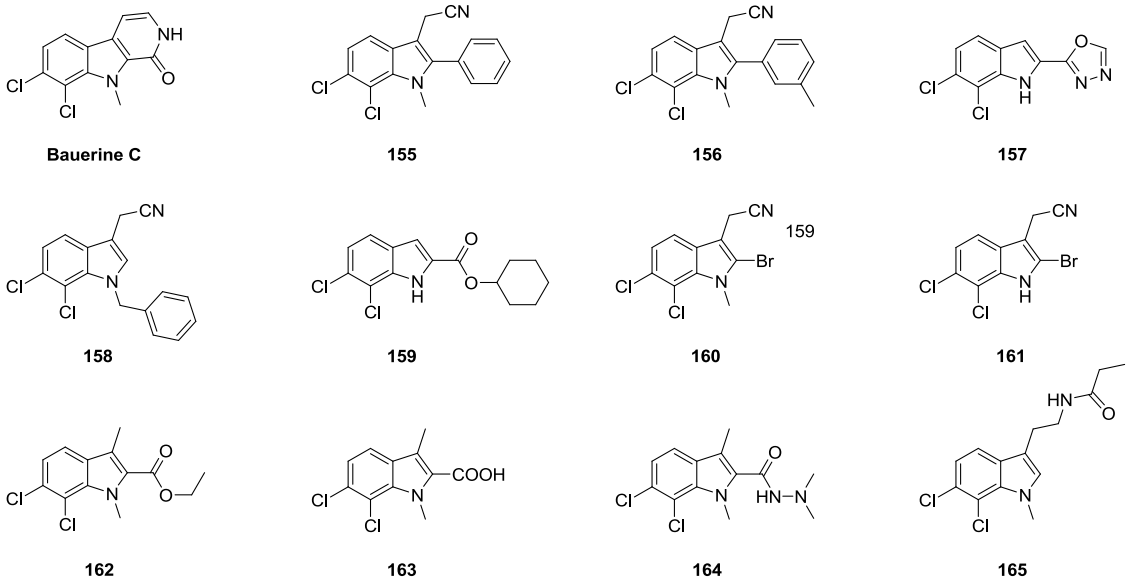


Figure 7.20 Structures of bauerine C and 6,7-dichloro-1*H*-indole derivatives **155**-**165**.

The hyaluronidase inhibitory activities of compounds **155**-**165** are summarized in Table 7.18.

Table 7.18 Inhibitory activity of 6,7-dichloro-1*H*-indole derivatives **155**-**165**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	log$D_{5.0}$[b]
155	inactive	inactive	3.8
156	inactive	inactive	4.3
157	152 ± 32	20% (1 mM)	2.7
158	inactive	inactive	4.2
159	196 ± 34	inactive	5.9
160	inactive	inactive	2.4

161	70% (1 mM)	inactive	4.1
162	inactive	inactive	3.9
163	172 ± 38	15% (1 mM)	1.0
164	40% (300 µM)	inactive	2.2
165	50% (200 µM)	inactive	2.1

[a] mean values ± SEM (N = 2, experiments performed in duplicate), highest concentration of the test compounds in the assay was 1 mM; IC_{50} values determined at pH 5.0 in the turbidimetric assay (cuvettes); [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

The compounds were kindly provided by Prof. Dr. F. Bracher (Department of Pharmacy, LMU Munich). Among the investigated small molecules, substances **157**, **159** and **163** were identified as moderate inhibitors of *Sag*Hyal$_{4755}$, whereas potent inhibitors of BTH were not observed identified among the 6,7-dichloro-1*H*-indoles.

7.3 Conclusion

For the current screening, heterogeneous synthetic organic molecules, bioactive compounds and peptide mimetics of hyaluronan were analyzed under standardized conditions. All substances were elucidated in the presence of *Sag*Hyal$_{4755}$ and BTH. It should be stressed that the selection of these compounds was not the result of random search, but inspired by reports from the literature and the results from our workgroup including the present doctoral project. Moreover, aiming at drug-like inhibitors, based on the experience with previously identified hyaluronidase inhibitors, the physicochemical properties and were taken into account. For example, compounds meeting the characteristic requirements of formerly discovered highly potent inhibitors, i.e. combining lipophilic moieties and negatively charged groups, were mostly excluded, because such compounds usually possess high plasma protein binding affinity as well as surfactant properties. In addition, with respect to the application of computer-assisted drug design methods, the idea was to identify promising molecular scaffolds or, if possible, lead compounds and pharmacophoric groups.

This strategy led to the discovery of novel inhibitors among the previously investigated 2-phenylindoles. Also, isosteric heterocycles, e.g. derivatives of benzo[*b*]thiophen were identified as streptococcal hyaluronidase inhibitors for the first time. Some of the most potent inhibitors of *Sag*Hyal$_{4755}$ (i.e. compounds **9**, **10**, **104**, **105** and **128**), discovered by this screening approach, are displayed in Figure 7.21.

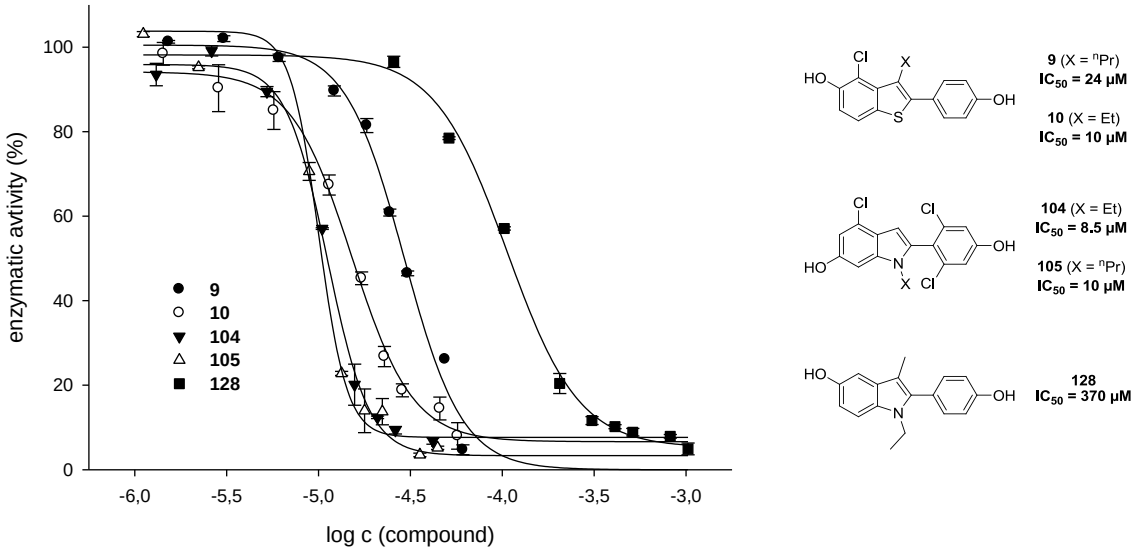


Figure 7.21 Concentration dependent activity of *Sag*Hyal$_{4755}$ in the presence of **9**, **10**, **104**, **105** and **128**.

Moreover, molecules bearing a *N*-benzylated 3-methyl-2-phenylindole scaffold were found as highly potent inhibitors of *Sag*Hyal$_{4755}$. Here, hydoxylated molecules (**134**-**136**) were found as equipotent to substances bearing one or two sulfamoyl residues (**138**, **140**) (Figure 7.22).

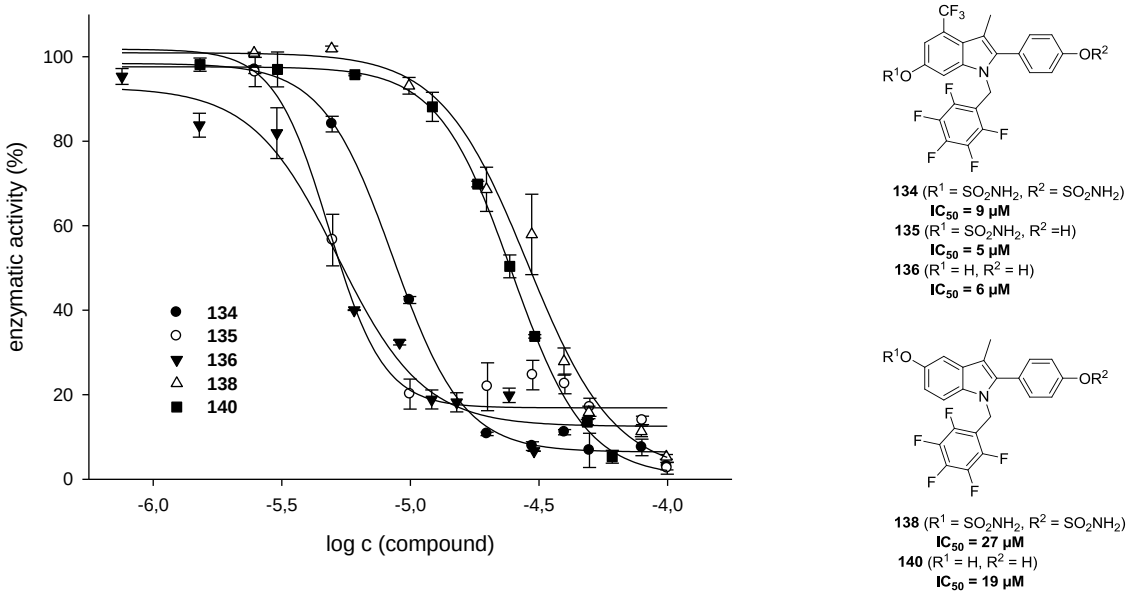


Figure 7.22 Concentration dependent activity of *Sag*Hyal$_{4755}$ in the presence of **134**-**136**, **138** and **140**.

Previously, the skeleton of the investigated 2-phenylindoles proved to be a versatile structure for the development of compounds with antiproliferative activity against breast cancer cells. Therefore, depending on the chemical nature and the position of the substituents, it must be kept in mind, that 2-phenylindoles inhibit the growth of tumor cells by different mechanisms.[50] Hence, chemical modifications of this pharmacophore must be evaluated to overcome the “off-target” estrogen receptor (ER) and to reduce the cytotoxic effects.

Under the current conditions, only inhibitors of *Sag*Hyal$_{4755}$ were identified. The mammalian hyaluronidase BTH was not affected by the small molecule inhibitors included in this section. This suggests inactivity of these compounds at the related human PH-20 hyaluronidase.

In summary, 4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol (**104**, IC_{50} = 8.5 µM) was classified as a novel lead compound for the inhibition of *Sag*Hyal$_{4755}$. Furthermore, the related benzo[*b*]thiophen derivatives **9** and **10** were identified as hits, but were not further investigated in this thesis. The 2-phenylindole scaffold is considered a pharmacophoric core structure of inhibitors of *Sag*Hyal$_{4755}$. Compared to previously developed inhibitors of the hyaluronate lyase *Sag*Hyal$_{4755}$, lead compound **104** is characterized by major structural differences, in particular, the lack of long, lipophilic carbon chains. This is of special relevance with respect to the development of selective hyaluronate lyase inhibitors with drug-like properties. The structural features of **104** inspired the rational development of related 3-methyl-2-phenylindoles. Aiming at overcoming side-effects due to nanomolar affinity to the estrogen receptor, the 6,7-dichloro-1*H*-indole motif (cf. inhibitors **157** (IC_{50} = 152 µM), **159** (IC_{50} = 196 µM) and **163** (IC_{50} = 172 µM)) is a promising discovery. Accordingly, the introduction of chloro substituents in positions 6 and 7 of the 2-phenylindole scaffold might provide access to potent inhibitors of *Sag*Hyal$_{4755}$ and simultaneously avoid anti-estrogenic activity.

7.4 Experimental data

7.4.1 Pharmacological parameters

The structures of all synthesized compounds were confirmed by means of NMR and HPLC-MS analysis prior the testing of hyaluronidase inhibition. All data are presented as mean values ± SEM of two independent experiments performed in duplicate. Inhibitory activities are given as IC_{50} values (molar concentration of the inhibitor causing 50 % inhibition of the enzymatic activity) or % inhibition of the inhibitor at concentration of the compound indicated in brackets. The maximum inhibitor concentration in the assay was set to 1 mM. Poorly soluble or colored compounds were used at lower concentrations. All measurements were performed in cuvettes, using the turbidimetric assay (pH 5.0).

7.4.2 Chemistry

4-Chloro-3-ethyl-2-(4-hydroxyphenyl)benzo[*b*]thiophen-5-ol (10)

Compound **10** was previously synthesized and published from our workgroup. Single crystals of **10** were grown from a solution of 10 mg product in anhydrous methanol (1.5 mL) in a 2 mL plastic vial. The solvent was slowly evaporated at room temperature over 7 days. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 10.06 (s, 1H, indole-O***H***-5), 9.79 (s, 1H, Ph-O***H***-4), 7.67 (d, *J* = 8.6 Hz, 1H, indole-***H***-7), 7.35 – 7.23 (m, 2H, Ph-***H***-2,6), 7.05 (d, *J* = 8.6 Hz, 1H, indole-***H***-6), 6.98 – 6.79 (m, 2H, Ph-***H***-3,5), 2.97 (q, *J* = 7.2 Hz, 2H, C***H***$_2$CH$_3$), 1.20 (t, *J* = 7.3 Hz, 3H, CH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 157.62 (C_{quat}, Ph-***C***-4), 150.99 (C_{quat}, indole-***C***-5), 140.62 (C_{quat}, indole-***C***), 136.12 (C_{quat}, indole-***C***), 133.24 (C_{quat}, indole-***C***), 130.71 (+, Ph-***C***-2,6), 130.66 (C_{quat}, indole-***C***), 124.22 (C_{quat}, Ph-***C***-1), 121.31 (+, indole-***C***-6), 115.42 (+, Ph-***C***-3,5), 114.39 (+, indole-***C***-7), 112.96 (C_{quat}, indole-***C***-4), 20.89 (-, ***C***H$_2$CH$_3$), 16.86 (+, CH$_2$***C***H$_3$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 306.2 (32), 304.2 ([M]$^{+\bullet}$, 86), 289.1 (55), 254.2 (100), 253.1 (25). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 305.0398, found [M + H]$^+$ 305.0396. $C_{16}H_{13}ClO_2S$ (M_r = 304.79 g/mol).

4-Chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol (104)

Compound **104** was previously synthesized and published from workgroup.[34, 51] Single crystals of **104** were grown from a solution of 10 mg product in dry methanol (1.5 mL) in a 2 mL plastic vial. The solvent was slowly evaporated at room temperature over 7 days. ^{1}H-

NMR (300 MHz, DMSO-d_6): δ [ppm] = 10.75 (s, 1H, indole-O***H***-5), 9.49 (s, 1H, Ph-O***H***-4), 7.03 (d, *J* = 9.1 Hz, 2H, Ph-***H***-3,5), 6.80 (d, *J* = 1.0 Hz, 1H, indole-***H***-5), 6.69 (d, *J* = 1.8 Hz, 1H, indole-***H***-7), 6.26 (s, 1H, indole-***H***-3), 3.79 (q, *J* = 7.0 Hz, 2H, NC***H***$_2$CH$_3$), 1.19 – 1.02 (m, 3H, NCH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 159.14 (C_{quat}, Ph-***C***-4 C), 153.54 (C_{quat}, indole-***C***-6), 136.92 (C_{quat}, indole-***C***), 136.32 (C_{quat}, Ph-***C***-2,6), 132.79 (C_{quat}, indole-***C***-4), 124.32 (C_{quat}, Ph-***C***-1), 120.22 (C_{quat}, indole-***C***), 119.33 (C_{quat}, indole-***C***), 115.27 (+, Ph-***C***-3,5), 109.37 (+, indole-***C***-5), 100.30 (+, indole-***C***-3), 94.45 (+, indole-***C***-7), 38.41 (-, N***C***H$_2$CH$_3$), 14.54 (+, NCH$_2$***C***H$_3$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 359.1 (33), 357.1 (100), 356.1 (25), 355.1 ([M]$^{+\bullet}$, 99), 342.1 (33), 340.0 (37). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 357.9979, found [M + H]$^+$ 357.9978. $C_{16}H_{12}Cl_3NO_2$ (M_r = 356.63 g/mol).

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (128)

Compound **128** was previously synthesized and published from our workgroup.[43] Single crystals of **5.48** were grown from a solution of 10 mg product in dry methanol (1.5 mL) in a 2 mL plastic vial. The solvent was slowly evaporated at room temperature over 5 days. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 9.75 (s, 1H, Ph-O***H***-4), 8.75 (s, 1H, indole-O***H***-5), 7.21 (d, *J* = 5.9 Hz, 1H, indole-***H***-7), 7.19 – 7.15 (m, 2H, Ph-***H***-2,6), 6.89 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3,5), 6.78 (d, *J* = 2.2 Hz, 1H, indole-***H***-4), 6.64 (dd, *J* = 8.6, 2.3 Hz, 1H, indole-***H***-6), 3.94 (q, *J* = 7.0 Hz, 2H, NC***H***$_2$CH$_3$), 2.04 (s, 3H, indole-C***H***$_3$-3), 1.13 – 0.94 (m, 3H, NCH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 156.92 (C_{quat}, Ph-***C***-4), 150.42 (C_{quat}, indole-***C***-5), 137.38 (C_{quat}, indole-***C***), 131.19 (+, Ph-***C***-2,6), 130.03 (C_{quat}, indole-***C***), 122.25 (C_{quat}, Ph-***C***-1), 115.25 (+, Ph-***C***-3,5), 110.95 (+, indole-***C***-6), 109.97 (+, indole-***C***-7), 105.97 (C_{quat}, indole-***C***), 102.32 (+, indole-***C***-4), 93.62 (C_{quat}, indole-***C***), 38.39 (-, N***C***H$_2$CH$_3$), 15.02 (+, NCH$_2$***C***H$_3$), 9.18 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 267.2 ([M]$^{+\bullet}$, 100), 266.2 (27), 252.1 (59). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 268.1332, found [M + H]$^+$ 268.1333. $C_{17}H_{17}NO_2$ (M_r = 267.32 g/mol).

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8 2-Phenylindole derivatives as inhibitors of streptococcal hyaluronidases

8.1 Introduction

In 1975, the anti-inflammatory drug indomethacin was reported for the first time as a hyaluronidase inhibitor.[1] As shown by Spickenreither, under standardized conditions, indomethacin is an inhibitor of the bacterial hyaluronidase *Sag*Hyal$_{4755}$ and the mammalian enzymes BTH, Hyal-1 and PH-20.[2] Accordingly, aiming at derivatives of indomethacin, several structurally related substances were synthesized and tested for inhibition of hyaluronidase in our laboratory. This approach led to the discovery of compounds, which were among the most potent inhibitors of bacterial and mammalian hyaluronidases known so far. Besides, retaining the indole skeleton, an additional structural motif was found with 2-phenylindoles. As shown for the bacterial hyaluronidase *Sag*Hyal$_{4755}$, compounds incorporating hydroxylated 2-phenylindoles displayed inhibitory activity in the lower micromolar range. Moreover, these molecules, as evidenced by X-ray crystallography, bind the active site of the streptococcal hyaluronidase *Spn*Hyl (PDB code: 2BRP).[3, 4]

A common structural feature of potent indole-based inhibitors includes aliphatic carbon chains, directly attached to the indole moiety. Typically, the potency correlated significantly with the length of the aliphatic chain. To illustrate indole-based inhibitors, some of the most potent molecules are displayed in Figure 8.1.

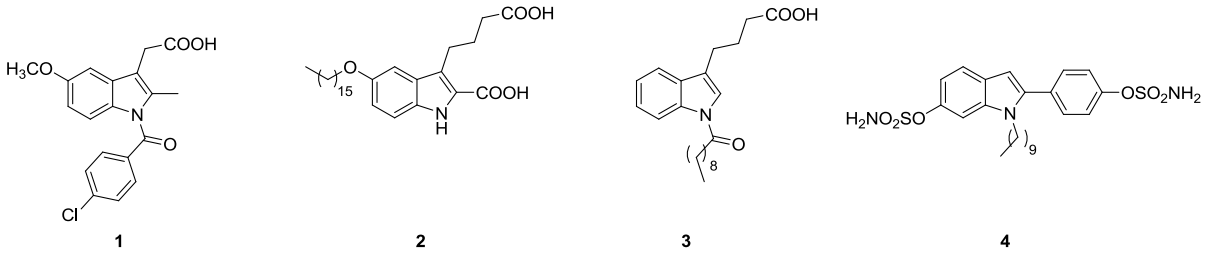


Figure 8.1 Structures of indole-type hyaluronidase inhibitors.

Interestingly, 2-phenylindole-type copmounds displayed selectivity for *Sag*Hyal$_{4755}$. The mammalian enzyme BTH was not or only very weakly affected by these molecules. In contrast, inhibitors derived from indomethacin, addressed both bacterial and mammalian enzymes. By trend, highest inhibitory potency at bacterial enzymes resided in indole-based substances.

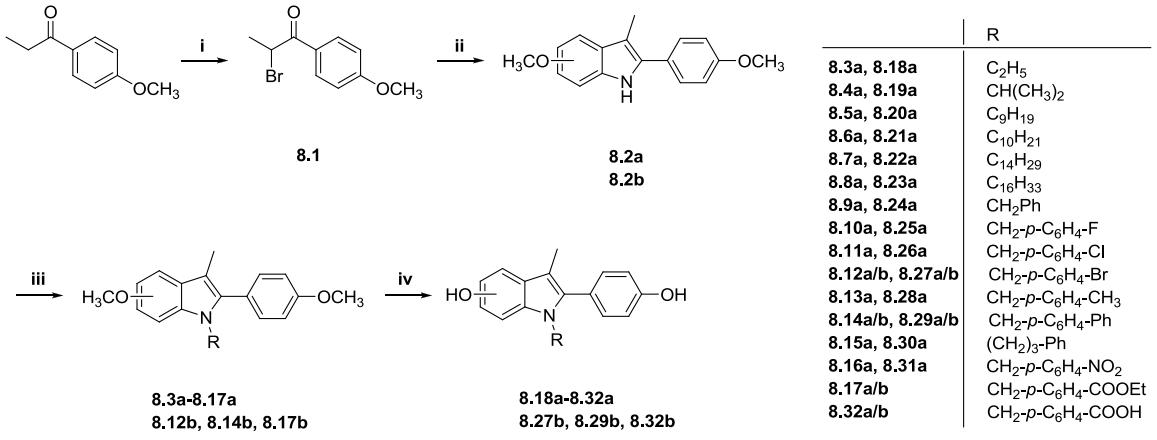
Despite the high potency of indole-based hyaluronidase inhibitors, the discussed compounds are most probably unfavorable for *in vivo* application by various reasons.

Firstly, in terms of drug-likeness, long lipophilic alkyl residues are unsuited, e.g., due to extremely high plasma protein binding of the respective compounds. This is a dilemma, as lipophilicity positively correlates with potency. Hence, the truncation of an alkyl chain will result in less active compounds. Secondly, *N*-alkylated indoles bearing negatively charged groups might possess surfactant properties. To avoid such unfavorable properties, hydroxylated 2-phenylindoles might harbor the potential as a template for the development of highly potent hyaluronidase inhibitors. However, depending on the *N*-substituent, compounds derived from the 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol scaffold are known for antiestrogenic activity in the low nanomolar range.[5-7] Hence, potential binding of hyaluronidase inhibitors bearing a hydroxylated 2-phenylindole motif to the estrogen receptor (ER) should be kept in mind.

Inspired by the compound **104** (see section 7.2.11) and *N*-benzylated 2-phenylindoles **134**-**142** (see section 7.2.13), new 2-phenylindole derivatives were explored in the current section. The intention was to elaborate new ideas for the design of structurally modified 2-phenylindole-based inhibitors of *Sag*Hyal$_{4755}$. For the current investigations, the 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol moiety was regarded as an appropriate core structure for the design of small molecules with drug-like properties. The synthesis and structure-activity relationships of *N*-alkylated and *N*-benzylated indoles will be reported in this chapter.

8.2 Chemistry

The *N*-substituted 3-methyl-2-phenylindoles were synthesized as outlined in Figure 8.2.



	R
8.3a, 8.18a	C_2H_5
8.4a, 8.19a	$CH(CH_3)_2$
8.5a, 8.20a	C_9H_{19}
8.6a, 8.21a	$C_{10}H_{21}$
8.7a, 8.22a	$C_{14}H_{29}$
8.8a, 8.23a	$C_{16}H_{33}$
8.9a, 8.24a	CH_2Ph
8.10a, 8.25a	CH_2-*p*-C_6H_4-F
8.11a, 8.26a	CH_2-*p*-C_6H_4-Cl
8.12a/b, 8.27a/b	CH_2-*p*-C_6H_4-Br
8.13a, 8.28a	CH_2-*p*-C_6H_4-CH_3
8.14a/b, 8.29a/b	CH_2-*p*-C_6H_4-Ph
8.15a, 8.30a	$(CH_2)_3$-Ph
8.16a, 8.31a	CH_2-*p*-C_6H_4-NO_2
8.17a/b	CH_2-*p*-C_6H_4-COOEt
8.32a/b	CH_2-*p*-C_6H_4-COOH

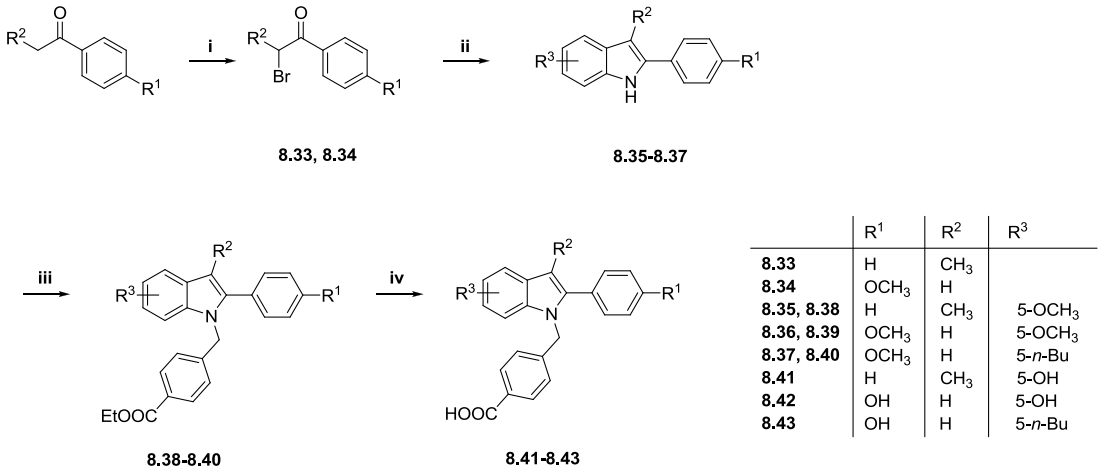
Figure 8.2 Synthesis of the hydroxylated 2-phenylindoles **8.18a**-**8.32a**, **8.27b**, **8.29b** and **8.32b**. Reagents and conditions: (i) acetic acid, 48 % HBr, Br_2, 0 °C; (ii) 4-methoxyaniline or 3-methoxyaniline, *N,N*-dimethylaniline, xylene, 170 °C; (iii) NaH, DMF, R-X (X= Cl, Br, I), 0 °C; (iv) BBr_3, DCM, -20 °C.

Bromination of 1-(4-methoxyphenyl)propan-1-one afforded the 2-bromo-1-(4-methoxyphenyl)propan-1-one derivative **8.1**.[8] For the synthesis of the 2-phenylindole building blocks (**8.2a, 8.2b**), the Bischler-Möhlau indole synthesis method was applied.[5, 9, 10] For this procedure, the reaction of primary aromatic amines (methoxyanilines) with the α-bromoacylbenzene afforded the desired 2-phenylindole scaffold.[8] The ring closure reaction was achieved at high temperature (170 °C) in the presence of *N,N*-dimethylaniline. For the preparation of the building block **8.2a** (5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole), 4-methoxyaniline was used. For the synthesis of **8.2b** (6-methoxy-2-(4-methoxyphenyl)-3-methyl-1H-indole), the primary amine 3-methoxyaniline was used. After removal of side-products by flash-chromatography and recrystallization, building blocks **8.2a**, **8.2b** were obtained in moderate yields.

The mechanism of the Bischler-method was described as a multi-step reaction.[11] In detail, in the primary step, nucleophilic substitution of α-bromoacylbenzene and methoxyaniline results in a α-aminoketone. The ketone is converted to a Schiff's base by condensation with a second methoxyaniline molecule. The cyclisation of the intermediate endiamine structure takes place under displacement of the “first” charged aniline molecule. After a 1,3-H-shift, aromatization yields the 2-phenylindole.

The *N*-alkylated indoles **8.3a**-**8.8a** were prepared from the parent indole **8.2a** by deprotonation with sodium hydride in dimethylformamide and subsequent addition of alkyl halide at 0 °C.[5] By analogy with this procedure, *N*-substitution of **8.2a** and **8.2b** with benzylic residues was accomplished to yield substances **8.9a**-**8.17a**, **8.12b**, **8.14b** and **8.17b**.[6] In the last step, cleavage of the methyl ether groups was performed with boron tribromide in anhydrous dichloromethane.[12] The corresponding hydroxylated 3-methyl-2-phenylindoles **8.18a**-**8.32a**, **8.27b**, **8.29b** and **8.32b** were purified by flash-chromatography.

The *N*-substituted 2-phenylindoles **8.41**-**8.43** were synthesized as outlined in Figure 8.3.



	R^1	R^2	R^3
8.33	H	CH_3	
8.34	OCH_3	H	
8.35, 8.38	H	CH_3	5-OCH_3
8.36, 8.39	OCH_3	H	5-OCH_3
8.37, 8.40	OCH_3	H	5-*n*-Bu
8.41	H	CH_3	5-OH
8.42	OH	H	5-OH
8.43	OH	H	5-*n*-Bu

Figure 8.3 Synthesis of the 2-phenylindoles **8.41**-**8.43**. Reagents and conditions: (i) **8.33**: acetic acid, 48 % HBr, Br_2, 0 °C; **8.34**: Et_2O, dioxane, Br_2, 0 °C; (ii) aniline, *N,N*-dimethylaniline, xylene, 170 °C; (iii) NaH, DMF, ethyl 4-(bromomethyl)benzoate, 0 °C; (iv) BBr_3, DCM, -20 °C.

Bromination of 1-(4-methoxyphenyl)ethanone **8.34** was achieved with bromine in a mixture of dioxane and diethyl ether at ambient temperature.[13] The 2-phenylindole building blocks **8.35**-**8.37** were obtained by the Bischler method and purified as described previously. Because of the harsh conditions of the ether cleavage with boron tribromide, at this stage the reaction was performed with the indolylmethylbenzoic esters **8.38**-**8.40**, which were obtained by reacting the 2-phenylindoles with ethyl 4-(bromomethyl)benzoate in the presence of sodium hydride.[14] Since the ester function was cleaved as well, the products **8.41**-**8.43** were obtained as the corresponding indolylmethylbenzoic acids after column chromatography.

The synthesis of the 6,7-dichloro-2-(4-methoxyphenyl)-3-methyl-1*H*-indole building block (**8.45**) was performed as displayed in Figure 8.4.

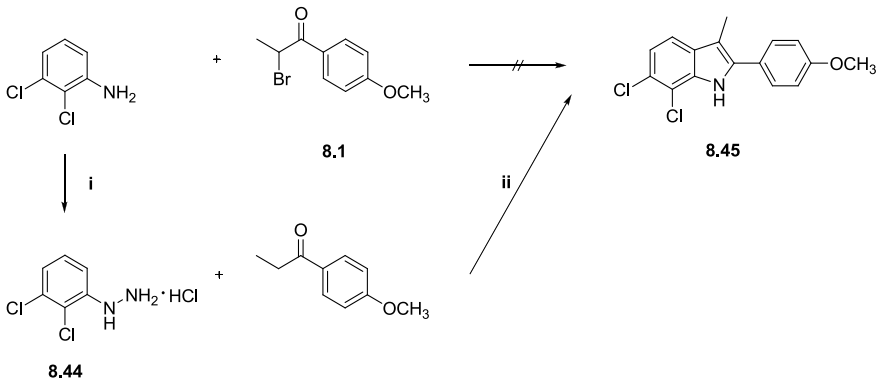


Figure 8.4 Synthesis of the 6,7-dichloro-2-(4-methoxyphenyl)-3-methyl-1*H*-indole **8.45**. Reagents and conditions: (i) a) $NaNO_2$, HCl; b) $SnCl_2 \cdot 2H_2O$, conc. HCl; (ii) EtOH, 32 % HCl, reflux, 2h.

The Bischler method, which involved the condensation of substituted anilines with α-bromoacyl derivatives, did not afford the building block 6,7-dichloro-2-(4-methoxyphenyl)-3-methyl-1*H*-indole **8.45** (Figure 8.4). A versatile route of synthesis was found in the Fischer indole synthesis.[15, 16] Commercially available 2,3-dichloroaniline was diazotized and reduced with stannous chloride in one pot to give the corresponding (2,3-dichlorophenyl)hydrazine **8.44** in good yield.[17-21] The building block **8.45** was separated from starting materials and isolated by flash-chromatography in acceptable yields.

The synthesis of the target compound **8.47** was performed as shown in Figure 8.5.

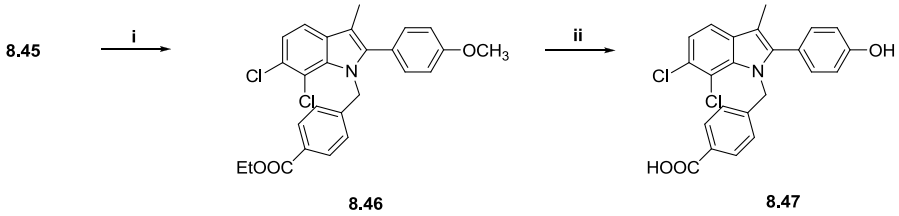


Figure 8.5 Synthesis of the 4-{[6,7-dichloro-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoic acid **8.47**. Reagents and conditions: (i) NaH, DMF, ethyl 4-(bromomethyl)benzoate, 0 °C; (ii) BBr_3, DCM, -20 °C.

N-Benzylation with ethyl 4-(bromomethyl)benzoate was performed as described before. Deprotection of the ether and the ester group in **8.46** with boron tribromide and subsequent flash-chromatography afforded **8.47**.[14]

8.3 Pharmacological results and discussion

8.3.1 General conditions

All synthesized 2-phenylindole derivatives were investigated for inhibition of the bacterial hyaluronan lyase *Sag*$Hyal_{4755}$ and the bovine testicular enzyme BTH (Neopermease®) in a turbidimetric assay based on the method of Di Ferrante[22] as described in chapter 3.5.3.
It is important to mention that the Morgan Elson assay is not suitable for the investigation of indole or 2-phenylindole derivatives.[3]

8.3.2 Inhibitory activities of *N*-alkylated 2-phenylindole derivatives

The IC_{50}-values determined for the *N*-alkylated 2-phenylindole (**8.18a**-**8.23a**) derivatives are summarized in Table 8.1.

Table 8.1 Inhibitory activity[a] and calculated $logD_{5.0}$ values[b] of *N*-alkylated 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol derivatives **8.18a**-**8.23a**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)[a]	$logD_{5.0}$[b]
8.18a	510 ± 47	inactive	3.2
8.19a	130 ± 15	inactive	4.1
8.20a	22 ± 4.1	inactive	6.2
8.21a	29 ± 3.3	inactive	7.3
8.22a	180 ± 20	inactive	9.8
8.23a	120 ± 53	inactive	10.8

[a] mean values ± SEM (N = 2, experiments performed in duplicate), IC_{50} values determined at pH 5.0 in the automated 96-well turbidimetric assay; [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00.

Previously, *N*-alkylated 3-methyl-2-phenylindoles were identified as selective inhibitors of *Sag*Hyal$_{4755}$. Inhibitory activity correlated with the length of the alkyl chain. This was examined for compounds bearing hydrogen, methyl, *n*-propyl, *n*-pentyl and *n*-heptyl substituents at the indole-nitrogen. The most potent compounds (**5**, **6**) are displayed in Figure 8.6.

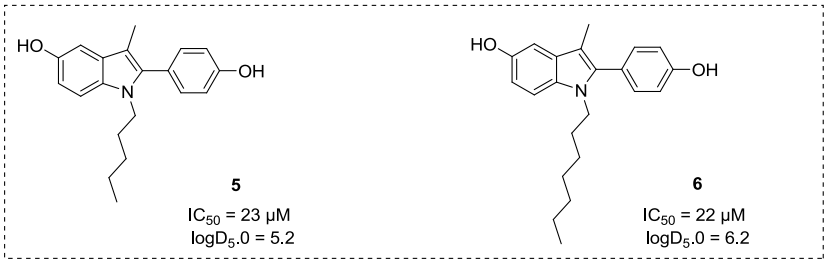


Figure 8.6 Structures of *N*-alkylated 3-methyl-2-phenylindole derivatives (**5**, **6**), previously determined as inhibitors of *Sag*Hyal$_{4755}$.[3]

To further explore the structure-activity relationships, the analogs **8.18a**-**8.23a** were analyzed. As expected, an increase in the length of the aliphatic chain led to a significant decrease in IC_{50} values. However, elongation of the aliphatic chain beyond 14 carbon atoms did not increase inhibitory potency in case of substances **8.22a** and **8.23a**. The latter highly lipophilic compounds suffered additionally from limited solubility. The

enzymatic activity of *Sag*Hyal$_{4755}$ in the presence of **8.18a**-**8.21a** is depicted as concentration-response curves in Figure 8.7.

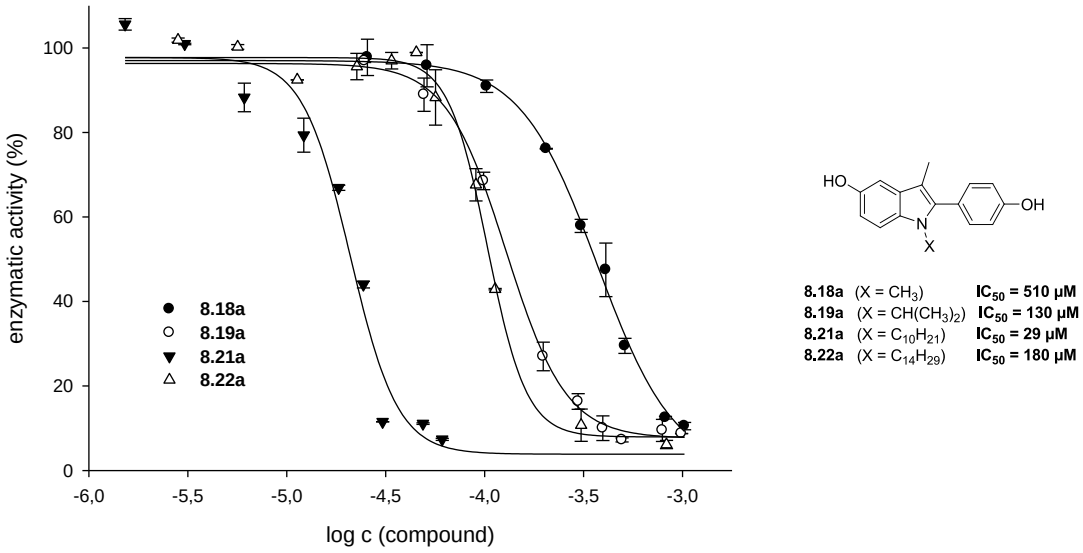


Figure 8.7 Enzymatic activity of *Sag*Hyal$_{4755}$ in the presence of **8.18a**-**8.21a**.

A positive correlation between inhibition of *Sag*Hyal$_{4755}$ and lipophilicity was observed for *N*-alkylated 3-methyl-2-phenylindoles comprising a chain of 5 to 10 carbon atoms (cf. Figure 8.6, Table 8.1). Taken together, the most potent 2-phenylindoles containing aliphatic chains, were inhibitors of the bacterial hyaluronate lyase with IC_{50} values of approximately 20 µM. The mammalian enzyme BTH was not inhibited by molecules analyzed in this section.

8.3.3 Inhibitory activity of *N*-benzylated 2-phenylindole derivatives

The IC_{50} values determined for the *N*-benzylated 2-phenylindole derivatives are summarized in Table 8.2.

Table 8.2 Inhibitory activity[a] and calculated $logD_{5.0}$ values[b] of *N*-benzylated 2-phenylndol derivatives **8.24a**-**8.32a**, **8.27b**, **8.29b**, **8.32b**, **8.40**-**8.43**, **8.46**, **8.47**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)[a]	$logD_{5.0}$[b]
8.24a	30 ± 4.1	inactive	5.5
8.25a	41 ± 3.3	inactive	5.6
8.26a	21 ± 2.1	inactive	6.0

8.27a	19 ± 1.7	inactive	6.3
8.27b	30 ± 2.6	inactive	6.2
8.28a	46 ± 4.4	inactive	6.0
8.29a	121 ± 7	inactive	7.4
8.29b	115 ± 10	inactive	7.2
8.30a	178 ± 34	inactive	5.9
8.31a	49 ± 17	inactive	5.2
8.17a	inactive	inactive	6.2[c]
8.17b	inactive	inactive	5.9[c]
8.32a	46.2 ± 2.2	605 ± 47	3.9
8.32b	33.1 ± 5.0	415 ± 23	3.8
8.40	575± 44	> 1000	8.8[c]
8.41	420 ± 23	> 1000	4.6
8.42	53.1 ± 6.7	465 ± 17	3.6
8.43	8.5 ± 0.4	113 ± 34	6.3
8.46	inactive	inactive	7.4[c]
8.47	6.3 ± 0.1	460 ± 53	5.0

[a] mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 in the automated 96-well turbidimetric assay; [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00; [c] the structure does not contain ionization centers calculated by ACD-Labs. LogP-value is indicated instead.

Systematic screening of *N*-benzylated compounds (see section 7.2.13) led to the discovery of potent inhibitors of *Sag*Hyal$_{4755}$. Two prototypical structures of this approach, **140** and **141**, are illustrated in Figure 8.8.

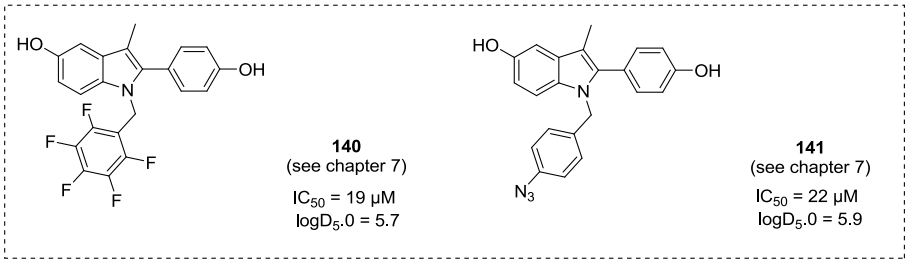


Figure 8.8 Structures of *N*-benzylated 3-methyl-2-phenylindole derivatives (**140**, **141** (see chapter 7)), identified as inhibitors of *Sag*Hyal$_{4755}$.

To study the influence of variously substituted benzyl residues, compounds **8.24a**-**8.32a**, **8.27b**, **8.29b** and **8.32b** were compared in the turbidimetric assay. Compounds **8.17a**,

8.17b, bearing methyl ether protecting groups and an ethyl benzoate moiety, were included to verify the contribution of the free phenolic and carboxylic groups, respectively.

The enzymatic activity of *Sag*Hyal$_{4755}$ in presence of **8.17a**, **8.26a**, **8.27a** and **8.32a** is depicted as concentrations-response curves in Figure 8.9.

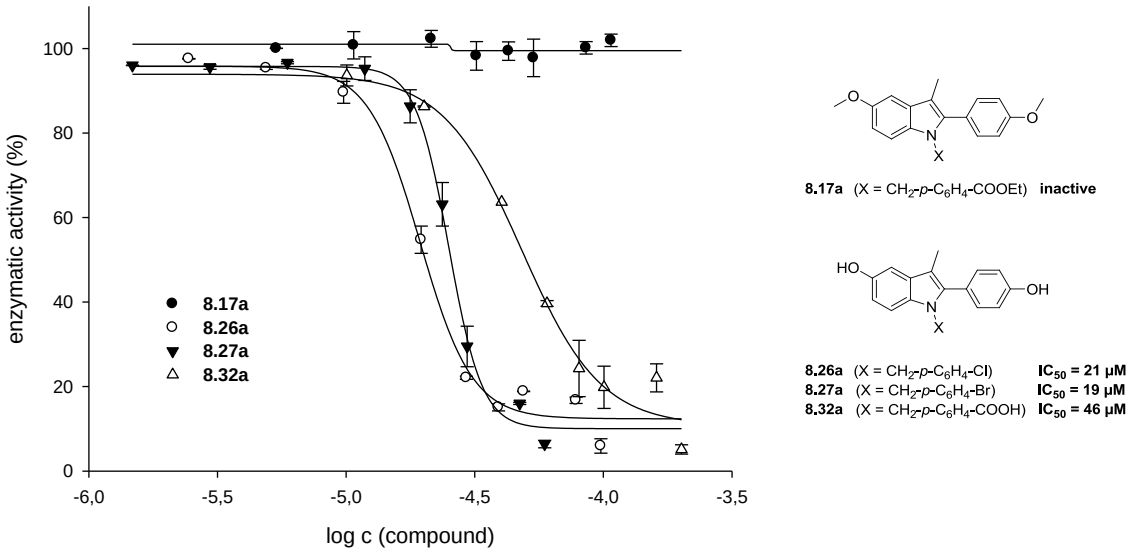


Figure 8.9 Enzymatic activity of *Sag*Hyal$_{4755}$ in the presence of **8.17a**, **8.26a**, **8.27a** and **8.32a**.

The benzylindoles **8.24a**-**8.28a** and **8.31a**, bearing different substitutents in *para*-position of the benzyl residues, were equipotent inhibitors of *Sag*Hyal$_{4755}$. For these inhibitors the calculated log$D_{5.0}$ values covered the range from 5.2 to 6.3. A shift of the hydroxyl group from position 5 (cf. **8.27a**, IC_{50} = 19 µM) to position 6 (cf. **8.27b**, IC_{50} = 30 µM) was tolerated. As predicted, compounds **8.17a**, **8.17b** were inactive. The introduction of bulky, lipophilic groups (cf. compounds **8.29a** (IC_{50} = 121 µM) and **8.29b** (IC_{50} = 115 µM)) resulted in a decrease in inhibitory activity on the *streptococcal* hyaluronidase, regardless of the position of the hydroxyl group on the indole skeleton. A similar effect was observed for **8.30a** (IC_{50} = 178 µM).

Interestingly, the introduction of a negatively charged group (cf. **8.32a**, **8.32b**), i.e. a carboxyl group in position 4 of the benzyl residue did not enhance the activity at the bacterial enzyme (IC_{50} values: 46 µM (**8.32a**) and 33 µM (**8.32b**)). However, in these cases weak inhibition of the mammalian enzyme BTH by 2-phenylindoles was observed for the first time. Compared to the previously discussed phenylindoles, the calculated log$D_{5.0}$ values of **8.32a**, **8.32b** were significantly lower and the solubility was improved.

Strikingly, the combination of lipophilic residues (*N*-benzyl substituents) and negatively charged groups (carboxylic group) was identified as conferring affinity to BTH, thus reducing selectivity for streptococcal hyaluronidases. This substantiates our working hypothesis regarding crucial structural features of inhibitors of bacterial and mammalian hyaluronidase inhibitors.

Further modifications of 2-phenylindoles were performed to combine *N*-benzyl moieties and charged residues. Aiming at more potent inhibitors, structural features of other highly potent inhibitors of the bacterial hyaluronidase were also taken into consideration. For example, as shown compounds **104**, **136** in Figure 8.10, electron withdrawing groups and bulky substituents at the indole core structure were investigated.

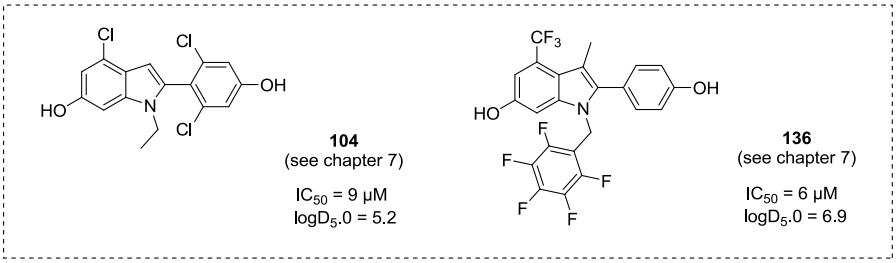


Figure 8.10 Structures of *N*-benzylated 2-phenylindole derivatives (**104**, **136** (see chapter 7)) determined as inhibitors of *Sag*Hyal$_{4755}$.

A similar substitution pattern was included in a series of 6,7-dichloro-1*H*-indole derivatives (cf. section 7.2.16). As illustrated by **157**, **163** (Figure 8.11), such compounds were confirmed to possess moderate inhibitory activity on *Sag*Hyal$_{4755}$.

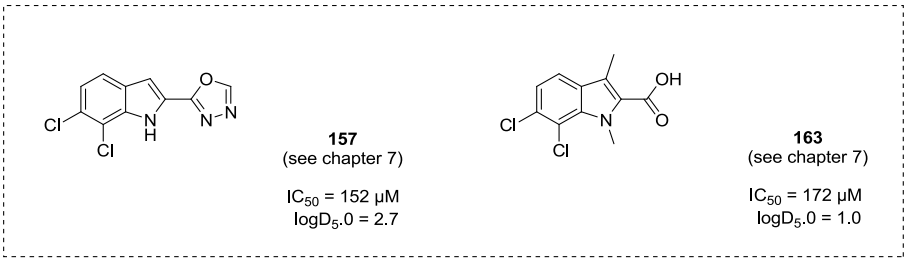


Figure 8.11 Structures of indole derivatives (**157**, **163** (see chapter 7)) determined as inhibitors of *Sag*Hyal$_{4755}$.

Therefore, compounds **8.43** (bulky substituent instead indole-5-OH) and **8.47** (6,7-dichloro substituents instead of indole-5-OH) were synthesized. The enzymatic activity of *Sag*Hyal$_{4755}$ in presence of **8.41**-**8.43** and **8.47** is depicted as concentration-response curves in Figure 8.12.

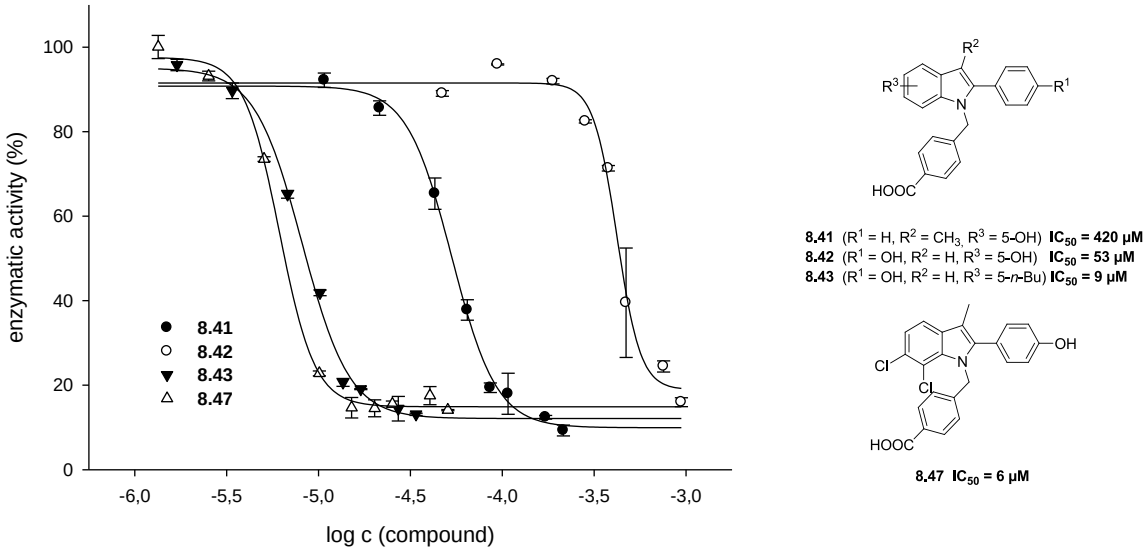


Figure 8.12 Enzymatic activity of *Sag*Hyal$_{4755}$ in the presence of **8.41**-**8.43** and **8.47**.

The IC_{50} values of compounds **8.42** and **8.43** were in the two- and in the one-digit µM range. The weak inhibition of *Sag*Hyal$_{4755}$ by **8.41** is mainly caused by the missing hydroxyl substitutent in position 4 of the phenyl residue. This is in agreement with results for structurally related potent inhibitors **8.32a**, **8.32b** and **8.42**, which incorporate a hydroxyl group on the respective position. The replacement of the hydroxyl group in position 5 (cf. **8.42**, IC_{50} = 53 µM, $logD_{5.0}$ = 3.6) of the indole skeleton by an *n*-butyl moiety (cf. **8.43**, IC_{50}= 9 µM, $logD_{5.0}$ = 6.3) was accompanied by a 5-fold increase in inhibitory activity. The introduction of chloro substituents in positions 6 and 7 (**8.47**, $logD_{5.0}$ = 5.0) resulted in an IC_{50} value of 6 µM. With regard to BTH, compound **8.42** (IC_{50} = 463 µM) and **8.47** (IC_{50} = 460 µM) were identified as weak inhibitors. The more lipophilic substances **8.43** showed an IC_{50} value of 113 µM for BTH. Compounds **8.42** and **8.43** were about 10-fold more potent on the bacterial enzyme, and the 6,7-dichloro-1*H*-indole motif in **8.47** resulted in 75-fold selectivity for *Sag*Hyal$_{4755}$ compared to BTH.

8.3.4 Inhibitory activities of selected compounds on *Spn*Hyl

To compare the results from *Sag*Hyal$_{4755}$ with data from an additional bacterial enzyme, an ensemble of four inhibitors was tested by Dr. J. Hamberger from our workgroup on hyaluronidase from *S. pneumonia*, *Spn*Hyl. Under identical assay conditions (including the pH value) these substances were inactive on *Spn*Hyl (Table 8.3) except for compound **8.47**, which was identified as inhibitor of the streptococcal hyaluronate lyase SpnHyl. Most

likely the inhibitory activity can be attributed to both, the lipophilic chloro substituent and the carboxylate group in **8.47**. With an IC_{50} value of 93 µM, compound **8.47** is one of the most potent inhibitors of *Spn*Hyl known to date.

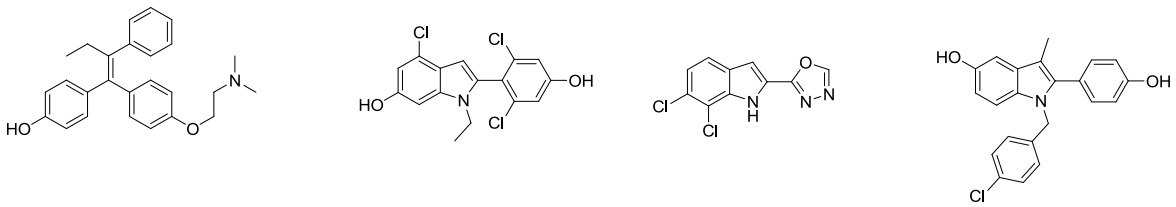
Table 8.3 Inhibitory activity[a] of **104** (see chapter 7) and 3-methyl-2-phenylindoles derivatives **8.26a**, **8.29a**, **8.47**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	*Spn*Hyl IC_{50} (µM)[a]
104[b]	8.5 ± 1.3[C]	inactive
8.26a	21 ± 2.1	inactive
8.29a	121 ± 7	inactive
8.47	6.3 ± 0.1	93 ± 1.1

[a] mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 in the automated 96-well turbidimetric assay; [b] see Figure 8.10; [c] see section 7.2.11.

8.3.5 Investigation of selected indole derivatives for anti-proliferative activity on MCF-7 mammary carcinoma cell

Several 2-phenylindole compounds were reported to possess antiestrogenic activities and to show cytostatic effects on hormone sensitive breast cancer cells.[5, 6, 23] Therefore three phenylindole-type hyaluronidase inhibitors were tested for antiproliferative activity on human MCF-7 mammary carcinoma cells (Figure 8.13). The selective estrogen receptor modulator 4-hydroxytamoxifen was used as reference substance.[24]



4-hydroxytamoxifen **104** (see chapter 7) **157** (see chapter 7) **8.26a**

Figure 8.13 Structures of compounds investigated for cytotoxicity on mammary carcinoma cells.

The investigation of the antiproliferative activity was performed in a kinetic cytotoxicity assay according to a well-established protocol.[25] In principle, cell growth in the presence and in the absence of the test compounds are compared. To determine cytostatic effects of the analyzed compounds (see Figure 8.13), the change of cell mass is quantified by staining cells with crystal violet. Test compounds were investigated in five different

concentrations (10 µM, 3 µM, 1 µM, 300 nM, 100 nM). During the 14 day incubation period, tumor cells were fixed after 70, 120, 260 and 330 hours, respectively. To calculate the effect of the test compounds on cell proliferation, the following equation was used (Equation 8.1):

$$T/C_{corr.}\,(\%) = \frac{T - C_0}{C - C_0} \times 100 \qquad \text{Equation 8.1}$$

T: mean ratio of the optical density of treated cells

C_o: mean ratio of the initial optical density (first addition of test compound, t = 0)

C: mean ratio of the optical density of non-treated cells

$T/C_{corr.}$ corrected value

To calculate the cell growth for the non-treated cell population, Equation 8.2 was used:

$$\frac{C_0 - T}{C_0} \times 100 \qquad \text{Equation 8.2}$$

T: mean ratio of the optical density of treated cells

C_o: mean ratio of the initial optical density (first addition of test compound, t = 0)

Corrected values ($T/C_{corr.}$) are calculated as the ratio of the optical densities of treated to non-treated cells. Each density is corrected by subtraction of the initial cell density T_0. Accordingly, corrected $T/C_{corr.}$ values, obtained after maximal incubation periods (end points) were plotted as a function of the concentration. The corresponding plots, indicating the cell growth of MCF-7 cells upon treatment with compounds (cf. Figure 8.13) compared to the vehicle control (non-treated cells) are given in Figure 8.14.

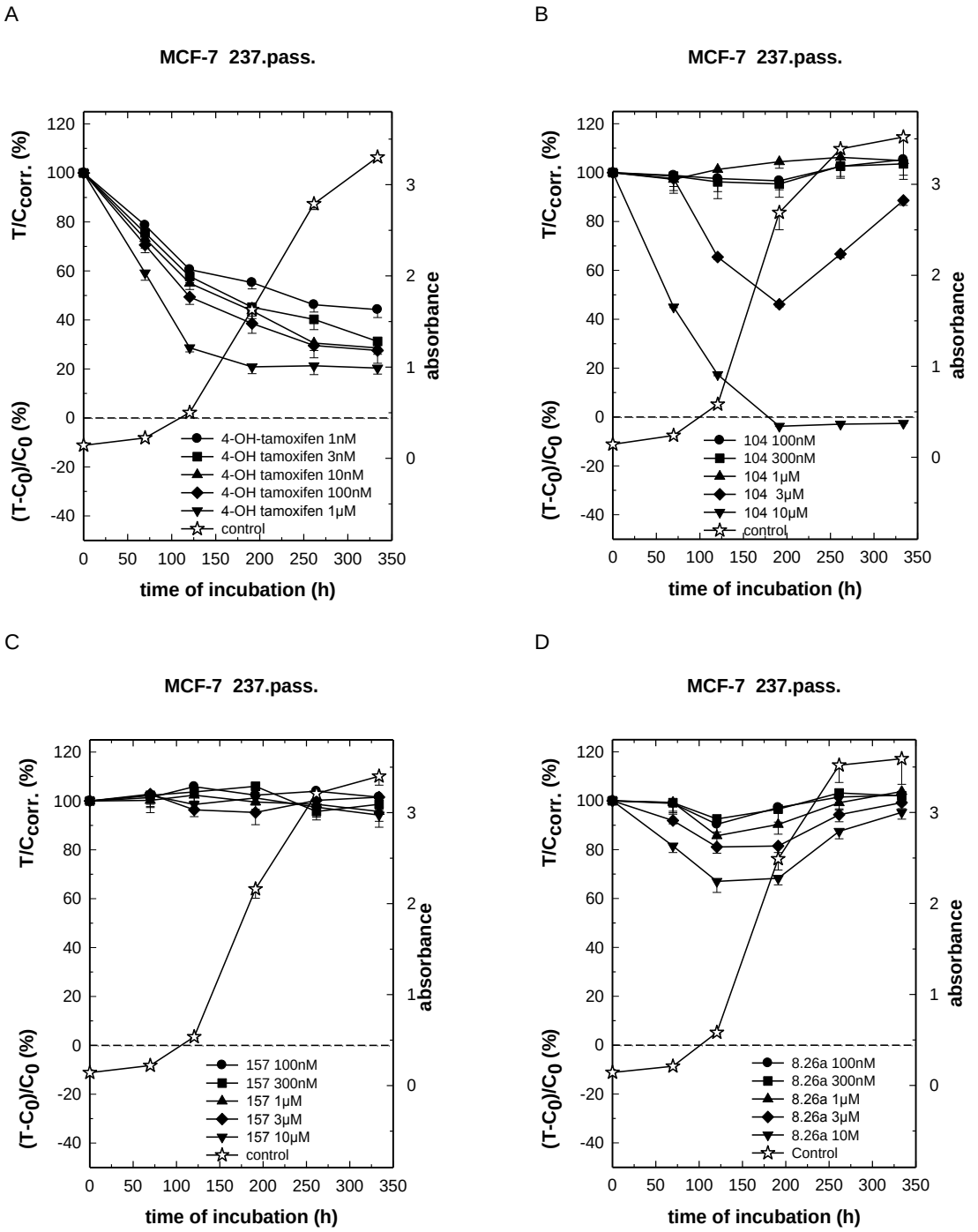


Figure 8.14 Sensitivity of MCF-7 cells against of 4-hydroxytamoxifen (A), 4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol (**104** (see chapter 7), B), 2-(6,7-dichloro-1*H*-indol-2-yl)-1,3,4-oxadiazole (**157**, C) and 1-(4-chlorobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (**8.26a**, D) compared to the vehicle control. MCF-7 passage 237; values represent means of at least 14 replicates ± SD; Errors of $T/C_{corr.}$ were calculated according to the Gaussian law of error propagation.

Compound **104** (see chapter 7, IC_{50} (*Sag*$Hyal_{4755}$) = 9 µM) showed strong growth inhibition on the estrogen-responsive MCF-7 cells at concentrations of 3 µM and 10 µM, respectively. Moderate inhibition of the cell growth was observed for **8.26a** (IC_{50} (*Sag*$Hyal_{4755}$) = 21 µM) in the same concentration range. Thus, cytotoxic effects were

found for the 2-phenylindoles **104** and **8.26a**, which were already described as mammary tumor inhibiting agents.[6, 23] By contrast, 2-(6,7-dichloro-1*H*-indol-2-yl)-1,3,4-oxadiazole (**157**, see chapter 7, IC_{50} (*Sag*Hyal$_{4755}$) = 152 µM) did not show cytotoxic effects against MCF-7 cells. Therefore, the 6,7-dichloroindole moiety may be considered a promising structural motif for the rational development of inhibitors of streptococcal hyaluronidases, devoid of effects on the estrogen receptor.

8.4 Outlook

As stated in the introduction, depending on the *N*-substituent, molecules incorporating a 2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol scaffold are known for antiestrogenic activity in the low nanomolar range. Hydroxylated 2-phenylindoles carrying substituted benzyl groups or similar residues at the indole nitrogen were previously synthesized and tested for estrogen receptor affinity.[6] The estrogenicity of the 1-benzylindoles is rather similar to that of 1-alkyl-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindoles.[5, 6] The compounds **8.24a**-**8.31a**, **8.27b** and **8.29b** turned out to be of interest as scaffolds for the development of hyaluronidase inhibitors. However, only **8.24a**, **8.26a** and **8.28a** were documented to show binding affinity for the calf uterine estrogen receptor.[6] Introducing negatively charged groups such as carboxylate (cf. **8.32a**, **8.32b**) appears to be an effective strategy to strongly decrease estrogen receptor binding. Furthermore, the substitution pattern of **157**, **163** (cf. Figure 8.11), bearing two chloro substituents in position 6 and 7, gives access to more potent and selective inhibitors of *Sag*Hyal$_{4755}$. An overview of selected indole-based inhibitors is shown in Figure 8.15.

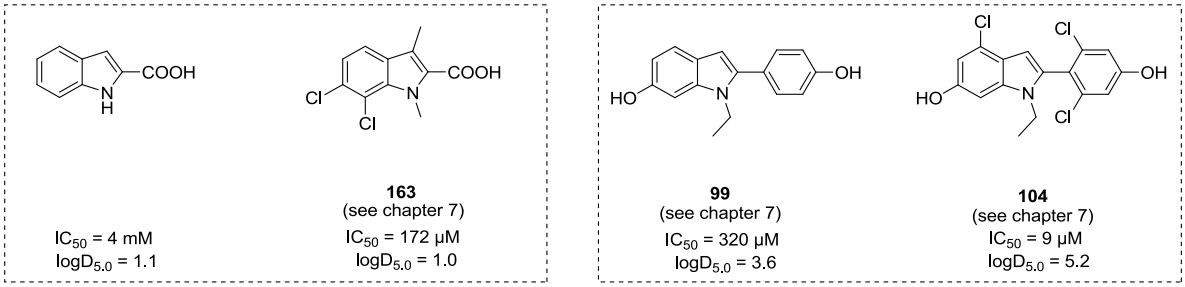


Figure 8.15 Comparison of indole-based inhibitors of *Sag*Hyal$_{4755}$ with focus on the contribution of chloro-substituents to inhibitory potency.

Previously, 1*H*-indole-2-carboxylic acid was scored as hit in a LUDI-based approach for the identification of hyaluronate lyase inhibitors.[26] This molecule, proposed by computer-assisted drug design (virtual screening), was confirmed as a weak inhibitor (IC_{50} = 4 mM)

of *Sag*Hyal$_{4755}$ in the turbidimetric assay.[3] As shown for **163** (see chapter 7), the introduction of chloro-substituents in combination with methyl residues in position 1 and 3, resulted in an IC_{50} value of 172 µM. Most probably, the increase in potency compared to the parent compound indole-3-carboxylic acid has to be attributed to the chloro substituents. This is corroborated by further examples, for instance, comparing 1-ethyl-2-(4-hydroxyphenyl)-1*H*-indol-6-ol (**99**) and 4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol (**104**). The latter was 35-fold more potent than the parent compound. Thus, the introduction of chloro substituents may be considered an alternative to aliphatic chains to provide the required level of lipophilicity for binding to hyaluronidase. However, the cytotoxic effects of **104** and interaction with the ER must be kept in mind. Inhibitors based on **157**, **163** (cf. Figure 8.11) might pave the way to overcome these limitations.

As an outlook for future developments, several structural modification of inhibitors containing the structural features of **157** (Figure 8.11) can be proposed. As outlined in Figure 8.16, three different concepts (concept 1-3) are exemplified in a schematic draft. In the current chapter, concept 1 was elaborated for the design of the potent inhibitor **8.47**. Besides, additional modifications, e.g. the insertion of the 1,3,4-oxadiazole moiety to a hydroxylated 2-phenylindole (concept 2) or substituents on the core structure of **157**(concept 3) can be suggested.

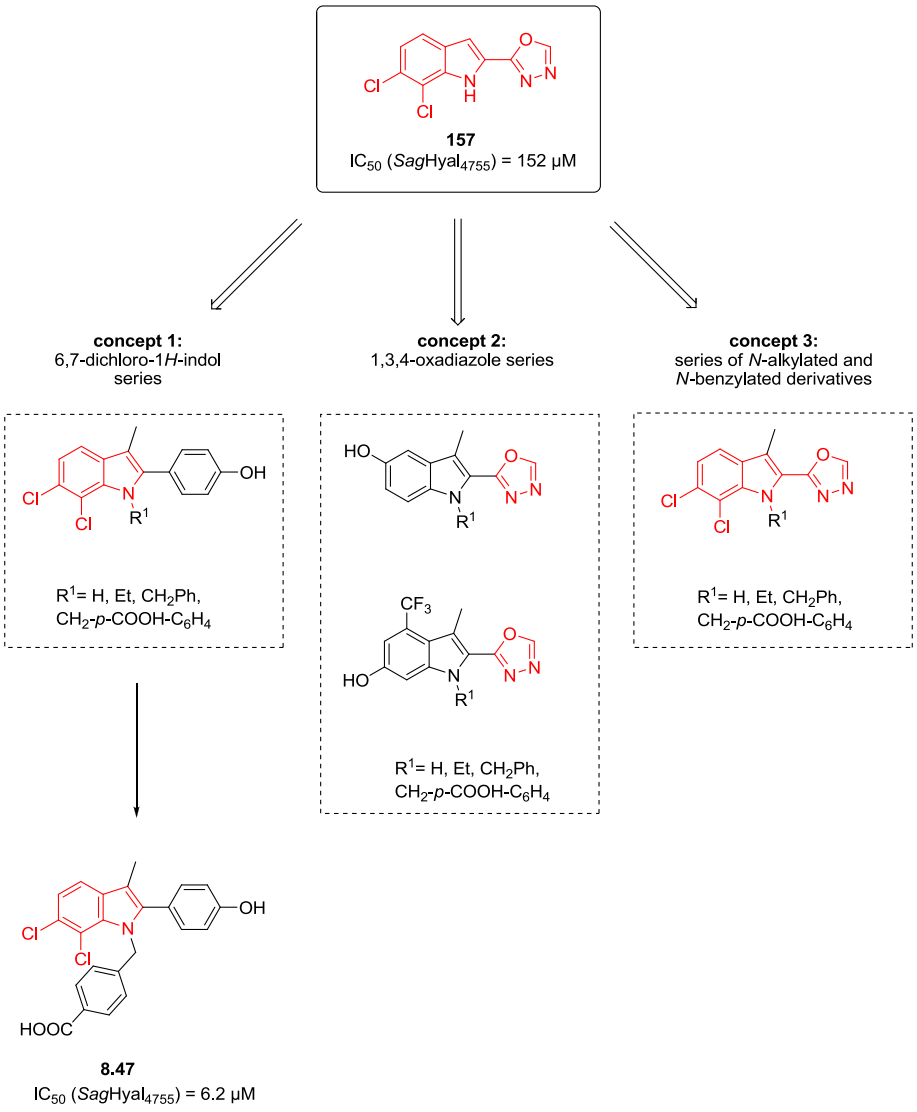


Figure 8.16 Proposed structural modifications of **157** for the development of potent inhibitors of *Sag*Hyal$_{4755}$.

8.5 Summary

Aiming at inhibitors of the bacterial hyaluronate lyase *Sag*Hyal$_{4755}$, the 3-methyl-2-phenylindole skeleton was used as a pharmacophoric moiety. Structural modifications were performed taking into account to obtain compounds with drug-like properties. Starting from extensive studies on *N*-alkylated 3-methyl-2-phenylindoles, crucial structural features of compounds with retained activity in the micromolar range but significantly reduced lipophilicity were elaborated.

This knowledge was transformed into the development of a library of *N*-benzylated 3-methyl-2-phenylindoles. A stepwise variation of the substructures resulted in inhibitors with IC_{50} values for inhibition of *Sag*Hyal$_{4755}$ in the lower micromolar range. Compared to *N*-alkylated analogs, the novel structures were not superior in terms of potency at the bacterial enzyme. In accordance to our expectations, these compounds were not capable of inhibiting the mammalian enzyme BTH. As structurally closely related molecules are known for antiestrogenic activity in the low nanomolar range, potential disadvantages, such as cytotoxic activity, have to be taken into account.

To improve the solubility and to obtain more potent and selective hyaluronidase inhibitors, a negatively charged group was introduced in position 4 of the benzyl residue. In this way inhibition of the target enzyme was not improved, however, estrogen receptor mediated off-target effects were prevented. As a side-effect, charged 2-phenylindole derivatives proved to be weak inhibitors of BTH. i.e., inhibition of a mammalian enzyme by 2-phenylindoles was observed for the first time.

Bioisosteric replacement of a hydroxyl moiety by chlorine residues on the indole skeleton, resulted in a significant increase in inhibitory activity for the bacterial hyaluronate lyase. For example, an IC_{50} value of 6 µM was determined for one of the most potent inhibitors of *Sag*Hyal$_{4755}$ known to date, 4-[(6,7-dichloro-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl)methyl]benzoic acid (**8.47**). Moreover, an IC_{50} value of 93 µM was determined on the structurally related streptococcal hyaluronidase *Spn*Hyl. The chloro-substituted 2-phenylindole **157** (2-(6,7-dichloro-1*H*-indol-2-yl)-1,3,4-oxadiazole) was devoid of cytotoxic effects on human estrogen-responsive MCF-7 breast cancer cells. In summary, compound **8.47** can be considered as a new lead structure for the development of inhibitors of bacterial hyaluronate lyases.

8.6 Experimental section

8.6.1 General conditions;

Cf. 5.8.1

Compounds **8.2a**-**8.4a**[5], **8.9a**[6], **8.11a**[6], **8.13a**[6], **8.24a**[6], **8.26a**[6], **8.28a**[6], **8.2b**[5] and **8.37**[27] have been described before. Compound **8.33** was commercially available (Sigma-Aldrich Chemie GmbH, Munich, Germany).

8.6.2 Chemistry

8.6.2.1. Preparation of the compounds 8.1, 8.34

2-Bromo-1-(4-methoxyphenyl)propan-1-one (8.1)[8]

To a cooled solution of 1-(4-methoxyphenyl)propan-1-one (15.4 g, 93.6 mmol) in 80 mL glacial acetic acid, a few drops of aqueous hydrobromic acid (48 %, v/v) and bromine (15.0 g, 93.6 mmol) were slowly added under vigorous stirring at a temperature below 20 °C. Subsequently, the reaction mixture was stirred at ambient temperature for 1 h. The mixture was poured into ice water (90 mL) and the precipitate was filtrated off and washed several times with ice-cold water. The product was purified by recrystallization from ethanol to give colorless crystals. Yield: 18.3 g (80 %, colorless crystals); mp 67 °C (ref:[8] 66-67 °C). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 8.06 – 7.99 (m, 2H, Ph-***H***-2,6), 7.11 – 7.04 (m, 2H, Ph-***H***-3,5), 5.79 (q, *J* = 6.5 Hz, 1H, COC***H***BrCH$_3$), 3.86 (s, 3H, OC***H***$_3$-4), 1.76 (d, *J* = 6.5 Hz, 3H, COCHBrC***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 191.92 (C$_{quat}$, ***C***OCHBrCH$_3$), 163.48 (C$_{quat}$, Ph-***C***-4), 131.15 (+, Ph-***C***-3,5), 126.27 (C$_{quat}$, Ph-***C***-1), 113.99 (+, Ph-***C***-2,6), 55.53 (+, O***C***H$_3$-4), 42.88 (+, CO***C***HBrCH$_3$), 19.99 (+, COCHBr***C***H$_3$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 224.0 ([M$^{+\bullet}$], 3), 135.0 ([*p*-OCH$_3$-C$_6$H$_4$-CO]$^+$, 100). C$_{10}$H$_{11}$BrO$_2$ (*M*$_r$ = 243.10 g/mol).

2-Bromo-1-(4-methoxyphenyl)ethanone (8.34)[13]

Bromine (4.8 g, 30.0 mmol) was added dropwise to a solution of 4-methoxyacetophenone (4.5 g, 30.0 mmol) in dioxane (100 mL) and dichloromethane (50 mL) under vigorous stirring. The solution was cooled in an ice-bath to maintain the temperatures below 20 °C. Subsequently, the reaction mixture was stirred at ambient temperature for 1 h. After

washing with water (2 x 200 mL) the aqueous solution was extracted with EtOAc (4 x 250 mL). The combined organic layers were combined and dried over Na_2SO_4 and the solvent was removed in vacuo. The crude product was recrystallized from ethanol to give a white solid. Yield: 4.8 g (70 %, colorless crystals); mp 71 °C (ref[28]: 73 °C). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 8.05 – 7.98 (m, 2H, Ph-***H***-2,6), 7.09 – 7.02 (m, 2H, Ph-***H***-3,5), 4.79 (s, 2H, COC***H***$_2$Br), 3.86 (s, 3H, OC***H***$_3$-4). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 191.73 (C_{quat}, ***C***OCHBrCH$_3$), 162.99 (C_{quat}, Ph-***C***-4), 131.11 (+, Ph-***C***-3,5), 126.11 (C_{quat}, Ph-***C***-1), 113.95 (+, Ph-***C***-2,6), 55.49 (+, O***C***H$_3$-4), 33.98 (-, CO***C***H$_2$Br). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 230.0 ([M$^{+\bullet}$], 10), 135.0 ([*p*-OCH$_3$-C$_6$H$_4$-CO]$^+$, 100). $C_9H_9BrO_2$ (M_r = 229.07 g/mol).

8.6.2.2. Preparation of the compounds 8.2a, 8.2b, 8.35-8.37

General procedure[27]

A solution of the pertinent ring-substituted 2-bromo-1-phenylethanone (1 eq) or 2-bromo-1-phenyl)propan-1-one (1 eq) in xylene (60 mL per 0.1 mol) was added dropwise to a solution of the substituted aniline (2.1 eq) in *N,N*-dimethylaniline (20 mL per 0.1 mol) at a temperature of 170 °C over a period of 1h. After addition, the mixture was kept at a temperature of 170 °C for additional 2 h. After cooling to room temperature, the dark brown solution was poured into 2N HCl (150 mL per 0.1 mol). EtOAc was added and the aqueous phase was extracted three more times with EtOAc. After washing with 2N HCl, water and sat. $NaHCO_3$, the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (SiO_2) as indicated.

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.2a)[8, 13]

The title compound was prepared from 4-methoxyaniline (11.7 g, 95 mmol), *N,N*-dimethylaniline (18.8 mL) and 2-bromo-1-(4-methoxyphenyl)propan-1-one **8.1** (10.9 g, 45 mmol) according to the general procedure. The crude product was purified by flash-chromatography (PE/EtOAc 80/20, v/v). Yield: 5.8 g (48 %, white solid); mp 137 °C (ref:[8] 139 °C). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 10.88 (s, 1H, indole-N***H***), 7.61 – 7.55 (m, 2H, Ph-***H***-2,6), 7.23 (dd, *J* = 8.7, 2.7 Hz, 1H, indole-***H***-7), 7.12 – 7.03 (m, 2H, Ph-***H***-3,5), 6.98 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.73 (dd, *J* = 8.7, 2.4 Hz, 1H, indole-***H***-6), 3.81 (s, 3H, OC***H***$_3$-4), 3.79 (s, 3H, indole-OC***H***$_3$-5), 2.36 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 158.20 (C_{quat}, Ph-***C***-4), 153.05 (C_{quat}, indole-***C***-5), 134.42

(C_{quat}, indole-***C***), 130.75 (C_{quat}, indole-***C***), 129.72 (C_{quat}, indole-***C***), 128.53 (+, Ph-***C***-2,6), 125.63 (C_{quat}, Ph-***C***-1), 114.04 (+, Ph-***C***-3,5), 111.40 (+, indole-***C***-6), 111.04 (+, indole-***C***-7), 105.37 (C_{quat}, indole-***C***), 99.90 (+, indole-***C***-4), 55.22 (+, O***C***H_3-4), 55.04 (+, indole-O***C***H_3-5), 9.81 (+, indole-***C***H_3-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 268.0 ($[M + H]^+$, 100). $C_{17}H_{17}NO_2$ (M_r = 267.32 g/mol).

6-Methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.2b)[13]

The title compound was prepared from 3-methoxyaniline (11.7 g, 95 mmol), *N,N*-dimethylaniline (18.8 mL) and 2-bromo-1-(4-methoxyphenyl)propan-1-one **8.1** (10.9 g, 45 mmol) according to the general procedure. The crude product was purified by flash-chromatography (PE/EtOAc 80/20, v/v). Yield: 3.9 g (32 %, white solid); mp 134 °C (ref:[8] 136 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 10.86 (s, 1H, indole-N***H***), 7.55 (d, *J* = 8.8 Hz, 2H, Ph-***H***-2,6), 7.36 (d, *J* = 8.6 Hz, 1H, indole-***H***-4), 7.06 (d, *J* = 8.8 Hz, 2H, Ph-***H***-3,5), 6.82 (d, *J* = 2.2 Hz, 1H, indole-***H***-7), 6.65 (dd, *J* = 8.6, 2.2 Hz, 1H, indole-***H***-6), 3.81 (s, 3H, OC***H***$_3$-4), 3.77 (s, 3H, indole-OC***H***$_3$-6), 2.33 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 158.14 (C_{quat}, Ph-***C***-4), 157.23 (C_{quat}, indole-***C***-6), 134.44 (C_{quat}, indole-***C***), 130.81 (C_{quat}, indole-***C***), 130.03 (C_{quat}, indole-***C***), 128.53 (+, Ph-***C***-2,6), 125.56 (C_{quat}, Ph-***C***-1), 114.03 (+, Ph-***C***-3,5), 110.77 (+, indole-***C***-7), 107.40 (+, indole-***C***-5), 105.37 (C_{quat}, indole-***C***), 99.87 (+, indole-***C***-4), 55.34 (+, O***C***H_3-4), 55.11 (+, indole-O***C***H_3-6), 9.77 (+, indole-***C***H_3-3). MS (ES-MS, DCM/MeOH + NH_4OAc) m/z (rel. int. in %) = 268.0 ($[M + H]^+$, 100). $C_{17}H_{17}NO_2$ (M_r = 267.32 g/mol).

5-Methoxy-3-methyl-2-phenyl-1*H*-indole (8.35)

The title compound was prepared from 4-methoxyaniline (5.2 g, 42 mmol), *N,N*-dimethylaniline (8.3 mL) and 2-bromo-1-phenylpropan-1-one **8.33** (4.3 g, 20 mmol) according to the general procedure. The crude product was purified by flash-chromatography (PE/EtOAc 80/20, v/v). Yield: 2.0 g (41 %, white solid); mp 117 °C (ref:[29] 114-116 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 10.99 (s, 1H, indole-N***H***), 7.65 (dt, *J* = 2.8, 1.6 Hz, 2H, Ph-***H***-3,5), 7.50 (dd, *J* = 10.5, 4.9 Hz, 2H, Ph-***H***-2,6), 7.38 – 7.30 (m, 1H, Ph-***H***-4), 7.25 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 7.01 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.75 (dd, *J* = 8.7, 2.4 Hz, 1H, indole-***H***-6), 3.79 (s, 3H, OC***H***$_3$-4), 2.39 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6) δ [ppm] = 153.09 (C_{quat}, indole-***C***-5), 134.31 (C_{quat}, indole-***C***), 133.09 (C_{quat}, Ph-***C***-1), 130.95 (C_{quat}, indole-***C***), 129.59 (C_{quat}, indole-***C***), 128.56 (+, Ph-***C***-3,5), 127.24 (+, Ph-***C***-2,6), 126.75 (+, Ph-***C***-4), 111.63 (+, indole-***C***-7), 108.20

(C_{quat}, indole-***C***), 106.50 (+, indole-***C***-6), 99.99 (+, indole-***C***-4), 55.22 (+, indole-O***C***H_3-5), 9.88 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 237.1 ([$M^{+\bullet}$], 100), 195.1 (12), 105.1 (10). $C_{16}H_{15}NO$ (M_r = 237.30 g/mol).

5-Methoxy-2-(4-methoxyphenyl)-1*H*-indole (8.36)[30]

The title compound was prepared from 4-methoxyaniline (5.2 g, 42 mmol), *N,N*-dimethylaniline (8.3 mL) and 2-bromo-1-(4-methoxyphenyl)ethanone **8.34** (4.6 g, 20 mmol) according to the general procedure. The crude product was purified by flash-chromatography (PE/EtOAc 80/20, v/v). Yield: 2.5 g (49 %, beige solid); mp 211 °C (ref:[31] 219-220 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 11.25 (s, 1H, indole-N***H***), 7.80 – 7.70 (m, 2H, Ph-***H***-2,6), 7.25 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 7.06 – 6.96 (m, 3H, indole-***H***-4, Ph-***H***-3,5), 6.74 – 6.64 (m, 2H, indole-***H***-3, indole-***H***-6), 3.80 (s, 3H, OC***H***$_3$-4), 3.75 (s, 3H, indole-OC***H***$_3$-6). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 158.57 (C_{quat}, Ph-***C***-4), 153.42 (C_{quat}, indole-***C***-5), 138.16 (C_{quat}, indole-***C***), 131.92 (C_{quat}, indole-***C***), 129.12 (C_{quat}, Ph-***C***-1), 126.10 (+, Ph-***C***-2,6), 124.90 (C_{quat}, indole-***C***), 114.20 (+, Ph-***C***-3,5), 111.57 (+, indole-***C***-7), 110.97 (+, indole-***C***-6), 101.28 (+, indole-***C***-4), 97.15 (+, indole-***C***-3), 55.32 (+, O***C***H_3-4), 55.09 (+, indole-O***C***H_3-5). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 253.1 ([$M^{+\bullet}$], 100), 238.1 (31), 210.1 (31), 195.1 (12), 167.1 (9), 126.6 (21), 105.1 (10). $C_{16}H_{15}NO_2$ (M_r = 253.30 g/mol).

5-*sec*-Butyl-2-(4-methoxyphenyl)-1*H*-indole (8.37)[27]

The title compound was prepared from 4-*sec*-butylaniline (6.3 g, 42 mmol), *N,N*-dimethylaniline (8.3 mL) and 2-bromo-1-(4-methoxyphenyl)ethanone **8.34** (4.6 g, 20 mmol) according to the general procedure. The crude product was purified by flash-chromatography (PE/EtOAc 80/20, v/v). Yield: 2.0 g (36 %, colorless crystals); mp 194 °C (ref:[27] 191 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 11.15 (s, 1H, indole-N***H***), 7.42 (m, 1H, indole-***H***-4), 7.25 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 7.06 – 6.96 (m, 4H, Ph-***H***-2,6, Ph-***H***-3,5), 6.94 – 6.82 (m, 1H, indole-***H***-6), 6.67 (s, 1H, indole-***H***-3), 3.80 (s, 3H, OC***H***$_3$-4), 2.68 (t, *J* = 7.1 Hz, 1H, C***H***(CH_3)CH_2CH_3), 1.65 (quin, *J* = 7 Hz, 2H, CH(CH_3)C***H***$_2$$CH_3$), 1.27 (*J* = 7.0 Hz, 3H, CH(CH_3)CH_2C***H***$_3$), 0.89 (t, *J* = 7.1 Hz, 3H, CH(C***H***$_3$)CH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 158.57 (C_{quat}, Ph-***C***-4), 138.54 (C_{quat}, indole-***C***), 133.32 (C_{quat}, indole-***C***-5), 131.94 (C_{quat}, indole-***C***), 129.12 (C_{quat}, Ph-***C***-1), 126.10 (+, Ph-***C***-2,6), 125.21 (C_{quat}, indole-***C***), 118.98 (+, indole-***C***-4), 114.56 (+, Ph-***C***-3,5), 111.41 (+, indole-***C***-7), 111.07 (+, indole-***C***-6), 98.05 (+, indole-***C***-3), 55.32 (+, O***C***H_3-4), 45.09

(+, ***C***H(CH_3)CH_2CH_3), 32.89 (-, CH(CH_3)***C***H_2CH_3), 21.01 (+, CH(***C***H_3)CH_2CH_3), 11.89 (+, CH(***C***H_3)CH_2CH_3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 279.1 ([$M^{+\bullet}$], 100), 126.6 (15), 105.1 (10). $C_{19}H_{21}NO_2$ (M_r = 279.38 g/mol).

8.6.2.3. Preparation of the compounds 8.44, 8.45

(2,3-Dichlorophenyl)hydrazine (8.44)[19]

A solution of sodium nitrite (2.6 g, 37.4 mmol) in water (8 mL) was added dropwise to a solution of 2,3-dichloroaniline (6.1 g, 37.4 mmol) in 24 mL water and 24 mL concentrated HCl at 0 °C. The solution was stirred for 10 min and then slowly added dropwise to a rapidly stirred mixture of $SnCl_2 \cdot 2H_2O$ (33.7 g, 149.5 mmol) in concentrated HCl (140 mL) at 0 °C. The reaction was allowed to warm to room temperature, stirred for 15 min, and filtered. The precipitate was washed on the filter with large portions of ice-cold ether and dried under reduced pressure to give the target compound, which was used in the next step without further purification. Yield: 5.2 g (78 %, white solid).

6,7-Dichloro-2-(4-methoxyphenyl)-3-methyl-1H-indole (8.45)[18, 20]

(2,3-Dichlorophenyl)hydrazine (2.0 g, 11.3 mmol) and 1-(4-methoxyphenyl)propan-1-one (1.9 g, 11.3 mmol) were dissolved in anhydrous EtOH (80 mL). After the addition of 40 mL of 32 % HCl, the solution was boiled under reflux for at least 48 h. Subsequently, the mixture was allowed to cool to room temperature (precipitation occurred). After concentration of the solution, the aqueous phase was extracted with EtOAc (3 x 250 mL). The organic layers were combined and washed with brine. The precipitate (white solid, not product), formed in the organic phase, was removed by filtration. The remaining organic phase was dried over Na_2SO_4 and the solvent was evaporated yielding dark red oil. The crude product was tediously purified by column chromatography (SiO_2). The product was finally isolated with 25-fold excess of SiO_2 (relative to crude substance) at a PE/EtOAc gradient of 97/3 (v/v). Yield: 5.2 g (15 %, yellow crystals). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 11.42 (s, 1H, indole-N***H***), 7.65 – 7.59 (m, 2H, Ph-***H***-2,6), 7.49 (d, *J* = 8.4 Hz, 1H, indole-***H***-4), 7.22 – 7.17 (m, 1H, indole-***H***-5), 7.12 – 7.05 (m, 2H, Ph-***H***-3,5), 3.83 (d, *J* = 2.1 Hz, 3H, OC***H***$_3$-4), 2.33 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 158.77 (C_{quat}, Ph-***C***), 136.59 (C_{quat}, indole-***C***), 133.42 (C_{quat}, indole-***C***), 129.74 (+, Ph-***C***-2,6), 129.55 (C_{quat}, indole-***C***-Cl), 124.13 (C_{quat}, Ph-***C***-1), 123.29 (C_{quat}, indole-***C***), 120.32 (+, indole-***C***-5), 117.87 (+, indole-***C***-4), 113.86 (+, Ph-***C***-3,5), 113.50 (C_{quat}, indole-***C***-Cl), 107.38 (C_{quat}, indole-***C***), 55.12 (+, O***C***H_3-4), 9.44 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV)

m/z (rel. int. in %) = 307.1 (62), 306.1 (38), 305.1 ([$M^{+\bullet}$], 100), 292.1 (15), 290.0 ([M - $CH_3^{+\bullet}$], 28). HRMS (ESI-MS) m/z calcd. $[M + H]^+$ 306.0447, found $[M + H]^+$ 306.0448. $C_{16}H_{13}Cl_2NO$ (M_r = 306.19 g/mol).

8.6.2.4. Preparation of the compounds 8.3a-8.8a

General procedure[5, 6]

With ice cooling, NaH (60 % in paraffin, 1.9 eq) was suspended in anhydrous DMF (15 mL per 10 mmol NaH) and stirred for 15 min under an atmosphere of nitrogen. Subsequently, 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (**8.2a**, 1.0 eq) in anhydrous DMF (22.5 mL per 10 mmol 2-phenylindole) was added dropwise at 0 °C under an atmosphere of nitrogen. After stirring for 45 min at 0 °C, the corresponding alkyl halide (1.2 eq) in anhydrous DMF (45 mL per 10 mmol alkyl halide) was slowly added. After removing of the cooling bath, the mixture was stirred for additional 2.5 h at ambient temperature and then poured into ice water. The aqueous solution was extracted at least three times with EtOAc. The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (SiO_2) as indicated.

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-ethyl-1*H*-indole (8.3a)[32]

The title compound was prepared from **8.2a** (400 mg, 1.5 mmol) and iodoethane (280 mg, 1.8 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (330 mg, 85 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.32 (ddd, *J* = 9.8, 6.0, 3.6 Hz, 3H, indole-***H***-7, Ph-***H***-2,6), 7.11 – 7.04 (m, 2H, Ph-***H***-3,5), 6.99 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.78 (dd, *J* = 8.8, 2.4 Hz, 1H, indole-***H***-6), 4.56 (q, *J* = 7.1 Hz, 2H, NC***H***$_2$$CH_3$), 3.82 (s, 3H, OC***H***$_3$-4), 3.79 (s, 3H, indole-OC***H***$_3$-5), 2.11 (s, 3H, indole-C***H***$_3$-3), 1.29 (t, *J* = 7.2 Hz, 3H, NCH_2C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 158.72 (C_{quat}, Ph-***C***-4), 153.22 (C_{quat}, indole-***C***-5), 137.46 (C_{quat}, indole-***C***), 131.32 (+, Ph-***C***-2,6), 131.16 (C_{quat}, indole-***C***), 128.25 (C_{quat}, Ph-***C***-1), 123.92 (C_{quat}, indole-***C***), 113.86 (+, Ph-***C***-3,5), 110.89 (+, indole-***C***-6), 110.56 (+, indole-***C***-7), 106.88 (C_{quat}, indole-***C***), 100.25 (+, indole-***C***-4), 55.29 (+, O***C***H_3-4), 55.00 (+, indole-O***C***H_3-5), 35.71 (-, N***C***H_2CH_3), 15.40 (+, NCH_2***C***H_3), 9.17 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 295.1 ([$M^{+\bullet}$], 100), 174.1 (20). $C_{19}H_{21}NO_2$ (M_r = 295.38 g/mol).

1-Isopropyl-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.4a)[5]

The title compound was prepared from **8.2a** (400 mg, 1.5 mmol) and 2-iodopropane (310 mg, 1.8 mmol) according to the general procedure. Flash chromatography yielded a dark yellow oil (330 mg, 85 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.42 (t, J = 7.2 Hz, 1H, indole-***H***-6), 7.15 (dd, J = 8.9, 2.3 Hz, 2H, Ph-***H***-2,6), 6.90 – 6.79 (m, 2H, Ph-***H***-3,5), 6.76 (d, J = 2.2 Hz, 1H, indole-***H***-4), 6.63 (dd, J = 8.8, 2.4 Hz, 1H, indole-***H***-7), 3.86 (s, 3H, OC***H***$_3$-4), 3.82 (s, 3H, indole-OC***H***$_3$-5), 4.41 – 4.27 (m, 1H, NC***H***$(CH_3)_2$), 1.99 (s, 3H, indole-C***H***$_3$-3), 1.42 (d, J = 7.0 Hz, 6H, NCH(C***H***$_3)_2$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 310.2 (25), 309.2 ([$M^{+\bullet}$], 100). $C_{20}H_{23}NO_2$ (M_r = 309.40 g/mol).

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-nonyl-1*H*-indole (8.5a)

The title compound was prepared from **8.2a** (300 mg, 1.1 mmol) and 1-chlorononane (210 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a dark orange oil (200 mg, 46 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.33-7.28 (m, 3H, indole-***H***-7, Ph-***H***-2,6), 7.09-7.04 (m, 2H, Ph-***H***-3,5), 6.78 (dd, J = 2.5, 7.9 Hz, 1H, indole-***H***-6), 6.98 (d, 1H, J = 2.5 Hz, indole-***H***-4), 3.98 (t, 2H, J = 7.1 Hz, NC***H***$_2CH_2(CH_2)_6CH_3$), 3.79 (s, 3H, OC***H***$_3$-4), 3.83 (s, 3H, indole-OC***H***$_3$-5), 2.11 (s, 3H, indole-C***H***$_3$-3), 1.46-1.35 (m, 2H, NCH_2C***H***$_2(CH_2)_6CH_3$), 1.26 - 1.18 (m, 8H, $NCH_2CH_2(C$***H***$_2)_6CH_3$), 1.16-1.00 (m, 4H, $NCH_2CH_2(C$***H***$_2)_6CH_3$), 0.81 – 0.74 (m, 3H, $NCH_2CH_2(CH_2)_6C$***H***$_3$). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 393.3 ([M + H]$^+$, 100). $C_{26}H_{35}NO_2$ (M_r = 393.56 g/mol).

1-Decyl-6-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.6a)

The title compound was prepared from **8.2a** (500 mg, 1.9 mmol) and 1-bromodecane (500 mg, 2.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (310 mg, 40 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.36 (d, J = 8.5 Hz, 1H, indole-***H***-7), 7.33 – 7.27 (m, 2H, Ph-***H***-2,6), 7.07 (t, J = 5.8 Hz, 2H, Ph-***H***-3,5), 6.95 (d, J = 2.1 Hz, 1H, indole-***H***-4), 6.69 (dd, J = 8.5, 2.2 Hz, 1H, indole-***H***-6), 3.99 (t, J = 7.2 Hz, 2H, NC***H***$_2CH_2(CH_2)_7CH_3$), 3.82 (s, 3H, OC***H***$_3$-4), 3.80 (s, 3H, indole-OC***H***$_3$-5), 2.09 (s, 3H, indole-C***H***$_3$), 1.45 – 1.35 (m, 2H, NCH_2C***H***$_2(CH_2)_7CH_3$), 1.28 – 1.20 (m, 10H, $NCH_2CH_2(C$***H***$_2)_7CH_3$), 1.06 – 0.99 (m, 4H, $NCH_2CH_2(C$***H***$_2)_7CH_3$), 0.87 – 0.83 (m, 3H, $NCH_2CH_2(CH_2)_7C$***H***$_3$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 408.4 (24), 407.3 ([$M^{+\bullet}$], 100), 280.1 (17). $C_{27}H_{37}NO_2$ (M_r = 407.59 g/mol).

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-tetradecyl-1*H*-indole (8.7a)

The title compound was prepared from **8.2a** (300 mg, 1.1 mmol) and 1-chlorotetradecane (310 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (170 mg, 37 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.32 – 7.27 (m, 2H, Ph-***H***-2,6), 7.23 (d, *J* = 8.8 Hz, 1H, indole-***H***-7), 7.04 – 7.01 (m, 2H, Ph-***H***-3,5), 7.00 – 6.98 (m, 1H, indole-***H***-4), 6.88 (dd, *J* = 8.5, 2.2 Hz, 1H, indole-***H***-6), 3.99 – 3.92 (m, 2H, $NC\boldsymbol{H}_2CH_2(CH_2)_{11}CH_3$), 3.89 (s, 3H, $OC\boldsymbol{H}_3$-4), 3.88 (s, 3H, indole-$OC\boldsymbol{H}_3$-5), 2.19 (s, 3H, indole-$C\boldsymbol{H}_3$), 2.05 (s, 2H, $NCH_2C\boldsymbol{H}_2(CH_2)_{11}CH_3$), 1.62 – 1.54 (m, 2H, $NCH_2CH_2(C\boldsymbol{H}_2)_{11}CH_3$), 1.27 – 1.20 (m, 16H, $NCH_2CH_2(C\boldsymbol{H}_2)_{11}CH_3$), 1.18 – 1.09 (m, 6H, $NCH_2CH_2(C\boldsymbol{H}_2)_{11}CH_3$), 0.90 – 0.85 (m, 3H, $NCH_2CH_2(CH_2)_{11}C\boldsymbol{H}_3$). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 512.2 (60), 496.1 (85), 480.1 ($[M^{+\bullet}] + NH_3$, 100), 464.2 ($[M + H]^+$, 20), 462.2 ($[M^{+\bullet}]$, 15). $C_{31}H_{45}NO_2$ (M_r = 463.69 g/mol).

1-Hexadecyl-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.8a)

The title compound was prepared from **8.2a** (300 mg, 1.1 mmol) and 1-chlorohexadecane (350 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded an orange oil (490 mg, 41 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.33 – 7.27 (m, 2H, Ph-***H***-2,6), 7.23 (d, *J* = 8.8 Hz, 1H, indole-***H***-7), 7.04 – 7.02 (m, 2H, Ph-***H***-3,5), 7.00 – 6.98 (m, 1H, indole-***H***-4), 6.88 (dd, *J* = 8.5, 2.5 Hz, 1H, indole-***H***-6), 3.99 – 3.92 (m, 2H, $NC\boldsymbol{H}_2CH_2(CH_2)_{13}CH_3$), 3.90 (s, 3H, $OC\boldsymbol{H}_3$-4), 3.89 (s, 3H, indole-$OC\boldsymbol{H}_3$-5), 2.20 (s, 3H, indole-$C\boldsymbol{H}_3$), 1.61 – 1.54 (s, 2H, $NCH_2C\boldsymbol{H}_2(CH_2)_{13}CH_3$), 1.39 – 1.22 (m, 4H, $NCH_2CH_2(C\boldsymbol{H}_2)_{13}CH_3$), 1.18 – 1.09 (m, 22H, $NCH_2CH_2(C\boldsymbol{H}_2)_{13}CH_3$), 0.92 – 0.85 (m, 3H, $NCH_2CH_2(CH_2)_{13}C\boldsymbol{H}_3$). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 540.2 (100), 524.2 (40), 508.2 ($[M^{+\bullet}] + NH_3$, 75, 491.2 ($[M + H]^+$, 10), 490.1 ($[M^{+\bullet}]$, 20). $C_{33}H_{49}NO_2$ (M_r = 491.75 g/mol).

8.6.2.5. Preparation of the compounds 8.9a-8.17a, 8.12b, 8.14b, 8.17b, 8.38-8.40, 8.46

General procedure[5, 6]

With ice cooling, NaH (60 % in paraffin, 1.9 eq) was suspended in anhydrous DMF (15 mL per 10 mmol NaH) and stirred for 15 min under an atmosphere of nitrogen. Subsequently, the corresponding 2-phenylindole (1.0 eq) in anhydrous DMF (22.5 mL per 10 mmol indole) was added dropwise at 0 °C under an atmosphere of nitrogen. After stirring for 45 min at 0 °C, the corresponding benzyl halide (1.2 eq) in anhydrous DMF (45 mL per

10 mmol alkyl halide) was slowly added. After removing of the cooling bath, the mixture stirred for additional 2.5 h at ambient temperature before it was poured into ice water. The aqueous solution was extracted three times with EtOAc. The combined organic layers were washed with water and dried (Na_2SO_4). The solvent was removed under vacuum and the crude product was purified by flash-chromatography (SiO_2) as indicated.

1-Benzyl-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.9a)[6]

The title compound was prepared from **8.2a** (900 mg, 3.4 mmol) and (bromomethyl)-benzene (690 mg, 4.0 mmol) according to the general procedure. Flash chromatography yielded a yellow, viscous oil (970 mg, 81 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.40 – 7.35 (m, 1H, indole-***H***-7), 7.27 – 7.18 (m, 5H, Ph-***H***-2,6, benzyl-***H***-3,4,5), 7.08 – 7.02 (m, 2H, benzyl-***H***-2,6), 6.97 (d, *J* = 2.0 Hz, 1H, indole-***H***-4), 6.96 – 6.91 (m, 2H, Ph-***H***-3,5), 6.81 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 5.19 (s, 2H, NC***H***$_2$), 3.89 (s, 3H, OC***H***$_3$-4), 3.84 (s, 3H, indole-OC***H***$_3$-5), 2.28 (s, 3H, indole-C***H***$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 358.2 (23), 357.1 ([$M^{+\bullet}$], 100), 266.1 ([$M^{+\bullet}$] – $^{\bullet}C_7H_7$, 30), 91.0 ($^{\bullet}C_7H_7$, 16). $C_{24}H_{23}NO_2$ (M_r = 357.44 g/mol).

1-(4-Fluorobenzyl)-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.10a)

The title compound was prepared from **8.2a** (250 mg, 0.9 mmol) and 1-(bromomethyl)-4-fluorobenzene (215 mg, 1.1 mmol) according to the general procedure. Flash chromatography yielded a colorless oil (130 mg, 37 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.25 – 7.18 (m, 2H, Ph-***H***-2,6), 7.08 – 7.01 (m, 2H, Ph-***H***-3,5), 6.98 – 6.92 (m, 2H, benzyl-***H***), 6.92 - 6.87 (m, 4H, indole-***H***-4, indole-***H***-7, benzyl-***H***), 6.82 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 5.14 (s, 2H, NC***H***$_2$), 3.89 (s, 3H, OC***H***$_3$-4), 3.84 (s, 3H, indole-OC***H***$_3$-5), 2.25 (s, 3H, indole-C***H***$_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. In %) = 377.1 (30), 376.1 ([M + H]$^+$, 100). $C_{24}H_{22}FNO_2$ (M_r = 375.44 g/mol).

1-(4-Chlorobenzyl)-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.11a)[6]

The title compound was prepared from **8.2a** (300 mg, 1.1 mmol) and 1-chloro-4-(chloromethyl)benzene (215 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (210 mg, 48 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.24 - 7.16 (s, 4H, benzyl-***H***, Ph-***H***-2,6), 7.06 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 7.02 (d, *J* = 8.8 Hz, 1H, indole-***H***-7), 6.99 – 6.90 (m, 2H, benzyl-***H***), 6.86 (d, *J* = 8.5 Hz,

2H, Ph-***H***-3,5), 6.82 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 5.14 (s, 2H, NC***H***$_2$), 3.89 (s, 3H, OC***H***$_3$-4), 3.84 (s, 3H, indole-OC***H***$_3$-5), 2.26 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 159.37 (C_{quat}, Ph-***C***-4), 154.19 (C_{quat}, indole-***C***-5), 138.27 (C_{quat}, benzyl-***C***), 137.21 (C_{quat}, benzyl-***C***), 132.73 (C_{quat}, indole-***C***), 131.61 (+, Ph-***C***-2,6), 129.22 (C_{quat}, indole-***C***), 128.74 (+, benzyl-***C***), 127.40 (+, benzyl-***C***), 124.13 (C_{quat}, Ph-***C***-1), 113.90 (+, Ph-***C***-3,5), 111.77 (+, indole-***C***-6), 110.68 (+, indole-***C***-7), 108.72 (C_{quat}, indole-***C***), 100.88 (+, indole-***C***-4), 55.98 (+, indole-O***C***H_3-5), 55.31 (+, O***C***H_3-4), 47.01 (-, N***C***H_2), 9.51 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 393.2 (30), 392.2 (25), 391.2 ([$M^{+\bullet}$], 100), 266.1 (68), 125.0 (16). $C_{24}H_{22}ClNO_2$ (M_r = 391.89 g/mol).

1-(4-Bromobenzyl)-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.12a)

The title compound was prepared from **8.2a** (300 mg, 1.1 mmol) and 1-bromo-4-(bromomethyl)benzene (330 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded an orange oil (290 mg, 60 %). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 7.43 – 7.35 (m, 2H, benzyl-***H***), 7.32 – 7.24 (m, 2H, Ph-***H***-2,6), 7.18 (d, *J* = 8.8 Hz, 1H, indole-***H***-7), 7.07 – 6.99 (m, 3H, benzyl-***H***, indole-***H***-4), 6.79 – 6.70 (m, 3H, Ph-***H***-3,5, indole-***H***-6), 5.21 (s, 2H, NC***H***$_2$), 3.78 (s, 6H, indole-OC***H***$_3$-5, OC***H***$_3$-4), 2.18 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 158.88 (C_{quat}, Ph-***C***-4), 153.58 (C_{quat}, indole-***C***-5), 138.02 (C_{quat}, benzyl-***C***), 137.63 (C_{quat}, indole-***C***), 131.27 (+, benzyl-***C***), 131.17 (+, benzyl-***C***), 128.67 (C_{quat}, indole-***C***), 127.99 (+, Ph-***C***-2,6), 123.29 (C_{quat}, Ph-***C***-1), 119.80 (C_{quat}, benzyl-***C***), 117.55 (C_{quat}, indole-***C***), 113.97 (+, Ph-***C***-3,5), 111.30 (+, indole-***C***-6), 110.90 (+, indole-***C***-7), 107.71 (C_{quat}, indole-***C***), 100.50 (+, indole-***C***-4), 55.29 (+, indole-O***C***H_3-5), 55.02 (+, O***C***H_3-4), 45.95 (-, N***C***H_2), 9.30 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 437.1 (70), 435.1 ([$M^{+\bullet}$], 72), 266.1 ([$M^{+\bullet}$] – $^{\bullet}CH_2C_6H_4Br$, 100), 223.1 (22), 171.0 (25), 169.0 (26), 90.1 (21). $C_{24}H_{22}BrNO_2$ (M_r = 436.34 g/mol).

1-(4-Bromobenzyl)-6-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.12b)

The title compound was prepared from **8.2b** (500 mg, 1.9 mmol) and 1-bromo-4-(bromomethyl)benzene (570 mg, 2.3 mmol) according to the general procedure. Flash chromatography yielded an yellow oil (290 mg, 57 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.54 – 7.45 (m, 1H, indole-***H***-4), 7.35 (d, *J* = 8.3 Hz, 2H, benzyl-***H***), 7.19 (d, *J* = 8.8 Hz, 2H, Ph-***H***-2,6), 6.93 (d, *J* = 8.8 Hz, 2H, Ph-***H***-3,5), 6.86 – 6.78 (m, 3H, benzyl-***H***, indole-***H***-7), 6.61 (d, 1H, indole-***H***-5), 5.10 (s, 2H, NC***H***$_2$), 3.84 (s, 3H, OC***H***$_3$-4), 3.79 (s,

3H, indole-OC***H***$_3$-6), 2.26 (s, 3H, indole-C***H***$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 437.1 (56), 435.1 ([$M^{+\bullet}$], 65), 266.1 ([$M^{+\bullet}$] – $^{\bullet}CH_2C_6H_4Br$, 100), 223.1 (12). $C_{24}H_{22}BrNO_2$ (M_r = 436.34 g/mol).

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-(4-methylbenzyl)-1*H*-indole (8.13a)[6]

The title compound was prepared from **8.2a** (250 mg, 0.9 mmol) and 1-(bromomethyl)-4-methylbenzene (210 mg, 1.1 mmol) according to the general procedure. Flash chromatography yielded a colorless oil (140 mg, 40 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.27 – 7.23 (m, 2H, Ph-***H***-2,6), 7.07 – 7.01 (m, 4H, indole-***H***-4, indole-***H***-7, benzyl-***H***), 6.96 – 6.90 (m, 2H, Ph-***H***-3,5), 6.85 (d, *J* = 8.1 Hz, 2H, benzyl-***H***), 6.80 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 5.14 (s, 2H, NC***H***$_2$), 3.89 (s, 3H, OC***H***$_3$-4), 3.84 (s, 3H, indole-OC***H***$_3$-5), 2.29 (s, 3H, benzyl-C***H***$_3$), 2.27 (s, 3H, indole-C***H***$_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 373.1 (25), 372.0 ([M + H]$^+$, 100). $C_{25}H_{25}NO_2$ (M_r = 371.47 g/mol).

1-(Biphenyl-4-ylmethyl)-5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.14a)

The title compound was prepared from **8.2a** (500 mg, 1.9 mmol) and 4-(chloromethyl)biphenyl (450 mg, 2.2 mmol) according to the general procedure. Flash chromatography yielded a dark yellow oil (530 mg, 64 %). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 7.71 – 7.64 (m, 1H, indole-***H***-7), 7.60 – 7.52 (m, 2H, Ph-***H***-2,6), 7.49 (dd, *J* = 7.6, 3.9 Hz, 3H, biphenyl-***H***), 7.45 – 7.38 (m, 2H, biphenyl-***H***), 7.37 – 7.31 (m, 2H, biphenyl-***H***), 7.26 – 7.19 (m, 1H, indole-***H***-4), 7.09 – 7.01 (m, 2H, biphenyl-***H***), 6.91 (d, *J* = 8.3 Hz, 2H, Ph-***H***-3,5), 6.74 (dd, *J* = 8.8, 2.4 Hz, 1H, indole-***H***-6), 5.29 (s, 2H, NC***H***$_2$), 3.80 (s, 3H, OC***H***$_3$-4), 3.79 (s, 3H, indole-OC***H***$_3$-5), 2.20 (s, 3H, indole-C***H***$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 433.3 ([$M^{+\bullet}$], 100), 167.1 (100). $C_{30}H_{27}NO_2$ (M_r = 433.54 g/mol).

1-(Biphenyl-4-ylmethyl)-6-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indole (8.14b)

The title compound was prepared from **8.2b** (350 mg, 1.3 mmol) and 4-(chloromethyl)biphenyl (450 mg, 1.6 mmol) according to the general procedure. Flash chromatography yielded a dark yellow oil (350 mg, 62 %). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 7.71 – 7.64 (m, 1H, indole-***H***-4), 7.61 – 7.56 (m, 2H, biphenyl-***H***), 7.55 – 7.47 (m, 2H, Ph-***H***-2,6), 7.46 – 7.37 (m, 4H, biphenyl-***H***), 7.36 – 7.26 (m, 3H, biphenyl-***H***), 6.97-

6.90 (m, 3H, indole-***H***-7, Ph-***H***-3,5), 6.74 (dd, *J* = 8.8, 2.4 Hz, 1H, indole-***H***-5), 5.31 (s, 2H, NC***H***$_2$), 3.79 (s, 3H, OC***H***$_3$-4), 3.72 (s, 3H, indole-OC***H***$_3$-6), 2.19 (s, 3H, indole-C***H***$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 433.3 ([$M^{+\bullet}$], 100), 266.1 (80), 167.0 (68). $C_{30}H_{27}NO_2$ (M_r = 433.54 g/mol).

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-(3-phenylpropyl)-1*H*-indole (8.15a)

The title compound was prepared from **8.2a** (350 mg, 1.3 mmol) and (3-chloropropyl)benzene (240 mg, 1.6 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (160 mg, 32 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.52 – 7.44 (m, 3H, indole-***H***-7, Ph-***H***), 7.27 – 7.18 (m, 5H, Ph-***H***), 6.97 (d, *J* = 2.0 Hz, 1H, indole-***H***-4), 6.96 – 6.91 (m, 2H, Ph-***H***), 6.81 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 4.38 (t, *J* = 7.1 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$), 3.83 (s, 3H, OC***H***$_3$-4), 3.79 (s, 3H, indole-OC***H***$_3$-5), 2.86 (t, 2H, NCH$_2$CH$_2$C***H***$_2$), 2.28 (s, 3H, indole-C***H***$_3$-3), 2.09 – 2.01 (m, 2H, NCH$_2$C***H***$_2$CH$_2$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 385.2 ([$M^{+\bullet}$], 100), 281.2 (60), 266.2 (80), 207.0 (55), 91.0 (30). $C_{26}H_{27}NO_2$ (M_r = 385.50 g/mol).

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-(4-nitrobenzyl)-1*H*-indole (8.16a)

The title compound was prepared from **8.2a** (500 mg, 1.9 mmol) and 1-(bromomethyl)-4-nitrobenzene (490 mg, 2.2 mmol) according to the general procedure. Flash chromatography yielded a dark yellow solid (170 mg, 22 %); mp 152 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.22 – 8.11 (m, 2H, benzyl-***H***-3,5), 7.51 – 7.44 (m, 1H, indole-***H***-7), 7.38 – 7.34 (m, 1H, indole-***H***-4), 7.22 (d, *J* = 8.7 Hz, 1H, indole-***H***-6), 7.02 – 6.86 (m, 4H, benzyl-***H***-2,6, Ph-***H***-2,6), 6.85 – 6.67 (m, 2H, Ph-***H***-3,5), 5.25 (s, 2H, NC***H***$_2$), 3.89 – 3.80 (m, 6H, indole-OC***H***$_3$-5, OC***H***$_3$-4), 1.59 – 1.53 (m, 3H, indole-C***H***$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 404.1 (20), 403.1 ([$M^{+\bullet}$], 100), 402.0 (25). $C_{24}H_{22}N_2O_4$ (M_r = 402.44 g/mol).

Ethyl 4-{[5-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoate (8.17a)

The title compound was prepared from **8.2a** (300 mg, 1.1 mmol) and ethyl 4-(bromomethyl)benzoate (330 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (320 mg, 68 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.94 – 7.85 (m, 2H, benzoate-***H***-3,5), 7.23 – 7.18 (m, 2H, Ph-***H***-2,6), 7.08 (d, *J* = 2.4 Hz, 1H,

indole-***H***-4), 7.00 (d, *J* = 6.5 Hz, 1H, indole-***H***-7), 6.97 (d, *J* = 6.1 Hz, 2H, benzoate-***H***-2,6), 6.94 – 6.88 (m, 2H, Ph-***H***-3,5), 6.80 (dd, *J* = 8.8, 2.4 Hz, 1H, indole-***H***-6), 5.19 (s, 2H, NC$\boldsymbol{H}_2$), 4.40 – 4.26 (m, 2H, COOC$\boldsymbol{H}_2$CH$_3$), 3.87 (s, 3H, OC$\boldsymbol{H}_3$-4), 3.79 (s, 3H, indole-OC$\boldsymbol{H}_3$-5), 2.28 (s, 3H, indole-C$\boldsymbol{H}_3$-3), 1.39 – 1.30 (m, 3H, COOCH$_2$C$\boldsymbol{H}_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 171.03 (C_{quat}, ***C***OOCH$_2$CH$_3$), 166.25 (C_{quat}, benzoate-***C***-4), 159.42 (C_{quat}, Ph-***C***-4), 154.28 (C_{quat}, indole-*C*-5), 143.92 (C_{quat}, benzoate-***C***-1), 138.30 (C_{quat}, indole-***C***), 131.92 (C_{quat}, indole-***C***), 131.59 (+, Ph-***C***-2,6), 129.88 (+, benzoate-***C***-3,5), 129.34 (C_{quat}, indole-***C***), 125.96 (+, benzoate-***C***-2,6), 124.05 (C_{quat}, Ph-***C***-1), 113.91 (+, Ph-***C***-3,5), 111.79 (+, indole-***C***-6), 110.65 (+, indole-***C***-7), 108.74 (C_{quat}, indole-***C***), 100.90 (+, indole-***C***-4), 60.86 (-, COO***C***H$_2$CH$_3$), 55.85 (+, indole-O***C***H$_3$-5), 55.19 (+, O***C***H$_3$-4), 47.43 (-, N***C***H$_2$), 14.24 (+, COOCH$_2$***C***H$_3$), 9.51 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 430.2 (34), 429.2 ([M$^{+\bullet}$], 100), 266.1 (62), 223.1 (23), 163.1 (23), 107.1 (22). $C_{27}H_{27}NO_4$ (M_r = 429.51 g/mol).

Ethyl 4-{[6-methoxy-2-(4-methoxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoate (8.17b)

The title compound was prepared from **8.2b** (300 mg, 1.1 mmol) and ethyl 4-(bromomethyl)benzoate (330 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (340 mg, 72 %). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.94 – 7.90 (m, 2H, benzoate-***H***-3,5), 7.52 – 7.48 (m, 1H, indole-***H***-4), 7.22 – 7.17 (m, 2H, Ph-***H***-2,6), 7.02 (d, *J* = 8.5 Hz, 2H, benzoate-***H***-2,6), 6.94 – 6.89 (m, 2H, Ph-***H***-3,5), 6.83 (dd, *J* = 8.6, 2.2 Hz, 1H, indole-***H***-5), 6.60 (d, *J* = 2.2 Hz, 1H, indole-***H***-7), 5.20 (s, 2H, NC$\boldsymbol{H}_2$), 4.35 (q, *J* = 7.1 Hz, 2H, COOC$\boldsymbol{H}_2$CH$_3$), 3.83 (s, 3H, OC$\boldsymbol{H}_3$-4), 3.77 (s, 3H, indole-OC$\boldsymbol{H}_3$-6), 2.27 (s, 3H, indole-C$\boldsymbol{H}_3$-3), 1.37 (t, *J* = 7.1 Hz, 3H, COOCH$_2$C$\boldsymbol{H}_3$). ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 171.11 (C_{quat}, ***C***OOCH$_2$CH$_3$), 166.34 (C_{quat}, benzoate-***C***-4), 159.22 (C_{quat}, Ph-***C***-4), 156.48 (C_{quat}, indole-***C***-6), 143.64 (C_{quat}, benzoate-***C***-1), 137.41 (C_{quat}, indole-***C***), 136.41 (C_{quat}, indole-***C***), 131.62 (+, Ph-***C***-2,6), 129.88 (+, benzonate-***C***-3,5), 125.97 (+, benzoate-***C***-2,6), 124.16 (C_{quat}, Ph-***C***-1), 123.44 (C_{quat}, indole-***C***), 119.46 (+, indole-***C***-4), 113.84 (+, Ph-***C***-3,5), 109.00 (C_{quat}, indole-***C***), 108.81 (+, indole-***C***-5), 93.96 (+, indole-***C***-7), 60.89 (-, COO***C***H$_2$CH$_3$), 55.73 (+, indole-O***C***H$_3$-6), 55.24 (+, O***C***H$_3$-4), 47.34 (-, N***C***H$_2$), 14.23 (+, COOCH$_2$***C***H$_3$), 9.44 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 430.2 (27), 429.2 ([M$^{+\bullet}$], 100), 266.1 (87), 220.1 (22), 163.1 (26), 107.1 (21). $C_{27}H_{27}NO_4$ (M_r = 429.51 g/mol).

Ethyl 4-[(5-methoxy-3-methyl-2-phenyl-1*H*-indol-1-yl)methyl]benzoate (8.38)

The title compound was prepared from **8.35** (270 mg, 1.1 mmol) and ethyl 4-(bromomethyl)benzoate (330 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (130 mg, 29 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.99 – 7.91 (m, 2H, benzoate-***H***-3,5), 7.47 – 7.38 (m, 3H, Ph-***H***-2,4,6), 7.37 – 7.31 (m, 2H, Ph-***H***-3,5), 7.13 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 7.08 – 6.09 (m, 3H, benzoate-***H***-2,6, indole-***H***-7), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H, indole-***H***-6), 5.26 (s, 2H, NC***H***$_2$), 4.38 (dt, *J* = 14.3, 5.3 Hz, 2H, COOC***H***$_2$CH$_3$), 3.92 (s, 3H, indole-OC***H***$_3$-5), 2.34 (s, 3H, indole-C***H***$_3$-3), 1.39 (t, *J* = 7.1 Hz, 3H, COOCH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 166.36 (C$_{quat}$, ***C***OOCH$_2$CH$_3$), 154.34 (C$_{quat}$, indole-***C***-5), 143.81 (C$_{quat}$, benzoate-***C***-1), 138.49 (C$_{quat}$, Ph-***C***-1), 132.10 (C$_{quat}$, indole-***C***), 131.92 (C$_{quat}$, indole-***C***), 130.44 (+, benzonate-***C***-3,5), 129.95 (+, Ph-***C***-3,5), 129.45 (C$_{quat}$, benzoate-***C***-1), 129.34 (C$_{quat}$, indole-***C***), 128.49 (+, benzonate-***C***-2,6), 128.03 (+, Ph-***C***-4), 126.01 (+, Ph-***C***-2,6), 112.13 (+, indole-***C***-6), 110.83 (+, indole-***C***-7), 109.19 (C$_{quat}$, indole-***C***), 101.03 (+, indole-***C***-4), 60.95 (-, COO***C***H$_2$CH$_3$), 55.97 (+, indole-O***C***H$_3$-6), 47.61 (-, N***C***H$_2$), 14.31 (+, COOCH$_2$***C***H$_3$), 9.58 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 400.3 (25), 399.2 ([M$^{+\bullet}$], 100), 236.2 (31), 193.2 (33), 163.1 (26). $C_{26}H_{25}NO_3$ (M_r = 399.48 g/mol).

Ethyl 4-{[5-methoxy-2-(4-methoxyphenyl)-1*H*-indol-1-yl]methyl}benzoate (8.39)

The title compound was prepared from **8.36** (290 mg, 1.1 mmol) and ethyl 4-(bromomethyl)benzoate (330 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (140 mg, 30 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.00 – 7.93 (m, 2H, benzoate-***H***-3,5), 7.36 – 7.28 (m, 2H, Ph-***H***-2,6), 7.13 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 7.08 (d, *J* = 8.4 Hz, 2H, benzoate-***H***-2,6), 7.04 – 6.98 (m, 1H, indole-***H***-7), 6.94 – 6.87 (m, 2H, Ph-***H***-3,5), 6.80 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 6.54 (s, 1H, indole-***H***-3), 5.34 (s, 2H, NC***H***$_2$), 4.36 (q, *J* = 7.1 Hz, 2H, COOC***H***$_2$CH$_3$), 3.86 (s, 3H, OC***H***$_3$-4), 3.82 (s, 3H, indole-OC***H***$_3$-5), 1.37 (t, *J* = 7.1 Hz, 3H, COOCH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 171.40 (C$_{quat}$, ***C***OOCH$_2$CH$_3$), 166.30 (C$_{quat}$, benzoate-***C***-4), 159.62 (C$_{quat}$, Ph-***C***-4), 154.63 (C$_{quat}$, indole-***C***-5), 143.55 (C$_{quat}$, benzoate-***C***-1), 132.98 (C$_{quat}$, indole-***C***), 130.35 (+, Ph-***C***-2,6), 130.07 (+, benzonate-***C***-3,5), 129.57 (C$_{quat}$, indole-***C***), 128.83 (C$_{quat}$, indole-***C***), 125.89 (+, benzonate-***C***-2,6), 124.94 (C$_{quat}$, Ph-***C***-1), 114.07 (+, Ph-***C***-3,5), 111.87 (+, indole-***C***-6), 110.95 (+, indole-***C***-7), 102.31 (+, indole-***C***-3), 101.72 (+, indole-***C***-4), 60.95 (-, COO***C***H$_2$CH$_3$), 55.83 (+, O***C***H$_3$-4), 55.30 (+, indole-O***C***H$_3$-5), 47.63 (-, N***C***H$_2$), 14.26 (+, COOCH$_2$***C***H$_3$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 416.3 (24), 415.2 ([M$^{+\bullet}$], 78), 252.2 (100), 209.2 (33), 163.1 (18), 107.1 (19). $C_{26}H_{25}NO_4$ (M_r = 415.48 g/mol).

Ethyl 4-{[5-*sec*-butyl-2-(4-methoxyphenyl)-1*H*-indol-1-yl]methyl}benzoate (8.40)

The title compound was prepared from **8.37** (300 mg, 1.1 mmol) and ethyl 4-(bromomethyl)benzoate (310 mg, 1.3 mmol) according to the general procedure. Flash chromatography yielded a yellow oil (150 mg, 31 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.84 (d, *J* = 8.2 Hz, 2H, benzoate-***H***-3,5), 7.42 – 7.34 (m, 3H, indole-***H***-4, Ph-***H***-2,6), 7.19 (d, *J* = 8.4 Hz, 1H, indole-***H***-7), 7.04 (d, *J* = 8.2 Hz, 2H, benzoate-***H***-2,6), 7.02 – 6.98 (m, 2H, Ph-***H***-3,5), 6.94 (dd, *J* = 8.5, 1.3 Hz, 1H, indole-***H***-6), 6.52 (s, 1H, indole-***H***-3), 5.46 (s, 2H, NC***H***$_2$), 4.27 (q, *J* = 7.1 Hz, 2H, COOC***H***$_2$CH$_3$), 3.77 (s, 3H, OC***H***$_3$-4), 2.70 – 2.57 (m, 1H, indole-C***H***(CH$_3$)(CH$_2$CH$_3$)), 2.02 – 1.97 (m, 3H, COOCH$_2$C***H***$_3$), 1.58 (p, *J* = 7.3 Hz, 2H, indole-CH(CH$_3$)(C***H***$_2$CH$_3$)), 1.27 (t, *J* = 7.1 Hz, 3H, indole-CH(C***H***$_3$)(CH$_2$CH$_3$)), 0.82 – 0.73 (m, 3H, indole-CH(CH$_3$)(CH$_2$C***H***$_3$)). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 172.10 (C$_{quat}$, ***C***OOCH$_2$CH$_3$), 166.30 (C$_{quat}$, benzoate-***C***-4), 159.62 (C$_{quat}$, Ph-***C***-4), 154.63 (C$_{quat}$, indole-***C***), 151.42 (C$_{quat}$, benzoate-***C***-1), 138.41 (C$_{quat}$, indole-***C***), 130.26 (+, Ph-***C***-2,6), 129.21 (+, benzonate-***C***-3,5), 129.17 (C$_{quat}$, indole-***C***), 127.38 (C$_{quat}$, indole-***C***), 125.96 (+, benzoate-***C***-2,6), 122.22 (C$_{quat}$, Ph-***C***-1), 121.11 (+, indole-***C***-4), 117.58 (+, indole-***C***-6), 115.49 (+, Ph-***C***-3,5), 110.23 (+, indole-***C***-7), 100.99 (+, indole-***C***-3), 60.95 (-, COO***C***H$_2$CH$_3$), 55.82 (+, O***C***H$_3$-4), 46.50 (-, N***C***H$_2$), 40.94 (+, indole-***C***H(CH$_3$)(CH$_2$CH$_3$)), 31.24 (-, indole-CH(CH$_3$)(***C***H$_2$CH$_3$)), 22.27 (+, indole-CH(***C***H$_3$)(CH$_2$CH$_3$)), 14.13 (+, COOCH$_2$***C***H$_3$), 12.17 (+, indole-CH(CH$_3$)(CH$_2$***C***H$_3$)). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 441.3 ([M$^{+\bullet}$], 48), 262.2 (18), 207.1 (22), 183.4 (51), 44.1 (100), 40.0 (55). $C_{29}H_{31}NO_3$ (M_r = 441.56 g/mol).

Ethyl 4-{[6,7-Dichloro-2-(4-methoxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoate (8.46)

The title compound was prepared from **8.45** (200 mg, 0.6 mmol) and ethyl 4-(bromomethyl)benzoate (190 mg, 0.8 mmol) according to the general procedure. Flash chromatography yielded an orange oil (150 mg, 48 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.92 – 7.86 (m, 2H, benzoate-***H***-3,5), 7.43 – 7.38 (m, 1H, indole-***H***-4), 7.24 – 7.20 (m, 1H, indole-***H***-5), 7.20 – 7.14 (m, 2H, Ph-***H***-2,6), 6.94 – 6.87 (m, 2H, benzoate-***H***-2,6), 6.82 (d, *J* = 8.4 Hz, 2H, Ph-***H***-3,5), 5.63 (s, 2H, NC***H***$_2$), 4.33 (q, *J* = 7.1 Hz, 2H, COOC***H***$_2$CH$_3$), 3.81 (s, 3H, OC***H***$_3$-4), 2.21 (s, 3H, indole-C***H***$_3$-3), 1.35 (t, *J* = 7.1 Hz, 3H, COOCH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 171.12 (C$_{quat}$, ***C***OOCH$_2$CH$_3$), 166.33 (C$_{quat}$, benzoate-***C***-4), 159.82 (C$_{quat}$, Ph-***C***-4), 145.23 (C$_{quat}$, benzoate-***C***-1), 140.51 (C$_{quat}$, indole-***C***), 132.90 (C$_{quat}$, indole-***C***), 131.88 (+, benzoate-***C***-3,5), 130.21 (+, Ph-***C***-2,6), 129.83 (+, indole-***C***), 129.20 (C$_{quat}$, indole-***C***-Cl), 127.08 (C$_{quat}$, indole-***C***), 125.37 (+, benzoate-***C***-2,6), 123.12 (C$_{quat}$, Ph-***C***-1), 117.81 (+, indole-***C***), 115.18 (C$_{quat}$, indole-***C***-Cl), 114.00 (+, Ph-***C***-3,5), 110.33 (C$_{quat}$, indole-***C***), 60.87 (-, COO***C***H$_2$CH$_3$), 55.33 (+, O***C***H$_3$-4), 48.77 (-, N***C***H$_2$), 14.32 (+, COOCH$_2$***C***H$_3$), 9.32 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) =

469.1 (59), 468.2 (29), 467.1 ([$M^{+\bullet}$], 88), 306.1 (35), 304.1 (54), 269.1 (25), 163.1 (100), 135.1 (36), 107.1 (46), 90.1 (25), 44.1 (36), 40.1 (23). $C_{26}H_{23}Cl_2NO_3$ (M_r = 468.37 g/mol).

8.6.2.6. Preparation of the compounds 8.18a-8.32a, 8.27b, 8.29b, 8.32b, 8.41-8.43, 8.47

General procedure 1[8]

A solution of 4.0 eq of BBr_3 (~ 1.0 M solution in CH_2Cl_2) in anhydrous CH_2Cl_2 (20 mL per 4 mmol BBr_3) was cooled to -20 °C under nitrogen atmosphere. A solution of the corresponding methoxy-substituted indole (1.0 eq) in anhydrous CH_2Cl_2 (20 mL per 1 mmol indole) was added at -20 °C under nitrogen atmosphere within 15 min. After removal of the cooling bath, the mixture was stirred overnight. Under a nitrogen atmosphere and cooling (ice bath), a saturated solution of $NaHCO_3$ was added slowly. The reaction mixture was extracted several times with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography (SiO_2) as indicated.

General procedure 2[14]

A solution of 5.5 eq of BBr_3 (~ 1.0 M solution in CH_2Cl_2) in anhydrous CH_2Cl_2 (20 mL per 4 mmol BBr_3) was cooled to -20 °C under nitrogen atmosphere. A solution of the corresponding methoxy-substituted indole (1.0 eq) in anhydrous CH_2Cl_2 (20 mL per 1 mmol indole) was added at -20 °C under nitrogen atmosphere within 15 min. After removal of the cooling bath, the mixture was stirred overnight. Under a nitrogen atmosphere and cooling with an ice bath, a saturated solution of $NaHCO_3$ was added slowly until the vigorous reaction ceased. By dropwise addition of 32 % hydrochloric acid, the pH value was carefully adjusted to < 3. The reaction mixture was extracted several times with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography (SiO_2) as indicated.

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.18a)[5]

The title compound was prepared from **8.3a** (210 mg, 0.7 mmol) according to general procedure 1. Flash chromatography yielded a yellow solid (160 mg, 85 %); mp 199 °C (ref:[5] 204 – 207 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 9.67 (s, 1H, Ph-O***H*** -4),

8.68 (s, 1H, indole-O***H***-5), 7.14 – 7.07 (m, 3H, Ph-***H***-2,6, indole-***H***-7), 6.90 (d, *J* = 8.6 Hz, 2H, Ph-***H***-3,5), 6.79 (d, *J* = 2.2 Hz, 1H, indole-***H***-4), 6.65 (dd, *J* = 8.6, 2.3 Hz, 1H, indole-***H***-6), 4.66 (q, *J* = 7.1 Hz, 2H, NC***H***$_2$CH$_3$), 2.09 (s, 3H, indole-C***H***$_3$-3), 1.28 (t, J = 7.1 Hz, 3H, NCH$_2$C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 156.89 (C$_{quat}$, Ph-***C***-4), 150.47 (C$_{quat}$, indole-***C***-5), 137.79 (C$_{quat}$, indole-***C***), 131.36 (+, Ph-***C***-2,6), 131.31 (C$_{quat}$, indole-***C***), 128.52 (C$_{quat}$, Ph-***C***-1), 121.99 (C$_{quat}$, indole-***C***), 115.17 (+, Ph-***C***-3,5), 111.00 (+, indole-***C***-6), 109.81 (+, indole-***C***-7), 105.43 (C$_{quat}$, indole-***C***), 102.16 (+, indole-***C***-4), 37.57 (-, N***C***H$_2$CH$_3$), 17.51 (+, NCH$_2$***C***H$_3$), 9.30 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 268.2 (16), 267.2 ([M$^{+\bullet}$], 100), 266.1 (73), 174.1 (27), 140.6 (15). $C_{17}H_{17}NO_2$ (M_r = 267.32 g/mol).

2-(4-Hydroxyphenyl)-1-isopropyl-3-methyl-1*H*-indol-5-ol (8.19a)[5]

The title compound was prepared from **8.4a** (200 mg, 0.6 mmol) according to general procedure 1. Flash chromatography yielded and subsequent recrystallization (solvent: anhydrous EtOH) yielded pale yellow crystals (120 mg, 71 %); mp 157 -162 °C (ref:[5] 150 - 161 °C). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 9.67 (s, 1H, O***H***-4), 8.66 (s, 1H, indole-O***H***-5), 7.38 (t, *J* = 7.2 Hz, 1H, indole-***H***-6), 7.14 (dd, *J* = 8.9, 2.3 Hz, 2H, Ph-***H***-2,6), 6.93 – 6.84 (m, 2H, Ph-***H***-3,5), 6.77 (d, *J* = 2.3 Hz, 1H, indole-***H***-4), 6.61 (dd, *J* = 8.8, 2.4 Hz, 1H, indole-***H***-7), 4.42 – 4.23 (m, 1H, NC***H***(CH$_3$)$_2$), 1.99 (s, 3H, indole-C***H***$_3$-3), 1.42 (d, *J* = 7.0 Hz, 6H, NCH(C***H***$_3$)$_2$). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 156.98 (C$_{quat}$, Ph-***C***-4), 150.16 (C$_{quat}$, indole-***C***-5), 137.77 (C$_{quat}$, indole-***C***), 131.34 (+, Ph-***C***-2,6), 129.69 (C$_{quat}$, indole-***C***), 128.32 (C$_{quat}$, indole-***C***), 122.74 (C$_{quat}$, Ph-***C***-1), 115.17 (+, Ph-***C***-3,5), 112.12 (+, indole-***C***-6), 110.60 (+, indole-***C***-7), 105.60 (C$_{quat}$, indole-***C***), 102.42 (+, indole-***C***-4), 46.95 (+, N***C***H(CH$_3$)$_2$), 21.17 (+, NCH(***C***H$_3$)$_2$), 9.22 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) =282.2 (20), 281.2 ([M$^{+\bullet}$], 100), 266.1 (44), 44.1 (67). $C_{18}H_{19}NO_2$ (M_r = 281.35 g/mol).

2-(4-Hydroxyphenyl)-3-methyl-1-nonyl-1*H*-indol-5-ol (8.20a)

The title compound was prepared from **8.5a** (150 mg, 0.4 mmol) according to general procedure 1. Flash chromatography yielded a yellow oil (100 mg, 68 %). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 9.62 (s, 1H, O***H***-4), 8.64 (s, 1H, indole-OH-***5***), 7.22 – 7.15 (m, 3H, Ph-***H***-2,6, indole-***H***-7), 6.98 – 6.88 (m, 2H, Ph-***H***-3,5), 6.82 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.68 (dd, *J* = 8.5, 2.5 Hz, 1H, indole-***H***-6), 3.96 – 3.89 (m, 2H, NC***H***$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 2.11 (s, 3H, indole-C***H***$_3$), 1.60 – 1.55 (m, 2H, NCH$_2$C***H***$_2$(CH$_2$)$_6$CH$_3$), 1.23 – 1.11 (m, 8H, NCH$_2$CH$_2$(C***H***$_2$)$_6$CH$_3$), 1.10 – 1.03 (m, 4H, NCH$_2$CH$_2$(C***H***$_2$)$_6$CH$_3$), 0.89 – 0.75 (m, 3H, NCH$_2$CH$_2$(CH$_2$)$_6$C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 158.08

(C_{quat}, Ph-***C***-4), 153.30 (C_{quat}, indole-***C***-5), 137.50 (C_{quat}, indole-***C***), 131.42 (+, Ph-***C***-2,6), 131.21 (C_{quat}, indole-***C***), 128.44 (C_{quat}, indole-***C***), 124.04 (C_{quat}, Ph-***C***-1), 113.87 (+, Ph-***C***-3,5), 110.89 (+, indole-***C***-6), 110.85 (+, indole-***C***-7), 106.85 (C_{quat}, indole-***C***), 100.42 (+, indole-***C***-4), 42.94 (-, N***C***$H_2CH_2(CH_2)_6CH_3$), 30.91 (-, NCH_2***C***$H_2(CH_2)_6CH_3$), 27.89 (-, NCH_2CH_2(***C***$H_2)_6CH_3$), 25.9 (-, NCH_2CH_2(***C***$H_2)_6CH_3$), 21.86 (-, NCH_2CH_2(***C***$H_2)_6CH_3$), 21.45 (-, NCH_2CH_2(***C***$H_2)_6CH_3$), 20.22 (-, NCH_2CH_2(***C***$H_2)_6CH_3$), 20.13 (-, NCH_2CH_2(***C***$H_2)_6CH_3$), 13.80 (+, $NCH_2CH_2(CH_2)_6$***C***H_3), 9.27 (+, indole-***C***H_3-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 366.2 ($[M + H]^+$, 100). $C_{24}H_{31}NO_2$ (M_r = 365.51 g/mol).

1-Decyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-6-ol (8.21a)

The title compound was prepared from **8.6a** (250 mg, 0.6 mmol) according to general procedure 1. Flash chromatography yielded an orange oil (120 mg, 53 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 9.61 (s, 1H, Ph-O***H***-4), 8.94 (s, 1H, indole-O***H***-5), 7.24 (d, *J* = 8.4 Hz, 1H, indole-***H***-7), 7.15 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2,6), 6.87 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3,5), 6.70 (d, *J* = 1.8 Hz, 1H, indole-***H***-4), 6.55 (dd, *J* = 8.4, 1.9 Hz, 1H, indole-***H***-6), 3.86 (t, *J* = 7.2 Hz, 2H, NC***H***$_2CH_2(CH_2)_7CH_3$), 2.07 (s, 3H, indole-C***H***$_3$), 1.48 – 1.37 (m, 2H, NCH_2C***H***$_2(CH_2)_7CH_3$), 1.25 – 1.13 (m, 10H, NCH_2CH_2(C***H***$_2)_7CH_3$), 1.10 – 1.02 (m, 4H, NCH_2CH_2(C***H***$_2)_7CH_3$), 0.87 – 0.81 (m, 3H, $NCH_2CH_2(CH_2)_7$C***H***$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 155.29 (C_{quat}, Ph-***C***-4), 148.87 (C_{quat}, indole-***C***-5), 138.22 (C_{quat}, indole-***C***), 131.80 (+, Ph-***C***-2,6), 128.67 (C_{quat}, indole-***C***), 125.01 (C_{quat}, Ph-***C***-1), 122.01 (C_{quat}, indole-***C***), 115.37 (+, Ph-***C***-3,5), 110.86 (+, indole-***C***-6), 110.26 (+, indole-***C***-7), 107.57 (C_{quat}, indole-***C***), 103.41 (+, indole-***C***-4), 43.97 (-, N***C***$H_2(CH_2)_8CH_3$), 31.94 (-, NCH_2(***C***$H_2)_8CH_3$), 30.06 (-, NCH_2(***C***$H_2)_8CH_3$), 29.73 (-, NCH_2(***C***$H_2)_8CH_3$), 29.69 (-, NCH_2(***C***$H_2)_8CH_3$), 29.46 (-, NCH_2(***C***$H_2)_8CH_3$), 29.36 (-, NCH_2(***C***$H_2)_8CH_3$), 29.13 (-, NCH_2(***C***$H_2)_8CH_3$), 22.71 (-, NCH_2(***C***$H_2)_8CH_3$), 14.14 (+, $NCH_2(CH_2)_8$***C***H_3), 9.30 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 380.3 (23), 379.2 ($[M^{+\bullet}]$, 100), 252.1 (35). $C_{25}H_{33}NO_2$ (M_r = 379.54 g/mol).

2-(4-Hydroxyphenyl)-3-methyl-1-tetradecyl-1*H*-indol-5-ol (8.22a)

The title compound was prepared from **8.7a** (100 mg, 0.2 mmol) according to general procedure 1. Flash chromatography yielded a yellow solid (80 mg, 92 %); mp 178 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.26 – 7.21 (m, 2H, Ph-***H***-2,6), 7.18 (d, *J* = 8.6 Hz, 1H, indole-***H***-7), 6.98 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.95 – 6.91 (m, 2H, Ph-***H***-3,5), 6.78 (dd, *J* = 8.5, 2.5 Hz, 1H, indole-***H***-6), 4.89 (s, 1H, Ph-O***H***-4), 4.48 (s, 1H, indole-O***H***-5), 3.97 – 3.88 (m, 2H, NC***H***$_2CH_2(CH_2)_{11}CH_3$), 2.15 (s, 3H, indole-C***H***$_3$), 1.60 – 1.55 (m, 2H,

$NCH_2C\boldsymbol{H}_2(CH_2)_{11}CH_3$), 1.28 – 1.21 (m, 18H, $NCH_2CH_2(C\boldsymbol{H}_2)_{11}CH_3$), 1.16 – 1.09 (m, 4H, $NCH_2CH_2(C\boldsymbol{H}_2)_{11}CH_3$), 0.91 – 0.85 (m, 3H, $NCH_2CH_2(CH_2)_{11}C\boldsymbol{H}_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 155.36 (C_{quat}, Ph-***C***-4), 148.98 (C_{quat}, indole-***C***-5), 138.35 (C_{quat}, indole-***C***), 131.82 (+, Ph-***C***-2,6), 129.17 (C_{quat}, indole-***C***), 124.91 (C_{quat}, Ph-***C***-1), 121.99 (C_{quat}, indole-***C***), 115.33 (+, Ph-***C***-3,5), 110.88 (+, indole-***C***-6), 110.27 (+, indole-***C***-7), 107.65 (C_{quat}, indole-***C***), 103.40 (+, indole-***C***-4), 43.97 (-, $N\boldsymbol{C}H_2(CH_3)_{12}CH_3$), 31.94 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 30.06 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.84 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.82 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.73 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.69 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.56 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.46 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.36 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 29.13 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 26.83 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 22.71 (-, $NCH_2(\boldsymbol{C}H_3)_{12}CH_3$), 14.14 (+, $NCH_2(CH_3)_{12}\boldsymbol{C}H_3$), 9.30 (+, indole-$\boldsymbol{C}H_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 437.2 (30), 436.1 ($[M + H]^+$, 100). $C_{29}H_{41}NO_2$ (M_r = 435.64 g/mol).

1-Hexadecyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.23a)

The title compound was prepared from **8.8a** (110 mg, 0.2 mmol) according to general procedure 1. Flash chromatography yielded a yellow solid (90 mg, 97 %); mp 189 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.27 – 7.21 (m, 2H, Ph-***H***-2,6), 7.19 (d, *J* = 8.6 Hz, 1H, indole-***H***-7), 6.96 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.94 – 6.90 (m, 2H, Ph-***H***-3,5), 6.80 (dd, *J* = 8.5, 2.5 Hz, 1H, indole-***H***-6), 3.97 – 3.91 (m, 2H, $NC\boldsymbol{H}_2CH_2(CH_2)_{13}CH_3$), 2.15 (s, 3H, indole-$C\boldsymbol{H}_3$), 1.63 – 1.50 (m, 2H, $NCH_2C\boldsymbol{H}_2(CH_2)_{13}CH_3$), 1.31 – 1.21 (m, 20H, $NCH_2CH_2(C\boldsymbol{H}_2)_{13}CH_3$), 1.17 – 1.10 (m, 6H, $NCH_2CH_2(C\boldsymbol{H}_2)_{13}CH_3$), 0.92 – 0.87 (m, 3H, $NCH_2CH_2(CH_2)_{13}C\boldsymbol{H}_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 155.20 (C_{quat}, Ph-***C***-4), 149.04 (C_{quat}, indole-***C***-5), 138.23 (C_{quat}, indole-***C***), 131.86 (+, Ph-***C***-2,6), 128.06 (C_{quat}, indole-***C***), 125.06 (C_{quat}, Ph-***C***-1), 121.99 (C_{quat}, indole-***C***), 115.32 (+, Ph-***C***-3,5), 110.92 (+, indole-***C***-6), 110.29 (+, indole-***C***-7), 107.67 (C_{quat}, indole-***C***), 103.38 (+, indole-***C***-4), 43.94 (-, $N\boldsymbol{C}H_2(CH_2)_{14}CH_3$), 31.93 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 30.04 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 30.01 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.69 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.64 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.66 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.62 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.53 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.42 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.37 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 29.12 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 28.57 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 26.79 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 22.74 (-, $NCH_2(\boldsymbol{C}H_2)_{14}CH_3$), 14.17 (+, $NCH_2(CH_2)_{14}\boldsymbol{C}H_3$), 9.31 (+, indole-$\boldsymbol{C}H_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 465.2 (30), 464.2 ($[M + H]^+$, 100). $C_{31}H_{45}NO_2$ (M_r = 463.69 g/mol).

1-Benzyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.24a)[6]

The title compound was prepared from **8.9a** (700 mg, 2.0 mmol) according to general procedure 1. Flash chromatography yielded a green oil (660 mg, 99 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 9.66 (s, 1H, O***H***-4), 8.71 (s, 1H, indole-O***H***-5), 7.22 – 7.11 (m, 5H, indole-***H***-7, Ph-***H***-2,6, benzyl-***H***-3,5), 7.05 (d, *J* = 8.7 Hz, 1H, indole-***H***-6), 6.89 – 6.77 (m, 5H, Ph-***H***-3,5, benzyl-***H***-2,4,6), 6.59 (td, *J* = 9.0, 2.3 Hz, 1H, indole-***H***-4), 5.18 (s, 2H, NC***H***$_2$), 2.15 – 2.08 (m, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 157.11 (C_{quat}, Ph-***C***-4), 150.75 (C_{quat}, indole-***C***-5), 138.69 (C_{quat}, benzyl-***C***), 131.27 (+, Ph-***C***-2,6), 130.57 (C_{quat}, indole-***C***), 128.98 (C_{quat}, indole-***C***), 128.22 (+, benzyl-***C***), 126.65 (+, benzyl-***C***), 125.88 (+, benzyl-***C***), 121.91 (C_{quat}, Ph-***C***-1), 115.23 (+, Ph-***C***-3,5), 111.19 (C_{quat}, indole-***C***), 110.62 (+, indole-***C***-6), 106.43 (+, indole-***C***-7), 102.37 (+, indole-***C***-4), 88.96 (C_{quat}, indole-***C***), 46.45 (-, N***C***H$_2$), 9.31 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 330.2 (21), 329.2 ([$M^{+\bullet}$], 100), 238.1 (33), 91.1 (50). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 330.1489, found $[M + H]^+$ 330.1492. $C_{22}H_{19}NO_2$ (M_r = 329.39 g/mol).

1-(4-Fluorobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.25a)

The title compound was prepared from **8.10a** (80 mg, 0.2 mmol) according to general procedure 1. Flash chromatography yielded a dark green oil (71 mg, 97 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.19 – 7.13 (m, 2H, Ph-***H***-2,6), 7.02 – 6.98 (m, 2H, Ph-***H***-3,5), 6.94 – 6.83 (m, 6H, indole-***H***-4, indole-***H***-7, benzyl-***H***), 6.72 (dd, *J* = 8.6, 2.5 Hz, 1H, indole-***H***-6), 5.12 (s, 2H, NC***H***$_2$), 2.21 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 155.47 (C_{quat}, Ph-***C***-4), 149.44 (C_{quat}, indole-***C***-5), 144.29 (C_{quat}, benzyl-***C***), 134.23 (C_{quat}, benzyl-***C***), 131.82 (+, Ph-***C***-2,6), 129.53 (C_{quat}, indole-***C***), 129.14 (C_{quat}, indole-***C***), 127.62 (+, benzyl-***C***), 127.56 (C_{quat}, indole-***C***), 124.26 (C_{quat}, Ph-***C***-1), 115.61 (C_{quat}, indole-***C***), 115.36 (+, Ph-***H***-3,5), 115.29 (+, benzyl-***C***), 111.30 (+, indole-***C***-6), 110.63 (+, indole-***C***-7), 103.50 (+, indole-***C***-4), 46.56 (-, N***C***H$_2$), 9.25 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 348.2 (20), 347.0 ([$M^{+\bullet}$], 100), 238.1 (66), 109.1 (82). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 348.1394, found $[M + H]^+$ 348.1394. $C_{22}H_{18}FNO_2$ (M_r = 347.38 g/mol).

1-(4-Chlorobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.26a)[6]

The title compound was prepared from **8.11a** (200 mg, 0.5 mmol) according to general procedure 1. Flash chromatography yielded a green oil (200 mg, 99 %). ^{1}H-NMR

(300 MHz, DMSO-d_6): δ [ppm] = 9.68 (s, 1H, O***H***-4), 8.74 (s, 1H, indole-OH-***5***), 7.32 – 7.21 (m, 2H, Ph-***H***-2,6), 7.18 – 7.09 (m, 2H, benzyl-***H***), 7.05 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 6.85 (d, *J* = 2.0 Hz, 1H, indole-***H***-4), 6.84 – 6.80 (m, 4H, Ph-***H***-3,5, benzyl-***H***), 6.58 (dd, *J* = 8.7, 2.3 Hz, 1H, indole-***H***-6), 5.17 (s, 2H, NC***H***$_2$), 2.10 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 155.97 (C_{quat}, Ph-***C***-4), 149.62 (C_{quat}, indole-***C***-5), 138.64 (C_{quat}, benzyl-***C***), 137.20 (C_{quat}, benzyl-***C***), 132.70 (C_{quat}, indole-***C***), 131.83 (+, Ph-***H***-2,6), 129.62 (C_{quat}, indole-***C***), 128.71 (+, benzyl-***C***), 127.43 (+, benzyl-***C***), 123.85 (C_{quat}, Ph-***C***-1), 115.44 (+, Ph-***C***-3,5), 111.39 (+, indole-***C***-6), 110.58 (+, indole-***C***-7), 108.32 (C_{quat}, indole-***C***), 103.57 (+, indole-***C***-4), 88.96 (C_{quat}, indole-***C***), 46.99 (-, N***C***H$_2$), 9.45 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 365.1 (24), 363.2 ([M$^{+\bullet}$], 76), 238.1 (54), 125.0 (33), 44.0 (37), 40.0 (100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 364.1099, found [M + H]$^+$ 364.1100. $C_{22}H_{18}ClNO_2$ (M_r = 363.84 g/mol).

1-(4-Bromobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.27a)[33]

The title compound was prepared from **8.12a** (250 mg, 0.6 mmol) according to general procedure 1. Flash chromatography yielded a green oil (220 mg, 91 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.37 – 7.30 (m, 2H, benzyl-***H***), 7.18 – 7.11 (m, 2H, Ph-***H***-2,6), 7.01 (d, *J* = 2.4 Hz, 1H, indole-***H***-4), 6.97 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 6.89 – 6.83 (m, 2H, Ph-***H***-3,5), 6.79 (d, *J* = 8.4 Hz, 2H, benzyl-***H***), 6.72 (dd, *J* = 8.6, 2.4 Hz, 1H, indole-***H***-6), 5.10 (s, 2H, NC***H***$_2$), 2.21 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 155.61 (C_{quat}, Ph-***C***-4), 149.53 (C_{quat}, indole-***C***-5), 137.69 (C_{quat}, benzyl-***C***), 131.98 (C_{quat}, indole-***C***), 131.79 (+, benzyl-***C***), 131.69 (+, Ph-***C***-2,6 Ar-C), 129.59 (C_{quat}, indole-***C***), 127.76 (+, benzyl-***C***), 124.12 (C_{quat}, Ph-***C***-1), 120.78 (C_{quat}, benzyl-***C***), 115.41 (+, Ph-***C***-3,5), 111.37 (+, indole-***C***-6), 110.59 (+, indole-***C***-7), 108.35 (C_{quat}, indole-***C***), 103.56 (+, indole-***C***-4), 89.65 (C_{quat}, indole-***C***), 47.06 (-, N***C***H$_2$), 9.44 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 409.1 (67), 408.2 (32), 407.1 ([M$^{+\bullet}$], 100), 238.1 (80), 171.0 (34), 169.0 (30). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 408.0594, found [M + H]$^+$ 408.0591. $C_{22}H_{18}BrNO_2$ (M_r = 408.29 g/mol).

1-(4-Bromobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-6-ol (8.27b)

The title compound was prepared from **8.12b** (250 mg, 0.6 mmol) according to general procedure 1. Flash chromatography yielded a yellow oil (220 mg, 90 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.43 (d, *J* = 8.5 Hz, 2H, benzyl-***H***), 7.34 – 7.27 (m, 1H, indole-***H***-4), 7.12 (d, *J* = 8.5 Hz, 1H, Ph-***H***-2,6), 6.86 – 6.77 (m, 4H, benzyl-***H***, Ph-***H***-3,5), 6.62 – 6.50 (m, 2H, indole-***H***-5, indole-***H***-7), 5.11 (s, 2H, NC***H***$_2$), 2.14 (s, 3H, indole-C***H***$_3$-

3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 155.59 (C_{quat}, Ph-***C***-4), 149.47 (C_{quat}, indole-***C***-5), 137.61 (C_{quat}, benzyl-***C***), 131.98 (C_{quat}, indole-***C***), 131.21 (+, benzyl-***C***), 131.69 (+, Ph-***C***-2,6 Ar-C), 129.66 (C_{quat}, indole-***C***), 128.03 (+, benzyl-***C***), 124.03 (C_{quat}, Ph-***C***-1), 120.28 (C_{quat}, benzyl-***C***), 114.41 (+, Ph-***C***-3,5), 111.31 (+, indole-***C***-4), 110.09 (+, indole-***C***-5), 108.35 (C_{quat}, indole-***C***), 99.42 (+, indole-***C***-7), 89.88 (C_{quat}, indole-***C***), 47.06 (-, N***C***H_2), 9.41 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 409.1 (46), 407.0 ([$M^{+\bullet}$], 100), 238.1 (100). $C_{22}H_{18}BrNO_2$ (M_r = 408.29 g/mol).

2-(4-Hydroxyphenyl)-3-methyl-1-(4-methylbenzyl)-1*H*-indol-5-ol (8.28a)[6]

The title compound was prepared from **8.13a** (100 mg, 0.3 mmol) according to general procedure 1. Flash chromatography yielded a green solid (85 mg, 91 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.23 – 7.15 (m, 2H, Ph-***H***-2,6), 7.09 – 6.97 (m, 4H, benzyl-***H***), 6.91 – 6.80 (m, 4H, indole-***H***-4, indole-***H***-7, Ph-***H***-3,5), 6.70 (dd, *J* = 8.6, 2.5 Hz, 1H, indole-***H***-6), 5.12 (s, 2H, NC***H***$_2$), 2.29 (s, 3H, benzyl-C***H***$_3$), 2.22 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 155.46 (C_{quat}, Ph-***C***-4), 149.31 (C_{quat}, indole-***C***-5), 138.61 (C_{quat}, benzyl-***C***), 136.57 (C_{quat}, benzyl-***C***), 135.59 (C_{quat}, indole-***C***), 132.14 (C_{quat}, indole-***C***), 131.85 (+, Ph-***C***-2,6), 129.26 (+, benzyl-***C***-3,5), 125.94 (+, benzyl-***C***-2,6), 124.41 (C_{quat}, Ph-***C***-1), 115.32 (+, Ph-***C***-3,5), 111.18 (+, indole-***C***-6), 110.87 (+, indole-***C***-7), 107.91 (C_{quat}, indole-***C***), 103.39 (+, indole-***C***-4), 92.82 (C_{quat}, indole-***C***), 47.40 (-, N***C***H_2), 14.00 (+, benzyl-***C***H_3), 9.50 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 344.2 (22), 343.2 ([$M^{+\bullet}$], 100), 238.1 (21), 105.1 (90). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 344.1645, found $[M + H]^+$ 344.1645. $C_{23}H_{21}NO_2$ (M_r = 343.42 g/mol).

1-(Biphenyl-4-ylmethyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (8.29a)

The title compound was prepared from **8.14a** (250 mg, 0.6 mmol) according to general procedure 1. Flash chromatography yielded a white solid (230 mg, 98 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 9.68 (s, 1H, O***H***-4), 8.73 (s, 1H, indole-O***H***-5), 7.58 (dd, *J* = 5.3, 3.3 Hz, 2H, biphenyl-***H***), 7.50 (d, *J* = 8.3 Hz, 2H, biphenyl-***H***), 7.45 – 7.37 (m, 2H, biphenyl-***H***), 7.35 – 7.30 (m, 1H, biphenyl-***H***), 7.19 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2,6), 7.09 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 6.92 (d, *J* = 8.3 Hz, 2H, biphenyl-***H***), 6.90 – 6.81 (m, 3H, indole-***H***-4, Ph-***H***-3,5), 6.60 (dt, *J* = 8.6, 4.3 Hz, 1H, indole-***H***-6), 5.23 (s, 2H, NC***H***$_2$), 2.13 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 157.10 (C_{quat}, Ph-***C***-4), 150.76 (C_{quat}, indole-***C***-5), 139.66 (C_{quat}, biphenyl-***C***), 138.57 (C_{quat}, biphenyl-***C***), 137.98 (C_{quat}, biphenyl-***C***), 131.30 (+, Ph-***C***-2,6), 130.65 (C_{quat}, indole-***C***), 129.12 (C_{quat}, indole-***C***), 128.76 (+, biphenyl-***C***), 127.23 (+, biphenyl-***C***), 126.54 (+, biphenyl-***C***), 126.48 (+, biphenyl-***C***), 126.40 (+, biphenyl-***C***), 121.92 (C_{quat}, Ph-***C***-1), 115.28 (+, Ph-***C***-3,5),

111.20 (+, indole-***C***-6), 110.60 (+, indole-***C***-7), 106.49 (C_{quat}, indole-***C***), 102.33 (+, indole-***C***-4), 93.53 (C_{quat}, indole-***C***), 45.97 (-, N***C***H_2), 9.53 (+, indole-3 ***C***H_3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 407.1 (30), 405.0 ([$M^{+\bullet}$], 100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 406.1802, found $[M + H]^+$ 406.1803. $C_{28}H_{23}NO_2$ (M_r = 405.49 g/mol).

1-(Biphenyl-4-ylmethyl)-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-6-ol (8.29b)

The title compound was prepared from **8.14b** (250 mg, 0.6 mmol) according to general procedure 1. Flash chromatography yielded a white solid (220 mg, 96 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.53 (dd, *J* = 7.6, 6.1 Hz, 2H, biphenyl-***H***), 7.50 (d, *J* = 8.3 Hz, 2H, biphenyl-***H***), 7.49 – 7.34 (m, 2H, biphenyl-***H***), 7.36 – 7.24 (m, 1H, biphenyl-***H***), 7.17 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2,6), 7.00 (d, *J* = 8.2 Hz, 1H, indole-***H***-7), 6.89 – 6.82 (m, 2H, biphenyl-***H***), 6.75 – 6.69 (m, 1H, indole-***H***-4), 6.65 (dd, *J* = 6.0, 2.5 Hz, 2H, Ph-***H***-3,5), 6.31 – 6.16 (m, 1H, indole-***H***-6), 5.12 (s, 2H, NC***H***$_2$), 2.27 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 155.61 (C_{quat}, Ph-***C***-4), 151.00 (C_{quat}, indole-***C***-5), 139.86 (C_{quat}, biphenyl-***C***), 138.41 (C_{quat}, biphenyl-***C***), 137.60 (C_{quat}, biphenyl-***C***), 131.89 (+, Ph-***C***-2,6), 132.03 (C_{quat}, indole-***C***), 128.79 (C_{quat}, indole-***C***), 127.68 (+, biphenyl-***C***), 127.31 (+, biphenyl-***C***), 127.00 (+, biphenyl-***C***), 126.93 (+, biphenyl-***C***), 126.56 (+, biphenyl-***C***), 120.81 (C_{quat}, Ph-***C***-1), 119.52 (+, Ph-***C***-3,5), 115.23 (+, indole-***C***-4), 109.22 (+, indole-***C***-5), 108.71 (C_{quat}, indole-***C***), 100.91 (C_{quat}, indole-***C***), 96.27 (+, indole-***C***-7), 47.23 (-, N***C***H_2), 9.56 (+, indole-***C***H_3-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 406.2 (22), 405.1 ([$M^{+\bullet}$], 88), 238.1 (25), 167.0 (100). HRMS (ESI-MS) *m/z* calcd. $[M + H]^+$ 406.1802, found $[M + H]^+$ 406.1798. $C_{28}H_{23}NO_2$ (M_r = 405.49 g/mol).

2-(4-Hydroxyphenyl)-3-methyl-1-(3-phenylpropyl)-1*H*-indol-5-ol (8.30a)

The title compound was prepared from **8.15a** (200 mg, 0.5 mmol) according to general procedure 1. Flash chromatography yielded a yellow oil (130 mg, 36 %). ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 7.50 – 7.41 (m, 3H, indole-***H***-7, Ph-***H***), 7.24 – 7.12 (m, 5H, Ph-***H***), 6.99 (d, *J* = 2.0 Hz, 1H, indole-***H***-4), 6.96 – 6.92 (m, 2H, Ph-***H***), 6.79 (dd, *J* = 8.8, 2.5 Hz, 1H, indole-***H***-6), 4.25 (t, *J* = 7.1 Hz, 2H, NC***H***$_2$$CH_2$$CH_2$), 2.83 (t, 2H, NCH_2CH_2C***H***$_2$), 2.24 (s, 3H, indole-C***H***$_3$-3), 2.00 – 1.96 (m, 2H, NCH_2C***H***$_2$$CH_2$). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 157.03 (C_{quat}, Ph-***C***-4), 150.40 (C_{quat}, indole-***C***-5), 141.13 (C_{quat}, benzyl-***C***), 132.68 (C_{quat}, indole-***C***), 131.27 (+, Ph-***C***-2,6), 130.57 (C_{quat}, indole-***C***), 127.67 (+, benzyl-***C***), 127.53 (+, benzyl-***C***), 121.85 (C_{quat}, Ph-***C***-1), 118.97 (+, benzyl-***C***), 115.29 (+, Ph-***C***-3,5), 111.30 (C_{quat}, indole-***C***), 110.55 (+, indole-***C***-6), 106.65 (+, indole-***C***-7), 104.39 (+, indole-***C***-4), 90.18 (C_{quat}, indole-***C***), 53.21 (-, N***C***H_2), 33.42 (-,

$NCH_2CH_2\boldsymbol{C}H_2$), 31.30 (-, $NCH_2\boldsymbol{C}H_2CH_2$), 9.30 (+, indole-$\boldsymbol{C}H_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 357.2 ([$M^{+\bullet}$], 100), 266.2 (25), 91.0 (30). $C_{24}H_{23}NO_2$ (M_r = 357.44 g/mol).

2-(4-Hydroxyphenyl)-3-methyl-1-(4-nitrobenzyl)-1*H*-indol-5-ol (8.31a)

The title compound was prepared from **8.16a** (300 mg, 0.7 mmol) according to general procedure 2. Flash chromatography yielded a pale yellow solid (240 mg, 92 %). Flash chromatography yielded a dark yellow solid (170 mg, 22 %); mp 152 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.20 – 8.12 (m, 2H, benzyl-***H***-3,5), 7.49 – 7.43 (m, 1H, indole-***H***-7), 7.36 – 7.32 (m, 1H, indole-***H***-4), 7.26 (d, *J* = 8.7 Hz, 1H, indole-***H***-6), 7.02 – 6.87 (m, 4H, benzyl-***H***-2,6, Ph-***H***-2,6), 6.84 – 6.67 (m, 2H, Ph-***H***-3,5), 5.26 (s, 2H, $NC\boldsymbol{H}_2$), 1.57 (s, 3H, indole-$C\boldsymbol{H}_3$-3). ^{13}C-NMR (75 MHz, $CDCl_3$) δ [ppm] = 156.95 (C_{quat}, Ph-***C***-4), 150.57 (C_{quat}, indole-***C***-5), 147.34 (C_{quat}, benzyl-***C***), 137.98 (C_{quat}, indole-***C***), 131.32 (+, benzyl-***C***), 129.00 (+, benzyl-***C***), 126.88 (C_{quat}, indole-***C***), 125.47 (+, Ph-***C***-2,6), 122.21 (C_{quat}, Ph-***C***-1), 121.08 C_{quat}, benzyl-***C***), 115.16 (C_{quat}, indole-***C***), 113.54 (+, Ph-***C***-3,5), 111.20 (+, indole-***C***-6), 110.93 (+, indole-***C***-7), 106.03 (C_{quat}, indole-***C***), 102.17 (+, indole-***C***-4), 47.43 (-, $N\boldsymbol{C}H_2$), 9.33 (+, indole-$\boldsymbol{C}H_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 374.1 (30), 373.1 ([$M^{+\bullet}$], 100. $C_{22}H_{18}N_2O_4$ (M_r = 374.39 g/mol).

4-{[5-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoic acid (8.32a)

The title compound was prepared from **8.14a** (320 mg, 0.7 mmol) according to general procedure 2. Flash chromatography yielded a yellow oil (150 mg, 54 %). ^{1}H-NMR (300 MHz, MeOD) δ [ppm] = 7.87 – 7.79 (m, 2H, benzyl-***H***-3,5), 7.13 – 7.07 (m, 2H, Ph-***H***-2,6), 6.99 (d, *J* = 8.7 Hz, 1H, indole-***H***-4), 6.93 (d, *J* = 3.1 Hz, 1H, indole-***H***-7), 6.91 (d, *J* = 2.7 Hz, 2H, benzyl-***H***-2,6), 6.84 – 6.78 (m, 2H, Ph-***H***-3,5), 6.65 (dd, *J* = 8.7, 2.3 Hz, 1H, indole-***H***-6), 5.25 (s, 2H, $NC\boldsymbol{H}_2$), 2.17 (s, 3H, indole-$C\boldsymbol{H}_3$-3). ^{13}C-NMR (75 MHz, MeOD) δ [ppm] = 168.07 (C_{quat}, ***C***OOH), 157.77 (C_{quat}, indole-***C***-6), 153.11 (C_{quat}, Ph-***C***-4), 147.01 (C_{quat}, benzyl-***C***-1), 139.83 (C_{quat}, indole-***C***), 132.68 (+, Ph-***C***-2,6), 130.93 (C_{quat}, indole-***C***), 130.80 (+, benzyl-***C***-3,5), 127.21 (C_{quat}, benzyl-***C***-4), 127.10 (+, benzyl-***C***-2,6), 124.44 (C_{quat}, Ph-***C***-1), 124.30 (C_{quat}, indole-***C***), 116.30 (+, indole-***C***-6), 116.17 (+, Ph-***C***-3,5), 112.31 (C_{quat}, indole-***C***), 111.23 (+, indole-***C***-7), 104.07 (+, indole-***C***-4), 47.82 (-, $N\boldsymbol{C}H_2$), 9.52 (+, indole-$\boldsymbol{C}H_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 375.0 (25), 374.0 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 374.1387, found [M + H]$^+$ 374.1387. $C_{23}H_{19}NO_4$ (M_r = 373.40 g/mol).

4-{[6-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoic acid (8.32b)

The title compound was prepared from **8.14b** (320 mg, 0.7 mmol) according to general procedure 2. Flash chromatography yielded a yellow oil (140 mg, 51 %). ^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 7.88 – 7.77 (m, 2H, benzyl-***H***-3,5), 7.34 (d, *J* = 8.4 Hz, 1H, indole-***H***-4), 7.11 – 7.01 (m, 2H, Ph-***H***-2,6), 6.93 (d, *J* = 8.3 Hz, 2H, benzyl-***H***-2,6), 6.83 – 6.74 (m, 2H, Ph-***H***-3,5), 6.64 (dd, *J* = 8.4, 2.1 Hz, 1H, indole-***H***-5), 6.55 (d, *J* = 2.0 Hz, 1H, indole-***H***-7), 5.13 (s, 2H, NC***H***$_2$), 2.18 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, MeOD) δ [ppm] = 169.67 (C_{quat}, ***C***OOH), 158.46 (C_{quat}, indole-***C***-6), 154.49 (C_{quat}, Ph-***C***-4), 145.82 (C_{quat}, benzyl-***C***-1), 139.20 (C_{quat}, indole-***C***), 137.62 (C_{quat}, indole-***C***), 132.92 (+, Ph-***C***-2,6), 131.00 (+, benzyl-***C***-3,5), 130.58 (C_{quat}, benzyl-***C***-4), 127.28 (+, benzyl-***C***-2,6), 124.44 (C_{quat}, Ph-***C***-1), 124.33 (C_{quat}, indole-***C***), 120.27 (+, indole-***C***-4), 116.31 (+, Ph-***C***-3,5), 110.37 (+, indole-***C***-5), 109.65 (C_{quat}, indole-***C***), 96.74 (+, indole-***C***-7), 48.13 (-, N***C***H$_2$), 9.69 (+, indole-***C***H$_3$-3). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 391.0 (20), 390.0 (100), 373.9 ([M$^{+\bullet}$], 70), 373.0 (15). HRMS (ESI-MS) *m/z* calcd. [M + H]$^+$ 373.1309, found [M + H]$^+$ 373.1304. $C_{23}H_{19}NO_4$ (M_r = 373.40 g/mol).

4-[(5-Hydroxy-3-methyl-2-phenyl-1*H*-indol-1-yl)methyl]benzoic acid (8.41)

The title compound was prepared from **8.38** (130 mg, 0.3 mmol) according to general procedure 2. Flash chromatography yielded a yellow solid (110 mg, 96 %); mp 158 °C. ^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 7.82 (d, *J* = 8.3 Hz, 2H, benzoate-***H***-3,5), 7.45 – 7.32 (m, 3H, Ph-***H***-2,4,6), 7.29 (dd, *J* = 7.7, 1.7 Hz, 2H, benzoate-***H***-2,6), 7.02 (d, *J* = 8.7 Hz, 1H, indole-***H***-7), 6.95 (d, *J* = 2.2 Hz, 1H, indole-***H***-4), 6.90 (d, *J* = 8.3 Hz, 2H, Ph-***H***-3,5), 6.68 (dd, *J* = 8.7, 2.4 Hz, 1H, indole-***H***-6), 5.24 (s, 2H, NC***H***$_2$), 2.19 (s, 3H, indole-3 C***H***$_3$). ^{13}C-NMR (75 MHz, MeOD): δ [ppm] = 169.56 (C_{quat}, ***C***OOH), 152.01 (C_{quat}, indole-***C***-5), 145.78 (C_{quat}, benzoate-***C***-1), 139.60 (C_{quat}, indole-***C***), 133.49 (C_{quat}, indole-***C***), 133.39 (C_{quat}, indole-***C***), 131.60 (+, benzoate-***C***-3,5), 131.08 (C_{quat}, Ph-***C***-1), 130.95 (+, Ph-***C***-3,5), 130.68 (C_{quat}, indole-***C***), 129.53 (+, Ph-***C***-4), 129.09 (+, benzoate-***C***-2,6), 127.18 (+, Ph-***C***-2,6), 112.86 (+, indole-***C***-6), 111.58 (+, indole-***C***-7), 109.61 (C_{quat}, indole-***C***), 104.19 (+, indole-***C***-4), 48.19 (-, N***C***H$_2$), 9.57 (+, indole-***C***H$_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 359.0 (26), 358.0 ([M + H]$^+$, 100). HRMS (ESI-MS) *m/z* calcd [M + H]$^+$ 358.1438, found [M + H]$^+$ 358.1440. $C_{23}H_{19}NO_3$ (M_r = 357.40 g/mol).

4-{[5-Hydroxy-2-(4-hydroxyphenyl)-1*H*-indol-1-yl]methyl}benzoic acid (8.42)

The title compound was prepared from **8.39** (40 mg, 0.1 mmol) according to general procedure 2. Flash chromatography yielded a yellow solid (30 mg, 83 %); mp 147 °C. ^{1}H-

NMR (300 MHz, MeOD): δ [ppm] = 7.88 (d, *J* = 8.2 Hz, 2H, benzoate-***H***-3,5), 7.24 – 7.18 (m, 2H, Ph-***H***-2,6), 7.01 (dd, *J* = 8.4, 2.0 Hz, 2H, benzoate-***H***-2,6), 6.95 (dd, *J* = 4.5, 2.1 Hz, 1H, indole-***H***-7), 6.79 (dd, *J* = 6.7, 2.0 Hz, 2H, Ph-***H***-3,5), 6.68 – 6.62 (m, 2H, indole-***H***-4, indole-***H***-6), 6.36 (s, 1H, indole-***H***-3), 5.36 (s, 2H, NC***H***$_2$). ^{13}C-NMR (75 MHz, MeOD): δ [ppm] = 172.88 (C_{quat}, ***C***OOH), 158.83 (C_{quat}, indole-***C***-5), 152.37 (C_{quat}, Ph-***C***-4), 145.54 (C_{quat}, benzoate-***C***-1), 134.23 (C_{quat}, indole-***C***), 131.53 (+, Ph-***C***-2,6), 131.08 (+, benzonate-***C***-3,5), 130.87 (C_{quat}, benzoate-***C***-4), 130.66 (C_{quat}, indole-***C***), 127.19 (+, benzoate-***C***-2,6), 125.26 (C_{quat}, Ph-***C***-1), 116.42 (+, Ph-***C***-3,5), 115.56 (C_{quat}, indole-***C***), 112.35 (+, indole-***C***-6), 111.73 (+, indole-***C***-7), 105.39 (+, indole-***C***-4), 85.18 (C_{quat}, indole-***C***), 48.41 (-, N***C***H$_2$). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 361.0 (35), 360.0 ($[M + H]^+$, 100). $C_{22}H_{17}NO_4$ (M_r = 359.37 g/mol).

4-{[(5-*sec*-Butyl-2-(4-hydroxyphenyl)-1*H*-indol-1-yl]methyl}benzoic acid (8.43)

The title compound was prepared from **8.40** (130 mg, 0.3 mmol) according to general procedure 2. Flash chromatography yielded yellow crystals (100 mg, 86 %); mp 137 °C. ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ [ppm] = 12.88 (s, 1H, COO***H***), 9.71 (s, 1H, O***H***-4), 7.82 (d, *J* = 8.2 Hz, 2H, benzoate-***H***-3,5), 7.36 (s, 1H, indole-***H***-4), 7.25 (d, *J* = 8.5 Hz, 2H, Ph-***H***-2,6), 7.17 (d, *J* = 8.4 Hz, 1H, indole-***H***-7), 7.03 (d, *J* = 8.2 Hz, 2H, benzoate-***H***-2,6), 6.92 (dd, *J* = 8.5, 1.3 Hz, 1H, indole-***H***-6), 6.82 (d, *J* = 8.5 Hz, 2H, Ph-***H***-3,5), 6.47 (s, 1H, indole-***H***-3), 5.43 (s, 2H, NC***H***$_2$), 2.63 (h, *J* = 7.0 Hz, 1H, indole-C***H***(CH_3)(CH_2CH_3)), 1.58 (p, *J* = 7.3 Hz, 2H, indole-CH(CH_3)(C***H***$_2$$CH_3$)), 1.22 (d, *J* = 6.9 Hz, 3H, indole-CH(C***H***$_3$)(CH_2CH_3)), 0.82 – 0.72 (m, 3H, indole-CH(CH_3)(CH_2C***H***$_3$)). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$): δ [ppm] = 166.89 (C_{quat}, ***C***OOH), 157.42 (C_{quat}, Ph-***C***-4), 143.56 (C_{quat}, benzoate-***C***-1), 141.41 (C_{quat}, indole-***C***), 138.46 (C_{quat}, indole-***C***), 135.89 (C_{quat}, benzoate-***C***-4), 130.06 (+, Ph-***C***-2,6), 129.51 (+, benzonate-***C***-3,5), 129.44 (C_{quat}, indole-***C***), 127.88 (C_{quat}, indole-***C***), 125.96 (+, benzoate-***C***-2,6), 122.67 (C_{quat}, Ph-***C***-1), 120.71 (+, indole-***C***-4), 117.56 (+, indole-***C***-6), 115.42 (+, Ph-***C***-3,5), 110.11 (+, indole-***C***-7), 100.98 (+, indole-***C***-3), 46.52 (-, N***C***H$_2$), 40.89 (+, indole-***C***H(CH_3)(CH_2CH_3)), 31.19 (-, indole-CH(CH_3)(***C***H$_2$$CH_3$)), 22.36 (+, indole-CH(***C***H$_3$)(CH_2CH_3)), 12.18 (+, indole-CH(CH_3)(CH_2***C***H$_3$)). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 401.0 (25), 400.0 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M - H]^-$ 398.1770, found $[M - H]^-$ 398.1762. $C_{22}H_{17}NO_4$ (M_r = 399.48 g/mol).

4-{[6,7-Dichloro-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoic acid (8.47)

The title compound was prepared from **8.46** (150 mg, 0.3 mmol) according to general procedure 2. Flash chromatography yielded orange crystals (120 mg, 88 %); mp 155 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 12.84 (s, 1H, COO***H***), 9.79 (s, 1H, O***H***-4), 7.79 (d, *J* = 8.3 Hz, 2H, benzoate-***H***-3,5), 7.58 (d, *J* = 8.4 Hz, 1H, indole-***H***-4), 7.30 (d, *J* = 8.4 Hz, 1H, indole-***H***-5), 7.16 (d, *J* = 8.4 Hz, 2H, Ph-***H***-2,6), 6.83 (dd, *J* = 8.4, 4.1 Hz, 4H, benzoate-***H***-2,6, Ph-***H***-3,5), 5.62 (s, 2H, NC***H***$_2$), 2.17 (s, 3H, indole-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 166.87 (C_{quat}, ***C***OOH), 157.77 (C_{quat}, Ph-***C***-4), 144.76 (C_{quat}, benzoate-***C***-1), 141.08 (C_{quat}, indole-***C***), 131.88 (C_{quat}, indole-***C***), 131.64 (+, benzoate-***C***-3,5), 130.12 (C_{quat}, indole-***C***), 129.52 (+, Ph-***C***-2,6), 129.30 (C_{quat}, indole-***C***-Cl), 125.40 (C_{quat}, benzoate-***C***-4), 125.04 (+, benzoate-***C***-2,6), 121.25 (+, indole-***C***-5), 120.38 (C_{quat}, Ph-***C***-1), 118.40 (+, indole-***C***-4), 115.38 (+, Ph-***C***-3,5), 113.38 (C_{quat}, indole-***C***-Cl), 109.26 (C_{quat}, indole-***C***), 48.11 (-, N***C***H$_2$), 9.07 (+, indole-***C***H$_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 428.9 (25), 427.6 (80), 425.9 ($[M + H]^+$, 100). HRMS (ESI-MS) *m/z* calcd. $[M - H]^-$ 424.0513, found $[M - H]^-$ 424.0520. $C_{23}H_{17}Cl_2NO_3$ (M_r = 425.29 g/mol).

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9 Indolizines: a bioisosteric approach to inhibitors of bacterial hyaluronidases

9.1 Introduction

A fundamental strategic approach in medicinal chemistry involves the development and application of bioisosteres.[1] The concept of bioisosterism can be summarized as the introduction of structural changes into a given active compound, leading to a derivative that broadly maintains the bioactivity on the biological target. The definition of bioisosteres implies compounds causing similar biological effects. Therefore, compounds bearing isosteric molecular features do not necessarily represent bioisosteres. A universal, rational prediction of bioisosteres is impossible as it strongly depends on the individual target, physicochemical parameters and biological mimicry. As a consequence, the evaluation of suitable structural modifications remains the most challenging step for a medicinal chemist. To quantify parameters of bioisosteric similarity, various methods and concepts such as size, shape, electronic distribution, polarizability, dipole, polarity, lipophilicity and pK_a values play key roles.[1]

For the current approach, a computer-assisted prediction of bioisosteric properties for novel inhibitors was performed. For this purpose, a compound library containing 32 potent inhibitors of the target enzyme served as foundation. The selection of suitable inhibitors was based on experience and intuition. To quantify inhibitor similarity, or ideally bioiosteric molecular properties, the surface polarities of the library compounds were computed. Surface polarity, that is conductor surface polarization charge density σ, represents an established parameter derived from quantum chemistry.[2] In detail, the σ-profile measurement was defined by the conductor-like screening model for realistic solvation (COMSO-RS). The acronym for the σ-profile based drug similarity measurement was termed COSMO*sim*.

Hence, the current approach was entirely based on the assumption that bioisosteres must have, to a certain extent, similar physiochemical properties that determine their interactions with different “environments” (solvents, membranes, biological targets). As a result, the COSMO*sim* based calculations proposed indolizines as novel, putatively bioisosteric molecules to possess inhibitory activity on the target enzyme.

Indolizine was discovered by Angeli in 1890 and first prepared by Scholz in 1912 from α-picoline and acetic anhydride.[3, 4] The generally accepted numbering system of indolizine is shown in Figure 9.1. Alternative notations for the molecular structure of indolizine derivatives are pyrrodine, pyrindole, 8-pyrrolopyridine, pyrrolo[1,2-*a*]pyridine or pyrrocoline.

indolizine

2-phenylindolizine

Figure 9.1 Molecular structure of indolizine (including atomic numbering system) and 2-phenylindolizine.

Indolizine seems to play a minor role for drug development and discovery in comparison to its isomer indole, despite the fact that it is one of the fundamental nitrogen heterocyclic systems.[4] Indolizine derivatives were discovered in natural products, for example among alkaloids such as pumiliotoxin or swainsonine. Synthetic organic molecules bearing an indolizine motif were reported as aromatase inhibitors,[5] agonist of the estrogen receptor,[6] antibacterial agents,[7] histamine H_3 receptor antagonists,[8] hypoglycemic agents,[9] inhibitors of 15-lipoxygenase[10] and of secretory phospholipase A2.[11] A comprehensive review on methods for the construction of the indolizine nucleus was published by Uchida and Matasumoto in 1976.[4] Recently, organo-metal catalyzed isomerization reaction synthesis[12-15] and multicomponent reaction synthesis[16, 17] of indolizine derivatives were reported.

For the virtual screening approach, structural requirements, e.g. drug-like properties, solubility and innovational character, were included. Commercially available or easily accessible starting materials were preferred for the construction of indolizine derivatives. Products available via multicomponent reaction synthesis (cf. chapter 5) were excluded. Accordingly, the target molecules, bearing an indolizine core structure, were mainly accessible via conventional synthesis. COSMO*sim* based calculations gave a total of 300 hits which were ranked in terms of expected binding affinity on the target protein. To prove the suitability of COSMO*sim* based indolizine derivatives in principal, a single compound was synthesized and tested for inhibition of *Sag*Hyal$_{4755}$. Besides, a "minimal pharmacophore", that is a 2-phenylindolizine without substituents was synthesized as reference compound. The results are presented in the following sections.

9.2 COSMO*sim* based modeling of bioisosteric inhibitors for *Sag*Hyal$_{4755}$

For the computer-assisted evaluation of new inhibitors, a compound library containing 32 selected inhibitors of *Sag*Hyal$_{4755}$ served as foundation (see section B.1.2, (appendix II)). As ligand-protein interactions were not elucidated, the project was conceptualized entirely as a ligand-based approach. The evaluation of the electronic properties (COSMO*sim*) was performed for all compounds of the library. Accordingly, structural information from well-established inhibitors was provided and abstracted for the *de novo* design of (bioisosteric) inhibitors. To illustrate the COSMO surface of such compounds, two examples were illustrated in this section. The surface models of 1-ethyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol is shown in Figure 9.2.

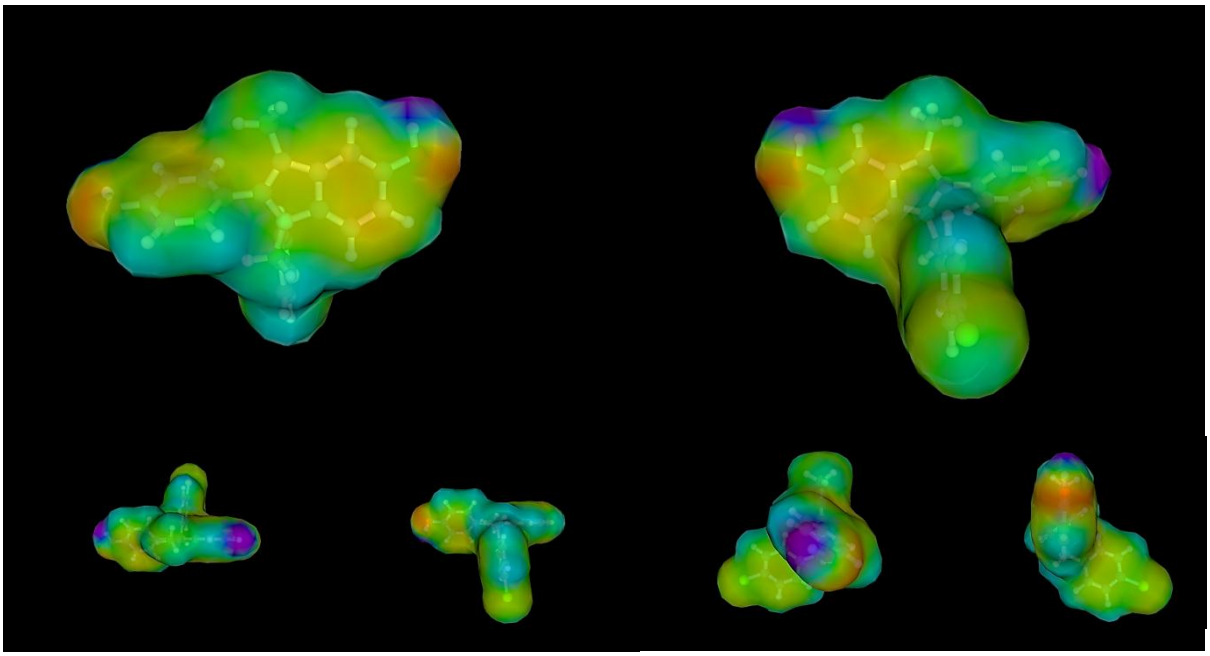

Figure 9.2 Surface polarity of 1-ethyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol [a]. The regions of strongly negative molecular polarity are displayed in red. The strongly positive molecular regions carrying negative σ are colored blue, while the neutral parts of the molecules with σ close to zero appear green; [a] cf. section 5.6.

As a second example, the COSMO surface color coded by the polarization charge density for 4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol is given in Figure 9.3.

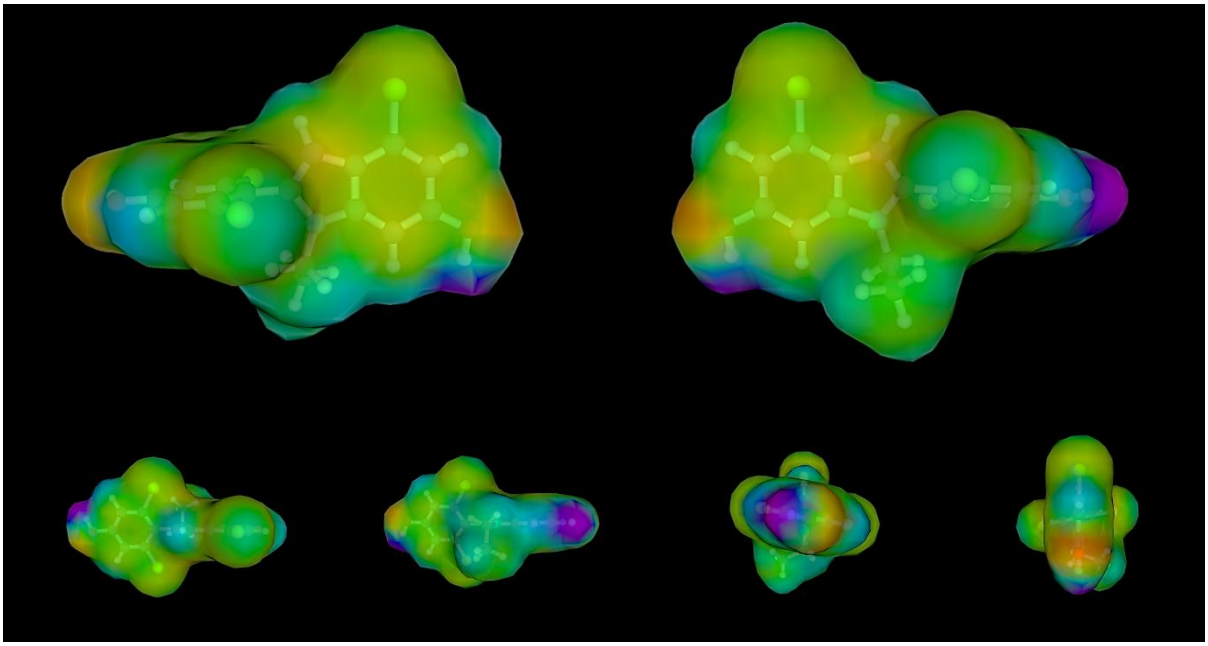

Figure 9.3 Surface polarity of 4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol [a]. The regions of strongly negative molecular polarity are displayed in red. The strongly positive molecular regions carrying negative σ are colored blue, while the neutral parts of the molecules with σ close to zero appear green; [a] cf. section 5.6.

The information on relevant parameters must be regarded as the most critical step and therefore strongly depends on an appropriate selection of compounds. This is even more significant for an ensemble of only 32 molecules. It must be reconsidered that an inappropriate selection of library molecules, e.g. molecules with low intermolecular similarity, might result in a failure of the entire method. To minimize such risks, the selection of ligands included two aspects. At first, substances bearing an identical chemical scaffold (2-phenylindoles) with IC_{50} values from 750 µM to 6 µM were selected. Hence, ligands of improved potency with a similar chemical scaffold were included. Secondly, benzo[*b*]thiophenes were selected as isosteric inhibitors to explore additional structural patterns.

The structural information on the ligands was abstracted for subsequent molecular similarity calculations. As a result, computer-assisted molecular design involved the indolizine skeleton as novel structural scaffold. With respect to structural diversity, three subclasses including different substitution patterns were elaborated. Besides, indolizine-based substances were ranked according to their proposed binding affinity on the target lyase. For each class of indolizines, the top 100 ranked molecules are illustrated in the following section.

The indolizine derivatives of the first compound class, ranked according to their proposed binding affinity on *Sag*Hyal$_{4755}$, are listed in Table 9.1.

Table 9.1 Indolizine derivatives proposed by virtual chemistry as inhibitors of *Sag*Hyal$_{4755}$ (compound class 1).

No.[a]	SMILES-code[b]
1	Cc1c(cc2cccc(C(=O)O)n12)c3ccc(Br)cc3
2	CN(C)c1c(c(C)n2cccc(O)c12)c3ccc(Br)cc3
3	Cc1c(cc2c(C(=O)O)c(C)ccn12)c3ccc(Br)cc3
4	Cc1c(c(CCc2ccccc2)c3ccccn13)c4ccc(Cl)cc4
5	Oc1ccn2c3CCc4ccccc4-c3cc2c1OCc5ccc(Cl)cc5
6	Cc1c(cc2ccc3cccc([N+](=O)[O])c3n12)c4ccc(Br)cc4
7	CCN(CC)c1c(c(C)n2cccc(O)c12)c3ccc(Br)cc3
8	Cc1c(c(C(=O)O)c2c(cccn12)C(F)(F)F)c3ccc(Br)cc3
9	Cc1c(c(n2cc(Br)cn2)c3ccccn13)c4ccc(Br)cc4
10	Cc1c(c(O)c2ccc(O)c(Cl)n12)c3ccc(Br)cc3
11	CCc1cc2c(OCc3ccc(Cl)cc3)c(O)ccn2c1C
12	Cc1c(cc2cc(C#N)c3ccccc3n12)c4ccc(Br)cc4
13	Cc1c(cc2cc(O)c(C(=O)O)c(O)n12)c3ccc(Br)cc3
14	Cc1c(c(C(=O)O)c2ccc(Cl)cn12)c3ccc(Br)cc3
15	Cc1c(cc2cc(ccn12)C(=O)O)c3ccc(Br)cc3
16	Cc1c(c(C(=O)O)c2ccccn12)c3ccc(Br)cc3
17	Cc1c(c(N2CCC[C@H](CO)C2)c3ccccn13)c4ccc(Br)cc4
18	Oc1c(c([C@H](Br)c2ccc(Br)cc2)n3ccc(Cl)cc13)c4ccccc4O
19	Cc1c(cc2c(OCc3ccc(Cl)cc3)c(O)ccn12)C(F)(F)F
20	Cc1c(cc2c(cccn12)C(=O)O)c3ccc(Br)cc3
21	Cc1c(c(C)n2c(S)c(C#N)cc(C)c12)c3ccc(Br)cc3
22	Cc1c(cc2c(cc3cccnc3n12)C(=O)O)c4ccc(Br)cc4
23	Cc1c(c(N2CCCC2)c3c(O)cccn13)c4ccc(Br)cc4
24	Cc1c(cc2c(Br)c(C)c(C#N)c(O)n12)c3ccc(Br)cc3
25	Cc1c(c(CN2CCCC2)c3ccc(cn13)C(=O)O)c4ccc(Br)cc4
26	Oc1ccn2c3CCc4nonc4-c3cc2c1OCc5ccc(Cl)cc5
27	Cc1c(cc2c3ccc(Cl)nc3ccn12)c4ccc(Br)cc4
28	Cc1c(c(O)c2cccc(CO)n12)c3ccc(Br)cc3
29	CCc1c(cc2c(OCc3ccc(Cl)cc3)c(O)ccn12)C(F)(F)F
30	C[C@@H](Br)c1cc2c(OCc3ccc(Cl)cc3)c(O)ccn2c1C
31	COC(=O)c1ccn2c(C)c(cc2c1)c3ccc(Br)cc3
32	Cc1c(c(C(=O)O)c2cccc(n12)C(F)(F)F)c3ccc(Br)cc3
33	Cc1c(cc2ccc(cn12)C(=O)O)c3ccc(Br)cc3
34	Cc1c(cc2c(O)cc3ccccc3n12)c4ccc(Br)cc4
35	Cc1c(cc2ccc3c(C)ccc(O)c3n12)c4ccc(Br)cc4
36	Cc1c(c(c2ccc(Cl)nn2)c3ccccn13)c4ccc(Br)cc4
37	Cc1c(cc2cc(O)c3ccc(C)c(C)c3n12)c4ccc(Br)cc4
38	Cc1c(cc2cc(O)c3ccc(Cl)cc3n12)c4ccc(Br)cc4
39	CCOC(=O)c1cccn2c(C)c(c(Br)c12)c3ccc(Br)cc3
40	Cc1c(c(C(=O)O)c2ccc(Br)cn12)c3ccc(Br)cc3
41	Oc1ccccc1c2c(Cl)c3cccc(Cl)n3c2[C@H](Br)c4ccc(Br)cc4
42	Cc1cccn2c([C@H](Br)c3ccc(Br)cc3)c(c(Cl)c12)c4ccccc4O
43	Cc1c(cc2cc(S)c3ccccc3n12)c4ccc(Br)cc4
44	Cc1c(cc2cc(O)c3cc(C)ccc3n12)c4ccc(Br)cc4
45	Cc1ccc(Cl)n2c([C@H](Br)c3ccc(Br)cc3)c(cc12)c4ccccc4O
46	Cc1c(cc2cc(O)c3cc(C)c(C)cc3n12)c4ccc(Br)cc4
47	CC(=O)Oc1c(c(C)n2ccc(Br)cc12)c3ccc(Br)cc3
48	Oc1ccn2c3CCc4nonc4-c3cc2c1OCc5ccc(Cl)cc5Cl
49	Cc1c(c(Br)c2cccc(C#N)n12)c3ccc(Br)cc3
50	Cc1c(c(C(=O)O)c2c(Br)cccn12)c3ccc(Br)cc3
51	Cc1cc2c(OCc3ccc(Cl)cc3)c(O)ccn2c1C

52	Cc1cc2c(OCc3ccc(Cl)cc3)c(O)ccn2c1c4ccccc4
53	Cc1c(cc2c(cc3cc(C)ccc3n12)C(=O)O)c4ccc(Br)cc4
54	Cc1c(cc2cc(C)cc(C(=O)O)n12)c3ccc(Br)cc3
55	Cc1c(cc2cc(Cl)c([N+](=O)[O])c(C)n12)c3ccc(Br)cc3
56	Cc1c(cc2cc(O)c3ccc4ccccc4c3n12)c5ccc(Br)cc5
57	Cc1nc(sc1C(=O)O)c2c(c(C)n3ccccc23)c4ccc(Br)cc4
58	CCc1c(cc2c(ccc(c3ccc(Br)cc3)n12)C(=O)O)C(F)(F)F
59	CC(=O)c1ccn2c(C)c(cc2c1)c3ccc(Br)cc3
60	Cc1c(c(N2CCC(CC2)C(=O)O)c3ccccn13)c4ccc(Br)cc4
61	Cc1c(cc2cc(O)ccn12)c3ccc(Br)cc3
62	Cc1c(cc2c3cc(Br)c(Cl)nc3ccn12)c4ccc(Br)cc4
63	Cc1c(cc2cc(Cl)c(C(=O)O)c(C)n12)c3ccc(Br)cc3
64	Cc1c(c(N2CCC[C@@H](C2)C(=O)O)c3ccccn13)c4ccc(Br)cc4
65	Oc1ccccc1c2c(Cl)c3ccccn3c2[C@H](Br)c4ccc(Br)cc4
66	Cc1c(c(SCC(=O)O)c2ccccn12)c3ccc(Br)cc3
67	Cc1c(cc2cc(C)c(O)cn12)c3ccc(Br)cc3
68	Cc1c(cc2ccc(O)c(CO)n12)c3ccc(Br)cc3
69	Cc1c(cc2cc(O)c3cccc(c3n12)C(F)(F)F)c4ccc(Br)cc4
70	COc1cn2c(C)c(c(O)c2c(I)c1O)c3ccc(Br)cc3
71	C[C@H](O)c1ccc2cc(c(C)n2c1)c3ccc(Br)cc3
72	Cc1c(cc2c(O)c(Br)c3ccccc3n12)c4ccc(Br)cc4
73	Cc1c(cc2ccc3oc(S)nc3n12)c4ccc(Br)cc4
74	CCOC(=O)c1ccn2c(C)c(cc2c1)c3ccc(Br)cc3
75	Cc1c(cc2cc(O)c3cc(ccc3n12)C(F)(F)F)c4ccc(Br)cc4
76	Cc1c(c(C(=O)c2ccsc2)c3ccccn13)c4ccc(Br)cc4
77	Cc1c(c(N2CCC(=NO)CC2)c3ccccn13)c4ccc(Br)cc4
78	Cc1c(c(n2cccc2)c3ccccn13)c4ccc(Br)cc4
79	Cc1c(cc2ccc(O)c(Br)n12)c3ccc(Br)cc3
80	Oc1ccn2c3C(=O)CCc3cc2c1OCc4ccc(Cl)cc4Cl
81	Cc1cccn2c([C@H](Br)c3ccc(Br)cc3)c(c(O)c12)c4ccccc4O
82	Oc1ccn2c3C(=O)CCCc3cc2c1OCc4ccc(Cl)cc4Cl
83	COc1ccc([N+](=O)[O])c2cc(c(C)n12)c3ccc(Br)cc3
84	Cc1c(c(C(=O)O)c2cccc(Cl)n12)c3ccc(Br)cc3
85	COc1ccc(Cl)c2c(O)cc3cc(c(C)n3c12)c4ccc(Br)cc4
86	Cc1c(cc2ccc(C(=O)O)c(O)n12)c3ccc(Br)cc3
87	Cc1c(cc2c(Br)c(C)c(cn12)C(=O)O)c3ccc(Br)cc3
88	Oc1ccn2c3CCCCc3cc2c1OCc4ccc(Cl)cc4
89	COc1ccc([N+](=O)[O])n2c(C)c(cc12)c3ccc(Br)cc3
90	Cc1cc(Br)n2c([C@H](Br)c3ccc(Br)cc3)c(cc2c1)c4ccccc4O
91	Cc1c(c(CCc2ccccc2)c3ccccn13)c4cccc(Cl)c4
92	Cc1c(c(Br)c2ccc3ccccc3n12)c4ccc(Br)cc4
93	Cc1c(cc2cccc(CC(=O)O)n12)c3ccc(Br)cc3
94	COC(=O)c1cc(Cl)n2c(C)c(c(C)c2c1)c3ccc(Br)cc3
95	Cc1c(c(C(=O)C(C)(C)C)c2ccccn12)c3ccc(Br)cc3
96	Cc1c(cc2cc(S)c3cc(C)ccc3n12)c4ccc(Br)cc4
97	Oc1ccccc1c2cc3ccc4cccnc4n3c2[C@H](Br)c5ccc(Br)cc5
98	COc1c(c([C@H](Br)c2ccc(Br)cc2)n3ccccc13)c4ccccc4O
99	Cc1cccc2c(Cl)c(c(c([C@H](Br)c3ccc(Br)cc3)n12)c4ccccc4O
100	Cc1c(cc2cc(O)c3ccc(cc3n12)C(F)(F)F)c4ccc(Br)cc4

[a] the listed molecules were ranked according to the calculated binding affinity to *Sag*Hyal$_{4755}$ (details not shown); accordingly, compound 1 represents the highest binding affinity, compound 100 the lowest binding affinity in this synopsis; [b] simplified molecular-input line-entry system (SMILES) code can be easily converted to the corresponding molecular structures e.g. with ChemDraw software (PerkinElmer Inc., Cambridge, USA), level: ultra, product version: 11.

The proposed molecules are encoded as SMILES-code. The SMILES (simplified molecular-input line-entry system) represents a coding system for simple or complex chemical structures that can be written down as a string of characters. The codes are designed to allow chemical structure definitions to be transferred between the various computer systems that are used to catalogue and design chemical compounds. A conversion to the corresponding molecular structure was simply achieved using ChemDraw software. The top ten ranked molecules of compound class 1 are illustrated in Figure 9.4.

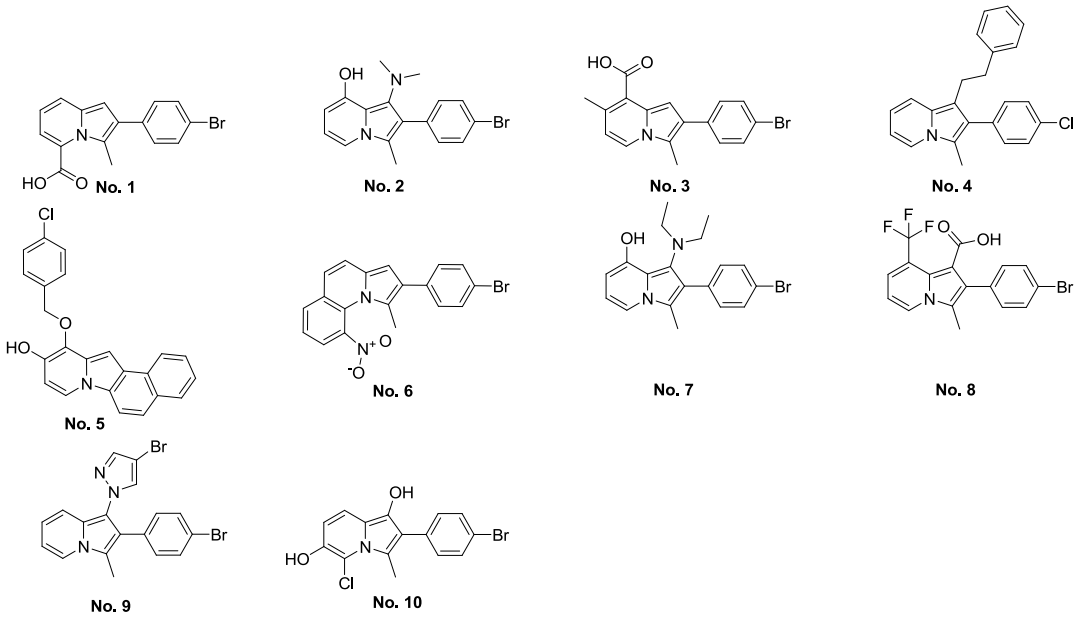

Figure 9.4 Top ten ranked indolizine derivatives proposed by virtual chemistry as inhibitors of *Sag*Hyal$_{4755}$ (compound class 1).

Compound 3, predicted to possess high binding affinity to the target enzyme, was selected as molecular basis for the synthesis of a first indolizine derivative. For economic reasons, the methyl group in position 7 was skipped, resulting in the modified target molecule 2-(4-bromophenyl)-3-methylindolizine-8-carboxylic acid (Figure 9.5).

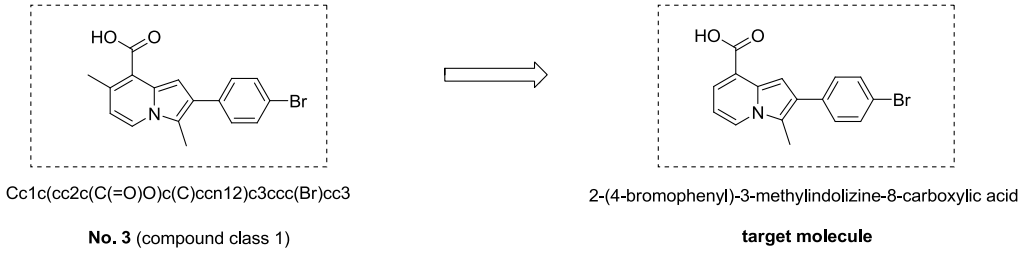

Figure 9.5 Illustration of the target molecule 2-(4-bromophenyl)-3-methylindolizine-8-carboxylic acid; The substance was essentially originated from molecule No. 3 (compound class 1) but modified by elimination of the methyl group in position 7.

The indolizine derivatives of the second compound class, ranked according to their proposed binding affinity on *Sag*Hyal$_{4755}$, are listed in Table 9.2.

Table 9.2 Indolizine derivatives proposed by virtual chemistry as inhibitors of *Sag*Hyal$_{4755}$ (compound class 2).

No.[a]	SMILES-code[b]
1	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(O)cc3
2	Cc1c(c(C#N)c2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
3	CCOC(=O)c1c(cc2c(Cl)cccn12)c3ccccc3F
4	CCOC(=O)c1c(cc2c(Br)cccn12)c3ccccc3F
5	Cc1c(cc2c(Br)cc(Br)c(Cl)n12)c3ccc(O)cc3
6	CCOC(=O)c1c(cc2c(Br)cc(Br)cn12)c3ccccc3F
7	CCOC(=O)c1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
8	Cc1c(cc2c(I)cccn12)c3ccc(O)cc3
9	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(Cl)cc3
10	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(Br)cc3
11	Cc1c(cc2c(Br)cccn12)c3ccc(O)cc3
12	Cc1c(c(C#N)c2c(Cl)cc(cn12)C(F)(F)F)c3cccc(Cl)c3
13	CCOC(=O)c1c(cc2c(Br)ccc(O)n12)c3ccccc3F
14	Cc1c(c(Br)c2c3ccccc3ccn12)c4ccc(O)cc4
15	COc1cc(C)c(cc1C)c2c(C)n3cc(F)cc(F)c3c2Br
16	CCOC(=O)c1c(cc2c(Br)cc(Br)cn12)c3ccccc3
17	COc1cc(C)c(cc1C)c2c(C)n3cc(F)cc(F)c3c2O
18	Cc1c(c(Cl)c2c(F)cc(F)cn12)c3ccccc3F
19	CCOC(=O)c1c(cc2ccc(F)cn12)c3ccccc3F
20	Cc1c(c(O)c2c(Br)cccn12)c3ccccc3F
21	CC1(C)C(=C(Br)c2cc3ccc4cc(O)ccc4n3c21)Br
22	Cc1c(c(Cl)c2ccc(Cl)cn12)c3ccc(O)cc3
23	CCOC(=O)c1c(cc2c(Cl)cccn12)c3cccc(F)c3
24	CCOC(=O)c1c(cc2c(Br)ccc(Cl)n12)c3ccccc3F
25	Cc1c(c(Br)c2c(F)cc(F)cn12)c3ccccc3F
26	CCOC(=O)c1c(cc2c(Cl)cccn12)c3cc(F)ccc3F
27	CCOC(=O)c1c(cc2c(Br)cccn12)c3cc(F)ccc3F
28	Cc1c(c(Cl)c2c3ccccc3ccn12)c4ccc(O)cc4
29	CCOC(=O)c1c(cc2c(I)cccn12)c3ccccc3
30	Cc1c(c(Cl)c2c(Cl)cc(cn12)C(F)(F)F)c3ccc(O)cc3
31	C[C@H](Br)c1cc2cc(C(=O)O)c3cccc(Br)c3n2c1C
32	Cc1c(cc2c(I)ccc(O)n12)c3ccccc3F
33	CCOC(=O)c1c(cc2c(I)ccc(O)n12)c3ccccc3F
34	CC1(C)C(=C(Br)c2cc3cc(O)c4ccc(Cl)cc4n3c21)Br
35	CCOC(=O)c1c(cc2ccc(Br)cn12)c3ccccc3F
36	Cc1c(c(Br)c2c(F)cc(F)cn12)c3ccc(OC(F)F)cc3
37	CCc1c(c(C)n2c(O)cc(cc12)C(F)(F)F)c3ccc(O)cc3
38	Fc1cccn2c(c(c(Br)c12)c3ccc(Cl)cc3)c4ccccc4
39	COc1cc(C)c(cc1C)c2cc3c(Cl)cc(cn3c2C)C(F)(F)F
40	Cc1c(c(O)c2c3ccccc3ccn12)c4ccc(O)cc4
41	Cc1c(cc2c(Br)ccc(O)n12)c3ccccc3F
42	Cc1c(c(C#N)c2c(Br)cccn12)c3ccccc3F
43	Cc1c(cc2c(I)cccn12)c3ccc(Br)cc3
44	CCOC(=O)c1c(cc2c(Cl)cccn12)c3ccccc3
45	CCc1c(cc2c(Br)cc(Br)c(Cl)n12)c3ccccc3
46	Cc1c(cc2c3ccccc3c(Br)cn12)c4ccc(O)cc4
47	CCOC(=O)c1c(c(Br)c2ccc(F)cn12)c3ccccc3F
48	CCOC(=O)c1c(c(Cl)c2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
49	Cc1c(cc2c(Cl)cccn12)c3ccc(Cl)cc3
50	Cc1c(c(C(=O)O)c2c(Cl)cc(cn12)C(F)(F)F)c3cccc(F)c3
51	Cc1c(c(C(=O)O)c2cc3ccccc3cn12)c4ccccc4F

52	CCOC(=O)c1c(c(O)c2c(F)cc(F)cn12)c3ccccc3F
53	Cc1c(cc2c(Br)cc(Br)cn12)c3ccc(O)cc3
54	CCOC(=O)c1c(cc2c(Br)cc(Br)cn12)c3cc(F)ccc3F
55	CCOC(=O)c1c(cc2c(I)cccn12)c3cc(F)ccc3F
56	CCOC(=O)c1c(c(Cl)c2c(F)cccn12)c3ccccc3F
57	Cc1c(cc2c(Br)cccn12)c3ccc(Br)cc3
58	CCOC(=O)c1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3cc(F)ccc3F
59	Cc1c(c(C#N)c2c(Br)cccn12)c3ccc(Cl)cc3
60	Cc1c(cc2c(Br)c(O)c3ccccc3n12)c4ccc(O)cc4
61	COc1ccc(cc1)c2cc3c(Br)cc(Br)cn3c2C
62	COc1ccc(cc1)c2cc3c(Cl)cc(cn3c2C)C(F)(F)F
63	Cc1c(cc2c(Br)ccc(Br)n12)c3ccccc3F
64	CCOC(=O)c1c(cc2c(Br)ccc(Br)n12)c3ccccc3
65	Cc1c(c(C(=O)O)c2c(F)cc(cn12)C(F)(F)F)c3ccc(Cl)cc3
66	COc1ccc2Cc3c(c(Cl)c4c(Cl)cc(cn34)C(F)(F)F)
67	COc1ccc(cc1)c2c(C)n3cc(cc(Cl)c3c2Cl)C(F)(F)F
68	Cc1c(c(Cl)c2c(F)cc(F)cn12)c3ccc(O)cc3
69	Cc1c(c(Cl)c2c(F)cc(F)cn12)c3ccc(cc3)c4ccccc4F
70	Cc1c(c(Cl)c2c(Cl)cc(cn12)C(F)(F)F)c3ccc(Br)cc3
71	Cc1c(c(O)c2c(Cl)cc(cn12)C(F)(F)F)c3ccc(O)cc3
72	CCc1c(c(C(=O)O)c2cccc(n12)C(F)(F)F)c3ccccc3
73	CC(C)c1c(cc2c(Br)cc(Br)c(O)n12)c3ccccc3
74	Cc1c(c(Br)c2ccc(F)cn12)c3ccccc3F
75	Cc1ccc2Cc3c(cc4c(O)cc5cc(Br)cc(Br)c5n34)-c2c1
76	CCOC(=O)c1c(c(O)c2c(cccn12)C(F)(F)F)c3ccccc3F
77	COc1ccc(Br)c2c(O)c(c(C)n12)c3ccccc3F
78	Cc1c(c(Cl)c2c(Cl)cc(cn12)C(F)(F)F)c3ccc(OC(F)F)cc3
79	CC(C)c1c(cc2c(Br)cc(Br)c(Cl)n12)c3ccccc3
80	Cc1c(cc2ccc(F)c(Br)n12)c3ccccc3F
81	Fc1ccc(cc1)c2cc3c(Cl)cc(cn3c2c4ccccc4)C(F)(F)F
82	Cc1c(cc2c(Cl)c(C)c(C#N)cn12)c3ccc(O)cc3
83	Cc1c(cc2c(Br)c(C)ccn12)c3ccc(O)cc3
84	Cc1cc(C)c2-c3c(O)c4c(F)cc(F)cn4c3C(C)(C)Cc2c1
85	Cc1c(c(C)n2cc(cc(Cl)c12)C(F)(F)F)c3ccc(O)cc3
86	Cc1c(c(C#N)c2c(Cl)cc(Cl)cn12)c3ccc(Cl)cc3
87	Fc1ccc2Cc3c(c(Cl)c4c(Cl)cc(cn34)C(F)(F)F)-c2c1
88	Cc1cc(C)c2-c3c(Cl)c4c(F)cc(F)cn4c3C(C)(C)Cc2c1
89	Cc1c(c(Br)c2c(F)cccn12)c3ccccc3F
90	Cc1cc(C)c2-c3cc4c(Br)cccn4c3C(C)(C)Cc2c1
91	Cc1c(cc2c(Br)ccc(Cl)n12)c3ccccc3F
92	CCOC(=O)c1c(c(Br)c2c(F)cccn12)c3ccccc3F
93	COc1cc(C)c(cc1C)c2c(C)n3cccc(Br)c3c2C#N
94	Cc1c(c(c2ccc(O)cc2)c3c(Cl)cc(cn13)C(F)(F)F)c4ccccc4F
95	Cc1c(cc2ccc(F)c(Br)n12)c3ccc(Cl)cc3
96	CCOC(=O)c1c(c(O)c2c(Br)cccn12)c3ccccc3F
97	Cc1c(c(C(=O)O)c2cc3ccccc3cn12)c4ccc(Cl)cc4
98	Cc1c(cc2c(Cl)c(C)c(C#N)cn12)c3ccccc3F
99	Cc1c(cc2ccc(Br)c(Br)n12)c3ccccc3F
100	Cc1c(c(O)c2cc3ccccc3cn12)c4ccc(Cl)cc4

[a] the listed molecules were ranked according to the calculated binding affinity to *Sag*Hyal$_{4755}$ (details not shown); accordingly, compound 1 represents the highest binding affinity, compound 100 the lowest binding affinity in this synopsis; [b] simplified molecular-input line-entry system (SMILES) code can be easily converted to the corresponding molecular structures e.g. with ChemDraw software (PerkinElmer Inc., Cambridge, USA), level: ultra, product version: 11.

The top ten ranked molecules of compound class 2 are illustrated in Figure 9.6.

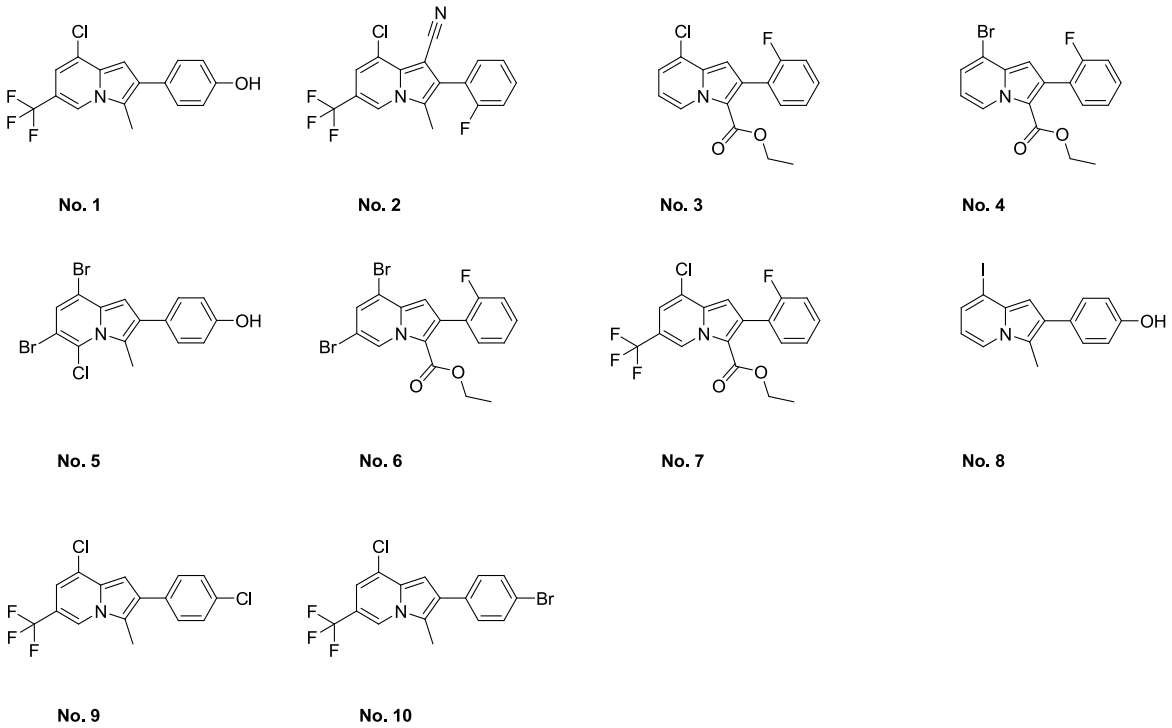

Figure 9.6 Top ten ranked indolizine derivatives proposed by virtual chemistry as inhibitors of *Sag*Hyal$_{4755}$ (compound class 2).

The indolizine derivatives of the third compound class, ranked according to their proposed binding affinity on *Sag*Hyal$_{4755}$, are listed in Table 9.3.

Table 9.3 Indolizine derivatives proposed by virtual chemistry as inhibitors of *Sag*Hyal$_{4755}$ (compound class 3).

No.[a]	SMILES-code[b]
1	Cc1c(c([C@H](Br)c2ccccc2)n3ccccc13)c4ccccc4
2	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(O)cc3
3	Fc1ccc(cc1)[C@@H](Br)c2c(cc3ccccn23)c4ccc(F)cc4
4	Oc1ccc2cc(c([C@H](Br)c3ccc(F)cc3)n2c1)c4ccc(F)cc4
5	OCc1c(c([C@H](Br)c2ccccc2)n3ccccc13)c4ccccc4
6	Cc1c(cc2cccc(C=NO)n12)c3ccc(Br)cc3
7	Cc1c(c(C#N)c2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
8	COc1cccn2c([C@H](Br)c3ccc(F)cc3)c(cc12)c4ccc(F)cc4
9	CCOC(=O)c1c(cc2c(Cl)cccn12)c3ccccc3F
10	CCOC(=O)c1c(cc2c(Br)cccn12)c3ccccc3F
11	Cc1c(c(C(=O)c2ccsc2)c3ccccn13)c4ccc(Br)cc4
12	Oc1ccn2c([C@H](Br)c3ccccc3)c(cc2c1)c4ccccc4
13	Cc1ccn2c([C@H](Br)c3ccc(F)cc3)c(cc2c1)c4ccc(F)cc4
14	Cc1cc2cc(c([C@H](Br)c3ccc(F)cc3)n2cc1O)c4ccc(F)cc4
15	Cc1c(cc2c(Br)cc(Br)c(Cl)n12)c3ccc(O)cc3
16	Fc1ccc(cc1)[C@@H](Br)c2c(cc3c(Cl)cccn23)c4ccc(F)cc4
17	Br[C@@H](c1c(cc2ccccn12)c3ccccc3)c4ccccc4
18	OCc1ccn2c([C@H](Br)c3ccc(F)cc3)c(cc2c1)c4ccc(F)cc4
19	Oc1ccccc1c2cc3c(O)cccn3c2[C@H](Br)c4ccc(F)cc4
20	Cc1c(cc2c(Br)c(C)c(cn12)C(=O)O)c3ccc(Br)cc3
21	Br[C@@H](c1c(c(Br)c2ccccn12)c3ccccc3)c4ccccc4
22	Cc1c(cc2cccc(C(=O)O)n12)c3ccc(Br)cc3
23	CCOC(=O)c1c(cc2c(Br)cc(Br)cn12)c3ccccc3F
24	Sc1cccc2cc(c(C(=O)c3ccc(Br)cc3)n12)c4ccc(Br)cc4
25	CCOC(=O)c1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
26	Cc1c(cc2c(Cl)c(C)c(C#N)cn12)c3ccc(Br)cc3
27	Cc1cc(C)c2cc(c([C@H](Br)c3ccc(F)cc3)n2c1)c4ccc(F)cc4
28	COCc1c(c([C@H](Br)c2ccccc2)n3ccccc13)c4ccccc4
29	COC(=O)c1cc(Cl)n2c(C)c(c(C)c2c1)c3ccc(Br)cc3
30	CN(C)c1c(c(C)n2cccc(O)c12)c3ccc(Br)cc3
31	Cc1c(cc2c(cc3ccc(C)cc3n12)C(=O)O)c4ccc(Br)cc4
32	Cc1c(cc2ccc3cccc([N+](=O)[O])c3n12)c4ccc(Br)cc4
33	Cc1c(cc2c(ccc(c3ccc(Br)cc3)n12)C(=O)O)C(F)(F)F
34	CN(C)Cc1c(c([C@H](Br)c2ccccc2)n3ccccc13)c4ccccc4
35	COC(=O)c1ccn2c(C)c(cc2c1)c3ccc(Br)cc3
36	Cc1c(cc2cc(O)c(cn12)C(=O)O)c3ccc(Br)cc3
37	CSc1c(C#N)cc(C)c2c(C)c(c(C)n12)c3ccc(Br)cc3
38	Cc1c(cc2c(C(=O)O)c(C)ccn12)c3ccc(Br)cc3
39	Cc1c(cc2c(I)cccn12)c3ccc(O)cc3
40	Cc1c(cc2cc(CO)c3ccccc3n12)c4ccc(Br)cc4
41	Cc1c(cc2cc(O)c(C(=O)O)c(O)n12)c3ccc(Br)cc3
42	Cc1c(cc2c(O)c(C)c(CO)cn12)c3ccc(Br)cc3
43	Cc1c(cc2c(cccn12)C(=O)O)c3ccc(Br)cc3
44	Cc1c(cc2ccc3cc(ccc3n12)C(=O)O)c4ccc(Br)cc4
45	Oc1ccn2c3CCc4ccccc4-c3cc2c1OCc5ccc(Cl)cc5
46	Cc1c(c(Oc2ccc(cc2)C(=O)O)c3ccccn13)c4ccc(Cl)cc4
47	Cc1c(c(Oc2cccc(c2)C(=O)O)c3ccccn13)c4ccc(Br)cc4
48	Cc1c(cc2ccc(CC(=O)O)cn12)c3ccc(Br)cc3
49	Cc1c(cc2cc(Cl)c(cn12)C(=O)O)c3ccc(Br)cc3
50	Cc1c(c(O)c2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
51	CCc1c(cc2c(OCc3ccc(Cl)cc3)c(O)ccn12)C(F)(F)F

52	CCN(CC)c1c(c(C)n2cccc(O)c12)c3ccc(Br)cc3
53	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(Cl)cc3
54	Cc1c(c(C(=O)O)c2c(cccn12)C(F)(F)F)c3ccc(Br)cc3
55	Cc1c(cc2cc(O)c3ccccc3n12)c4ccc(Br)cc4
56	Cc1c(c(n2cc(Br)cn2)c3ccccn13)c4ccc(Br)cc4
57	Cc1c(c(O)c2ccc(O)c(Cl)n12)c3ccc(Br)cc3
58	CCc1c(cc2c(ccc(c3ccc(Br)cc3)n12)C(=O)O)C(F)(F)F
59	Cc1cccn2c([C@H](Br)c3ccccc3)c(cc12)c4ccccc4
60	Cc1c(cc2cc(C)c(O)cn12)c3ccc(Br)cc3
61	CCc1cc2c(OCc3ccc(Cl)cc3)c(O)ccn2c1C
62	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(Br)cc3
63	Cc1c(cc2c(Cl)cc(cn12)C(F)(F)F)c3ccc(F)cc3F
64	Cc1c(c(C(=O)C(C)(C)C)c2ccccn12)c3ccc(Br)cc3
65	Cc1c(cc2c(cc3cccnc3n12)C(=O)O)c4ccc(Br)cc4
66	CCc1c(c([C@H](Br)c2ccccc2)n3ccc(Cl)cc13)c4ccccc4
67	Cc1c(cc2cc(C#N)c3ccccc3n12)c4ccc(Br)cc4
68	Cc1c(cc2cc(O)c3cc(C)ccc3n12)c4ccc(Br)cc4
69	Cc1ccn2c(C(=O)c3ccc(Br)cc3)c(c(O)c2c1)c4ccc(Br)cc4
70	CC(=O)c1ccc(c2ccc(Br)cc2)n3c(C)c(cc13)C(F)(F)F
71	CN(CC(=O)O)c1c(c(C)n2ccccc12)c3ccc(Br)cc3
72	COC(=O)c1cccc2c(Br)c(c(C)n12)c3ccc(Br)cc3
73	Cc1c(c(N2CCC[C@H]2CO)c3ccccn13)c4ccc(Br)cc4
74	Cc1c(c(C(=O)O)c2ccccn12)c3ccc(Br)cc3
75	Cc1c(cc2c(Br)cccn12)c3ccc(O)cc3
76	Cc1nc(sc1C(=O)O)c2c(c(C)n3ccccc23)c4ccc(Br)cc4
77	Fc1ccc(cc1)[C@@H](Br)c2c(cc3c4ccccc4ccn23)c5ccc(F)cc5
78	OCc1cccn2c([C@H](Br)c3ccc(F)cc3)c(cc12)c4ccc(F)cc4
79	Cc1c(c(Cl)c2c(Cl)cc(cn12)C(F)(F)F)c3ccccc3F
80	Cc1c(c(C(=O)O)c2ccc(Cl)cn12)c3ccc(Br)cc3
81	Cc1c(cc2cc(ccn12)C(=O)O)c3ccc(Br)cc3
82	Oc1ccccc1c2cc3cc(Br)ccn3c2[C@H](Br)c4ccc(Br)cc4
83	Cc1c(cc2cc(O)c3ccc(F)cc3n12)c4ccc(Br)cc4
84	Cc1c(c(N2CCC[C@H](CO)C2)c3ccccn13)c4ccc(Br)cc4
85	Oc1ccn2c3CCc4nonc4-c3cc2c1OCc5ccc(Cl)cc5
86	Cc1c(c(C(=O)C2CCC2)c3ccccn13)c4ccc(Br)cc4
87	[O][N+](=O)c1ccn2c([C@H](Br)c3ccc(F)cc3)c(cc2c1)c4ccc(F)cc4
88	Cc1c(cc2c(OCc3ccc(Cl)cc3)c(O)ccn12)C(F)(F)F
89	OCc1ccc2cc(c([C@H](Br)c3ccc(F)cc3)n2c1)c4ccc(F)cc4
90	Cc1c(c(C)n2c(S)c(C#N)cc(C)c12)c3ccc(Br)cc3
91	Cc1c(c(c2ccc(Cl)cc2)c3ccccn13)c4ccc(Br)cc4
92	Cc1c(cc2cc(C)c([N+](=O)[O])c(C)n12)c3ccc(Br)cc3
93	CCc1ccc2cc(c([C@H](Br)c3ccc(F)cc3)n2c1)c4ccc(F)cc4
94	Cc1c(c(N2CCCC2)c3c(O)cccn13)c4ccc(Br)cc4
95	COc1c(C#N)c(C)cc2cc(c(C)n12)c3ccc(Br)cc3
96	Cc1c(cc2c(Br)c(C)c(C#N)c(O)n12)c3ccc(Br)cc3
97	Cc1c(cc2ccc3cc(O)ccc3n12)c4ccc(Br)cc4
98	Cc1ccc(cc1)c2c(CO)c3ccccn3c2[C@H](Br)c4cccs4
99	Cc1c(cc2ccc(O)c(C)n12)c3ccc(Br)cc3
100	Cc1c(cc2c3ccc(Cl)nc3ccn12)c4ccc(Br)cc4

[a] the listed molecules were ranked according to the calculated binding affinity to *Sag*Hyal$_{4755}$ (details not shown); accordingly, compound 1 represents the highest binding affinity, compound 100 the lowest binding affinity in this synopsis; [b] simplified molecular-input line-entry system (SMILES) code can be easily converted to the corresponding molecular structures e.g. with ChemDraw software (PerkinElmer Inc., Cambridge, USA), level: ultra, product version: 11.

The top ten ranked molecules of compound class 3 are illustrated in Figure 9.7.

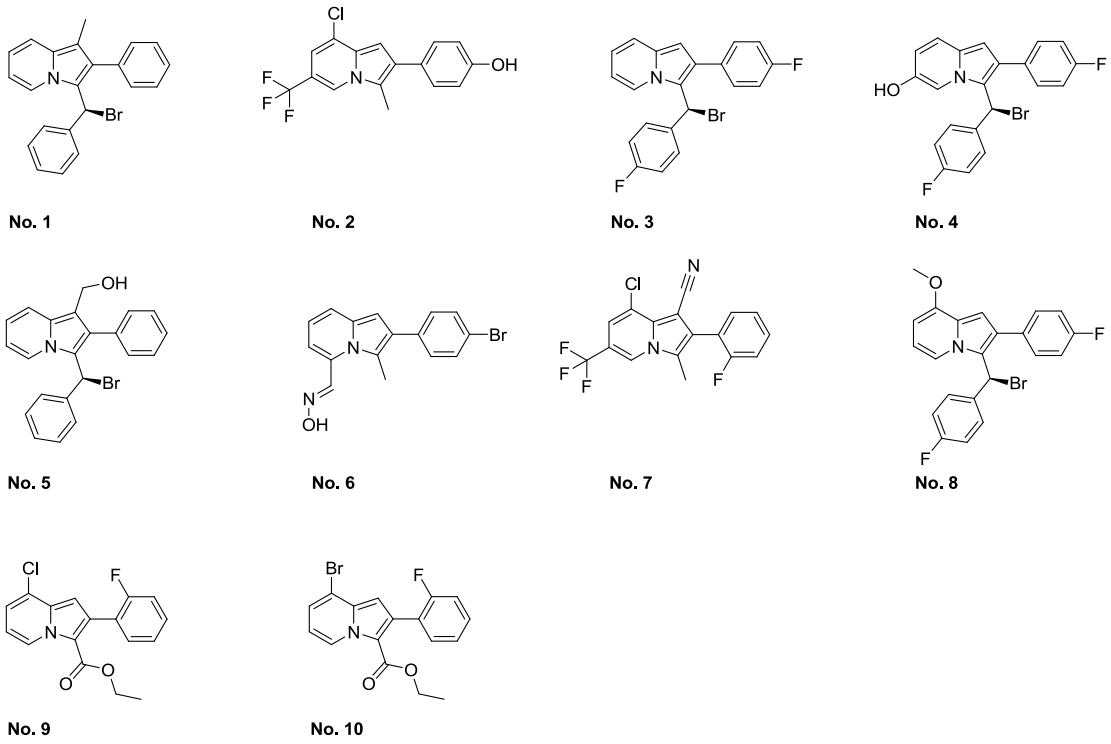

Figure 9.7 Top ten ranked indolizine derivatives proposed by virtual chemistry as inhibitors of *Sag*Hyal$_{4755}$ (compound class 3).

9.3 Chemistry

The synthesis of 2-(4-methoxyphenyl)indolizine (**9.1**) was performed as a condensation reaction as illustrated in Figure 9.8.

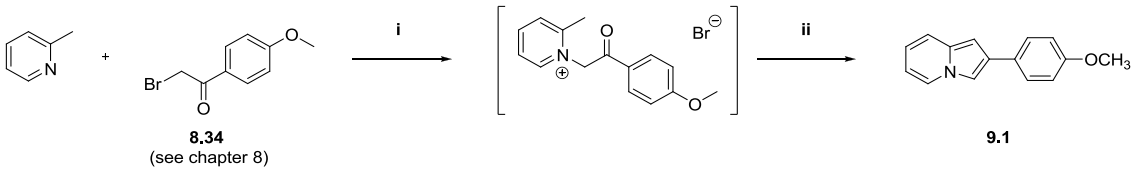

Figure 9.8 Synthesis of 2-(4-methoxyphenyl)indolizine **9.1**. Reagents and conditions: (i) acetone, microwave irradiation (130 °C, 4 min); (ii) K_2CO_3, H_2O, microwave irradiation (150 °C, 4 min).

A general method for the preparation of 2-alkyl- and 2-arylindolizines was discovered by Tschitschibabin in 1927.[18] In detail, a reaction of quaternary pyridinium halides leads to the cyclization resulting in indolizines. In a two-step protocol, 2-methylpyridine was alkylated with 2-bromo-1-(4-methoxyphenyl)ethanone (**8.34**, cf. chapter 8), followed by ring closure in the presence of a base (potassium carbonate).[4, 8] The synthesis was modified and optimized as a microwave assisted reaction, to promote the preparation of the product **9.1**.

Synthesis of ethyl 2-(4-bromophenyl)-3-methylindolizine-8-carboxylate was accomplished by the procedure outlined in Figure 9.9.

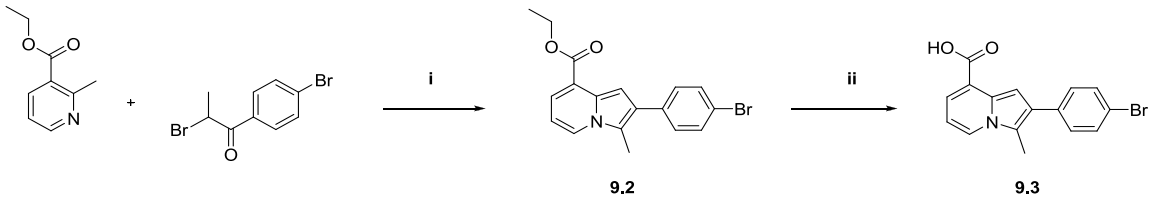

Figure 9.9 Synthesis of 2-(4-bromophenyl)-3-methylindolizine-8-carboxylic acid **9.3**. Reagents and conditions: (i) acetone, microwave irradiation (140 °C, 60 min); (ii) LiOH, THF/EtOH/H_2O = 3:0.2:1 (v/v/v).

Following a modified (one-step) and optimized (microwave assisted) reaction, ethyl 2-(4-bromophenyl)-3-methylindolizine-8-carboxylate (**9.2**) became directly accessible without the addition of base.[19] Saponification of the ester moiety with lithium hydroxide yielded the target molecule **9.3**.

It is important to mention, that the indolizine core structure was reported to be generally unstable against oxidation because of its electron-rich character. As reported by Hagishita et al., electron withdrawing groups on the indolizine nucleus make the compounds much

less susceptible to oxidation by air.[11] As a fact, for the synthesized indolizines **9.1**-**9.3**, autoxidation or decomposition was not observed. However, the documentation of chemical stability remains an important issue with regard to the indolizine derivatives proposed by virtual chemistry.

Single crystals of **9.3**, suitable for X-ray diffraction studies were grown by evaporation of the solvent (methanol). The obtained X-ray structure of **9.3** is shown in detail in section A.3.5 (appendix I). The crystal structure showed a disorder, which was calculated as “part 1” and “part 2”. However, anisotropic calculations for the corresponding carbon atoms were not accessible. Consequently, the crystal structure showed a double arrangement for the 2-phenylindole residue. The packing arrangement of a single crystalline phase contained symmetric dimers in which two molecules were present. To illustrate the configuration, an ORTEP-style plot and labeling scheme of **9.3** is given in Figure 9.10.

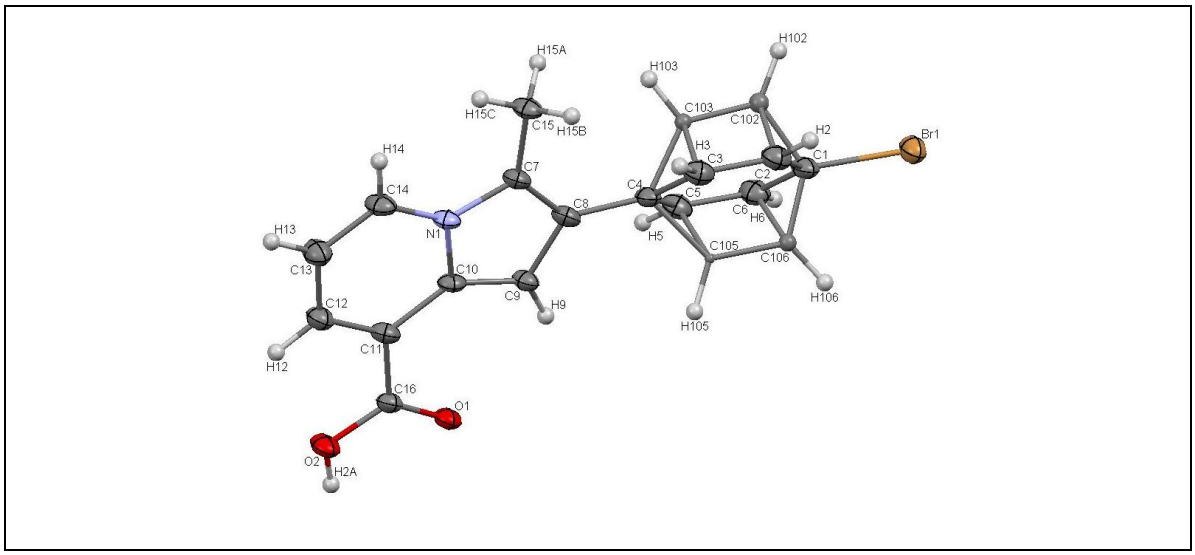

Figure 9.10 Molecular structure (ORTEP-style) of **9.3** including atomic numbering scheme (disorder in the crystal induces two calculations of the phenyl residue).

9.4 Pharmacological results and discussion

9.4.1 General conditions

All synthesized 2-phenylindolizine derivatives were investigated for inhibition of the bacterial hyaluronate lyase *Sag*Hyal$_{4755}$ and the bovine testicular enzyme BTH (Neopermease®) in a turbidimetric assay based on the method of Di Ferrante[20] as described in chapter 3.5.3.

9.4.2 Hyaluronidase inhibitory activities of 2-phenylindolizine derivatives

The IC_{50}-values determined for the 2-phenylindolizine derivatives are summarized in Table 9.4.

Table 9.4 Inhibitory activity[a] and calculated $logD_{5.0}$ values[b] of 2-phenylindolizine derivatives **9.1**-**9.3**.

Compound	*Sag*Hyal$_{4755}$ IC_{50} (µM)[a]	BTH IC_{50} (µM)	$logD_{5.0}$[b]
9.1	inactive[c]	inactive	4.4
9.2	inactive[c]	inactive	6.0[d]
9.3	85 ± 4.9	inactive	2.3

[a] mean values ± SEM (N = 2, experiments performed in duplicate), IC_{50} values determined at pH 5.0 in the automated 96-well turbidimetric assay; [b] calculated with ACD-Labs (Advanced Chemistry Development Inc., Toronto, Canada) product version 12.00; [c] at concentrations between 1 µM and 200 µM, inhibition was pretended due to colored compounds. [d] the structure does not contain ionization centers calculated by ACD-Labs, LogP-value is indicated instead.

The concentration dependent enzymatic activity of *Sag*Hyal$_{4755}$ in presence of **9.1**-**9.3** is depicted in Figure 9.11.

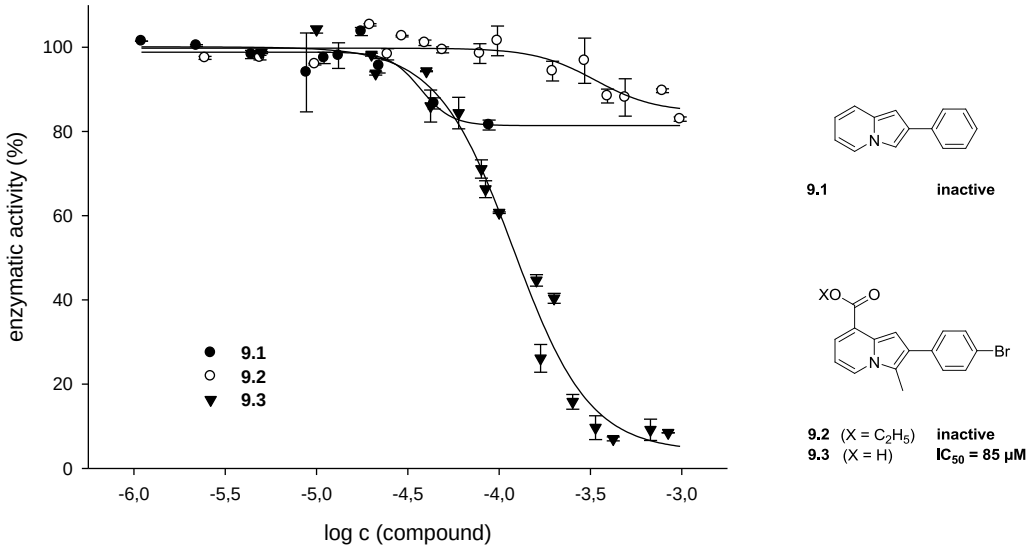

Figure 9.11 Enzymatic activity of *Sag*Hyal$_{4755}$ in the presence of **9.1**-**9.3**.

As a result, inhibitory activity on the target enzyme was confirmed for the indolizine derivative **9.3**. The "minimal pharmacophore" 2-phenylindolizine (**9.1**) and ethyl 2-(4-bromophenyl)-3-methylindolizine-8-carboxylate (**9.2**) were inactive. Most likely, the negatively charged residue of **9.3** plays an important role for inhibitory activity, as the corresponding ester **9.2** was inactive on *Sag*Hyal$_{4755}$. A similar behavior was observed for

2-phenylindoles (see chapter 8) and for indole-derivatives in previous studies.[21-24] Based on these observations, 2-phenylindolizines bearing COSMO*sim* proposed substitution patterns represent a new and promising class of inhibitors of *Sag*Hyal$_{4755}$. In particular, future investigations should consider indolizine derivatives with and without carboxylic groups. Such molecules should be closely related to compounds proposed for compound classes 1 to 3.

9.5 Summary

The molecules of the present chapter were designed with the aid of computational methods as bioisosteric molecules of previously identified potent hyaluronidase inhibitors. To minimize structural variability, a condensed inhibitor library of 32 substances, mainly representing 2-phenylindolizine derivatives, was selected. The *streptococcal* hyaluronate lyase *Sag*Hyal$_{4755}$ was set as target enzyme.

To quantify bioiosteric molecules, the electronic properties of the library compounds were investigated and served as templates for virtual chemistry. The corresponding COSMO*sim* based calculations suggested an indolizine skeleton as core structure of bioisosteric inhibitors. Consequently, the prediction of bioisosteric compounds was entirely based on physicochemical properties.

By the aid of virtual chemistry, indolizine derivatives were validated according to their calculated binding affinity to the target enzyme *Sag*Hyal$_{4755}$ *in silico*. The top ranked indolizine derivatives, predicted to possess the highest binding affinities, were selected as promising molecular structures for the design of novel inhibitors. As a result, a total of 300 compounds, subdivided into 3 different classes were listed in the present chapter.

To determine the ability of indolizine derivatives to inhibit the target enzyme under *in vitro* conditions, a single compound was selected and synthesized. As a result, in case of 2-(4-bromophenyl)-3-methylindolizine-8-carboxylic acid (**9.3**), an IC_{50} value of 85 µM was determined for *Sag*Hyal$_{4755}$ in the turbidimetric assay.

This results may be regarded as promising starting point for the development of additional inhibitors. For this purpose, indolizine derivatives, bearing structural patterns suggested by virtual drug design, might be a valuable source for novel hyaluronidase inhibitors. Indolizines and indoles represent chemical isomers. Depending on the results of future investigations, indolizines might also be classified as bioisosteric hyaluronidase inhibitors.

9.6 Experimental section

9.6.1 General conditions

Cf. section 5.8.1

Compound **8.34** has been described in section 8.6.2.1

Compound **9.1** has been described before.[18]

9.6.2 Chemistry

2-(4-Methoxyphenyl)indolizine (9.1)

Experimental details essentially adopted from ref[8, 25]; the procedure was modified and optimized for microwave irradiation.

A mixture of 2-methylpyridine (0.09 mL, 1 mmol) and 2-bromo-1-(4-methoxyphenyl)-ethanone **8.34** (229 mg, 1.0 mmol) was dissolved in 2 mL of anhydrous acetone. After microwave irradiation (130 °C, 4 min), the quaternary pyridinium halide was filtered off and collected to yield 270 mg of a pale gray solid. The precipitate was re-dissolved in 2 mL of hot H_2O with the aid of sonication. Subsequently, K_2CO_3 (138 mg, 1mmol) was added to the solution. After microwave irradiation (150 °C, 4 min), the remaining precipitate was solved in DCM. The organic layer was washed with H_2O and dried (Na_2SO_4). After evaporation of the solvent, the product was directly received from white crystals. Yield: 190 mg (85 %, white crystals); mp 179 – 181 °C (ref[25]: 184 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.92 (d, J = 7.0 Hz, 1H, indolizine-***H***), 7.64 – 7.59 (m, 2H, Ph-***H***-2,6), 7.45 (br s, indolizine-***H***), 7.38 – 7.37 (m, 1H, indolizine-***H***), 7.24 (s, 1H, indolizine-***H***), 6.64 – 6.59 (m, 2H, Ph-***H***-3,5), 6.44 (t, J = 6.7 Hz, 1H, indolizine-***H***), 3.86 (s, 3H, OC***H***$_3$-4). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 359.0 (90), 356.9 ([$M^{+\bullet}$], 100), 330.0 (35), 328.0 (35), 285.0 (20), 248.0 (30), 204.1 (40), 101.6 (30). $C_{15}H_{13}BrNO$ (M_r = 223.27 g/mol).

Ethyl 2-(4-bromophenyl)-3-methylindolizine-8-carboxylate (9.2)

Experimental details essentially adopted from ref[19]; the procedure was modified and optimized for microwave irradiation.

A mixture of ethyl 2-methylnicotinate (165 mg, 1.0 mmol) and 2-bromo-1-(4-bromophenyl)propan-1-one (292 mg, 1.0 mmol) was dissolved in 1.5 mL of anhydrous acetone. After microwave irradiation (140 °C, 60 min), the solvent was evaporated (rotary

evaporator) to yield the crude product as a dark yellow oil. The product was purified by flash-chromatography (PE/EtOAc 80/20, v/v). Yield: 80 mg (20 %, yellow solid); mp 169 – 171 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.91 (d, J = 7.0 Hz, 1H, indolizine-***H***-5), 7.61 (dd, J = 6.9, 0.7 Hz, 1H, indolizine-***H***-7), 7.59 – 7.53 (m, 2H, Ph-***H***-3,5), 7.43 – 7.37 (m, 2H, Ph-***H***-2,6), 7.24 (d, J = 11.7 Hz, 1H, indolizine-***H***-1), 6.64 (t, 1H, J = 7.0 Hz, indolizine-***H***-6), 4.44 (q, J = 7.1 Hz, 2H, COOC***H***$_2$CH$_3$), 2.55 (s, 3H, indolizine-C***H***$_3$-3), 1.44 (t, J = 7.1 Hz, 3H, 3H, COOCH$_2$C***H***$_3$). MS (EI-MS, 70 eV) *m/z* (rel. int. in %) = 359.0 (90), 356.9 ([M$^{+\bullet}$], 100), 330.0 (35), 328.0 (35), 285.0 (20), 248.0 (30), 204.1 (40), 101.6 (30). $C_{18}H_{16}BrNO_2$ (M_r = 358.23 g/mol).

2-(4-Bromophenyl)-3-methylindolizine-8-carboxylic acid (9.3)

A suspension of ethyl 2-(4-bromophenyl)-3-methylindolizine-8-carboxylate **9.2** (60 mg, 0.2 mmol) and LiOH (8 mg, 0.4 mmol) in a mixture 3:0.2:1 mixture of THF, EtOH, H_2O (v/v/v) was stirred at room temperature for 14 days. Purification by flash chromatography (PE/EtOAc 80/20, v/v) gave **9.3** as a yellow powder. Yield: 50 mg (76 %, yellow solid); mp 151 - 154 °C. Single crystals of **9.3** were grown from a solution of 10 mg product in anhydrous methanol (1.5 mL) in a 2 mL plastic vial. The solvent was slowly evaporated at room temperature over 14 days. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 13.00 (s, 1H, COO***H***), 8.32 (d, J = 7.1 Hz, 1H, indolizine-***H***-5), 7.66 – 7.60 (m, 2H, Ph-***H***-3,5), 7.53 (d, J = 6.5 Hz, 1H, indolizine-***H***-7), 7.51 – 7.45 (m, 2H, Ph-***H***-2,6), 7.12 (s, 1H, indolizine-***H***-1), 6.76 (t, J = 7.0 Hz, 1H, indolizine-***H***-6), 2.56 (s, 3H, indolizine-C***H***$_3$-3). ^{13}C-NMR (75 MHz, DMSO-d_6) δ [ppm] = 166.18 (C_{quat}, ***C***OOH), 135.03 (C_{quat}, indolizine-***C***), 131.46 (+, Ph-***C***-2,6), 130.54 (+, Ph-***C***-3,5), 128.31 (C_{quat}, Ph-***C***), 127.27 (+, indolizine-***C***-5), 126.22 (C_{quat}, indolizine-***C***), 122.63 (+, indolizine-***C***-7), 120.15 (C_{quat}, Ph-***C***), 119.51 (C_{quat}, indolizine-***C***), 117.13 (C_{quat}, indolizine-***C***), 109.07 (+, indolizine-***C***-6), 100.01 (+, indolizine-***C***-1), 10.21 (+, indolizine-***C***H$_3$-3). MS (ES-MS, DCM/MeOH + NH_4OAc) *m/z* (rel. int. in %) = 329.9 ([M + H]$^+$, 100), 328.8 (15). $C_{16}H_{12}BrNO_2$ (M_r = 330.18 g/mol).

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10 Summary

Hyaluronidases are enzymes that degrade hyaluronan, a major constituent of the extracellular matrix. As the role of hyaluronidases is far from being understood, potent and selective inhibitors are required as pharmacological tools. Moreover, inhibitors of the bacterial and mammalian enzymes might be useful agents in the treatment of various diseases, e.g. bacterial infections.

Previously, we identified hyaluronidase inhibitors among lipophilic vitamin C, indole and benzoxazole derivatives. Unfortunately, such substances proved to be inappropriate for *in vivo* studies due to the lack of drug-like properties, in particular, due to extremely high plasma protein binding. Furthermore, substances bearing a 2-phenylindole scaffold were chemically derived from known antiestrogens. Hence, (anti)estrogenicity of phenylindole-type hyaluronidase inhibitors had to be taken into account.

The goal of this thesis was the design, synthesis, identification and pharmacological characterization of novel lead inhibitors for the bacterial hyaluronidase *Sag*Hyal$_{4755}$. To give access to inhibitors suitable for *in vivo* characterization, the focus was set on small molecules with drug-like properties.

In a first attempt, a target-based approach led to the development of novel lead inhibitors of the streptococcal lyase *Sag*Hyal$_{4755}$. For this purpose, a convenient strategy, inspired by industrial pharmaceutical research, i.e. a computer-assisted and multicomponent synthesis approach was pursued in an interdisciplinary project. An existing turbidimetric assay for the investigation of hyaluronidase inhibitors was modified and adapted to automated screening in the 96 well and 384 well format. Decision criteria for the acceptance of “hit” compounds were elaborated. A compound library comprising 347 substances was analyzed for inhibition of *Sag*Hyal$_{4755}$. The structure activity relationships (SAR) were elucidated and models of the enzyme in complex with potential inhibitors were generated *in silico*. The suggested compounds were synthesized by parallel synthesis, analyzed and tested for biological activity in medium-throughput. Among 2640 screened samples, 4-amino-imidazolidine-2-thiones were identified as promising hits to develop inhibitors of *Sag*Hyal$_{4755}$. Synthesis on the preparative scale and purification of hit compounds revealed inhibitory activity in the micromolar range. However, some derivatives proved to be prone to autoxidation and decomposition. To cope with this problem, the oxidation process was studied in detail by means of [^{18}O] labeling and HPLC-MS analysis. The results paved the way to the synthesis of more stable molecules. 4-{1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-2-thioxo-4-[4-(trifluoromethyl)benzylamino]-2,5-dihydro-1*H*-imidazol-5-yl}benzoic acid (UR-CT574 with an IC_{50} value of 8 µM) was identified as one of the most potent inhibitors of the target enzyme known so far.

Furthermore, 1352 compounds with imidazopyridine scaffold were synthesized and tested. Screening for inhibition of the bacterial hyaluronate lyase revealed several hits, which will be subject of future studies.

A subproject focused on the synthesis and pharmacological characterization of a small series of 2-phenylindoles bearing a benzyl substituent in position 1, aiming at less lipophilic compounds compared to the previously described *N*-alkylated indole-type inhibitors. The IC_{50} values of the novel compounds, especially when bearing chloro substituents, were in the micromolar range. For example, an IC_{50} value of 9 µM for inhibition of *Sag*$Hyal_{4755}$ was determined for 4-{[6,7-dichloro-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-1-yl]methyl}benzoic acid (UR-CT619). In addition, this compound proved to be the most potent inhibitor of a related bacterial hyaluronidase from *S. pneumonia* (*Spn*Hyl) known so far (IC_{50} = 93 µM). These compounds are devoid of cytotoxicity against estrogen sensitive MCF-7 human breast cancer cells.

Taken together, molecules from two different chemical classes were discovered as one-digit micromolar inhibitors of the target enzyme *Sag*$Hyal_{4755}$. Inhibitory activity was also observed for the streptococcal hyaluronidase *Spn*Hyl. These compounds can be considered as lead structures for the development of inhibitors of bacterial hyaluronate lyases. Such agents might be useful in combination with antibiotics to combat bacteria producing hyaluronidase as a putative virulence factor.

A Appendix I

A.1 Abbreviations

A	Ala, alanine
Å	Angstrom (unit)
abs	absolute
Anal.	Analysis
aq	aqueous
Ar	aromatic
AU	absorption units
BSA	bovine serum albumin
BTH	bovine testicular hyaluronidase
c	concentration
calcd.	calculated
cat.	catalytical amounts
CD44	cluster of differentiation 44
CI	chemical ionization
conc.	concentrated
COSY	correlated spectroscopy
cpd	compound
C_{quat}	quaternary carbon atom
CTAB	cetrimonium bromide, cetyltrimethylammonium bromide
CV	column volume
4CC	four component condensation
d	day(s) or doublet
δ	chemical shift
Da	Dalton
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
DMAB	4-(dimethylamino)benzaldehyde
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide

DMSO-d_6	per-deuterated DMSO
EC	enzyme commission
ECM	extracellular matrix
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetic acid
EI	electron impact ionization
eq	equivalent(s)
ER	estrogen receptor
ESI	electrospray ionization
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
EWG	electron withdrawing group
F	Phe, phenylalanine
GAG	glycosaminoglycan
GlcNAc	*N*-acetyl-D-glycosamine
GlcUA	D-glucuronic acid
GPI	glycosylphospatidylinositol
h	hour(s)
HA	hyaluronic acid, hyaluronan
HAS	hyaluronan synthase
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum correlation
HTS	high-throughput screening
Hyal-1	(human) hyaluronidase 1
IC_{50}	concentration of an inhibitor required to give 50% inhibition of enzymatic activity
IMAC	immobilized metal affinity chromatography
IU	international units

J	coupling constant
LB	lysogeny broth
m	milli or multiplet
M	molar
M	mass
M_r	molecular mass
MCF-7	Michigan Cancer Foundation – 7 (human breast cancer cell line)
MCR	multicomponent reaction
Me	methyl
MeCN	acetonitrile
MeOH	methanol
min	minute(s)
mp	melting point
MS	mass spectral or mass spectrum
MTP	microtiter plate
mw	molecular weight
MWCO	molecular weight cutoff
m/z	mass-to-charge ratio
N	number of experiments
NCE	new chemical entities
NFU	national formulatory unit
NMR	nuclear magnetic resonance
NP	normal phase
NSAID	non-steroidal anti-inflammatory drug
OAc	acetate
OD	optical density
PAD	proton acceptance and donation
PAGE	polyacrylamide gel electrophoresis
PDB	protein data bank
PE	petroleum ether
PEGME	poly(ethylene glycol)monoethyl ether

pH	negative logarithm of the hydrogen ion concentration
Ph	phenyl
ppm	part per million
q	quartet
ref	reference
RHAMM	receptor for hyaluronate-mediated motility
RP	reversed phase
rpm	revolutions per minute
rt	room temperature
s	singulet
*Sag*Hyal$_{4755}$	hyaluronate lyase from *Streptococcus agalagtiae* strain 4755
SAR	structure-activity relationships
sat.	saturated
SDS	sodium dodecylsulfate
SEM	standard error of the mean
SPAM1	spam adhesion molecule 1 (also termed PH-20)
*Spn*Hyl	hyaluronate lyase from *Streptococcus pneumoniae*
t	triplet
t_0	dead time
t_R	retention time
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIC	total ion count
TLC	thin layer chromatography
TMS	trimethylsilyl
(r)TRU	(relative) turbidity reducing unit
U	unit(s)
UV	ultraviolet
V	Val, valine
W	Trp, tryptophan
Y	Tyr, tyrosine

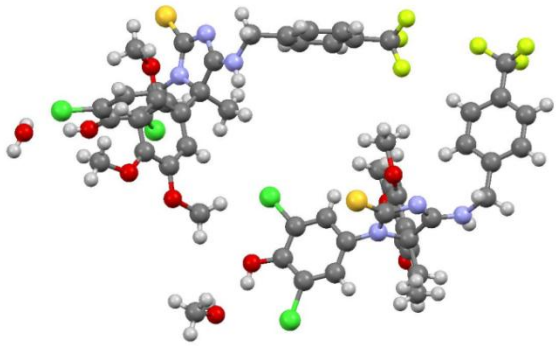
A.2 HPLC purity data

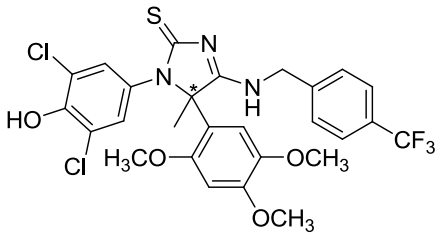
Cpd	t_R (min)	k'	purity (%)	Cpd	t_R (min)	k'	purity (%)
5.26[a]	12.33	4.29	90.3	**5.62**[b]	27.26	7.21	95.0
5.27[a]	11.26	3.83	94.9	**5.63**[b]	24.80	6.47	95.8
5.28[a]	10.87	3.67	95.1	**5.64**[b]	19.63	4.91	95.7
5.29[a]	11.71	4.03	96.3	**5.65**[a]	13.79	4.92	92.9
5.30[a]	12.14	4.21	94.8	**5.66**[a]	12.29	4.27	94.8
5.31[a]	12.15	4.21	94.1	**5.67**[b]	26.72	7.05	97.5
5.32[a]	10.93	3.69	95.8	**5.68**[b]	23.66	6.13	95.8
5.33[a]	9.83	3.22	98.2	**5.69**[a]	11.54	3.95	95.3
5.34[a]	12.73	4.46	99.1	**5.70**[a]	9.70	3.16	95.8
5.35[a]	9.84	3.22	93.9	**5.71**[a]	9.95	3.27	97.8
5.43[a]	8.30	2.56	95.0	**5.72**[a]	11.42	3.90	97.3
5.44[a]	9.82	3.21	97.4	**5.73**[a]	12.13	4.21	99.1
5.45[a]	9.17	2.94	95.1	**8.24a**[a]	12.14	4.21	95.9
5.46[a]	9.79	3.20	96.7	**8.25a**[a]	12.91	4.54	99.5
5.47[b]	24.26	6.31	95.1	**8.26a**[a]	12.78	4.48	97.7
5.48[a]	11.71	4.03	98.9	**8.27a**[a]	11.19	3.80	98.3
5.49[a]	11.35	3.87	98.1	**8.27b**[a]	8.54	2.67	95.7
5.50[a]	12.09	4.19	94.9	**8.28a**[a]	12.97	4.57	97.4
5.51[a]	10.37	3.45	99.8	**8.29a**[a]	13.61	4.84	98.3
5.52[b]	24.82	6.48	95.4	**8.29b**[a]	9.74	3.18	95.3
5.53[a]	11.34	3.87	95.7	**8.30a**[a]	11.89	4.10	94.8
5.54[b]	20.20	5.08	96.1	**8.31a**[a]	12.67	4.44	97.0
5.55[b]	24.13	6.27	95.0	**8.32a**[a]	10.25	3.40	94.7
5.56[a]	9.82	3.21	96.2	**8.32b**[a]	10.58	3.54	97.4
5.57[b]	21.54	5.49	95.9	**8.41**[a]	12.17	4.22	93.9
5.58[b]	23.24	6.00	94.9	**8.42**[a]	12.18	4.23	95.1
5.59[b]	24.82	6.48	95.4	**8.43**[a]	14.57	5.25	98.6
5.60[b]	26.24	6.90	95.2	**8.47**[a]	14.63	5.28	97.8
5.61[b]	26.58	7.01	96.2	**9.3**[a]	8.14	2.49	96.6

[a] Eurospher-100 C18, 250 x 4.0 mm, 5 µm (Knauer, Berlin, Germany), t0 = 2.33 min, gradient mode: MeCN/H_2O + 0.025% TFA: 0 min: 20/80, 15 min: 95/5, 21 min: 20/80, 25 min: 20/80; [b] Eurospher-100 C18, 250 x 4.0 mm, 5 µm (Knauer, Berlin, Germany), t_0 = 3.32 min, gradient mode: MeCN/H_2O + 0.05% TFA: 0 min: 20/80, 20 min: 90/10, 21 min: 95/5, 30 min: 95/5, 31 min: 10/90, 40 min 10/90.

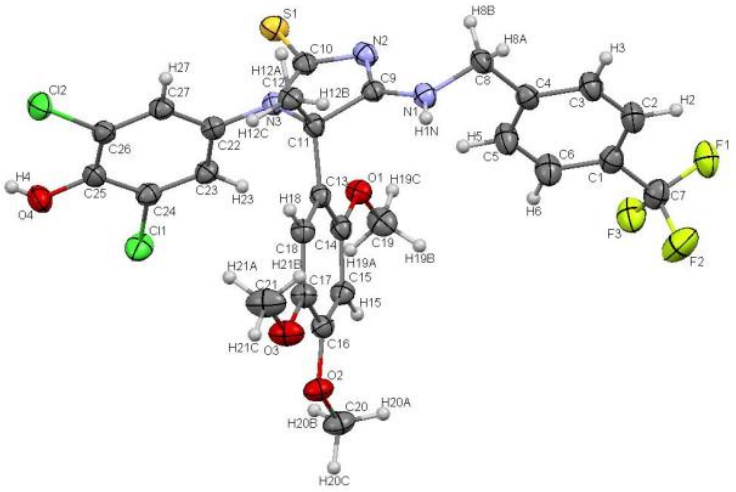
A.3 X-ray crystallographic data

A.3.1 *rac*-1-(3,5-Dichloro-4-hydroxyphenyl)-5-methyl-4-(4-(trifluoromethyl)benzylamino)-5-(2,4,5-trimethoxy-phenyl)-1*H*-imidazole-2(5*H*)-thione (5.45)





· H_2O · MeOH



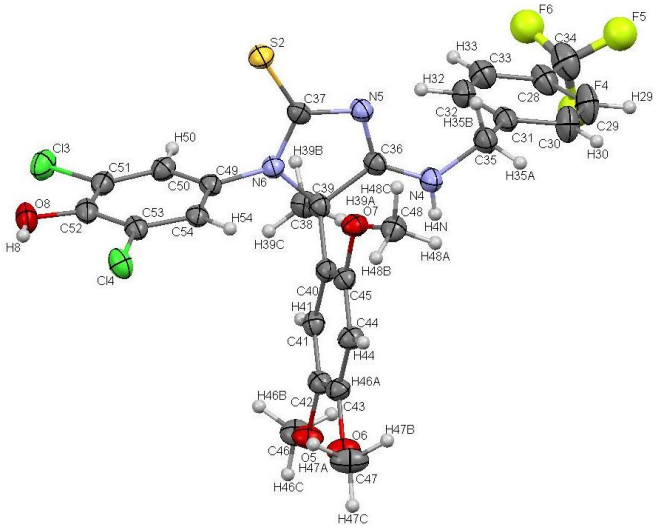


Table 1 Crystal data and structure refinement for 5.45.

Crystal Data	
Empirical formula	$C_{55}H_{54}Cl_4F_6N_6O_{10}S_2$
Formula weight	1278.98
Crystal size	0.1371 x 0.0373 x 0.0135 mm
Crystal description	plate
Crystal colour	colourless
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 11.6049(5) Å alpha = 61.372(4) deg.
	b = 17.0403(7) Å beta = 79.516(4) deg.
	c = 17.3915(7) Å gamma = 74.160(4) deg.
Volume	2898.8(2) $Å^3$
Z, Calculated density	2, 1.465 Mg/m^3
Absorption coefficient	3.239 mm^{-1}
F(000)	1320
Data Collection	
Measurement device type	SuperNova, Single source at offset), Atlas
Measurement method	\w scans
Temperature	123 K
Wavelength	1.54184 Å
Monochromator	graphite

Theta range for data collection	3.03 to 76.60 deg.
Index ranges	-14<=h<=10, -21<=k<=21, -21<=l<=21
Reflections collected / unique	21495 / 11732 [R(int) = 0.0340]
Reflections greater I>2\s(I)	9352
Absorption correction	Analytical
Max. and min. transmission	0.960 and 0.767
Refinement	---
Refinement method	Full-matrix least-squares on F^2
Hydrogen treatment	---
Data / restraints / parameters	11732 / 2 / 746
Goodness-of-fit on F^2	1.033
Final R indices [I>2sigma(I)]	R1 = 0.0546, wR2 = 0.1445
R indices (all data)	R1 = 0.0682, wR2 = 0.1572
Absolute structure parameter	---
Largest diff. peak and hole	1.073 and -0.648 e.A^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 5.45. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
Cl(1)	-1425(1)	1016(1)	2294(1)	39(1)
Cl(2)	-3793(1)	4515(1)	569(1)	40(1)
S(1)	1732(1)	3318(1)	290(1)	34(1)
F(1)	8258(2)	1222(1)	4064(1)	45(1)
F(2)	6975(2)	933(2)	5162(1)	61(1)
F(3)	7030(2)	380(1)	4279(2)	49(1)
O(1)	1769(2)	2187(1)	2960(1)	30(1)
O(2)	-866(2)	1470(1)	5607(1)	34(1)
O(3)	-1916(2)	3167(1)	5044(1)	40(1)
O(4)	-3402(2)	2429(1)	1414(1)	37(1)
N(1)	2431(2)	4287(2)	2432(2)	29(1)
N(2)	2370(2)	3869(2)	1344(2)	29(1)
N(3)	449(2)	3701(1)	1566(1)	26(1)
C(1)	6233(2)	1971(2)	3769(2)	33(1)
C(2)	6447(3)	2833(2)	3477(2)	37(1)

C(3)	5616(3)	3611(2)	2993(2)	35(1)
C(4)	4565(2)	3530(2)	2788(2)	29(1)
C(5)	4360(3)	2665(2)	3076(2)	41(1)
C(6)	5192(3)	1889(2)	3561(2)	41(1)
C(7)	7110(3)	1136(2)	4308(2)	38(1)
C(8)	3677(2)	4384(2)	2230(2)	31(1)
C(9)	1878(2)	4050(2)	2005(2)	26(1)
C(10)	1500(2)	3633(2)	1092(2)	28(1)
C(11)	557(2)	4010(2)	2209(2)	25(1)
C(12)	-196(2)	4982(2)	1943(2)	28(1)
C(13)	257(2)	3329(2)	3148(2)	25(1)
C(14)	860(2)	2415(2)	3488(2)	26(1)
C(15)	527(2)	1774(2)	4321(2)	28(1)
C(16)	-421(2)	2046(2)	4813(2)	28(1)
C(17)	-1014(2)	2971(2)	4498(2)	28(1)
C(18)	-669(2)	3594(2)	3671(2)	27(1)
C(19)	2438(3)	1269(2)	3288(2)	37(1)
C(20)	-267(3)	535(2)	5964(2)	39(1)
C(21)	-2549(3)	4097(2)	4732(2)	48(1)
C(22)	-571(2)	3408(2)	1534(2)	26(1)
C(23)	-529(2)	2472(2)	1912(2)	28(1)
C(24)	-1490(2)	2183(2)	1858(2)	28(1)
C(25)	-2536(2)	2788(2)	1450(2)	29(1)
C(26)	-2538(2)	3716(2)	1094(2)	29(1)
C(27)	-1581(2)	4033(2)	1126(2)	28(1)
Cl(3)	-3727(1)	-3312(1)	5082(1)	40(1)
Cl(4)	-3551(1)	-3885(1)	2258(1)	36(1)
S(2)	933(1)	-4819(1)	3808(1)	32(1)
F(4)	5423(3)	275(2)	2832(3)	118(2)
F(5)	6966(2)	-732(2)	3067(3)	87(1)
F(6)	5551(4)	-884(4)	4044(2)	132(2)
O(5)	-2390(2)	527(1)	88(1)	34(1)
O(6)	-1958(2)	-204(1)	-942(1)	37(1)
O(7)	630(2)	-2882(1)	1155(1)	30(1)
O(8)	-4572(2)	-3870(2)	4001(2)	38(1)
N(4)	2346(2)	-1944(2)	1735(2)	32(1)

N(5)	1973(2)	-3386(2)	2752(2)	29(1)
N(6)	-57(2)	-3035(1)	2885(1)	26(1)
C(28)	5257(3)	-1061(2)	2841(2)	41(1)
C(29)	5673(3)	-1009(3)	2027(3)	51(1)
C(30)	5145(3)	-1374(2)	1656(2)	45(1)
C(31)	4200(2)	-1806(2)	2104(2)	31(1)
C(32)	3801(3)	-1868(2)	2930(2)	37(1)
C(33)	4318(3)	-1496(2)	3297(2)	40(1)
C(34)	5793(3)	-622(3)	3211(3)	50(1)
C(35)	3653(2)	-2217(2)	1698(2)	34(1)
C(36)	1624(2)	-2504(2)	2235(2)	26(1)
C(37)	939(2)	-3721(2)	3132(2)	26(1)
C(38)	275(2)	-2145(2)	2304(2)	27(1)
C(39)	57(3)	-1583(2)	2815(2)	32(1)
C(40)	-311(2)	-1635(2)	1434(2)	26(1)
C(41)	-1078(2)	-769(2)	1176(2)	27(1)
C(42)	-1612(2)	-314(2)	388(2)	28(1)
C(43)	-1395(2)	-720(2)	-174(2)	28(1)
C(44)	-642(2)	-1578(2)	63(2)	29(1)
C(45)	-103(2)	-2027(2)	864(2)	26(1)
C(46)	-2535(3)	1015(2)	586(2)	36(1)
C(47)	-1981(3)	-638(2)	-1461(2)	45(1)
C(48)	1220(3)	-3180(2)	518(2)	31(1)
C(49)	-1256(2)	-3170(2)	3129(2)	26(1)
C(50)	-1843(2)	-3158(2)	3894(2)	30(1)
C(51)	-2973(2)	-3359(2)	4139(2)	31(1)
C(52)	-3523(2)	-3614(2)	3672(2)	32(1)
C(53)	-2915(2)	-3592(2)	2893(2)	28(1)
C(54)	-1802(2)	-3368(2)	2614(2)	26(1)
O(9)	-5408(2)	3225(2)	650(2)	53(1)
C(55)	-5319(17)	3216(13)	-147(13)	325(11)
O(10)	-5872(2)	-4571(2)	3466(2)	37(1)

Table 3. Bond lengths [Å] and angles [deg.] for 5.45.

Cl(1)-C(24)	1.740(3)	S(1)-C(10)-N(2)	123.8(2)
Cl(2)-C(26)	1.737(3)	N(3)-C(11)-C(12)	109.5(2)
Cl(3)-C(51)	1.740(3)	N(3)-C(11)-C(9)	97.8(2)
Cl(4)-C(53)	1.742(3)	C(9)-C(11)-C(13)	114.3(2)
S(1)-C(10)	1.674(3)	N(3)-C(11)-C(13)	111.7(2)
S(2)-C(37)	1.667(3)	C(9)-C(11)-C(12)	108.9(2)
F(1)-C(7)	1.349(4)	C(12)-C(11)-C(13)	113.5(2)
F(2)-C(7)	1.342(4)	C(14)-C(13)-C(18)	118.3(2)
F(3)-C(7)	1.342(4)	C(11)-C(13)-C(18)	121.1(3)
F(4)-C(34)	1.317(7)	C(11)-C(13)-C(14)	120.5(2)
F(5)-C(34)	1.316(5)	O(1)-C(14)-C(15)	122.7(3)
F(6)-C(34)	1.295(6)	C(13)-C(14)-C(15)	120.8(3)
O(1)-C(19)	1.420(4)	O(1)-C(14)-C(13)	116.5(2)
O(1)-C(14)	1.369(3)	C(14)-C(15)-C(16)	119.8(3)
O(2)-C(16)	1.363(3)	C(15)-C(16)-C(17)	120.4(2)
O(2)-C(20)	1.418(4)	O(2)-C(16)-C(15)	124.7(3)
O(3)-C(17)	1.364(4)	O(2)-C(16)-C(17)	114.9(2)
O(3)-C(21)	1.425(4)	C(16)-C(17)-C(18)	118.7(3)
O(4)-C(25)	1.335(4)	O(3)-C(17)-C(16)	115.6(2)
O(4)-H(4)	0.8400	O(3)-C(17)-C(18)	125.7(3)
O(5)-C(42)	1.374(4)	C(13)-C(18)-C(17)	121.8(3)
O(5)-C(46)	1.424(4)	C(23)-C(22)-C(27)	120.1(2)
O(6)-C(43)	1.364(3)	N(3)-C(22)-C(27)	121.1(3)
O(6)-C(47)	1.423(4)	N(3)-C(22)-C(23)	118.7(2)
O(7)-C(45)	1.370(4)	C(22)-C(23)-C(24)	119.2(2)
O(7)-C(48)	1.424(4)	Cl(1)-C(24)-C(25)	117.4(2)
O(8)-C(52)	1.340(3)	Cl(1)-C(24)-C(23)	119.5(2)
O(8)-H(8)	0.8400	C(23)-C(24)-C(25)	123.1(3)
O(9)-C(55)	1.38(2)	O(4)-C(25)-C(26)	126.7(3)
O(9)-H(9)	0.8400	C(24)-C(25)-C(26)	115.5(2)
O(10)-H(10O)	0.87(3)	O(4)-C(25)-C(24)	117.8(3)
O(10)-H(10P)	0.83(2)	C(25)-C(26)-C(27)	123.1(3)
N(1)-C(9)	1.314(4)	Cl(2)-C(26)-C(27)	118.2(2)
N(1)-C(8)	1.460(4)	Cl(2)-C(26)-C(25)	118.7(2)
N(2)-C(10)	1.388(4)	C(22)-C(27)-C(26)	119.1(3)

N(2)-C(9)	1.323(4)	C(3)-C(2)-H(2)	120.00
N(3)-C(22)	1.424(4)	C(1)-C(2)-H(2)	120.00
N(3)-C(10)	1.353(3)	C(4)-C(3)-H(3)	120.00
N(3)-C(11)	1.482(4)	C(2)-C(3)-H(3)	120.00
N(1)-H(1N)	0.78(5)	C(6)-C(5)-H(5)	120.00
N(4)-C(35)	1.459(4)	C(4)-C(5)-H(5)	120.00
N(4)-C(36)	1.322(4)	C(5)-C(6)-H(6)	120.00
N(5)-C(36)	1.317(4)	C(1)-C(6)-H(6)	120.00
N(5)-C(37)	1.388(4)	C(4)-C(8)-H(8B)	109.00
N(6)-C(49)	1.427(3)	N(1)-C(8)-H(8B)	109.00
N(6)-C(38)	1.476(4)	N(1)-C(8)-H(8A)	109.00
N(6)-C(37)	1.360(4)	C(4)-C(8)-H(8A)	109.00
N(4)-H(4N)	0.83(5)	H(8A)-C(8)-H(8B)	108.00
C(1)-C(7)	1.489(5)	H(12B)-C(12)-H(12C)	110.00
C(1)-C(6)	1.382(4)	C(11)-C(12)-H(12A)	110.00
C(1)-C(2)	1.384(5)	H(12A)-C(12)-H(12B)	109.00
C(2)-C(3)	1.387(5)	C(11)-C(12)-H(12C)	110.00
C(3)-C(4)	1.390(4)	C(11)-C(12)-H(12B)	109.00
C(4)-C(8)	1.520(4)	H(12A)-C(12)-H(12C)	109.00
C(4)-C(5)	1.386(5)	C(14)-C(15)-H(15)	120.00
C(5)-C(6)	1.387(5)	C(16)-C(15)-H(15)	120.00
C(9)-C(11)	1.522(4)	C(17)-C(18)-H(18)	119.00
C(11)-C(13)	1.527(4)	C(13)-C(18)-H(18)	119.00
C(11)-C(12)	1.531(4)	O(1)-C(19)-H(19B)	110.00
C(13)-C(18)	1.400(4)	H(19A)-C(19)-H(19B)	109.00
C(13)-C(14)	1.392(4)	H(19A)-C(19)-H(19C)	109.00
C(14)-C(15)	1.397(4)	O(1)-C(19)-H(19C)	109.00
C(15)-C(16)	1.384(4)	O(1)-C(19)-H(19A)	109.00
C(16)-C(17)	1.406(4)	H(19B)-C(19)-H(19C)	110.00
C(17)-C(18)	1.384(4)	H(20A)-C(20)-H(20B)	109.00
C(22)-C(27)	1.382(4)	O(2)-C(20)-H(20A)	109.00
C(22)-C(23)	1.394(4)	H(20A)-C(20)-H(20C)	109.00
C(23)-C(24)	1.369(4)	O(2)-C(20)-H(20B)	109.00
C(24)-C(25)	1.403(4)	H(20B)-C(20)-H(20C)	109.00
C(25)-C(26)	1.395(4)	O(2)-C(20)-H(20C)	110.00
C(26)-C(27)	1.381(4)	H(21A)-C(21)-H(21C)	109.00

C(2)-H(2)	0.9500	O(3)-C(21)-H(21C)	109.00
C(3)-H(3)	0.9500	H(21B)-C(21)-H(21C)	109.00
C(5)-H(5)	0.9500	H(21A)-C(21)-H(21B)	110.00
C(6)-H(6)	0.9500	O(3)-C(21)-H(21A)	109.00
C(8)-H(8A)	0.9900	O(3)-C(21)-H(21B)	109.00
C(8)-H(8B)	0.9900	C(24)-C(23)-H(23)	120.00
C(12)-H(12B)	0.9800	C(22)-C(23)-H(23)	120.00
C(12)-H(12A)	0.9800	C(22)-C(27)-H(27)	121.00
C(12)-H(12C)	0.9800	C(26)-C(27)-H(27)	120.00
C(15)-H(15)	0.9500	C(29)-C(28)-C(34)	119.6(3)
C(18)-H(18)	0.9500	C(33)-C(28)-C(34)	120.6(3)
C(19)-H(19B)	0.9800	C(29)-C(28)-C(33)	119.8(3)
C(19)-H(19A)	0.9800	C(28)-C(29)-C(30)	120.3(4)
C(19)-H(19C)	0.9800	C(29)-C(30)-C(31)	120.4(3)
C(20)-H(20C)	0.9800	C(32)-C(31)-C(35)	121.5(3)
C(20)-H(20B)	0.9800	C(30)-C(31)-C(35)	119.7(3)
C(20)-H(20A)	0.9800	C(30)-C(31)-C(32)	118.8(3)
C(21)-H(21A)	0.9800	C(31)-C(32)-C(33)	120.8(3)
C(21)-H(21C)	0.9800	C(28)-C(33)-C(32)	119.8(3)
C(21)-H(21B)	0.9800	F(5)-C(34)-C(28)	113.3(4)
C(23)-H(23)	0.9500	F(4)-C(34)-F(5)	102.8(4)
C(27)-H(27)	0.9500	F(4)-C(34)-C(28)	112.0(4)
C(28)-C(29)	1.378(6)	F(6)-C(34)-C(28)	114.6(4)
C(28)-C(33)	1.389(5)	F(4)-C(34)-F(6)	105.6(5)
C(28)-C(34)	1.491(6)	F(5)-C(34)-F(6)	107.7(4)
C(29)-C(30)	1.384(6)	N(4)-C(35)-C(31)	111.6(3)
C(30)-C(31)	1.390(4)	N(4)-C(36)-N(5)	125.3(3)
C(31)-C(32)	1.389(4)	N(5)-C(36)-C(38)	113.7(2)
C(31)-C(35)	1.507(4)	N(4)-C(36)-C(38)	120.9(3)
C(32)-C(33)	1.380(5)	N(5)-C(37)-N(6)	110.9(2)
C(36)-C(38)	1.523(4)	S(2)-C(37)-N(6)	125.0(2)
C(38)-C(39)	1.540(4)	S(2)-C(37)-N(5)	124.1(2)
C(38)-C(40)	1.517(4)	N(6)-C(38)-C(39)	109.1(2)
C(40)-C(45)	1.395(4)	N(6)-C(38)-C(36)	97.5(2)
C(40)-C(41)	1.402(4)	C(36)-C(38)-C(40)	114.3(2)
C(41)-C(42)	1.375(4)	N(6)-C(38)-C(40)	113.5(2)

C(42)-C(43)	1.401(4)	C(39)-C(38)-C(40)	113.8(3)
C(43)-C(44)	1.388(4)	C(36)-C(38)-C(39)	107.3(2)
C(44)-C(45)	1.396(4)	C(38)-C(40)-C(45)	120.2(3)
C(49)-C(50)	1.387(4)	C(41)-C(40)-C(45)	117.9(2)
C(49)-C(54)	1.388(4)	C(38)-C(40)-C(41)	121.9(3)
C(50)-C(51)	1.386(4)	C(40)-C(41)-C(42)	121.5(3)
C(51)-C(52)	1.391(4)	O(5)-C(42)-C(41)	125.7(3)
C(52)-C(53)	1.398(4)	C(41)-C(42)-C(43)	119.8(3)
C(53)-C(54)	1.383(4)	O(5)-C(42)-C(43)	114.6(2)
C(29)-H(29)	0.9500	C(42)-C(43)-C(44)	120.0(2)
C(30)-H(30)	0.9500	O(6)-C(43)-C(44)	125.1(3)
C(32)-H(32)	0.9500	O(6)-C(43)-C(42)	114.8(3)
C(33)-H(33)	0.9500	C(43)-C(44)-C(45)	119.4(3)
C(35)-H(35A)	0.9900	C(40)-C(45)-C(44)	121.4(3)
C(35)-H(35B)	0.9900	O(7)-C(45)-C(40)	115.5(2)
C(39)-H(39A)	0.9800	O(7)-C(45)-C(44)	123.0(3)
C(39)-H(39C)	0.9800	C(50)-C(49)-C(54)	120.4(2)
C(39)-H(39B)	0.9800	N(6)-C(49)-C(50)	120.6(2)
C(41)-H(41)	0.9500	N(6)-C(49)-C(54)	118.9(2)
C(44)-H(44)	0.9500	C(49)-C(50)-C(51)	118.8(3)
C(46)-H(46A)	0.9800	Cl(3)-C(51)-C(52)	118.4(2)
C(46)-H(46C)	0.9800	Cl(3)-C(51)-C(50)	118.8(2)
C(46)-H(46B)	0.9800	C(50)-C(51)-C(52)	122.8(3)
C(47)-H(47A)	0.9800	O(8)-C(52)-C(53)	125.2(3)
C(47)-H(47B)	0.9800	C(51)-C(52)-C(53)	116.4(2)
C(47)-H(47C)	0.9800	O(8)-C(52)-C(51)	118.4(3)
C(48)-H(48B)	0.9800	C(52)-C(53)-C(54)	122.3(3)
C(48)-H(48C)	0.9800	Cl(4)-C(53)-C(54)	118.3(2)
C(48)-H(48A)	0.9800	Cl(4)-C(53)-C(52)	119.4(2)
C(50)-H(50)	0.9500	C(49)-C(54)-C(53)	119.3(2)
C(54)-H(54)	0.9500	C(30)-C(29)-H(29)	120.00
C(55)-H(55C)	0.9800	C(28)-C(29)-H(29)	120.00
C(55)-H(55A)	0.9800	C(29)-C(30)-H(30)	120.00
C(55)-H(55B)	0.9800	C(31)-C(30)-H(30)	120.00
		C(33)-C(32)-H(32)	120.00
C(14)-O(1)-C(19)	118.7(2)	C(31)-C(32)-H(32)	119.00

C(16)-O(2)-C(20)	116.8(2)	C(32)-C(33)-H(33)	120.00
C(17)-O(3)-C(21)	116.7(2)	C(28)-C(33)-H(33)	120.00
C(25)-O(4)-H(4)	110.00	C(31)-C(35)-H(35A)	109.00
C(42)-O(5)-C(46)	116.7(2)	N(4)-C(35)-H(35A)	109.00
C(43)-O(6)-C(47)	117.8(3)	N(4)-C(35)-H(35B)	109.00
C(45)-O(7)-C(48)	117.9(2)	C(31)-C(35)-H(35B)	109.00
C(52)-O(8)-H(8)	109.00	H(35A)-C(35)-H(35B)	108.00
C(55)-O(9)-H(9)	109.00	C(38)-C(39)-H(39A)	109.00
H(10O)-O(10)-H(10P)	123(3)	H(39A)-C(39)-H(39B)	109.00
C(8)-N(1)-C(9)	123.8(3)	H(39A)-C(39)-H(39C)	109.00
C(9)-N(2)-C(10)	106.7(2)	C(38)-C(39)-H(39C)	109.00
C(11)-N(3)-C(22)	124.2(2)	C(38)-C(39)-H(39B)	109.00
C(10)-N(3)-C(11)	110.5(2)	H(39B)-C(39)-H(39C)	110.00
C(10)-N(3)-C(22)	124.9(2)	C(40)-C(41)-H(41)	119.00
C(9)-N(1)-H(1N)	119(3)	C(42)-C(41)-H(41)	119.00
C(8)-N(1)-H(1N)	117(3)	C(45)-C(44)-H(44)	120.00
C(35)-N(4)-C(36)	124.3(3)	C(43)-C(44)-H(44)	120.00
C(36)-N(5)-C(37)	106.7(2)	O(5)-C(46)-H(46C)	109.00
C(38)-N(6)-C(49)	125.0(2)	H(46A)-C(46)-H(46B)	109.00
C(37)-N(6)-C(49)	124.2(2)	O(5)-C(46)-H(46B)	110.00
C(37)-N(6)-C(38)	110.8(2)	H(46B)-C(46)-H(46C)	109.00
C(36)-N(4)-H(4N)	119(3)	H(46A)-C(46)-H(46C)	110.00
C(35)-N(4)-H(4N)	117(3)	O(5)-C(46)-H(46A)	109.00
C(6)-C(1)-C(7)	120.0(3)	O(6)-C(47)-H(47A)	110.00
C(2)-C(1)-C(7)	120.5(3)	H(47A)-C(47)-H(47B)	109.00
C(2)-C(1)-C(6)	119.5(3)	H(47A)-C(47)-H(47C)	109.00
C(1)-C(2)-C(3)	120.5(3)	O(6)-C(47)-H(47C)	109.00
C(2)-C(3)-C(4)	120.1(3)	O(6)-C(47)-H(47B)	109.00
C(5)-C(4)-C(8)	120.9(3)	H(47B)-C(47)-H(47C)	109.00
C(3)-C(4)-C(8)	119.9(3)	O(7)-C(48)-H(48B)	109.00
C(3)-C(4)-C(5)	119.1(3)	O(7)-C(48)-H(48A)	109.00
C(4)-C(5)-C(6)	120.6(3)	H(48A)-C(48)-H(48C)	109.00
C(1)-C(6)-C(5)	120.2(3)	H(48B)-C(48)-H(48C)	109.00
F(3)-C(7)-C(1)	113.6(3)	H(48A)-C(48)-H(48B)	110.00
F(1)-C(7)-C(1)	112.8(3)	O(7)-C(48)-H(48C)	109.00
F(1)-C(7)-F(3)	106.4(3)	C(49)-C(50)-H(50)	121.00

F(2)-C(7)-C(1)	112.7(3)	C(51)-C(50)-H(50)	121.00
F(1)-C(7)-F(2)	105.2(3)	C(53)-C(54)-H(54)	120.00
F(2)-C(7)-F(3)	105.6(3)	C(49)-C(54)-H(54)	120.00
N(1)-C(8)-C(4)	113.8(2)	O(9)-C(55)-H(55B)	110.00
N(2)-C(9)-C(11)	113.4(2)	O(9)-C(55)-H(55C)	109.00
N(1)-C(9)-C(11)	121.7(2)	O(9)-C(55)-H(55A)	109.00
N(1)-C(9)-N(2)	124.8(2)	H(55A)-C(55)-H(55C)	109.00
S(1)-C(10)-N(3)	124.8(2)	H(55B)-C(55)-H(55C)	109.00
N(2)-C(10)-N(3)	111.4(2)	H(55A)-C(55)-H(55B)	110.00

Symmetry transformations used to generate equivalent atoms

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 5.45. The anisotropic displacement factor exponent takes the form:-2 pi^2 [h^2 a^{*2} U11 +...+ 2 h k a* b* U12].

	U11	U22	U33	U23	U13	U12
Cl(1)	52(1)	24(1)	42(1)	-10(1)	-14(1)	-10(1)
Cl(2)	27(1)	35(1)	45(1)	-8(1)	-9(1)	-1(1)
S(1)	38(1)	35(1)	34(1)	-19(1)	3(1)	-11(1)
F(1)	29(1)	47(1)	64(1)	-30(1)	-11(1)	0(1)
F(2)	61(1)	66(1)	40(1)	-23(1)	-14(1)	13(1)
F(3)	44(1)	33(1)	66(1)	-20(1)	-15(1)	-3(1)
O(1)	27(1)	22(1)	33(1)	-11(1)	0(1)	-1(1)
O(2)	45(1)	26(1)	27(1)	-9(1)	-1(1)	-9(1)
O(3)	46(1)	30(1)	35(1)	-14(1)	8(1)	-3(1)
O(4)	31(1)	34(1)	44(1)	-14(1)	-5(1)	-11(1)
N(1)	26(1)	28(1)	34(1)	-15(1)	-2(1)	-5(1)
N(2)	25(1)	26(1)	33(1)	-12(1)	0(1)	-5(1)
N(3)	26(1)	24(1)	28(1)	-11(1)	-3(1)	-5(1)
C(1)	29(1)	35(1)	34(1)	-18(1)	-4(1)	-2(1)
C(2)	29(1)	40(2)	47(2)	-22(1)	-8(1)	-7(1)
C(3)	32(1)	33(1)	44(2)	-19(1)	-4(1)	-10(1)
C(4)	27(1)	31(1)	32(1)	-17(1)	1(1)	-7(1)
C(5)	33(2)	32(2)	58(2)	-18(1)	-13(1)	-7(1)
C(6)	39(2)	31(1)	56(2)	-18(1)	-12(1)	-5(1)
C(7)	34(2)	42(2)	40(2)	-21(1)	-7(1)	-2(1)

C(8)	27(1)	29(1)	37(1)	-13(1)	-2(1)	-11(1)
C(9)	26(1)	21(1)	27(1)	-7(1)	-3(1)	-5(1)
C(10)	28(1)	22(1)	30(1)	-8(1)	-3(1)	-6(1)
C(11)	22(1)	23(1)	30(1)	-12(1)	-2(1)	-4(1)
C(12)	27(1)	21(1)	31(1)	-10(1)	-2(1)	-2(1)
C(13)	22(1)	22(1)	29(1)	-10(1)	-4(1)	-4(1)
C(14)	23(1)	23(1)	32(1)	-12(1)	-3(1)	-4(1)
C(15)	31(1)	21(1)	30(1)	-10(1)	-6(1)	-5(1)
C(16)	33(1)	26(1)	27(1)	-11(1)	-3(1)	-10(1)
C(17)	31(1)	26(1)	29(1)	-14(1)	0(1)	-5(1)
C(18)	28(1)	22(1)	31(1)	-13(1)	-3(1)	-4(1)
C(19)	34(1)	23(1)	48(2)	-16(1)	-1(1)	0(1)
C(20)	52(2)	26(1)	31(1)	-7(1)	-3(1)	-7(1)
C(21)	54(2)	34(2)	43(2)	-17(1)	13(1)	2(1)
C(22)	27(1)	25(1)	25(1)	-12(1)	-1(1)	-4(1)
C(23)	29(1)	23(1)	28(1)	-9(1)	-5(1)	-2(1)
C(24)	34(1)	23(1)	27(1)	-9(1)	-3(1)	-6(1)
C(25)	26(1)	34(1)	27(1)	-14(1)	0(1)	-9(1)
C(26)	25(1)	29(1)	27(1)	-9(1)	-1(1)	-5(1)
C(27)	29(1)	23(1)	27(1)	-9(1)	-1(1)	-4(1)
Cl(3)	42(1)	41(1)	34(1)	-18(1)	10(1)	-8(1)
Cl(4)	27(1)	41(1)	48(1)	-26(1)	0(1)	-10(1)
S(2)	32(1)	24(1)	35(1)	-10(1)	-2(1)	-6(1)
F(4)	112(3)	73(2)	215(5)	-98(3)	-81(3)	20(2)
F(5)	38(1)	120(2)	162(3)	-111(2)	0(2)	-19(1)
F(6)	165(4)	227(5)	92(2)	-103(3)	43(2)	-150(4)
O(5)	40(1)	24(1)	38(1)	-16(1)	-11(1)	2(1)
O(6)	48(1)	27(1)	34(1)	-13(1)	-14(1)	0(1)
O(7)	34(1)	23(1)	29(1)	-12(1)	-3(1)	0(1)
O(8)	27(1)	39(1)	47(1)	-21(1)	9(1)	-13(1)
N(4)	25(1)	27(1)	38(1)	-9(1)	-3(1)	-8(1)
N(5)	24(1)	27(1)	34(1)	-13(1)	-3(1)	-6(1)
N(6)	25(1)	24(1)	27(1)	-11(1)	-1(1)	-5(1)
C(28)	29(1)	41(2)	59(2)	-28(2)	-1(1)	-8(1)
C(29)	39(2)	58(2)	72(2)	-38(2)	16(2)	-30(2)
C(30)	39(2)	54(2)	52(2)	-29(2)	12(1)	-23(2)

C(31)	25(1)	26(1)	41(1)	-13(1)	-3(1)	-6(1)
C(32)	31(1)	37(2)	39(2)	-13(1)	0(1)	-13(1)
C(33)	34(2)	42(2)	45(2)	-21(1)	-1(1)	-8(1)
C(34)	39(2)	53(2)	72(2)	-41(2)	2(2)	-13(2)
C(35)	26(1)	35(1)	39(1)	-16(1)	-1(1)	-9(1)
C(36)	24(1)	27(1)	30(1)	-14(1)	-4(1)	-7(1)
C(37)	25(1)	28(1)	27(1)	-13(1)	-4(1)	-4(1)
C(38)	24(1)	26(1)	32(1)	-14(1)	0(1)	-7(1)
C(39)	36(1)	31(1)	34(1)	-18(1)	-4(1)	-7(1)
C(40)	23(1)	24(1)	29(1)	-11(1)	2(1)	-8(1)
C(41)	26(1)	24(1)	32(1)	-15(1)	2(1)	-8(1)
C(42)	26(1)	22(1)	36(1)	-13(1)	-3(1)	-5(1)
C(43)	30(1)	24(1)	29(1)	-9(1)	-4(1)	-6(1)
C(44)	33(1)	23(1)	30(1)	-12(1)	-2(1)	-5(1)
C(45)	26(1)	21(1)	30(1)	-11(1)	-1(1)	-6(1)
C(46)	44(2)	25(1)	42(2)	-17(1)	-5(1)	-4(1)
C(47)	61(2)	34(2)	42(2)	-20(1)	-20(2)	2(1)
C(48)	34(1)	25(1)	34(1)	-15(1)	3(1)	-5(1)
C(49)	23(1)	23(1)	29(1)	-11(1)	-1(1)	-3(1)
C(50)	33(1)	27(1)	29(1)	-12(1)	-1(1)	-7(1)
C(51)	31(1)	25(1)	31(1)	-10(1)	4(1)	-3(1)
C(52)	27(1)	21(1)	40(1)	-11(1)	4(1)	-4(1)
C(53)	25(1)	22(1)	37(1)	-14(1)	-1(1)	-5(1)
C(54)	24(1)	21(1)	29(1)	-11(1)	0(1)	-3(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 5.45.

	x	y	z	U(eq)
H(1N)	2080(30)	4390(30)	2810(30)	35
H(2)	7168	2892	3610	45
H(3)	5765	4200	2802	42
H(4)	-4001	2849	1193	44
H(5)	3643	2604	2940	49
H(6)	5046	1299	3751	50
H(8A)	3751	4901	2317	37

H(8B)	3891	4537	1604	37
H(12A)	-49	5362	1314	34
H(12B)	28	5243	2274	34
H(12C)	-1049	4963	2071	34
H(15)	951	1154	4550	33
H(18)	-1072	4219	3452	32
H(19A)	1898	857	3436	44
H(19B)	2827	1109	3814	44
H(19C)	3050	1208	2839	44
H(20A)	571	481	6044	47
H(20B)	-294	289	5563	47
H(20C)	-663	189	6533	47
H(21A)	-2921	4291	4189	58
H(21B)	-1988	4485	4618	58
H(21C)	-3172	4155	5176	58
H(23)	158	2040	2202	33
H(27)	-1617	4671	870	33
H(4N)	2050(30)	-1390(30)	1450(30)	39
H(8)	-4817	-3998	3657	45
H(29)	6325	-722	1719	62
H(30)	5430	-1328	1092	54
H(32)	3164	-2171	3246	44
H(33)	4032	-1538	3860	48
H(35A)	3962	-2018	1079	40
H(35B)	3897	-2893	2013	40
H(39A)	474	-1075	2498	38
H(39B)	365	-1976	3400	38
H(39C)	-805	-1339	2871	38
H(41)	-1233	-490	1555	32
H(44)	-494	-1856	-315	35
H(46A)	-1759	1126	606	43
H(46B)	-2842	653	1184	43
H(46C)	-3104	1601	310	43
H(47A)	-2387	-1150	-1120	54
H(47B)	-1158	-868	-1633	54
H(47C)	-2415	-195	-1988	54

H(48A)	1733	-2760	111	37
H(48B)	619	-3186	192	37
H(48C)	1713	-3798	813	37
H(50)	-1477	-3013	4243	35
H(54)	-1415	-3349	2077	31
H(9)	-5460	2703	1057	64
H(55A)	-5891	3742	-537	390
H(55B)	-5499	2648	-59	390
H(55C)	-4502	3253	-413	390
H(10O)	-6510(20)	-4240(20)	3180(20)	45
H(10P)	-5900(30)	-4920(20)	3996(13)	45

Table 6. Torsion angles [deg.] for 5.45.

C(19)-O(1)-C(14)-C(13)	178.0(3)	C(11)-C(13)-C(18)-C(17)	174.9(2)
C(19)-O(1)-C(14)-C(15)	-3.0(4)	O(1)-C(14)-C(15)-C(16)	-178.3(2)
C(20)-O(2)-C(16)-C(15)	3.2(4)	C(13)-C(14)-C(15)-C(16)	0.7(4)
C(20)-O(2)-C(16)-C(17)	-177.8(3)	C(14)-C(15)-C(16)-C(17)	-3.1(4)
C(21)-O(3)-C(17)-C(16)	-178.7(3)	C(14)-C(15)-C(16)-O(2)	175.8(3)
C(21)-O(3)-C(17)-C(18)	-0.3(4)	C(15)-C(16)-C(17)-C(18)	3.1(4)
C(46)-O(5)-C(42)-C(43)	-173.1(2)	O(2)-C(16)-C(17)-O(3)	2.5(4)
C(46)-O(5)-C(42)-C(41)	8.3(4)	C(15)-C(16)-C(17)-O(3)	-178.4(2)
C(47)-O(6)-C(43)-C(42)	-167.5(3)	O(2)-C(16)-C(17)-C(18)	-176.0(2)
C(47)-O(6)-C(43)-C(44)	13.4(4)	O(3)-C(17)-C(18)-C(13)	-178.9(3)
C(48)-O(7)-C(45)-C(40)	-157.9(3)	C(16)-C(17)-C(18)-C(13)	-0.6(4)
C(48)-O(7)-C(45)-C(44)	23.9(4)	N(3)-C(22)-C(27)-C(26)	178.6(2)
C(9)-N(1)-C(8)-C(4)	-94.8(3)	N(3)-C(22)-C(23)-C(24)	-177.8(2)
C(8)-N(1)-C(9)-C(11)	-176.6(3)	C(23)-C(22)-C(27)-C(26)	-0.4(4)
C(8)-N(1)-C(9)-N(2)	0.0(5)	C(27)-C(22)-C(23)-C(24)	1.2(4)
C(9)-N(2)-C(10)-S(1)	-178.2(2)	C(22)-C(23)-C(24)-Cl(1)	177.8(2)
C(10)-N(2)-C(9)-N(1)	179.0(3)	C(22)-C(23)-C(24)-C(25)	-1.1(4)
C(10)-N(2)-C(9)-C(11)	-4.2(3)	C(23)-C(24)-C(25)-O(4)	178.0(3)
C(9)-N(2)-C(10)-N(3)	2.5(3)	Cl(1)-C(24)-C(25)-C(26)	-178.7(2)
C(10)-N(3)-C(11)-C(9)	-2.3(3)	C(23)-C(24)-C(25)-C(26)	0.2(4)
C(11)-N(3)-C(10)-N(2)	0.1(3)	Cl(1)-C(24)-C(25)-O(4)	-0.8(3)
C(11)-N(3)-C(10)-S(1)	-179.1(2)	C(24)-C(25)-C(26)-C(27)	0.6(4)

C(11)-N(3)-C(22)-C(23)	-99.7(3)	C(24)-C(25)-C(26)-Cl(2)	179.5(2)
C(22)-N(3)-C(11)-C(9)	170.4(2)	O(4)-C(25)-C(26)-C(27)	-177.0(3)
C(22)-N(3)-C(11)-C(13)	50.3(3)	O(4)-C(25)-C(26)-Cl(2)	2.0(4)
C(11)-N(3)-C(22)-C(27)	81.3(3)	Cl(2)-C(26)-C(27)-C(22)	-179.5(2)
C(10)-N(3)-C(11)-C(13)	-122.4(2)	C(25)-C(26)-C(27)-C(22)	-0.5(4)
C(22)-N(3)-C(10)-N(2)	-172.5(2)	C(34)-C(28)-C(33)-C(32)	177.5(4)
C(10)-N(3)-C(22)-C(27)	-107.1(3)	C(29)-C(28)-C(33)-C(32)	-0.5(6)
C(10)-N(3)-C(22)-C(23)	71.9(3)	C(33)-C(28)-C(34)-F(6)	17.4(6)
C(22)-N(3)-C(10)-S(1)	8.3(4)	C(33)-C(28)-C(34)-F(4)	-102.8(4)
C(10)-N(3)-C(11)-C(12)	111.0(2)	C(34)-C(28)-C(29)-C(30)	-176.8(4)
C(22)-N(3)-C(11)-C(12)	-76.3(3)	C(33)-C(28)-C(34)-F(5)	141.5(4)
C(35)-N(4)-C(36)-N(5)	-1.6(5)	C(29)-C(28)-C(34)-F(6)	-164.6(5)
C(36)-N(4)-C(35)-C(31)	-110.6(3)	C(29)-C(28)-C(34)-F(5)	-40.6(6)
C(35)-N(4)-C(36)-C(38)	173.8(3)	C(33)-C(28)-C(29)-C(30)	1.2(6)
C(36)-N(5)-C(37)-S(2)	177.4(2)	C(29)-C(28)-C(34)-F(4)	75.1(5)
C(37)-N(5)-C(36)-C(38)	6.4(3)	C(28)-C(29)-C(30)-C(31)	-0.8(6)
C(36)-N(5)-C(37)-N(6)	-3.3(3)	C(29)-C(30)-C(31)-C(32)	-0.3(5)
C(37)-N(5)-C(36)-N(4)	-177.9(3)	C(29)-C(30)-C(31)-C(35)	-178.7(4)
C(37)-N(6)-C(49)-C(54)	-86.6(3)	C(30)-C(31)-C(35)-N(4)	-131.7(3)
C(49)-N(6)-C(37)-S(2)	-4.3(4)	C(30)-C(31)-C(32)-C(33)	1.0(5)
C(49)-N(6)-C(37)-N(5)	176.4(2)	C(35)-C(31)-C(32)-C(33)	179.4(3)
C(49)-N(6)-C(38)-C(40)	-52.6(3)	C(32)-C(31)-C(35)-N(4)	49.9(4)
C(38)-N(6)-C(49)-C(54)	90.5(3)	C(31)-C(32)-C(33)-C(28)	-0.6(5)
C(37)-N(6)-C(49)-C(50)	89.7(4)	N(5)-C(36)-C(38)-C(40)	-126.7(3)
C(38)-N(6)-C(37)-S(2)	178.3(2)	N(4)-C(36)-C(38)-C(40)	57.4(4)
C(49)-N(6)-C(38)-C(39)	75.6(3)	N(4)-C(36)-C(38)-N(6)	177.5(3)
C(38)-N(6)-C(37)-N(5)	-1.1(3)	N(5)-C(36)-C(38)-N(6)	-6.6(3)
C(37)-N(6)-C(38)-C(40)	124.9(2)	N(4)-C(36)-C(38)-C(39)	-69.7(3)
C(37)-N(6)-C(38)-C(39)	-107.0(3)	N(5)-C(36)-C(38)-C(39)	106.2(3)
C(49)-N(6)-C(38)-C(36)	-173.2(2)	C(36)-C(38)-C(40)-C(45)	50.6(3)
C(38)-N(6)-C(49)-C(50)	-93.2(3)	C(39)-C(38)-C(40)-C(41)	-6.3(4)
C(37)-N(6)-C(38)-C(36)	4.3(3)	C(36)-C(38)-C(40)-C(41)	-130.0(3)
C(6)-C(1)-C(7)-F(3)	23.1(4)	N(6)-C(38)-C(40)-C(41)	119.4(3)
C(2)-C(1)-C(7)-F(3)	-157.3(3)	C(39)-C(38)-C(40)-C(45)	174.3(2)
C(6)-C(1)-C(7)-F(1)	144.2(3)	N(6)-C(38)-C(40)-C(45)	-60.1(3)
C(6)-C(1)-C(2)-C(3)	1.2(5)	C(41)-C(40)-C(45)-O(7)	-178.6(2)

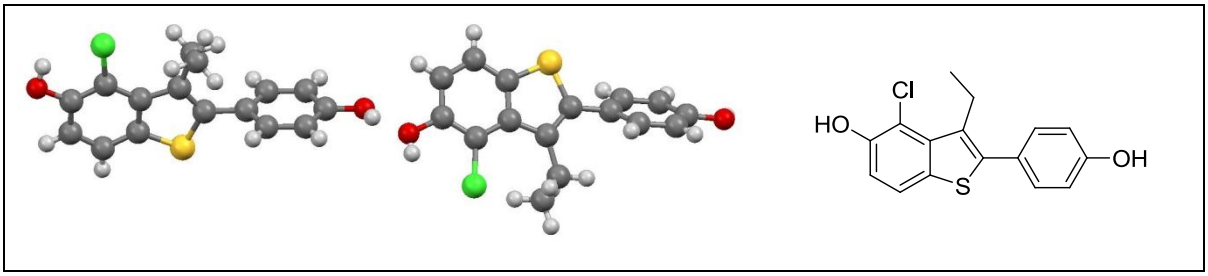
C(6)-C(1)-C(7)-F(2)	-96.9(4)	C(45)-C(40)-C(41)-C(42)	-0.1(4)
C(2)-C(1)-C(7)-F(2)	82.7(4)	C(38)-C(40)-C(41)-C(42)	-179.6(2)
C(7)-C(1)-C(6)-C(5)	178.6(3)	C(41)-C(40)-C(45)-C(44)	-0.3(4)
C(2)-C(1)-C(7)-F(1)	-36.2(4)	C(38)-C(40)-C(45)-C(44)	179.2(2)
C(7)-C(1)-C(2)-C(3)	-178.4(3)	C(38)-C(40)-C(45)-O(7)	1.0(4)
C(2)-C(1)-C(6)-C(5)	-1.1(5)	C(40)-C(41)-C(42)-O(5)	178.8(2)
C(1)-C(2)-C(3)-C(4)	-0.8(5)	C(40)-C(41)-C(42)-C(43)	0.3(4)
C(2)-C(3)-C(4)-C(8)	-177.9(3)	O(5)-C(42)-C(43)-C(44)	-178.8(2)
C(2)-C(3)-C(4)-C(5)	0.3(5)	C(41)-C(42)-C(43)-O(6)	-179.2(2)
C(3)-C(4)-C(5)-C(6)	-0.2(5)	C(41)-C(42)-C(43)-C(44)	-0.1(4)
C(8)-C(4)-C(5)-C(6)	178.0(3)	O(5)-C(42)-C(43)-O(6)	2.1(3)
C(5)-C(4)-C(8)-N(1)	32.6(4)	O(6)-C(43)-C(44)-C(45)	178.8(3)
C(3)-C(4)-C(8)-N(1)	-149.3(3)	C(42)-C(43)-C(44)-C(45)	-0.3(4)
C(4)-C(5)-C(6)-C(1)	0.6(5)	C(43)-C(44)-C(45)-C(40)	0.5(4)
N(1)-C(9)-C(11)-C(13)	-60.9(4)	C(43)-C(44)-C(45)-O(7)	178.6(2)
N(2)-C(9)-C(11)-C(12)	-109.8(3)	N(6)-C(49)-C(50)-C(51)	-175.3(3)
N(1)-C(9)-C(11)-C(12)	67.2(3)	C(54)-C(49)-C(50)-C(51)	1.0(4)
N(2)-C(9)-C(11)-C(13)	122.1(3)	N(6)-C(49)-C(54)-C(53)	173.4(3)
N(1)-C(9)-C(11)-N(3)	-179.0(3)	C(50)-C(49)-C(54)-C(53)	-2.9(4)
N(2)-C(9)-C(11)-N(3)	4.0(3)	C(49)-C(50)-C(51)-Cl(3)	-178.3(2)
N(3)-C(11)-C(13)-C(18)	-121.4(3)	C(49)-C(50)-C(51)-C(52)	3.0(5)
C(9)-C(11)-C(13)-C(18)	128.8(3)	Cl(3)-C(51)-C(52)-O(8)	-4.3(4)
C(12)-C(11)-C(13)-C(18)	3.1(4)	Cl(3)-C(51)-C(52)-C(53)	176.5(2)
N(3)-C(11)-C(13)-C(14)	55.3(3)	C(50)-C(51)-C(52)-O(8)	174.4(3)
C(12)-C(11)-C(13)-C(14)	179.7(2)	C(50)-C(51)-C(52)-C(53)	-4.8(5)
C(9)-C(11)-C(13)-C(14)	-54.6(4)	O(8)-C(52)-C(53)-Cl(4)	1.8(4)
C(14)-C(13)-C(18)-C(17)	-1.8(4)	O(8)-C(52)-C(53)-C(54)	-176.4(3)
C(18)-C(13)-C(14)-C(15)	1.8(4)	C(51)-C(52)-C(53)-Cl(4)	-179.0(2)
C(18)-C(13)-C(14)-O(1)	-179.2(2)	C(51)-C(52)-C(53)-C(54)	2.7(4)
C(11)-C(13)-C(14)-C(15)	-175.0(2)	Cl(4)-C(53)-C(54)-C(49)	-177.3(2)
C(11)-C(13)-C(14)-O(1)	4.1(4)	C(52)-C(53)-C(54)-C(49)	1.0(4)

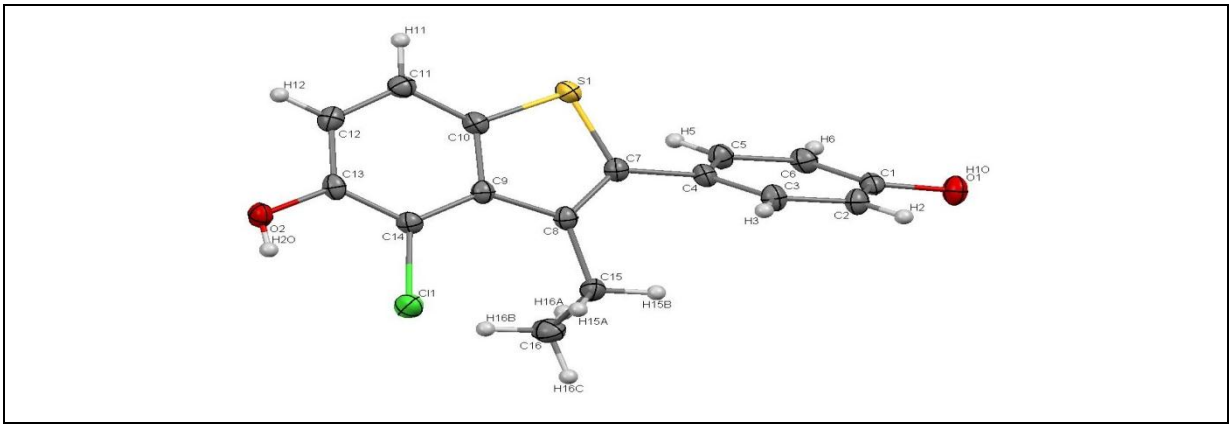
Symmetry transformations used to generate equivalent atoms

Table 7. Hydrogen-bonds for 5.45 [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1N)...S(2)#1	0.78(5)	2.65(5)	3.406(3)	165(5)
O(4)-H(4)...Cl(2)	0.8400	2.5700	3.059(3)	118.00
O(4)-H(4)...O(9)	0.8400	1.8400	2.588(3)	148.00
N(4)-H(4N)...O(5)#2	0.83(5)	2.37(5)	2.925(3)	124(4)
N(4)-H(4N)...O(6)#2	0.83(5)	2.40(5)	3.160(4)	153(4)
O(8)-H(8)...Cl(4)	0.8400	2.5500	3.055(3)	119.00
O(8)-H(8)...O(10)	0.8400	1.9200	2.670(4)	149.00
O(10)-H(10O)...N(5)#3	0.87(3)	1.95(3)	2.801(4)	166(3)
O(10)-H(10P)...Cl(3)#4	0.83(2)	2.77(3)	3.371(3)	132(3)
C(5)-H(5)...O(1)	0.9500	2.4600	3.385(4)	166.00
C(8)-H(8B)...N(2)	0.9900	2.5500	2.884(4)	100.00
C(33)-H(33)...F(2)#5	0.9500	2.3700	3.251(4)	154.00
C(35)-H(35B)...N(5)	0.9900	2.5100	2.906(4)	104.00
C(39)-H(39B)...O(2)#6	0.9800	2.4800	3.161(4)	127.00
C(39)-H(39C)...F(5)#3	0.9800	2.5400	3.516(5)	178.00

A.3.2 4-Chloro-3-ethyl-2-(4-hydroxyphenyl)benzo[*b*]thiophen-5-ol (10)





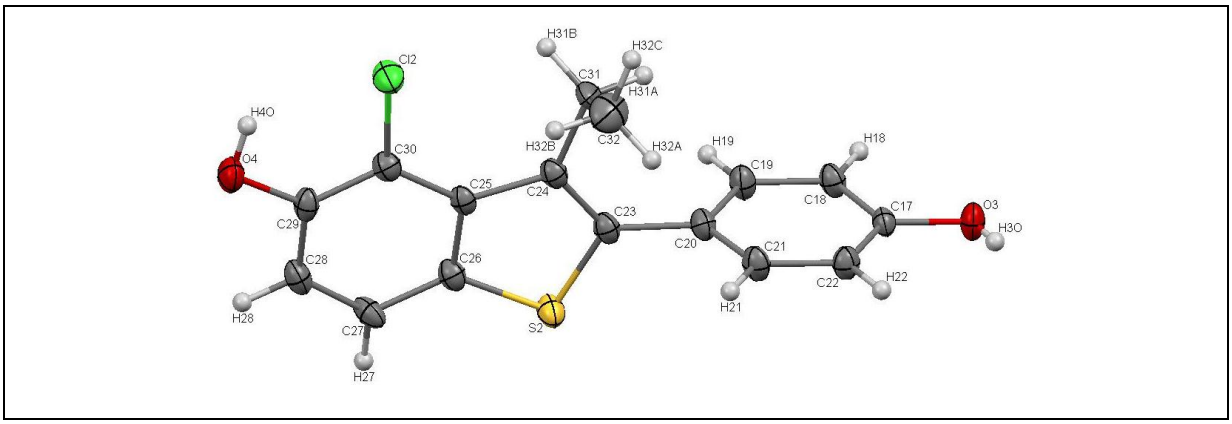


Table 1. Crystal data and structure refinement for 10.

Crystal Data	
Empirical formula	$C_{16}H_{13}ClO_2S$
Formula weight	304.78
Crystal size	0.2548 x 0.1468 x 0.0543 mm
Crystal description	plate
Crystal colour	colourless
Crystal system	Triclinic

Space group	P -1
Unit cell dimensions	a = 9.2721(4) Å alpha = 84.939(4) deg.
	b = 9.4576(4) Å beta = 86.032(3) deg.
	c = 16.7403(7) Å gamma = 69.579(4) deg.
Volume	1369.21(11) $Å^3$
Z, Calculated density	4, 1.479 Mg/m^3
Absorption coefficient	3.875 mm^{-1}
F(000)	632
Data Collection	
Measurement device type	SuperNova, Single source at offset), Atlas
Measuremnet method	\w scans
Temperature	123 K
Wavelength	1.54184 Å
Monochromator	graphite
Theta range for data collection	5.00 to 76.76 deg.
Index ranges	-11<=h<=11, -11<=k<=11, -20<=l<=21
Reflections collected / unique	24243 / 5651 [R(int) = 0.0251]
Reflections greater I>2\s(I)	5474
Absorption correction	Analytical
Max. and min. transmission	0.900 and 0.721
Refinement	
Refinement method	Full-matrix least-squares on F^2
Hydrogen treatment	---
Data / restraints / parameters	5651 / 0 / 373
Goodness-of-fit on F^2	1.059
Final R indices [I>2sigma(I)]	R1 = 0.0364, wR2 = 0.0979
R indices (all data)	R1 = 0.0372, wR2 = 0.0986
Absolute structure parameter	---
Largest diff. peak and hole	0.670 and -0.496 e. $Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 10. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
Cl(1)	4280(1)	1407(1)	-2351(1)	28(1)
S(1)	3528(1)	3044(1)	682(1)	22(1)
O(1)	10472(2)	2045(2)	1927(1)	25(1)
O(2)	886(2)	2499(1)	-2295(1)	22(1)
C(1)	9192(2)	2197(2)	1520(1)	20(1)
C(2)	9126(2)	904(2)	1213(1)	22(1)
C(3)	7879(2)	988(2)	774(1)	21(1)
C(4)	6674(2)	2356(2)	641(1)	19(1)
C(5)	6743(2)	3639(2)	964(1)	21(1)
C(6)	7997(2)	3568(2)	1399(1)	21(1)
C(7)	5325(2)	2427(2)	190(1)	19(1)
C(8)	5288(2)	2068(2)	-581(1)	18(1)
C(9)	3726(2)	2321(2)	-798(1)	17(1)
C(10)	2644(2)	2862(2)	-160(1)	19(1)
C(11)	1064(2)	3215(2)	-221(1)	21(1)
C(12)	516(2)	3085(2)	-943(1)	21(1)
C(13)	1537(2)	2572(2)	-1597(1)	19(1)
C(14)	3118(2)	2161(2)	-1521(1)	18(1)
C(15)	6704(2)	1610(2)	-1130(1)	23(1)
C(16)	6796(2)	2900(2)	-1727(1)	29(1)
Cl(2)	-10996(1)	7199(1)	-4670(1)	29(1)
S(2)	-5897(1)	8584(1)	-5387(1)	24(1)
O(3)	-1155(2)	5517(2)	-2435(1)	24(1)
O(4)	-11994(2)	8800(2)	-6224(1)	27(1)
C(17)	-2378(2)	5957(2)	-2929(1)	19(1)
C(18)	-3448(2)	7404(2)	-2870(1)	22(1)
C(19)	-4668(2)	7921(2)	-3379(1)	23(1)
C(20)	-4840(2)	6999(2)	-3945(1)	21(1)
C(21)	-3774(2)	5536(2)	-3980(1)	24(1)
C(22)	-2544(2)	5013(2)	-3478(1)	22(1)
C(23)	-6130(2)	7562(2)	-4504(1)	21(1)

C(24)	-7519(2)	7366(2)	-4416(1)	20(1)
C(25)	-8456(2)	8028(2)	-5107(1)	18(1)
C(26)	-7703(2)	8739(2)	-5686(1)	21(1)
C(27)	-8393(2)	9510(2)	-6404(1)	24(1)
C(28)	-9811(2)	9502(2)	-6555(1)	24(1)
C(29)	-10585(2)	8783(2)	-6007(1)	21(1)
C(30)	-9927(2)	8066(2)	-5296(1)	21(1)
C(31)	-7973(2)	6545(2)	-3679(1)	26(1)
C(32)	-7653(3)	4891(2)	-3755(1)	38(1)

Table 3. Bond lengths [Å] and angles [deg] for 10.

Cl(1)-C(14)	1.7414(18)	C(8)-C(9)-C(10)	111.95(15)
Cl(2)-C(30)	1.7391(19)	C(10)-C(9)-C(14)	116.16(17)
S(1)-C(10)	1.7281(18)	C(8)-C(9)-C(14)	131.87(16)
S(1)-C(7)	1.7349(18)	C(9)-C(10)-C(11)	123.18(16)
S(2)-C(26)	1.735(2)	S(1)-C(10)-C(9)	111.62(14)
S(2)-C(23)	1.7371(18)	S(1)-C(10)-C(11)	125.20(14)
O(1)-C(1)	1.367(2)	C(10)-C(11)-C(12)	118.75(17)
O(2)-C(13)	1.371(2)	C(11)-C(12)-C(13)	120.27(18)
O(1)-H(1O)	0.73(3)	O(2)-C(13)-C(14)	123.17(16)
O(2)-H(2O)	0.73(3)	O(2)-C(13)-C(12)	116.20(17)
O(3)-C(17)	1.372(2)	C(12)-C(13)-C(14)	120.63(16)
O(4)-C(29)	1.374(2)	Cl(1)-C(14)-C(9)	122.59(14)
O(3)-H(3O)	0.71(3)	C(9)-C(14)-C(13)	120.92(16)
O(4)-H(4O)	0.91(3)	Cl(1)-C(14)-C(13)	116.48(13)
C(1)-C(6)	1.390(3)	C(8)-C(15)-C(16)	112.80(15)
C(1)-C(2)	1.390(3)	C(1)-C(2)-H(2)	120.00
C(2)-C(3)	1.387(3)	C(3)-C(2)-H(2)	120.00
C(3)-C(4)	1.396(2)	C(4)-C(3)-H(3)	120.00
C(4)-C(5)	1.394(2)	C(2)-C(3)-H(3)	120.00
C(4)-C(7)	1.484(3)	C(4)-C(5)-H(5)	120.00
C(5)-C(6)	1.393(3)	C(6)-C(5)-H(5)	120.00
C(7)-C(8)	1.368(2)	C(1)-C(6)-H(6)	120.00
C(8)-C(15)	1.505(3)	C(5)-C(6)-H(6)	120.00
C(8)-C(9)	1.450(3)	C(12)-C(11)-H(11)	121.00
C(9)-C(10)	1.414(2)	C(10)-C(11)-H(11)	121.00

C(9)-C(14)	1.411(2)	C(11)-C(12)-H(12)	120.00
C(10)-C(11)	1.393(3)	C(13)-C(12)-H(12)	120.00
C(11)-C(12)	1.376(3)	C(8)-C(15)-H(15A)	109.00
C(12)-C(13)	1.399(3)	C(8)-C(15)-H(15B)	109.00
C(13)-C(14)	1.391(3)	C(16)-C(15)-H(15A)	109.00
C(15)-C(16)	1.529(3)	C(16)-C(15)-H(15B)	109.00
C(2)-H(2)	0.9500	H(15A)-C(15)-H(15B)	108.00
C(3)-H(3)	0.9500	C(15)-C(16)-H(16C)	109.00
C(5)-H(5)	0.9500	H(16A)-C(16)-H(16C)	109.00
C(6)-H(6)	0.9500	H(16B)-C(16)-H(16C)	110.00
C(11)-H(11)	0.9500	H(16A)-C(16)-H(16B)	109.00
C(12)-H(12)	0.9500	C(15)-C(16)-H(16A)	109.00
C(15)-H(15A)	0.9900	C(15)-C(16)-H(16B)	109.00
C(15)-H(15B)	0.9900	C(18)-C(17)-C(22)	120.61(17)
C(16)-H(16B)	0.9800	O(3)-C(17)-C(22)	121.88(16)
C(16)-H(16C)	0.9800	O(3)-C(17)-C(18)	117.51(15)
C(16)-H(16A)	0.9800	C(17)-C(18)-C(19)	119.58(16)
C(17)-C(22)	1.388(2)	C(18)-C(19)-C(20)	120.70(17)
C(17)-C(18)	1.389(2)	C(19)-C(20)-C(21)	118.71(17)
C(18)-C(19)	1.385(3)	C(19)-C(20)-C(23)	120.83(16)
C(19)-C(20)	1.396(3)	C(21)-C(20)-C(23)	120.46(16)
C(20)-C(23)	1.487(3)	C(20)-C(21)-C(22)	121.01(17)
C(20)-C(21)	1.395(3)	C(17)-C(22)-C(21)	119.36(16)
C(21)-C(22)	1.384(3)	S(2)-C(23)-C(24)	113.78(13)
C(23)-C(24)	1.360(3)	C(20)-C(23)-C(24)	127.69(16)
C(24)-C(31)	1.513(3)	S(2)-C(23)-C(20)	118.53(14)
C(24)-C(25)	1.455(2)	C(25)-C(24)-C(31)	126.12(17)
C(25)-C(30)	1.410(3)	C(23)-C(24)-C(25)	111.61(15)
C(25)-C(26)	1.413(2)	C(23)-C(24)-C(31)	122.27(16)
C(26)-C(27)	1.419(2)	C(26)-C(25)-C(30)	116.13(15)
C(27)-C(28)	1.359(3)	C(24)-C(25)-C(30)	132.03(16)
C(28)-C(29)	1.399(3)	C(24)-C(25)-C(26)	111.84(16)
C(29)-C(30)	1.385(2)	S(2)-C(26)-C(27)	125.64(14)
C(31)-C(32)	1.502(3)	S(2)-C(26)-C(25)	111.47(13)
C(18)-H(18)	0.9500	C(25)-C(26)-C(27)	122.86(18)
C(19)-H(19)	0.9500	C(26)-C(27)-C(28)	118.24(17)

C(21)-H(21)	0.9500	C(27)-C(28)-C(29)	120.81(17)
C(22)-H(22)	0.9500	C(28)-C(29)-C(30)	120.82(18)
C(27)-H(27)	0.9500	O(4)-C(29)-C(28)	116.48(16)
C(28)-H(28)	0.9500	O(4)-C(29)-C(30)	122.67(17)
C(31)-H(31A)	0.9900	Cl(2)-C(30)-C(25)	122.49(13)
C(31)-H(31B)	0.9900	Cl(2)-C(30)-C(29)	116.43(15)
C(32)-H(32A)	0.9800	C(25)-C(30)-C(29)	121.07(17)
C(32)-H(32B)	0.9800	C(24)-C(31)-C(32)	114.69(16)
C(32)-H(32C)	0.9800	C(17)-C(18)-H(18)	120.00
		C(19)-C(18)-H(18)	120.00
C(7)-S(1)-C(10)	91.34(9)	C(18)-C(19)-H(19)	120.00
C(23)-S(2)-C(26)	91.27(9)	C(20)-C(19)-H(19)	120.00
C(1)-O(1)-H(1O)	110(2)	C(20)-C(21)-H(21)	119.00
C(13)-O(2)-H(2O)	110(2)	C(22)-C(21)-H(21)	119.00
C(17)-O(3)-H(3O)	112(2)	C(17)-C(22)-H(22)	120.00
C(29)-O(4)-H(4O)	104.5(18)	C(21)-C(22)-H(22)	120.00
C(2)-C(1)-C(6)	120.00(17)	C(26)-C(27)-H(27)	121.00
O(1)-C(1)-C(2)	116.99(16)	C(28)-C(27)-H(27)	121.00
O(1)-C(1)-C(6)	123.01(16)	C(27)-C(28)-H(28)	120.00
C(1)-C(2)-C(3)	119.93(16)	C(29)-C(28)-H(28)	120.00
C(2)-C(3)-C(4)	120.89(16)	C(24)-C(31)-H(31A)	109.00
C(3)-C(4)-C(7)	120.51(15)	C(24)-C(31)-H(31B)	109.00
C(5)-C(4)-C(7)	120.90(16)	C(32)-C(31)-H(31A)	109.00
C(3)-C(4)-C(5)	118.56(17)	C(32)-C(31)-H(31B)	109.00
C(4)-C(5)-C(6)	120.93(16)	H(31A)-C(31)-H(31B)	108.00
C(1)-C(6)-C(5)	119.68(16)	C(31)-C(32)-H(32A)	109.00
S(1)-C(7)-C(4)	117.52(12)	C(31)-C(32)-H(32B)	109.00
S(1)-C(7)-C(8)	113.75(14)	C(31)-C(32)-H(32C)	109.00
C(4)-C(7)-C(8)	128.74(17)	H(32A)-C(32)-H(32B)	109.00
C(7)-C(8)-C(15)	122.30(17)	H(32A)-C(32)-H(32C)	109.00
C(9)-C(8)-C(15)	126.15(15)	H(32B)-C(32)-H(32C)	110.00
C(7)-C(8)-C(9)	111.34(16)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 10. The anisotropic displacement factor exponent takes the form:-2 pi^2 [h^2 a^{*2} U11 +...+ 2 h k a* b* U12].

	U11	U22	U33	U23	U13	U12
Cl(1)	23(1)	36(1)	23(1)	-13(1)	-3(1)	-6(1)
S(1)	21(1)	29(1)	16(1)	-3(1)	-2(1)	-8(1)
O(1)	23(1)	25(1)	30(1)	-2(1)	-11(1)	-10(1)
O(2)	20(1)	24(1)	21(1)	-4(1)	-7(1)	-7(1)
C(1)	20(1)	23(1)	17(1)	2(1)	-4(1)	-9(1)
C(2)	21(1)	18(1)	24(1)	2(1)	-6(1)	-4(1)
C(3)	25(1)	16(1)	23(1)	0(1)	-7(1)	-7(1)
C(4)	20(1)	20(1)	16(1)	1(1)	-5(1)	-7(1)
C(5)	22(1)	17(1)	21(1)	-1(1)	-6(1)	-3(1)
C(6)	26(1)	19(1)	21(1)	-2(1)	-5(1)	-8(1)
C(7)	19(1)	17(1)	19(1)	0(1)	-5(1)	-5(1)
C(8)	19(1)	15(1)	20(1)	0(1)	-5(1)	-4(1)
C(9)	18(1)	14(1)	19(1)	0(1)	-4(1)	-5(1)
C(10)	21(1)	19(1)	17(1)	0(1)	-3(1)	-7(1)
C(11)	22(1)	21(1)	21(1)	0(1)	0(1)	-7(1)
C(12)	18(1)	19(1)	26(1)	1(1)	-4(1)	-6(1)
C(13)	22(1)	15(1)	20(1)	0(1)	-8(1)	-8(1)
C(14)	20(1)	16(1)	18(1)	-2(1)	-3(1)	-5(1)
C(15)	17(1)	28(1)	21(1)	-5(1)	-4(1)	-4(1)
C(16)	27(1)	37(1)	26(1)	-5(1)	2(1)	-15(1)
Cl(2)	27(1)	36(1)	26(1)	4(1)	-4(1)	-17(1)
S(2)	25(1)	29(1)	22(1)	4(1)	-6(1)	-14(1)
O(3)	22(1)	20(1)	28(1)	-1(1)	-11(1)	-4(1)
O(4)	22(1)	33(1)	27(1)	-2(1)	-8(1)	-8(1)
C(17)	19(1)	21(1)	17(1)	2(1)	-6(1)	-7(1)
C(18)	27(1)	20(1)	20(1)	-3(1)	-7(1)	-7(1)
C(19)	25(1)	19(1)	23(1)	-3(1)	-8(1)	-3(1)
C(20)	22(1)	22(1)	20(1)	-1(1)	-6(1)	-8(1)
C(21)	27(1)	22(1)	22(1)	-6(1)	-7(1)	-7(1)
C(22)	23(1)	17(1)	24(1)	-3(1)	-5(1)	-3(1)
C(23)	24(1)	20(1)	18(1)	-3(1)	-5(1)	-7(1)
C(24)	24(1)	18(1)	18(1)	-3(1)	-3(1)	-6(1)

C(25)	22(1)	15(1)	16(1)	-4(1)	-4(1)	-5(1)
C(26)	26(1)	19(1)	19(1)	-2(1)	-5(1)	-9(1)
C(27)	34(1)	29(1)	15(1)	-1(1)	-4(1)	-18(1)
C(28)	31(1)	21(1)	19(1)	0(1)	-8(1)	-7(1)
C(29)	20(1)	19(1)	23(1)	-6(1)	-7(1)	-4(1)
C(30)	23(1)	20(1)	21(1)	-3(1)	-2(1)	-8(1)
C(31)	22(1)	34(1)	17(1)	8(1)	-2(1)	-6(1)
C(32)	38(1)	29(1)	44(1)	9(1)	0(1)	-11(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 10.

	x	y	z	U(eq)
H(1O)	10480(30)	2790(30)	2012(15)	30
H(2)	9935	-37	1303	26
H(2O)	1490(30)	2130(30)	-2591(15)	26
H(3)	7844	101	562	25
H(5)	5924	4575	886	25
H(6)	8035	4451	1612	26
H(11)	379	3540	229	26
H(12)	-560	3346	-999	25
H(15A)	6707	754	-1431	27
H(15B)	7629	1257	-803	27
H(16A)	6815	3744	-1433	35
H(16B)	5895	3239	-2062	35
H(16C)	7736	2544	-2068	35
H(3O)	-680(30)	4750(30)	-2480(15)	29
H(4O)	-12360(30)	8370(30)	-5788(16)	33
H(18)	-3344	8035	-2483	26
H(19)	-5396	8913	-3342	28
H(21)	-3894	4889	-4353	28
H(22)	-1820	4018	-3510	27
H(27)	-7878	10019	-6769	29
H(28)	-10283	9990	-7039	29
H(31A)	-7413	6654	-3219	31

H(31B)	-9087	7041	-3556	31
H(32A)	-6549	4382	-3867	45
H(32B)	-8234	4768	-4195	45
H(32C)	-7967	4445	-3252	45

Table 6. Torsion angles [deg] for 10.

C(10)-S(1)-C(7)-C(8)	-0.89(14)
C(7)-S(1)-C(10)-C(11)	-178.55(16)
C(7)-S(1)-C(10)-C(9)	0.61(13)
C(10)-S(1)-C(7)-C(4)	179.06(13)
C(23)-S(2)-C(26)-C(27)	178.90(16)
C(26)-S(2)-C(23)-C(24)	-1.55(14)
C(26)-S(2)-C(23)-C(20)	177.93(14)
C(23)-S(2)-C(26)-C(25)	0.66(13)
C(2)-C(1)-C(6)-C(5)	-0.6(3)
C(6)-C(1)-C(2)-C(3)	1.1(3)
O(1)-C(1)-C(6)-C(5)	178.74(16)
O(1)-C(1)-C(2)-C(3)	-178.24(16)
C(1)-C(2)-C(3)-C(4)	-0.6(3)
C(2)-C(3)-C(4)-C(5)	-0.5(3)
C(2)-C(3)-C(4)-C(7)	-178.37(17)
C(5)-C(4)-C(7)-S(1)	-57.7(2)
C(3)-C(4)-C(7)-S(1)	120.17(16)
C(3)-C(4)-C(5)-C(6)	1.0(3)
C(5)-C(4)-C(7)-C(8)	122.3(2)
C(3)-C(4)-C(7)-C(8)	-59.9(3)
C(7)-C(4)-C(5)-C(6)	178.91(16)
C(4)-C(5)-C(6)-C(1)	-0.5(3)
C(4)-C(7)-C(8)-C(9)	-179.04(16)
S(1)-C(7)-C(8)-C(9)	0.91(18)
C(4)-C(7)-C(8)-C(15)	-4.2(3)
S(1)-C(7)-C(8)-C(15)	175.80(13)
C(15)-C(8)-C(9)-C(14)	3.2(3)
C(7)-C(8)-C(15)-C(16)	-100.6(2)

C(9)-C(8)-C(15)-C(16)	73.6(2)
C(15)-C(8)-C(9)-C(10)	-175.08(16)
C(7)-C(8)-C(9)-C(10)	-0.4(2)
C(7)-C(8)-C(9)-C(14)	177.79(17)
C(8)-C(9)-C(14)-C(13)	-175.84(17)
C(8)-C(9)-C(14)-Cl(1)	5.7(3)
C(8)-C(9)-C(10)-C(11)	178.96(16)
C(10)-C(9)-C(14)-Cl(1)	-176.13(13)
C(8)-C(9)-C(10)-S(1)	-0.22(18)
C(14)-C(9)-C(10)-S(1)	-178.75(12)
C(14)-C(9)-C(10)-C(11)	0.4(2)
C(10)-C(9)-C(14)-C(13)	2.3(2)
S(1)-C(10)-C(11)-C(12)	176.71(14)
C(9)-C(10)-C(11)-C(12)	-2.4(3)
C(10)-C(11)-C(12)-C(13)	1.5(3)
C(11)-C(12)-C(13)-C(14)	1.2(3)
C(11)-C(12)-C(13)-O(2)	-179.14(15)
C(12)-C(13)-C(14)-Cl(1)	175.37(13)
O(2)-C(13)-C(14)-C(9)	177.16(15)
O(2)-C(13)-C(14)-Cl(1)	-4.3(2)
C(12)-C(13)-C(14)-C(9)	-3.2(2)
O(3)-C(17)-C(22)-C(21)	-177.86(17)
C(18)-C(17)-C(22)-C(21)	1.2(3)
C(22)-C(17)-C(18)-C(19)	-1.7(3)
O(3)-C(17)-C(18)-C(19)	177.38(16)
C(17)-C(18)-C(19)-C(20)	0.5(3)
C(18)-C(19)-C(20)-C(23)	-178.74(17)
C(18)-C(19)-C(20)-C(21)	1.1(3)
C(21)-C(20)-C(23)-S(2)	-94.68(19)
C(19)-C(20)-C(23)-S(2)	85.2(2)
C(19)-C(20)-C(23)-C(24)	-95.4(2)
C(19)-C(20)-C(21)-C(22)	-1.7(3)
C(23)-C(20)-C(21)-C(22)	178.22(17)
C(21)-C(20)-C(23)-C(24)	84.7(2)
C(20)-C(21)-C(22)-C(17)	0.5(3)
C(20)-C(23)-C(24)-C(25)	-177.45(16)

S(2)-C(23)-C(24)-C(25)	1.96(19)
S(2)-C(23)-C(24)-C(31)	-178.27(13)
C(20)-C(23)-C(24)-C(31)	2.3(3)
C(31)-C(24)-C(25)-C(26)	178.81(16)
C(31)-C(24)-C(25)-C(30)	-2.0(3)
C(23)-C(24)-C(25)-C(30)	177.75(18)
C(25)-C(24)-C(31)-C(32)	85.6(2)
C(23)-C(24)-C(31)-C(32)	-94.1(2)
C(23)-C(24)-C(25)-C(26)	-1.4(2)
C(24)-C(25)-C(26)-S(2)	0.29(18)
C(30)-C(25)-C(26)-C(27)	2.7(2)
C(24)-C(25)-C(30)-Cl(2)	-1.2(3)
C(24)-C(25)-C(30)-C(29)	-180.00(17)
C(26)-C(25)-C(30)-Cl(2)	177.95(13)
C(26)-C(25)-C(30)-C(29)	-0.8(2)
C(24)-C(25)-C(26)-C(27)	-178.00(16)
C(30)-C(25)-C(26)-S(2)	-179.04(12)
S(2)-C(26)-C(27)-C(28)	178.90(14)
C(25)-C(26)-C(27)-C(28)	-3.1(3)
C(26)-C(27)-C(28)-C(29)	1.5(3)
C(27)-C(28)-C(29)-O(4)	-178.25(16)
C(27)-C(28)-C(29)-C(30)	0.2(3)
O(4)-C(29)-C(30)-Cl(2)	-1.1(2)
O(4)-C(29)-C(30)-C(25)	177.79(16)
C(28)-C(29)-C(30)-Cl(2)	-179.42(14)
C(28)-C(29)-C(30)-C(25)	-0.6(3)

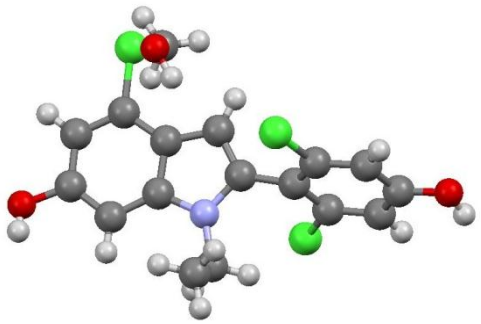
Symmetry transformations used to generate equivalent atoms:

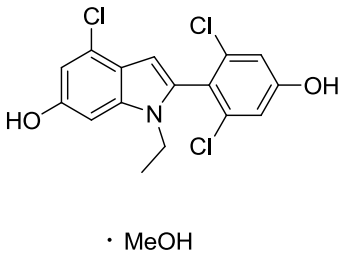
Table 7. Hydrogen-bonds for 10 [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1O)...O(3)#1	0.73(3)	2.10(3)	2.808(2)	162(3)
O(2)-H(2O)...Cl(1)	0.73(3)	2.49(3)	2.9475(15)	123(2)
O(2)-H(2O)...O(4)#2	0.73(3)	2.20(3)	2.8308(19)	146(3)
O(3)-H(3O)...O(2)	0.71(3)	2.13(3)	2.8212(19)	165(3)

O(4)-H(4O)...Cl(2)	0.91(3)	2.30(3)	2.9280(14)	126(2)
C(15)-H(15A)...Cl(1)	0.9900	2.6800	3.205(2)	114.00
C(16)-H(16B)...Cl(1)	0.9800	2.7500	3.380(2)	123.00
C(31)-H(31B)...Cl(2)	0.9900	2.6200	3.201(2)	118.00

A.3.3 4-Chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1*H*-indol-6-ol (104)





· MeOH

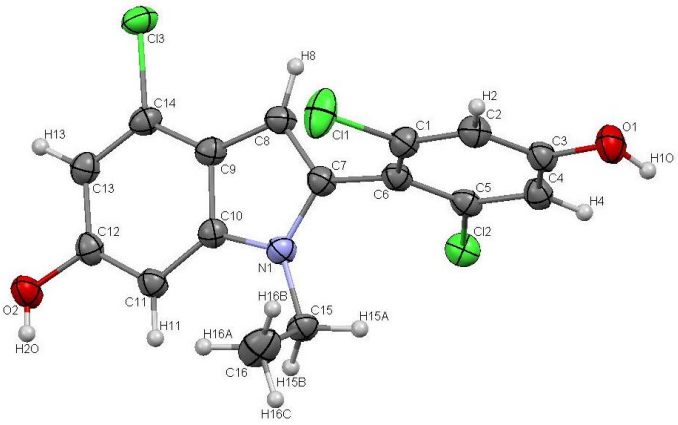


Table 1. Crystal data and structure refinement for 104.

Crystal Data	
Empirical formula	$C_{16}H_{12}Cl_3NO_2$, CH_4O
Formula weight	388.66
Crystal size	0.1335 x 0.1171 x 0.1137 mm
Crystal description	rod
Crystal colour	colourless
Crystal system	Orthorhombic
Space group	P b c a
Unit cell dimensions	a = 17.0072(6) Å alpha = 90 deg.
	b = 9.0354(3) Å beta = 90 deg.
	c = 23.0271(8) Å gamma = 90 deg.
Volume	3538.5(2) $Å^3$
Z, Calculated density	8, 1.459 Mg/m^3

Absorption coefficient	4.825 mm^{-1}
F(000)	1600
Data Collection	
Measurement device type	SuperNova, Single source at offset), Atlas
Measurement method	\w scans
Temperature	123 K
Wavelength	1.54184 Å
Monochromator	graphite
Theta range for data collection	3.84 to 76.71 deg.
Index ranges	-20<=h<=21, -11<=k<=11, -28<=l<=26
Reflections collected / unique	12713 / 3665 [R(int) = 0.0262]
Reflections greater I>2\s(I)	3519
Absorption correction	Analytical
Max. and min. transmission	0.683 and 0.600
Refinement	---
Refinement method	Full-matrix least-squares on F^2
Hydrogen treatment	---
Data / restraints / parameters	3665 / 0 / 227
Goodness-of-fit on F^2	1.054
Final R indices [I>2sigma(I)]	R1 = 0.0655, wR2 = 0.1878
R indices (all data)	R1 = 0.0667, wR2 = 0.1899
Absolute structure parameter	---
Largest diff. peak and hole	2.071 and -0.287 e. $Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 104. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
Cl(1)	4979(1)	2279(1)	5564(1)	48(1)
Cl(2)	1906(1)	3825(1)	5643(1)	32(1)
Cl(3)	4877(1)	8389(1)	6507(1)	36(1)
O(1)	3067(1)	620(3)	4040(1)	38(1)
O(2)	3835(1)	6480(2)	8418(1)	35(1)

N(1)	3359(1)	3721(2)	6615(1)	27(1)
C(1)	4035(2)	2286(3)	5270(1)	30(1)
C(2)	3918(2)	1474(3)	4771(1)	31(1)
C(3)	3165(2)	1409(3)	4537(1)	30(1)
C(4)	2543(2)	2130(3)	4809(1)	30(1)
C(5)	2693(2)	2939(3)	5308(1)	27(1)
C(6)	3438(2)	3070(3)	5553(1)	27(1)
C(7)	3602(2)	4040(3)	6054(1)	27(1)
C(8)	4003(2)	5354(3)	6049(1)	28(1)
C(9)	4030(2)	5869(3)	6631(1)	27(1)
C(10)	3623(2)	4824(3)	6977(1)	26(1)
C(11)	3535(2)	4978(3)	7579(1)	28(1)
C(12)	3878(2)	6209(3)	7828(1)	28(1)
C(13)	4289(2)	7268(3)	7505(1)	29(1)
C(14)	4355(2)	7092(3)	6912(1)	28(1)
C(15)	2969(2)	2365(3)	6809(1)	30(1)
C(16)	3546(2)	1263(4)	7064(2)	48(1)
O(3)	6711(2)	4266(3)	6414(1)	46(1)
C(17)	6144(2)	5343(4)	6284(2)	55(1)

Table 3. Bond lengths [Å] and angles [deg.] for 104.

Cl(1)-C(1)	1.742(3)	O(1)-C(3)-C(2)	117.5(3)
Cl(2)-C(5)	1.739(3)	C(3)-C(4)-C(5)	118.7(3)
Cl(3)-C(14)	1.741(3)	Cl(2)-C(5)-C(4)	117.9(2)
O(1)-C(3)	1.359(4)	Cl(2)-C(5)-C(6)	118.9(2)
O(2)-C(12)	1.382(3)	C(4)-C(5)-C(6)	123.3(3)
O(1)-H(1O)	0.88(5)	C(5)-C(6)-C(7)	122.6(2)
O(2)-H(2O)	0.75(4)	C(1)-C(6)-C(7)	121.8(2)
O(3)-C(17)	1.402(4)	C(1)-C(6)-C(5)	115.5(2)
O(3)-H(3O)	0.89(5)	N(1)-C(7)-C(6)	123.3(2)
N(1)-C(10)	1.374(3)	N(1)-C(7)-C(8)	109.6(2)
N(1)-C(15)	1.463(3)	C(6)-C(7)-C(8)	127.1(2)
N(1)-C(7)	1.388(3)	C(7)-C(8)-C(9)	107.0(2)
C(1)-C(2)	1.378(4)	C(10)-C(9)-C(14)	117.5(2)
C(1)-C(6)	1.399(4)	C(8)-C(9)-C(14)	135.3(3)

C(2)-C(3)	1.390(4)	C(8)-C(9)-C(10)	107.2(2)
C(3)-C(4)	1.392(4)	N(1)-C(10)-C(9)	107.7(2)
C(4)-C(5)	1.386(4)	N(1)-C(10)-C(11)	129.5(2)
C(5)-C(6)	1.391(4)	C(9)-C(10)-C(11)	122.8(2)
C(6)-C(7)	1.476(4)	C(10)-C(11)-C(12)	116.4(2)
C(7)-C(8)	1.370(4)	C(11)-C(12)-C(13)	122.8(3)
C(8)-C(9)	1.419(4)	O(2)-C(12)-C(11)	121.9(2)
C(9)-C(14)	1.395(4)	O(2)-C(12)-C(13)	115.3(2)
C(9)-C(10)	1.416(4)	C(12)-C(13)-C(14)	119.2(3)
C(10)-C(11)	1.403(4)	Cl(3)-C(14)-C(9)	119.1(2)
C(11)-C(12)	1.380(4)	Cl(3)-C(14)-C(13)	119.6(2)
C(12)-C(13)	1.399(4)	C(9)-C(14)-C(13)	121.2(3)
C(13)-C(14)	1.380(4)	N(1)-C(15)-C(16)	112.0(2)
C(15)-C(16)	1.517(5)	C(3)-C(2)-H(2)	121.00
C(2)-H(2)	0.9500	C(1)-C(2)-H(2)	121.00
C(4)-H(4)	0.9500	C(3)-C(4)-H(4)	121.00
C(8)-H(8)	0.9500	C(5)-C(4)-H(4)	121.00
C(11)-H(11)	0.9500	C(7)-C(8)-H(8)	126.00
C(13)-H(13)	0.9500	C(9)-C(8)-H(8)	126.00
C(15)-H(15B)	0.9900	C(12)-C(11)-H(11)	122.00
C(15)-H(15A)	0.9900	C(10)-C(11)-H(11)	122.00
C(16)-H(16C)	0.9800	C(14)-C(13)-H(13)	120.00
C(16)-H(16A)	0.9800	C(12)-C(13)-H(13)	120.00
C(16)-H(16B)	0.9800	N(1)-C(15)-H(15A)	109.00
C(17)-H(17A)	0.9800	C(16)-C(15)-H(15A)	109.00
C(17)-H(17B)	0.9800	C(16)-C(15)-H(15B)	109.00
C(17)-H(17C)	0.9800	N(1)-C(15)-H(15B)	109.00
		H(15A)-C(15)-H(15B)	108.00
C(3)-O(1)-H(1O)	109(3)	C(15)-C(16)-H(16B)	109.00
C(12)-O(2)-H(2O)	114(3)	C(15)-C(16)-H(16C)	110.00
C(17)-O(3)-H(3O)	94(4)	C(15)-C(16)-H(16A)	109.00
C(7)-N(1)-C(10)	108.4(2)	H(16A)-C(16)-H(16C)	109.00
C(7)-N(1)-C(15)	126.4(2)	H(16B)-C(16)-H(16C)	110.00
C(10)-N(1)-C(15)	124.8(2)	H(16A)-C(16)-H(16B)	109.00
Cl(1)-C(1)-C(2)	117.1(2)	O(3)-C(17)-H(17A)	109.00
Cl(1)-C(1)-C(6)	119.3(2)	O(3)-C(17)-H(17B)	109.00

C(2)-C(1)-C(6)	123.6(3)	O(3)-C(17)-H(17C)	109.00
C(1)-C(2)-C(3)	118.6(3)	H(17A)-C(17)-H(17B)	110.00
C(2)-C(3)-C(4)	120.4(3)	H(17A)-C(17)-H(17C)	110.00
O(1)-C(3)-C(4)	122.1(3)	H(17B)-C(17)-H(17C)	109.00

Symmetry transformations used to generate equivalent atoms

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 104. The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a^{*2} U11 +...+ 2 h k a* b* U12].

	U11	U22	U33	U23	U13	U12
Cl(1)	32(1)	53(1)	60(1)	-23(1)	-10(1)	9(1)
Cl(2)	33(1)	29(1)	34(1)	0(1)	3(1)	6(1)
Cl(3)	40(1)	28(1)	39(1)	-2(1)	9(1)	-10(1)
O(1)	43(1)	43(1)	29(1)	-10(1)	-2(1)	1(1)
O(2)	50(1)	29(1)	25(1)	-2(1)	-1(1)	-4(1)
N(1)	34(1)	21(1)	26(1)	0(1)	1(1)	-3(1)
C(1)	31(1)	28(1)	31(1)	-1(1)	0(1)	0(1)
C(2)	33(1)	27(1)	31(1)	-2(1)	4(1)	2(1)
C(3)	39(2)	27(1)	23(1)	-1(1)	1(1)	-1(1)
C(4)	34(1)	28(1)	27(1)	2(1)	-4(1)	1(1)
C(5)	33(1)	22(1)	26(1)	3(1)	2(1)	2(1)
C(6)	34(1)	23(1)	25(1)	1(1)	1(1)	-1(1)
C(7)	31(1)	24(1)	25(1)	-1(1)	1(1)	1(1)
C(8)	33(1)	24(1)	27(1)	0(1)	5(1)	-2(1)
C(9)	30(1)	23(1)	29(1)	0(1)	2(1)	1(1)
C(10)	28(1)	22(1)	27(1)	0(1)	-1(1)	1(1)
C(11)	34(1)	24(1)	25(1)	1(1)	0(1)	0(1)
C(12)	33(1)	26(1)	26(1)	-2(1)	-3(1)	3(1)
C(13)	32(1)	25(1)	32(1)	-4(1)	-1(1)	0(1)
C(14)	29(1)	22(1)	34(1)	-1(1)	3(1)	-1(1)
C(15)	37(1)	22(1)	31(1)	2(1)	0(1)	-6(1)
C(16)	47(2)	27(1)	68(2)	11(1)	-9(2)	0(1)
O(3)	48(1)	34(1)	57(1)	2(1)	18(1)	2(1)
C(17)	49(2)	40(2)	76(3)	9(2)	4(2)	2(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 104.

	x	y	z	U(eq)
H(1O)	2560(30)	520(50)	3973(17)	46
H(2)	4342	969	4590	37
H(2O)	3680(30)	5840(50)	8590(18)	42
H(4)	2025	2069	4655	35
H(8)	4222	5830	5719	33
H(11)	3255	4272	7804	33
H(13)	4520	8098	7692	35
H(15A)	2695	1904	6475	36
H(15B)	2569	2616	7105	36
H(16A)	3813	1711	7398	57
H(16B)	3936	993	6769	57
H(16C)	3263	375	7190	57
H(3O)	6370(30)	3520(60)	6390(20)	55
H(17A)	5696	5235	6548	66
H(17B)	5966	5216	5882	66
H(17C)	6375	6330	6330	66

Table 6. Torsion angles [deg] for 104.

C(7)-N(1)-C(10)-C(11)	-179.0(3)
C(15)-N(1)-C(10)-C(11)	-5.5(4)
C(7)-N(1)-C(15)-C(16)	96.3(3)
C(10)-N(1)-C(15)-C(16)	-76.0(3)
C(15)-N(1)-C(7)-C(6)	5.1(4)
C(7)-N(1)-C(10)-C(9)	1.0(3)
C(15)-N(1)-C(10)-C(9)	174.4(2)
C(10)-N(1)-C(7)-C(6)	178.5(2)
C(10)-N(1)-C(7)-C(8)	-1.4(3)
C(15)-N(1)-C(7)-C(8)	-174.8(2)
C(2)-C(1)-C(6)-C(5)	2.3(4)
Cl(1)-C(1)-C(6)-C(7)	7.5(4)
C(6)-C(1)-C(2)-C(3)	-0.9(4)
Cl(1)-C(1)-C(6)-C(5)	-176.1(2)

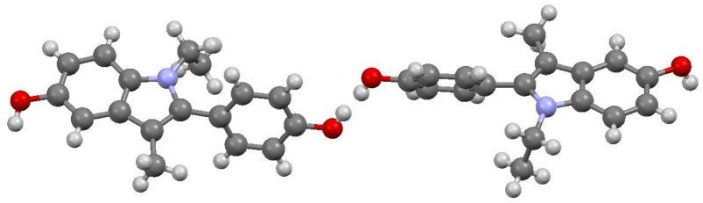
Cl(1)-C(1)-C(2)-C(3)	177.6(2)
C(2)-C(1)-C(6)-C(7)	-174.1(3)
C(1)-C(2)-C(3)-C(4)	-1.1(4)
C(1)-C(2)-C(3)-O(1)	179.0(3)
O(1)-C(3)-C(4)-C(5)	-178.6(3)
C(2)-C(3)-C(4)-C(5)	1.5(4)
C(3)-C(4)-C(5)-C(6)	0.1(4)
C(3)-C(4)-C(5)-Cl(2)	-179.7(2)
Cl(2)-C(5)-C(6)-C(1)	177.9(2)
Cl(2)-C(5)-C(6)-C(7)	-5.7(4)
C(4)-C(5)-C(6)-C(1)	-1.9(4)
C(4)-C(5)-C(6)-C(7)	174.5(3)
C(5)-C(6)-C(7)-C(8)	-107.3(3)
C(5)-C(6)-C(7)-N(1)	72.8(4)
C(1)-C(6)-C(7)-C(8)	68.8(4)
C(1)-C(6)-C(7)-N(1)	-111.0(3)
C(6)-C(7)-C(8)-C(9)	-178.6(3)
N(1)-C(7)-C(8)-C(9)	1.3(3)
C(7)-C(8)-C(9)-C(14)	178.7(3)
C(7)-C(8)-C(9)-C(10)	-0.7(3)
C(8)-C(9)-C(10)-N(1)	-0.2(3)
C(10)-C(9)-C(14)-C(13)	0.7(4)
C(8)-C(9)-C(10)-C(11)	179.8(2)
C(14)-C(9)-C(10)-N(1)	-179.7(2)
C(14)-C(9)-C(10)-C(11)	0.2(4)
C(8)-C(9)-C(14)-Cl(3)	-0.6(5)
C(8)-C(9)-C(14)-C(13)	-178.7(3)
C(10)-C(9)-C(14)-Cl(3)	178.8(2)
N(1)-C(10)-C(11)-C(12)	179.2(3)
C(9)-C(10)-C(11)-C(12)	-0.8(4)
C(10)-C(11)-C(12)-C(13)	0.4(4)
C(10)-C(11)-C(12)-O(2)	-179.7(2)
O(2)-C(12)-C(13)-C(14)	-179.4(2)
C(11)-C(12)-C(13)-C(14)	0.5(4)
C(12)-C(13)-C(14)-C(9)	-1.0(4)
C(12)-C(13)-C(14)-Cl(3)	-179.2(2)

Symmetry transformations used to generate equivalent atoms

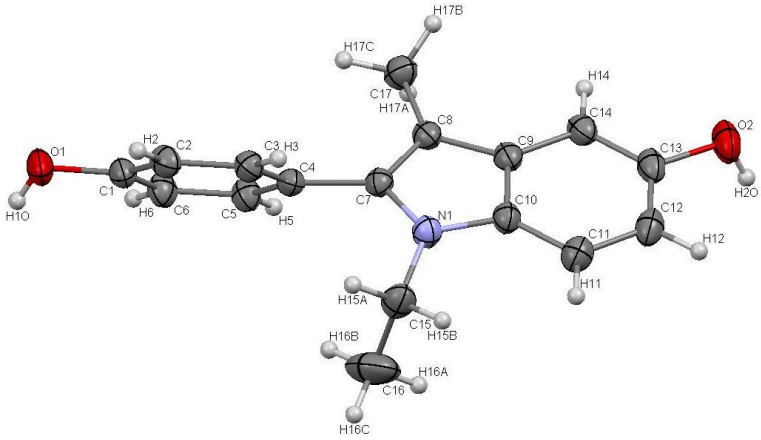
Table 7. Hydrogen-bonds for 104 [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1O)...O(3)#1	0.88(5)	1.71(5)	2.534(4)	155(4)
O(2)-H(2O)...O(1)#2	0.75(4)	1.98(5)	2.712(3)	168(5)
O(3)-H(3O)...O(2)#3	0.89(5)	1.93(5)	2.711(3)	146(5)

A.3.4 1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol (128)



1-ethyl-2-(4-hydroxyphenyl)-3-methyl-1*H*-indol-5-ol



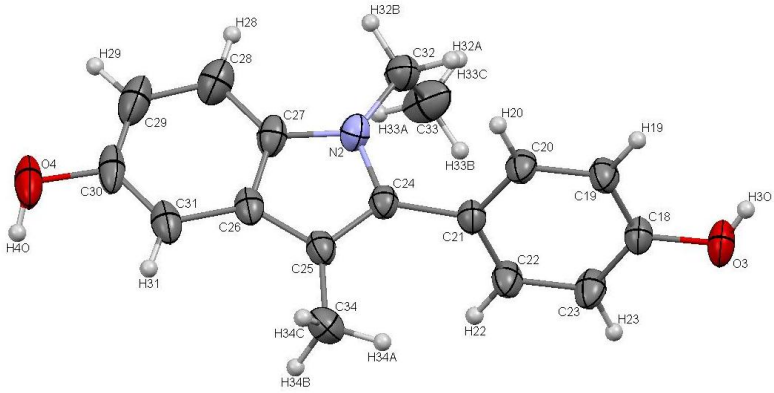


Table 1. Crystal data and structure refinement for 128.

Crystal Data	
Empirical formula	$C_{17}H_{17}NO_2$
Formula weight	267.32
Crystal size	0.2090 x 0.1217 x 0.0337 mm
Crystal description	plate
Crystal colour	colourless to faint green
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 8.7637(5) Å alpha = 100.284(5) deg.
	b = 9.3986(5) Å beta = 100.946(5) deg.
	c = 17.7622(10) Å gamma = 90.609(5) deg.
Volume	1411.82(14) $Å^3$
Z, Calculated density	4, 1.258 Mg/m^3
Absorption coefficient	0.657 mm^{-1}
F(000)	568
Data Collection	
Measurement device type	SuperNova, Single source at offset), Atlas
Measuremnet method	\w scans
Temperature	123 K
Wavelength	1.54184 Å
Monochromator	graphite
Theta range for data collection	4.79 to 76.99 deg.
Index ranges	-11<=h<=10, -11<=k<=10, -20<=l<=22
Reflections collected / unique	8380 / 5315 [R(int) = 0.0229]
Reflections greater I>2\s(I)	4684
Absorption correction	Analytical
Max. and min. transmission	0.978 and 0.923
Refinement	
Refinement method	Full-matrix least-squares on F^2
Hydrogen treatment	---
Data / restraints / parameters	5315 / 4 / 375
Goodness-of-fit on F^2	1.057
Final R indices [I>2sigma(I)]	R1 = 0.0576, wR2 = 0.1640

R indices (all data)	R1 = 0.0638, wR2 = 0.1750
Absolute structure parameter	---
Largest diff. peak and hole	0.351 and -0.337 e. $Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 128. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(1)	586(2)	8140(2)	1702(1)	44(1)
O(2)	-8978(2)	-883(2)	380(1)	44(1)
N(1)	-3149(2)	1859(2)	1203(1)	28(1)
C(1)	-402(2)	6937(2)	1580(1)	29(1)
C(2)	-160(2)	5715(2)	1051(1)	30(1)
C(3)	-1163(2)	4499(2)	916(1)	27(1)
C(4)	-2408(2)	4487(2)	1303(1)	24(1)
C(5)	-2609(2)	5720(2)	1839(1)	29(1)
C(6)	-1615(2)	6941(2)	1976(1)	31(1)
C(7)	-3555(2)	3257(2)	1133(1)	24(1)
C(8)	-5140(2)	3291(2)	886(1)	26(1)
C(9)	-5752(2)	1851(2)	804(1)	27(1)
C(10)	-4500(2)	985(2)	1019(1)	28(1)
C(11)	-4732(2)	-483(2)	1031(1)	33(1)
C(12)	-6248(2)	-1071(2)	814(1)	34(1)
C(13)	-7481(2)	-223(2)	590(1)	33(1)
C(14)	-7276(2)	1230(2)	582(1)	29(1)
C(15)	-1650(2)	1456(2)	1604(1)	37(1)
C(16)	-1434(3)	1985(4)	2474(2)	67(1)
C(17)	-6063(2)	4562(2)	719(1)	34(1)
O(3)	1535(2)	9616(2)	3224(1)	43(1)
O(4)	9507(3)	19911(2)	4227(1)	63(1)
N(2)	4693(2)	15789(2)	3275(1)	34(1)
C(18)	2532(2)	10806(2)	3320(1)	32(1)
C(19)	3003(2)	11193(2)	2683(1)	35(1)
C(20)	3967(2)	12415(2)	2781(1)	34(1)
C(21)	4466(2)	13274(2)	3512(1)	30(1)

C(22)	4020(3)	12838(2)	4152(1)	37(1)
C(23)	3067(3)	11608(2)	4058(1)	40(1)
C(24)	5379(2)	14648(2)	3598(1)	30(1)
C(25)	6884(2)	15035(2)	3966(1)	33(1)
C(26)	7133(2)	16512(2)	3896(1)	33(1)
C(27)	5754(2)	16953(2)	3477(1)	35(1)
C(28)	5604(3)	18352(3)	3316(2)	48(1)
C(29)	6874(4)	19315(3)	3590(2)	53(1)
C(30)	8266(3)	18874(3)	3989(1)	46(1)
C(31)	8429(3)	17506(2)	4147(1)	41(1)
C(32)	3066(2)	15847(2)	2906(1)	40(1)
C(33)	1999(3)	16117(3)	3484(2)	57(1)
C(34)	8040(3)	14115(3)	4361(1)	45(1)

Table 3. Bond lengths [Å] and angles [deg] for 128.

O(1)-C(1)	1.377(3)	C(8)-C(9)-C(10)	107.70(16)
O(2)-C(13)	1.400(2)	C(10)-C(9)-C(14)	119.78(17)
O(1)-H(1O)	0.77(2)	C(8)-C(9)-C(14)	132.50(17)
O(2)-H(2O)	0.85(2)	C(9)-C(10)-C(11)	121.57(17)
O(3)-C(18)	1.378(2)	N(1)-C(10)-C(11)	130.72(17)
O(4)-C(30)	1.403(4)	N(1)-C(10)-C(9)	107.71(16)
O(3)-H(3O)	0.78(2)	C(10)-C(11)-C(12)	117.65(17)
O(4)-H(4O)	0.86(3)	C(11)-C(12)-C(13)	120.82(18)
N(1)-C(7)	1.385(2)	O(2)-C(13)-C(12)	117.88(17)
N(1)-C(10)	1.386(2)	C(12)-C(13)-C(14)	122.33(17)
N(1)-C(15)	1.459(2)	O(2)-C(13)-C(14)	119.79(17)
N(2)-C(32)	1.458(3)	C(9)-C(14)-C(13)	117.84(17)
N(2)-C(24)	1.393(3)	N(1)-C(15)-C(16)	110.88(18)
N(2)-C(27)	1.379(3)	C(1)-C(2)-H(2)	120.00
C(1)-C(6)	1.382(3)	C(3)-C(2)-H(2)	120.00
C(1)-C(2)	1.393(3)	C(4)-C(3)-H(3)	120.00
C(2)-C(3)	1.392(3)	C(2)-C(3)-H(3)	120.00
C(3)-C(4)	1.396(2)	C(6)-C(5)-H(5)	119.00
C(4)-C(5)	1.399(2)	C(4)-C(5)-H(5)	119.00
C(4)-C(7)	1.474(3)	C(1)-C(6)-H(6)	120.00
C(5)-C(6)	1.391(3)	C(5)-C(6)-H(6)	120.00

C(7)-C(8)	1.378(3)	C(10)-C(11)-H(11)	121.00
C(8)-C(9)	1.423(3)	C(12)-C(11)-H(11)	121.00
C(8)-C(17)	1.492(3)	C(11)-C(12)-H(12)	120.00
C(9)-C(14)	1.408(3)	C(13)-C(12)-H(12)	120.00
C(9)-C(10)	1.408(3)	C(9)-C(14)-H(14)	121.00
C(10)-C(11)	1.397(3)	C(13)-C(14)-H(14)	121.00
C(11)-C(12)	1.390(3)	N(1)-C(15)-H(15B)	110.00
C(12)-C(13)	1.389(3)	N(1)-C(15)-H(15A)	109.00
C(13)-C(14)	1.379(3)	C(16)-C(15)-H(15A)	109.00
C(15)-C(16)	1.511(4)	C(16)-C(15)-H(15B)	109.00
C(2)-H(2)	0.9500	H(15A)-C(15)-H(15B)	108.00
C(3)-H(3)	0.9500	C(15)-C(16)-H(16C)	109.00
C(5)-H(5)	0.9500	H(16A)-C(16)-H(16B)	109.00
C(6)-H(6)	0.9500	H(16A)-C(16)-H(16C)	109.00
C(11)-H(11)	0.9500	C(15)-C(16)-H(16B)	110.00
C(12)-H(12)	0.9500	H(16B)-C(16)-H(16C)	109.00
C(14)-H(14)	0.9500	C(15)-C(16)-H(16A)	109.00
C(15)-H(15A)	0.9900	H(17A)-C(17)-H(17C)	110.00
C(15)-H(15B)	0.9900	H(17B)-C(17)-H(17C)	109.00
C(16)-H(16B)	0.9800	C(8)-C(17)-H(17A)	110.00
C(16)-H(16A)	0.9800	C(8)-C(17)-H(17B)	109.00
C(16)-H(16C)	0.9800	H(17A)-C(17)-H(17B)	109.00
C(17)-H(17B)	0.9800	C(8)-C(17)-H(17C)	109.00
C(17)-H(17C)	0.9800	C(19)-C(18)-C(23)	120.04(18)
C(17)-H(17A)	0.9800	O(3)-C(18)-C(23)	119.79(18)
C(18)-C(23)	1.383(3)	O(3)-C(18)-C(19)	120.17(17)
C(18)-C(19)	1.380(3)	C(18)-C(19)-C(20)	119.83(17)
C(19)-C(20)	1.384(3)	C(19)-C(20)-C(21)	121.23(17)
C(20)-C(21)	1.389(3)	C(20)-C(21)-C(24)	120.64(17)
C(21)-C(24)	1.481(3)	C(20)-C(21)-C(22)	118.07(17)
C(21)-C(22)	1.396(3)	C(22)-C(21)-C(24)	121.22(17)
C(22)-C(23)	1.386(3)	C(21)-C(22)-C(23)	120.89(18)
C(24)-C(25)	1.370(3)	C(18)-C(23)-C(22)	119.85(19)
C(25)-C(34)	1.494(3)	N(2)-C(24)-C(21)	119.70(16)
C(25)-C(26)	1.434(3)	N(2)-C(24)-C(25)	110.24(17)
C(26)-C(31)	1.414(3)	C(21)-C(24)-C(25)	130.07(17)

C(26)-C(27)	1.402(3)	C(24)-C(25)-C(26)	106.00(16)
C(27)-C(28)	1.397(3)	C(26)-C(25)-C(34)	126.62(18)
C(28)-C(29)	1.382(4)	C(24)-C(25)-C(34)	127.39(19)
C(29)-C(30)	1.396(4)	C(25)-C(26)-C(31)	133.28(18)
C(30)-C(31)	1.367(3)	C(27)-C(26)-C(31)	118.84(18)
C(32)-C(33)	1.507(3)	C(25)-C(26)-C(27)	107.87(16)
C(19)-H(19)	0.9500	C(26)-C(27)-C(28)	122.28(19)
C(20)-H(20)	0.9500	N(2)-C(27)-C(26)	107.91(17)
C(22)-H(22)	0.9500	N(2)-C(27)-C(28)	129.81(19)
C(23)-H(23)	0.9500	C(27)-C(28)-C(29)	117.6(2)
C(28)-H(28)	0.9500	C(28)-C(29)-C(30)	120.5(3)
C(29)-H(29)	0.9500	O(4)-C(30)-C(31)	121.0(2)
C(31)-H(31)	0.9500	O(4)-C(30)-C(29)	116.6(2)
C(32)-H(32A)	0.9900	C(29)-C(30)-C(31)	122.4(3)
C(32)-H(32B)	0.9900	C(26)-C(31)-C(30)	118.3(2)
C(33)-H(33A)	0.9800	N(2)-C(32)-C(33)	113.04(19)
C(33)-H(33B)	0.9800	C(18)-C(19)-H(19)	120.00
C(33)-H(33C)	0.9800	C(20)-C(19)-H(19)	120.00
C(34)-H(34A)	0.9800	C(19)-C(20)-H(20)	119.00
C(34)-H(34B)	0.9800	C(21)-C(20)-H(20)	119.00
C(34)-H(34C)	0.9800	C(21)-C(22)-H(22)	120.00
		C(23)-C(22)-H(22)	119.00
C(1)-O(1)-H(1O)	116(2)	C(18)-C(23)-H(23)	120.00
C(13)-O(2)-H(2O)	111.2(17)	C(22)-C(23)-H(23)	120.00
C(18)-O(3)-H(3O)	116(2)	C(27)-C(28)-H(28)	121.00
C(30)-O(4)-H(4O)	111(2)	C(29)-C(28)-H(28)	121.00
C(7)-N(1)-C(10)	108.07(15)	C(28)-C(29)-H(29)	120.00
C(10)-N(1)-C(15)	124.54(16)	C(30)-C(29)-H(29)	120.00
C(7)-N(1)-C(15)	125.36(16)	C(26)-C(31)-H(31)	121.00
C(24)-N(2)-C(27)	107.89(16)	C(30)-C(31)-H(31)	121.00
C(27)-N(2)-C(32)	124.80(16)	N(2)-C(32)-H(32A)	109.00
C(24)-N(2)-C(32)	126.54(16)	N(2)-C(32)-H(32B)	109.00
O(1)-C(1)-C(2)	119.06(16)	C(33)-C(32)-H(32A)	109.00
C(2)-C(1)-C(6)	120.48(17)	C(33)-C(32)-H(32B)	109.00
O(1)-C(1)-C(6)	120.46(17)	H(32A)-C(32)-H(32B)	108.00
C(1)-C(2)-C(3)	119.53(17)	C(32)-C(33)-H(33A)	109.00

C(2)-C(3)-C(4)	120.93(17)	C(32)-C(33)-H(33B)	109.00
C(3)-C(4)-C(7)	122.28(16)	C(32)-C(33)-H(33C)	109.00
C(5)-C(4)-C(7)	119.30(16)	H(33A)-C(33)-H(33B)	110.00
C(3)-C(4)-C(5)	118.34(17)	H(33A)-C(33)-H(33C)	109.00
C(4)-C(5)-C(6)	121.12(16)	H(33B)-C(33)-H(33C)	109.00
C(1)-C(6)-C(5)	119.59(17)	C(25)-C(34)-H(34A)	109.00
C(4)-C(7)-C(8)	127.01(17)	C(25)-C(34)-H(34B)	109.00
N(1)-C(7)-C(4)	123.02(16)	C(25)-C(34)-H(34C)	110.00
N(1)-C(7)-C(8)	109.98(16)	H(34A)-C(34)-H(34B)	109.00
C(9)-C(8)-C(17)	125.67(16)	H(34A)-C(34)-H(34C)	109.00
C(7)-C(8)-C(9)	106.51(16)	H(34B)-C(34)-H(34C)	109.00
C(7)-C(8)-C(17)	127.82(17)		

Symmetry transformations used to generate equivalent atoms

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 128. The anisotropic displacement factor exponent takes the form:-2 pi^2 [h^2 a^{*2} U11 +...+ 2 h k a* b* U12].

	U11	U22	U33	U23	U13	U12
O(1)	47(1)	44(1)	36(1)	7(1)	-2(1)	-24(1)
O(2)	41(1)	51(1)	38(1)	1(1)	10(1)	-23(1)
N(1)	25(1)	27(1)	33(1)	6(1)	5(1)	0(1)
C(1)	29(1)	31(1)	25(1)	6(1)	1(1)	-9(1)
C(2)	23(1)	41(1)	29(1)	8(1)	6(1)	-3(1)
C(3)	25(1)	30(1)	26(1)	4(1)	6(1)	0(1)
C(4)	24(1)	26(1)	22(1)	6(1)	3(1)	-1(1)
C(5)	28(1)	33(1)	27(1)	3(1)	9(1)	-3(1)
C(6)	36(1)	29(1)	27(1)	-1(1)	7(1)	-5(1)
C(7)	27(1)	25(1)	22(1)	4(1)	7(1)	-1(1)
C(8)	25(1)	31(1)	23(1)	6(1)	5(1)	-2(1)
C(9)	27(1)	32(1)	22(1)	5(1)	6(1)	-4(1)
C(10)	29(1)	27(1)	27(1)	4(1)	7(1)	-5(1)
C(11)	37(1)	29(1)	36(1)	6(1)	11(1)	-1(1)
C(12)	44(1)	28(1)	33(1)	2(1)	14(1)	-9(1)
C(13)	33(1)	39(1)	26(1)	2(1)	8(1)	-13(1)
C(14)	26(1)	38(1)	24(1)	6(1)	5(1)	-4(1)

C(15)	27(1)	35(1)	50(1)	16(1)	5(1)	4(1)
C(16)	54(2)	100(2)	50(2)	36(2)	-5(1)	13(2)
C(17)	28(1)	37(1)	40(1)	14(1)	8(1)	2(1)
O(3)	47(1)	31(1)	51(1)	-2(1)	18(1)	-16(1)
O(4)	79(1)	61(1)	45(1)	-7(1)	21(1)	-46(1)
N(2)	31(1)	32(1)	41(1)	8(1)	10(1)	-4(1)
C(18)	33(1)	27(1)	35(1)	1(1)	11(1)	-7(1)
C(19)	42(1)	33(1)	27(1)	-1(1)	6(1)	-10(1)
C(20)	42(1)	35(1)	27(1)	4(1)	10(1)	-8(1)
C(21)	30(1)	28(1)	30(1)	3(1)	7(1)	-4(1)
C(22)	48(1)	34(1)	27(1)	-2(1)	9(1)	-11(1)
C(23)	55(1)	37(1)	30(1)	1(1)	17(1)	-13(1)
C(24)	33(1)	30(1)	27(1)	2(1)	9(1)	-6(1)
C(25)	33(1)	36(1)	27(1)	0(1)	6(1)	-8(1)
C(26)	36(1)	37(1)	25(1)	-3(1)	13(1)	-11(1)
C(27)	38(1)	31(1)	39(1)	1(1)	18(1)	-6(1)
C(28)	55(1)	36(1)	56(1)	9(1)	21(1)	-3(1)
C(29)	72(2)	34(1)	60(1)	5(1)	31(1)	-14(1)
C(30)	57(1)	43(1)	37(1)	-9(1)	25(1)	-28(1)
C(31)	41(1)	46(1)	32(1)	-6(1)	14(1)	-17(1)
C(32)	35(1)	43(1)	45(1)	15(1)	6(1)	-1(1)
C(33)	41(1)	74(2)	70(2)	35(1)	23(1)	14(1)
C(34)	43(1)	49(1)	38(1)	6(1)	-3(1)	-7(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for 128.

	x	y	z	U(eq)
H(1O)	880(30)	8500(30)	2133(11)	53
H(2)	683	5712	785	37
H(2O)	-9290(30)	-1080(30)	773(13)	53
H(3)	-998	3666	556	32
H(5)	-3439	5724	2114	35
H(6)	-1768	7772	2341	38
H(11)	-3884	-1060	1183	40
H(12)	-6443	-2065	820	41

H(14)	-8136	1795	431	35
H(15A)	-803	1880	1404	44
H(15B)	-1589	390	1496	44
H(16A)	-2265	1556	2673	80
H(16B)	-1474	3042	2582	80
H(16C)	-423	1700	2731	80
H(17A)	-6649	4864	1134	41
H(17B)	-6788	4295	218	41
H(17C)	-5358	5362	695	41
H(3O)	1210(30)	9240(30)	2791(11)	52
H(4O)	10070(40)	19800(40)	4663(13)	76
H(19)	2666	10622	2179	42
H(20)	4294	12670	2341	41
H(22)	4375	13391	4660	44
H(23)	2781	11317	4500	49
H(28)	4663	18631	3028	57
H(29)	6798	20285	3506	64
H(31)	9390	17231	4418	49
H(32A)	2735	14919	2545	48
H(32B)	2967	16626	2594	48
H(33A)	2308	17043	3838	69
H(33B)	2069	15335	3786	69
H(33C)	926	16150	3205	69
H(34A)	7542	13171	4352	54
H(34B)	8423	14592	4903	54
H(34C)	8914	13977	4088	54

Table 6. Torsion angles [deg] for 128.

C(15)-N(1)-C(7)-C(4)	14.2(3)
C(7)-N(1)-C(10)-C(9)	2.21(19)
C(15)-N(1)-C(10)-C(9)	166.69(17)
C(7)-N(1)-C(10)-C(11)	-176.96(19)
C(15)-N(1)-C(10)-C(11)	-12.5(3)
C(7)-N(1)-C(15)-C(16)	68.6(3)

C(10)-N(1)-C(15)-C(16)	-93.2(2)
C(10)-N(1)-C(7)-C(4)	178.55(15)
C(10)-N(1)-C(7)-C(8)	-1.7(2)
C(15)-N(1)-C(7)-C(8)	-166.03(17)
C(32)-N(2)-C(24)-C(25)	-173.48(18)
C(27)-N(2)-C(32)-C(33)	-93.1(2)
C(24)-N(2)-C(27)-C(28)	-177.9(2)
C(27)-N(2)-C(24)-C(25)	-3.2(2)
C(27)-N(2)-C(24)-C(21)	176.57(16)
C(32)-N(2)-C(24)-C(21)	6.3(3)
C(32)-N(2)-C(27)-C(26)	173.25(18)
C(24)-N(2)-C(32)-C(33)	75.6(3)
C(32)-N(2)-C(27)-C(28)	-7.4(3)
C(24)-N(2)-C(27)-C(26)	2.8(2)
O(1)-C(1)-C(6)-C(5)	179.42(17)
C(6)-C(1)-C(2)-C(3)	0.9(3)
C(2)-C(1)-C(6)-C(5)	-0.7(3)
O(1)-C(1)-C(2)-C(3)	-179.26(17)
C(1)-C(2)-C(3)-C(4)	0.0(3)
C(2)-C(3)-C(4)-C(7)	175.63(17)
C(2)-C(3)-C(4)-C(5)	-1.0(3)
C(7)-C(4)-C(5)-C(6)	-175.57(17)
C(5)-C(4)-C(7)-N(1)	-125.35(19)
C(3)-C(4)-C(7)-N(1)	58.1(2)
C(3)-C(4)-C(5)-C(6)	1.2(3)
C(5)-C(4)-C(7)-C(8)	55.0(2)
C(3)-C(4)-C(7)-C(8)	-121.7(2)
C(4)-C(5)-C(6)-C(1)	-0.3(3)
C(4)-C(7)-C(8)-C(17)	1.1(3)
N(1)-C(7)-C(8)-C(17)	-178.59(17)
C(4)-C(7)-C(8)-C(9)	-179.76(16)
N(1)-C(7)-C(8)-C(9)	0.5(2)
C(17)-C(8)-C(9)-C(10)	180.00(17)
C(17)-C(8)-C(9)-C(14)	-1.7(3)
C(7)-C(8)-C(9)-C(14)	179.16(19)
C(7)-C(8)-C(9)-C(10)	0.9(2)

C(14)-C(9)-C(10)-C(11)	-1.2(3)
C(8)-C(9)-C(10)-C(11)	177.36(16)
C(8)-C(9)-C(10)-N(1)	-1.90(19)
C(14)-C(9)-C(10)-N(1)	179.54(16)
C(8)-C(9)-C(14)-C(13)	-177.62(18)
C(10)-C(9)-C(14)-C(13)	0.5(3)
N(1)-C(10)-C(11)-C(12)	179.83(18)
C(9)-C(10)-C(11)-C(12)	0.8(3)
C(10)-C(11)-C(12)-C(13)	0.3(3)
C(11)-C(12)-C(13)-O(2)	-179.90(17)
C(11)-C(12)-C(13)-C(14)	-1.0(3)
C(12)-C(13)-C(14)-C(9)	0.6(3)
O(2)-C(13)-C(14)-C(9)	179.43(16)
O(3)-C(18)-C(19)-C(20)	178.05(17)
O(3)-C(18)-C(23)-C(22)	-177.4(2)
C(19)-C(18)-C(23)-C(22)	2.9(3)
C(23)-C(18)-C(19)-C(20)	-2.3(3)
C(18)-C(19)-C(20)-C(21)	-0.5(3)
C(19)-C(20)-C(21)-C(22)	2.6(3)
C(19)-C(20)-C(21)-C(24)	-174.45(17)
C(20)-C(21)-C(22)-C(23)	-2.0(3)
C(24)-C(21)-C(22)-C(23)	175.1(2)
C(20)-C(21)-C(24)-N(2)	65.5(2)
C(20)-C(21)-C(24)-C(25)	-114.7(2)
C(22)-C(21)-C(24)-C(25)	68.3(3)
C(22)-C(21)-C(24)-N(2)	-111.4(2)
C(21)-C(22)-C(23)-C(18)	-0.8(4)
N(2)-C(24)-C(25)-C(26)	2.3(2)
N(2)-C(24)-C(25)-C(34)	-177.67(19)
C(21)-C(24)-C(25)-C(26)	-177.46(18)
C(21)-C(24)-C(25)-C(34)	2.6(3)
C(34)-C(25)-C(26)-C(27)	179.42(19)
C(24)-C(25)-C(26)-C(31)	-179.5(2)
C(24)-C(25)-C(26)-C(27)	-0.6(2)
C(34)-C(25)-C(26)-C(31)	0.5(4)
C(25)-C(26)-C(31)-C(30)	-179.2(2)

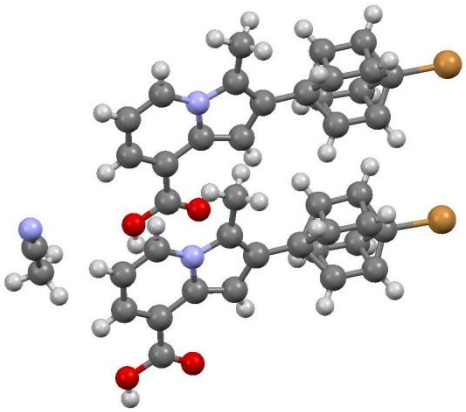
C(27)-C(26)-C(31)-C(30)	1.9(3)
C(25)-C(26)-C(27)-N(2)	-1.4(2)
C(25)-C(26)-C(27)-C(28)	179.2(2)
C(31)-C(26)-C(27)-N(2)	177.76(18)
C(31)-C(26)-C(27)-C(28)	-1.7(3)
C(26)-C(27)-C(28)-C(29)	-0.4(4)
N(2)-C(27)-C(28)-C(29)	-179.7(2)
C(27)-C(28)-C(29)-C(30)	2.2(4)
C(28)-C(29)-C(30)-O(4)	178.3(2)
C(28)-C(29)-C(30)-C(31)	-2.0(4)
C(29)-C(30)-C(31)-C(26)	-0.2(4)
O(4)-C(30)-C(31)-C(26)	179.6(2)

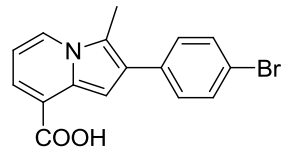
Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen-bonds for 128 [A and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1O)...O(3)	0.77(2)	2.01(2)	2.776(2)	174(3)
O(2)-H(2O)...O(1)#1	0.85(2)	1.94(2)	2.759(2)	162(3)
O(3)-H(3O)...O(1)	0.78(2)	2.00(2)	2.776(2)	174(3)
O(4)-H(4O)...O(4)#2	0.86(3)	1.91(2)	2.693(3)	152(3)

A.3.5 2-(4-Bromophenyl)-3-methylindolizine-8-carboxylic acid (9.3)





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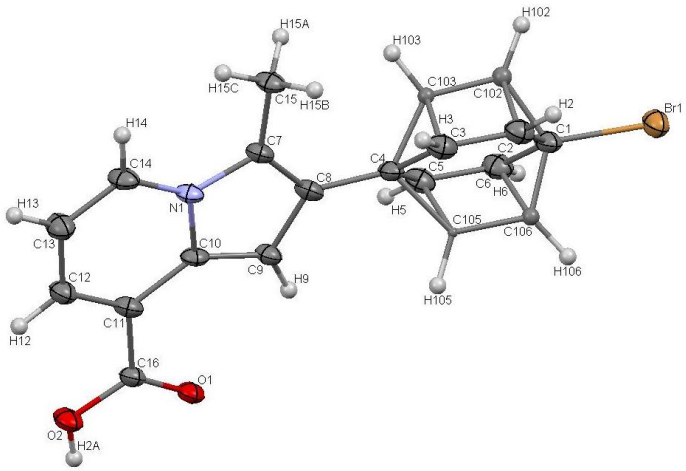


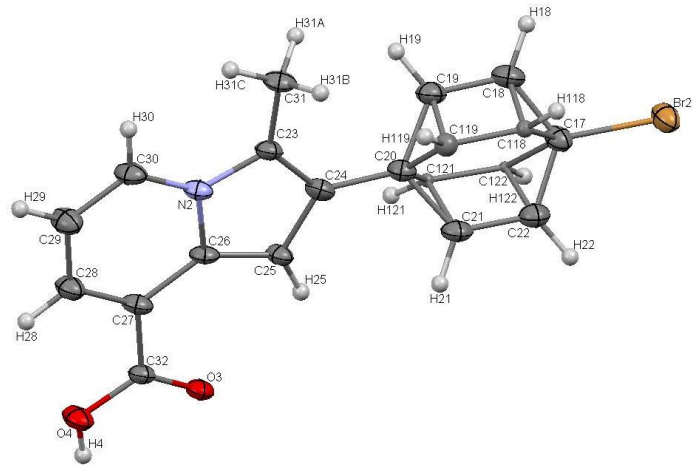


Table 1. Crystal data and structure refinement for 9.3.

Crystal Data	
Empirical formula	$C_{34}H_{27}Br_2N_3O_4$
Formula weight	701.39
Crystal size	0.2223 x 0.0487 x 0.0177 mm
Crystal description	rod
Crystal colour	yellow
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 9.9171(5) Å alpha = 71.961(5) deg.
	b = 10.9238(6) Å beta = 87.812(4) deg.
	c = 14.5789(8) Å gamma = 86.322(5) deg.
Volume	1498.36(15) $Å^3$
Z, Calculated density	2, 1.555 Mg/m^3
Absorption coefficient	3.792 mm^{-1}
F(000)	708
Data Collection	
Measurement device type	SuperNova, Single source at offset), Atlas
Measuremnet method	\w scans
Temperature	123 K
Wavelength	1.54184 Å
Monochromator	graphite
Theta range for data collection	3.19 to 76.83 deg.
Index ranges	-12<=h<=11, -13<=k<=13, -17<=l<=18
Reflections collected / unique	10891 / 6112 [R(int) = 0.0195]
Reflections greater I>2\s(I)	4978
Absorption correction	Analytical
Max. and min. transmission	0.937 and 0.679
Refinement	
Refinement method	Full-matrix least-squares on F^2
Hydrogen treatment	---
Data / restraints / parameters	6112 / 0 / 423
Goodness-of-fit on F^2	1.090
Final R indices [I>2sigma(I)]	R1 = 0.0506, wR2 = 0.1527

R indices (all data)	R1 = 0.0576, wR2 = 0.1627
Absolute structure parameter	---
Largest diff. peak and hole	1.178 and -0.882 e. $Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 9.3. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
Br(1)	5938(1)	9130(1)	4343(1)	26(1)
O(1)	11191(1)	5089(1)	679(1)	21(1)
O(2)	11974(1)	3355(1)	256(1)	25(1)
N(1)	8276(1)	2782(1)	2511(1)	18(1)
C(1)	6493(1)	7754(1)	3844(1)	21(1)
C(2)	6598(1)	6497(1)	4458(1)	24(1)
C(3)	7029(1)	5508(1)	4098(1)	24(1)
C(4)	7346(1)	5739(1)	3127(1)	19(1)
C(5)	7223(1)	7008(1)	2507(1)	25(1)
C(6)	6806(1)	8018(1)	2854(1)	24(1)
C(7)	7476(1)	3432(1)	3034(1)	19(1)
C(8)	7863(1)	4695(1)	2748(1)	19(1)
C(9)	8916(1)	4826(1)	2039(1)	19(1)
C(10)	9172(1)	3645(1)	1895(1)	18(1)
C(11)	10122(1)	3112(1)	1339(1)	20(1)
C(12)	10116(1)	1832(1)	1396(1)	23(1)
C(13)	9160(1)	1030(1)	2003(1)	26(1)
C(14)	8268(1)	1515(1)	2553(1)	23(1)
C(15)	6412(1)	2767(1)	3723(1)	22(1)
C(16)	11138(1)	3949(1)	728(1)	19(1)
C(106)	7790(7)	7725(6)	3636(5)	10(1)
C(105)	8242(6)	6701(6)	3301(4)	3(1)
C(102)	5519(7)	6901(7)	3818(5)	13(1)
C(103)	6013(7)	5955(6)	3449(5)	9(1)
Br(2)	873(1)	9118(1)	4371(1)	29(1)
O(3)	6164(1)	5106(1)	690(1)	21(1)
O(4)	6970(1)	3372(1)	282(1)	27(1)

N(2)	3245(1)	2791(1)	2519(1)	19(1)
C(17)	1472(1)	7742(1)	3888(1)	22(1)
C(18)	554(1)	6871(1)	3816(1)	27(1)
C(19)	1019(1)	5879(1)	3467(1)	25(1)
C(20)	2378(1)	5719(1)	3188(1)	20(1)
C(21)	3261(1)	6636(1)	3296(1)	24(1)
C(22)	2817(1)	7645(1)	3636(1)	25(1)
C(23)	2460(1)	3434(1)	3054(1)	20(1)
C(24)	2887(1)	4689(1)	2801(1)	21(1)
C(25)	3957(1)	4815(1)	2104(1)	19(1)
C(26)	4170(1)	3647(1)	1925(1)	19(1)
C(27)	5102(1)	3126(1)	1352(1)	21(1)
C(28)	5063(1)	1860(1)	1376(1)	25(1)
C(29)	4087(1)	1063(1)	1964(1)	28(1)
C(30)	3204(1)	1540(1)	2527(1)	24(1)
C(31)	1437(1)	2744(1)	3769(1)	24(1)
C(32)	6122(1)	3966(1)	746(1)	19(1)
C(122)	1854(6)	7989(6)	2862(4)	4(1)
C(121)	2261(6)	6970(6)	2524(5)	6(1)
C(118)	1594(7)	6425(7)	4475(5)	10(1)
C(119)	2038(8)	5453(8)	4087(6)	17(2)
N(3)	7828(3)	-228(2)	337(2)	119(1)
C(33)	8206(3)	627(2)	-249(1)	82(1)
C(34)	8743(3)	1790(2)	-1025(1)	90(1)

Table 3. Bond lengths [Å] and angles [deg] for 9.3.

Br(1)-C(1)	1.9063(10)	C(8)-C(9)-C(10)	107.78(8)
Br(2)-C(17)	1.9015(10)	N(1)-C(10)-C(9)	107.24(7)
O(1)-C(16)	1.2292(12)	C(9)-C(10)-C(11)	136.14(9)
O(2)-C(16)	1.3221(12)	N(1)-C(10)-C(11)	116.54(9)
O(2)-H(2A)	0.8400	C(12)-C(11)-C(16)	120.60(9)
O(3)-C(32)	1.2253(12)	C(10)-C(11)-C(16)	118.94(9)
O(4)-C(32)	1.3206(12)	C(10)-C(11)-C(12)	120.43(9)
O(4)-H(4)	0.8400	C(11)-C(12)-C(13)	120.19(9)
N(1)-C(10)	1.4163(11)	C(12)-C(13)-C(14)	120.15(10)

N(1)-C(7)	1.3892(12)	N(1)-C(14)-C(13)	120.12(9)
N(1)-C(14)	1.3676(13)	O(1)-C(16)-C(11)	122.25(8)
N(2)-C(26)	1.4183(12)	O(2)-C(16)-C(11)	113.87(9)
N(2)-C(23)	1.3892(13)	O(1)-C(16)-O(2)	123.88(8)
N(2)-C(30)	1.3671(13)	C(3)-C(2)-H(2)	120.00
N(3)-C(33)	1.127(3)	C(1)-C(2)-H(2)	120.00
C(1)-C(102)	1.394(7)	C(1)-C(102)-C(103)	112.6(6)
C(1)-C(106)	1.311(7)	C(4)-C(3)-H(3)	119.00
C(1)-C(2)	1.3871(16)	C(2)-C(3)-H(3)	119.00
C(1)-C(6)	1.4081(15)	C(4)-C(103)-C(102)	128.6(6)
C(2)-C(3)	1.3790(17)	C(4)-C(105)-C(106)	124.7(5)
C(3)-C(4)	1.3863(15)	C(6)-C(5)-H(5)	120.00
C(4)-C(103)	1.416(7)	C(4)-C(5)-H(5)	119.00
C(4)-C(5)	1.4019(15)	C(1)-C(106)-C(105)	114.1(6)
C(4)-C(105)	1.504(7)	C(1)-C(6)-H(6)	121.00
C(4)-C(8)	1.4702(14)	C(5)-C(6)-H(6)	120.00
C(5)-C(6)	1.3827(17)	C(10)-C(9)-H(9)	126.00
C(7)-C(15)	1.4889(13)	C(8)-C(9)-H(9)	126.00
C(7)-C(8)	1.3869(14)	C(13)-C(12)-H(12)	120.00
C(8)-C(9)	1.4223(13)	C(11)-C(12)-H(12)	120.00
C(9)-C(10)	1.3769(14)	C(14)-C(13)-H(13)	120.00
C(10)-C(11)	1.4303(13)	C(12)-C(13)-H(13)	120.00
C(11)-C(12)	1.3753(15)	N(1)-C(14)-H(14)	120.00
C(11)-C(16)	1.4802(13)	C(13)-C(14)-H(14)	120.00
C(12)-C(13)	1.4177(14)	C(7)-C(15)-H(15C)	109.00
C(13)-C(14)	1.3624(14)	H(15B)-C(15)-H(15C)	109.00
C(102)-C(103)	1.363(10)	C(7)-C(15)-H(15B)	109.00
C(2)-H(2)	0.9500	H(15A)-C(15)-H(15C)	109.00
C(3)-H(3)	0.9500	C(7)-C(15)-H(15A)	109.00
C(5)-H(5)	0.9500	H(15A)-C(15)-H(15B)	109.00
C(105)-C(106)	1.396(10)	C(1)-C(102)-H(102)	124.00
C(6)-H(6)	0.9500	C(103)-C(102)-H(102)	124.00
C(9)-H(9)	0.9500	C(4)-C(103)-H(103)	116.00
C(12)-H(12)	0.9500	C(102)-C(103)-H(103)	116.00
C(13)-H(13)	0.9500	C(106)-C(105)-H(105)	118.00
C(14)-H(14)	0.9500	C(4)-C(105)-H(105)	118.00

C(15)-H(15A)	0.9800	C(105)-C(106)-H(106)	123.00
C(15)-H(15C)	0.9800	C(1)-C(106)-H(106)	123.00
C(15)-H(15B)	0.9800	Br(2)-C(17)-C(18)	119.39(8)
C(102)-H(102)	0.9500	Br(2)-C(17)-C(118)	123.5(3)
C(103)-H(103)	0.9500	Br(2)-C(17)-C(122)	120.9(3)
C(105)-H(105)	0.9500	C(18)-C(17)-C(22)	122.64(10)
C(106)-H(106)	0.9500	C(118)-C(17)-C(122)	115.6(4)
C(17)-C(118)	1.429(8)	Br(2)-C(17)-C(22)	117.96(8)
C(17)-C(122)	1.475(6)	C(17)-C(18)-C(19)	118.07(10)
C(17)-C(18)	1.3871(16)	C(18)-C(19)-C(20)	122.70(11)
C(17)-C(22)	1.3771(15)	C(19)-C(20)-C(24)	123.57(9)
C(18)-C(19)	1.3805(18)	C(21)-C(20)-C(24)	120.44(9)
C(19)-C(20)	1.4087(14)	C(24)-C(20)-C(119)	120.0(4)
C(20)-C(21)	1.4198(15)	C(24)-C(20)-C(121)	116.3(3)
C(20)-C(24)	1.4621(14)	C(119)-C(20)-C(121)	123.6(5)
C(20)-C(119)	1.288(8)	C(19)-C(20)-C(21)	115.98(10)
C(20)-C(121)	1.410(7)	C(20)-C(21)-C(22)	122.41(10)
C(21)-C(22)	1.3830(17)	C(17)-C(22)-C(21)	118.18(11)
C(23)-C(24)	1.3940(14)	N(2)-C(23)-C(24)	107.15(8)
C(23)-C(31)	1.4882(13)	C(24)-C(23)-C(31)	132.33(9)
C(24)-C(25)	1.4233(13)	N(2)-C(23)-C(31)	120.47(9)
C(25)-C(26)	1.3818(14)	C(20)-C(24)-C(23)	127.27(9)
C(26)-C(27)	1.4300(13)	C(20)-C(24)-C(25)	124.16(9)
C(27)-C(32)	1.4812(13)	C(23)-C(24)-C(25)	108.54(9)
C(27)-C(28)	1.3757(15)	C(24)-C(25)-C(26)	107.77(9)
C(28)-C(29)	1.4181(15)	N(2)-C(26)-C(25)	107.36(8)
C(29)-C(30)	1.3655(15)	C(25)-C(26)-C(27)	135.82(9)
C(118)-C(119)	1.391(12)	N(2)-C(26)-C(27)	116.72(9)
C(18)-H(18)	0.9500	C(26)-C(27)-C(32)	118.88(9)
C(19)-H(19)	0.9500	C(28)-C(27)-C(32)	120.73(9)
C(121)-C(122)	1.383(10)	C(26)-C(27)-C(28)	120.39(9)
C(21)-H(21)	0.9500	C(27)-C(28)-C(29)	120.21(10)
C(22)-H(22)	0.9500	C(28)-C(29)-C(30)	120.12(10)
C(25)-H(25)	0.9500	N(2)-C(30)-C(29)	120.18(9)
C(28)-H(28)	0.9500	O(3)-C(32)-C(27)	122.36(8)
C(29)-H(29)	0.9500	O(4)-C(32)-C(27)	113.86(9)

C(30)-H(30)	0.9500
C(31)-H(31B)	0.9800
C(31)-H(31C)	0.9800
C(31)-H(31A)	0.9800
C(118)-H(118)	0.9500
C(119)-H(119)	0.9500
C(121)-H(121)	0.9500
C(122)-H(122)	0.9500
C(33)-C(34)	1.526(3)
C(34)-H(34A)	0.9800
C(34)-H(34B)	0.9800
C(34)-H(34C)	0.9800
C(16)-O(2)-H(2A)	109.00
C(32)-O(4)-H(4)	109.00
C(10)-N(1)-C(14)	122.52(8)
C(7)-N(1)-C(10)	109.29(8)
C(7)-N(1)-C(14)	128.19(8)
C(26)-N(2)-C(30)	122.35(8)
C(23)-N(2)-C(26)	109.18(8)
C(23)-N(2)-C(30)	128.47(8)
C(102)-C(1)-C(106)	130.2(4)
Br(1)-C(1)-C(102)	117.1(3)
C(2)-C(1)-C(6)	120.24(10)
Br(1)-C(1)-C(6)	119.86(8)
Br(1)-C(1)-C(2)	119.90(8)
Br(1)-C(1)-C(106)	112.5(3)
C(1)-C(2)-C(3)	119.70(10)
C(2)-C(3)-C(4)	121.36(11)
C(8)-C(4)-C(105)	122.7(2)
C(103)-C(4)-C(105)	109.4(4)
C(8)-C(4)-C(103)	127.7(3)
C(3)-C(4)-C(8)	121.45(9)
C(3)-C(4)-C(5)	118.68(10)
C(5)-C(4)-C(8)	119.81(9)
C(4)-C(5)-C(6)	121.00(10)

O(3)-C(32)-O(4)	123.78(8)
C(17)-C(118)-C(119)	121.3(6)
C(17)-C(18)-H(18)	121.00
C(19)-C(18)-H(18)	121.00
C(20)-C(119)-C(118)	120.8(7)
C(20)-C(19)-H(19)	119.00
C(18)-C(19)-H(19)	119.00
C(22)-C(21)-H(21)	119.00
C(20)-C(121)-C(122)	118.7(5)
C(20)-C(21)-H(21)	119.00
C(17)-C(122)-C(121)	119.9(5)
C(17)-C(22)-H(22)	121.00
C(21)-C(22)-H(22)	121.00
C(24)-C(25)-H(25)	126.00
C(26)-C(25)-H(25)	126.00
C(27)-C(28)-H(28)	120.00
C(29)-C(28)-H(28)	120.00
C(28)-C(29)-H(29)	120.00
C(30)-C(29)-H(29)	120.00
N(2)-C(30)-H(30)	120.00
C(29)-C(30)-H(30)	120.00
C(23)-C(31)-H(31A)	109.00
C(23)-C(31)-H(31B)	109.00
C(23)-C(31)-H(31C)	109.00
H(31A)-C(31)-H(31B)	109.00
H(31A)-C(31)-H(31C)	110.00
H(31B)-C(31)-H(31C)	109.00
C(17)-C(118)-H(118)	119.00
C(119)-C(118)-H(118)	119.00
C(20)-C(119)-H(119)	119.00
C(118)-C(119)-H(119)	120.00
C(20)-C(121)-H(121)	121.00
C(122)-C(121)-H(121)	121.00
C(17)-C(122)-H(122)	120.00
C(121)-C(122)-H(122)	120.00
N(3)-C(33)-C(34)	178.5(3)

C(1)-C(6)-C(5)	119.01(11)	C(33)-C(34)-H(34A)	109.00
N(1)-C(7)-C(15)	121.00(9)	C(33)-C(34)-H(34B)	109.00
N(1)-C(7)-C(8)	106.96(8)	C(33)-C(34)-H(34C)	109.00
C(8)-C(7)-C(15)	132.01(9)	H(34A)-C(34)-H(34B)	109.00
C(4)-C(8)-C(9)	123.98(9)	H(34A)-C(34)-H(34C)	109.00
C(7)-C(8)-C(9)	108.73(9)	H(34B)-C(34)-H(34C)	110.00
C(4)-C(8)-C(7)	127.24(9)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 9.3. The anisotropic displacement factor exponent takes the form:-2 pi^2 [h^2 a^{*2} U11 +...+ 2 h k a* b* U12].

	U11	U22	U33	U23	U13	U12
Br(1)	26(1)	22(1)	33(1)	-11(1)	8(1)	-4(1)
O(1)	16(1)	25(1)	23(1)	-8(1)	7(1)	-4(1)
O(2)	20(1)	26(1)	29(1)	-8(1)	12(1)	-6(1)
N(1)	13(1)	20(1)	19(1)	-2(1)	4(1)	-4(1)
C(1)	15(1)	22(1)	26(1)	-7(1)	5(1)	-4(1)
C(2)	20(1)	29(1)	22(1)	-7(1)	3(1)	-2(1)
C(3)	21(1)	27(1)	22(1)	-5(1)	3(1)	-2(1)
C(4)	13(1)	19(1)	25(1)	-7(1)	4(1)	-3(1)
C(5)	22(1)	27(1)	22(1)	-5(1)	6(1)	-3(1)
C(6)	22(1)	23(1)	26(1)	-4(1)	5(1)	-4(1)
C(7)	13(1)	23(1)	21(1)	-6(1)	4(1)	-2(1)
C(8)	13(1)	23(1)	20(1)	-4(1)	3(1)	1(1)
C(9)	14(1)	19(1)	22(1)	-5(1)	5(1)	-2(1)
C(10)	14(1)	22(1)	17(1)	-2(1)	4(1)	-5(1)
C(11)	13(1)	24(1)	22(1)	-4(1)	3(1)	-2(1)
C(12)	18(1)	25(1)	25(1)	-9(1)	6(1)	-2(1)
C(13)	25(1)	22(1)	32(1)	-8(1)	8(1)	-5(1)
C(14)	17(1)	22(1)	27(1)	-2(1)	7(1)	-5(1)
C(15)	17(1)	23(1)	23(1)	-2(1)	7(1)	-4(1)
C(16)	13(1)	23(1)	20(1)	-6(1)	3(1)	-2(1)
Br(2)	29(1)	23(1)	35(1)	-10(1)	10(1)	-3(1)
O(3)	15(1)	25(1)	23(1)	-7(1)	7(1)	-4(1)

O(4)	21(1)	26(1)	31(1)	-7(1)	12(1)	-5(1)
N(2)	14(1)	20(1)	19(1)	-1(1)	3(1)	-3(1)
C(17)	22(1)	20(1)	22(1)	-4(1)	6(1)	-3(1)
C(18)	16(1)	32(1)	30(1)	-7(1)	4(1)	-3(1)
C(19)	17(1)	32(1)	26(1)	-9(1)	3(1)	-5(1)
C(20)	16(1)	23(1)	21(1)	-5(1)	3(1)	-3(1)
C(21)	16(1)	29(1)	25(1)	-6(1)	5(1)	-6(1)
C(22)	19(1)	28(1)	27(1)	-7(1)	3(1)	-6(1)
C(23)	12(1)	26(1)	21(1)	-6(1)	4(1)	-3(1)
C(24)	14(1)	24(1)	22(1)	-5(1)	2(1)	0(1)
C(25)	14(1)	19(1)	22(1)	-4(1)	5(1)	-2(1)
C(26)	13(1)	22(1)	19(1)	-1(1)	4(1)	-5(1)
C(27)	14(1)	25(1)	23(1)	-3(1)	5(1)	-3(1)
C(28)	19(1)	25(1)	30(1)	-7(1)	8(1)	-3(1)
C(29)	26(1)	21(1)	34(1)	-5(1)	9(1)	-4(1)
C(30)	18(1)	24(1)	27(1)	-3(1)	6(1)	-5(1)
C(31)	17(1)	27(1)	23(1)	-2(1)	7(1)	-6(1)
C(32)	12(1)	24(1)	20(1)	-6(1)	3(1)	-3(1)
N(3)	219(3)	66(1)	67(1)	-20(1)	26(1)	9(1)
C(33)	125(2)	75(1)	45(1)	-26(1)	-9(1)	35(1)
C(34)	136(2)	79(1)	47(1)	-13(1)	-6(1)	29(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for 9.3.

	x	y	z	U(eq)
H(2A)	12526	3875	-74	30
H(3)	7111	4651	4524	29
H(5)	7430	7178	1839	30
H(6)	6732	8876	2430	29
H(14)	7639	971	2967	28
H(15A)	5524	3181	3508	26
H(15B)	6580	2823	4366	26
H(15C)	6430	1859	3748	26
H(9)	9362	5592	1722	23
H(12)	10754	1484	1029	28

H(13)	9142	153	2025	32
H(2)	6373	6319	5124	29
H(102)	4610	6972	4035	15
H(103)	5379	5362	3401	11
H(105)	9183	6605	3174	4
H(106)	8371	8347	3707	12
H(18)	-367	6955	4002	32
H(19)	397	5280	3413	30
H(21)	4190	6554	3129	29
H(22)	3424	8254	3694	30
H(30)	2558	1000	2926	29
H(31A)	547	3190	3612	29
H(31B)	1683	2730	4417	29
H(31C)	1409	1858	3748	29
H(25)	4438	5568	1814	23
H(28)	5691	1518	998	30
H(29)	4047	195	1965	33
H(4)	7493	3905	-72	32
H(118)	1367	6208	5143	12
H(119)	2092	4582	4489	21
H(121)	2457	7110	1857	8
H(122)	1821	8846	2435	5
H(34A)	9478	2132	-763	108
H(34B)	8013	2458	-1230	108
H(34C)	9084	1527	-1579	108

Table 6. Torsion angles [deg] for 9.3.

C(14)-N(1)-C(7)-C(8)	179.41(8)
C(10)-N(1)-C(7)-C(15)	178.39(8)
C(14)-N(1)-C(7)-C(15)	-2.33(13)
C(7)-N(1)-C(10)-C(11)	176.83(7)
C(7)-N(1)-C(14)-C(13)	-178.01(9)
C(10)-N(1)-C(14)-C(13)	1.18(13)
C(7)-N(1)-C(10)-C(9)	-0.25(9)

C(10)-N(1)-C(7)-C(8)	0.13(10)
C(14)-N(1)-C(10)-C(11)	-2.50(12)
C(14)-N(1)-C(10)-C(9)	-179.58(8)
C(30)-N(2)-C(26)-C(27)	-2.18(13)
C(23)-N(2)-C(26)-C(25)	0.50(10)
C(23)-N(2)-C(26)-C(27)	177.37(8)
C(26)-N(2)-C(30)-C(29)	1.16(14)
C(26)-N(2)-C(23)-C(31)	-177.62(8)
C(30)-N(2)-C(26)-C(25)	-179.04(8)
C(23)-N(2)-C(30)-C(29)	-178.28(10)
C(26)-N(2)-C(23)-C(24)	-0.06(10)
C(30)-N(2)-C(23)-C(31)	1.88(14)
C(30)-N(2)-C(23)-C(24)	179.45(9)
C(2)-C(1)-C(6)-C(5)	-0.31(15)
Br(1)-C(1)-C(2)-C(3)	-178.56(8)
Br(1)-C(1)-C(6)-C(5)	179.22(8)
C(6)-C(1)-C(2)-C(3)	0.97(15)
C(1)-C(2)-C(3)-C(4)	-0.90(16)
C(2)-C(3)-C(4)-C(8)	177.23(10)
C(2)-C(3)-C(4)-C(5)	0.16(15)
C(5)-C(4)-C(8)-C(9)	37.60(14)
C(8)-C(4)-C(5)-C(6)	-176.60(10)
C(3)-C(4)-C(8)-C(9)	-139.43(10)
C(3)-C(4)-C(8)-C(7)	37.60(15)
C(3)-C(4)-C(5)-C(6)	0.51(15)
C(5)-C(4)-C(8)-C(7)	-145.37(10)
C(4)-C(5)-C(6)-C(1)	-0.43(16)
N(1)-C(7)-C(8)-C(9)	0.05(10)
C(15)-C(7)-C(8)-C(4)	4.64(17)
N(1)-C(7)-C(8)-C(4)	-177.36(9)
C(15)-C(7)-C(8)-C(9)	-177.96(9)
C(7)-C(8)-C(9)-C(10)	-0.20(10)
C(4)-C(8)-C(9)-C(10)	177.30(8)
C(8)-C(9)-C(10)-N(1)	0.27(10)
C(8)-C(9)-C(10)-C(11)	-175.96(10)
C(9)-C(10)-C(11)-C(16)	-0.35(16)

C(9)-C(10)-C(11)-C(12)	177.60(10)
N(1)-C(10)-C(11)-C(12)	1.63(13)
N(1)-C(10)-C(11)-C(16)	-176.33(7)
C(10)-C(11)-C(16)-O(1)	-0.31(13)
C(12)-C(11)-C(16)-O(2)	1.25(13)
C(10)-C(11)-C(12)-C(13)	0.47(14)
C(16)-C(11)-C(12)-C(13)	178.39(9)
C(10)-C(11)-C(16)-O(2)	179.19(8)
C(12)-C(11)-C(16)-O(1)	-178.26(9)
C(11)-C(12)-C(13)-C(14)	-1.89(15)
C(12)-C(13)-C(14)-N(1)	1.06(15)
C(18)-C(17)-C(22)-C(21)	0.15(17)
C(22)-C(17)-C(18)-C(19)	-0.69(17)
Br(2)-C(17)-C(22)-C(21)	179.19(8)
Br(2)-C(17)-C(18)-C(19)	-179.72(8)
C(17)-C(18)-C(19)-C(20)	0.25(17)
C(18)-C(19)-C(20)-C(24)	-178.87(10)
C(18)-C(19)-C(20)-C(21)	0.67(16)
C(19)-C(20)-C(21)-C(22)	-1.24(16)
C(21)-C(20)-C(24)-C(25)	-32.06(15)
C(19)-C(20)-C(24)-C(23)	-34.53(16)
C(19)-C(20)-C(24)-C(25)	147.46(10)
C(24)-C(20)-C(21)-C(22)	178.32(10)
C(21)-C(20)-C(24)-C(23)	145.96(10)
C(20)-C(21)-C(22)-C(17)	0.86(17)
N(2)-C(23)-C(24)-C(20)	-178.67(9)
C(31)-C(23)-C(24)-C(20)	-1.51(17)
N(2)-C(23)-C(24)-C(25)	-0.41(11)
C(31)-C(23)-C(24)-C(25)	176.76(10)
C(20)-C(24)-C(25)-C(26)	179.06(9)
C(23)-C(24)-C(25)-C(26)	0.73(11)
C(24)-C(25)-C(26)-C(27)	-176.72(10)
C(24)-C(25)-C(26)-N(2)	-0.74(10)
N(2)-C(26)-C(27)-C(32)	-177.84(8)
N(2)-C(26)-C(27)-C(28)	1.28(13)
C(25)-C(26)-C(27)-C(28)	176.98(10)

C(25)-C(26)-C(27)-C(32)	-2.14(16)
C(26)-C(27)-C(28)-C(29)	0.55(15)
C(26)-C(27)-C(32)-O(4)	177.08(8)
C(28)-C(27)-C(32)-O(3)	178.14(9)
C(28)-C(27)-C(32)-O(4)	-2.04(13)
C(26)-C(27)-C(32)-O(3)	-2.74(13)
C(32)-C(27)-C(28)-C(29)	179.65(9)
C(27)-C(28)-C(29)-C(30)	-1.65(16)
C(28)-C(29)-C(30)-N(2)	0.79(15)

Symmetry transformations used to generate equivalent atoms:

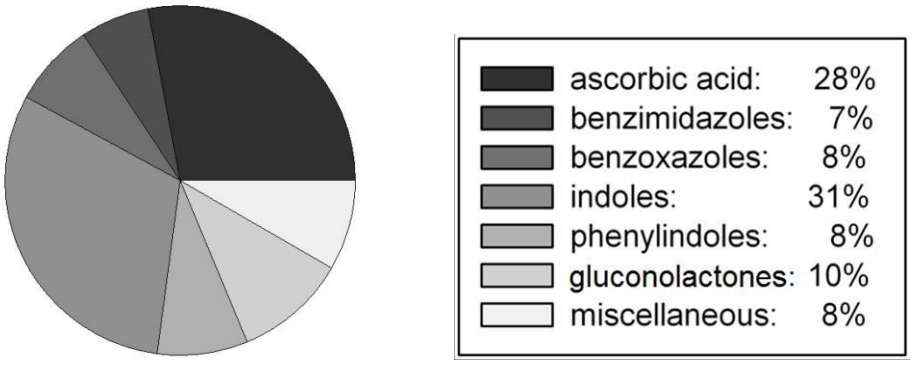
Table 7. Hydrogen-bonds for 9.3 [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2A)...O(3)#1	0.8400	1.7900	2.6288(10)	174.00
O(4)-H(4)...O(1)#1	0.8400	1.7900	2.6181(10)	171.00
C(9)-H(9)...O(1)	0.9500	2.4700	2.9151(11)	109.00
C(12)-H(12)...O(2)	0.9500	2.3900	2.7155(12)	100.00
C(15)-H(15C)...Br(1)#2	0.9800	2.9000	3.8480(10)	162.00
C(25)-H(25)...O(3)	0.9500	2.4600	2.9122(11)	109.00
C(28)-H(28)...O(4)	0.9500	2.3900	2.7188(13)	100.00
C(31)-H(31C)...Br(2)#2	0.9800	2.9200	3.8553(11)	159.00

B Appendix II

B.1 Compound library

B.1.1 Compound library of hyaluronidase inhibitors (library 1)



Compound library of hyaluronidase inhibitors (347 inhibitors, target molecule: *Sag*Hyal$_{4755}$) tabulated according to the corresponding chemical classes. The substances served as templates for molecules synthesized on plates **ori.hya.1**-**35**.

Index	Name	ID	IC_{50} (μM) *Sag*Hyal$_{4755}$
1	4-(Trifluoromethyl)-2-(4-sulfamoyloxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-6-yl sulfamate	UR-CT183	9
2	4-(Trifluoromethyl)-2-(4-hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-6-yl sulfamate	UR-CT184	5
3	2-(4-Sulfamoyloxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-5-yl sulfamate	UR-CT185	27
4	1-Ethyl-3-methyl-2-phenyl-1H-indol-6-yl sulfamate	UR-CT186	550
5	1-Ethyl-2-(4-sulfamoyloxyphenyl)-3-methyl-1H-indol-5-yl sulfamate	UR-CT192	124
6	1-Ethyl-2-(4-sulfamoyloxyphenyl)-3-methyl-1H-indol-6-yl sulfamate	UR-CT193	109
7	1-Ethyl-2-phenyl-1H-indol-5-yl sulfamate	UR-CT194	62
8	2-(4-Sulfamoyloxyphenyl)-3-methyl-1-(6-(pyrrolidin-1-yl)hexyl)-1H-indol-6-yl sulfamate	UR-CT195	219
9	1-Decyl-2-(4-hydroxyphenyl)-3-methyl-1H-indol-6-ol	UR-CT201	17.8
10	4-((E)-4-(4-Hydroxyphenyl)hex-3-en-3-yl)phenyl sulfamate	UR-CT204	49
11	2-(4-Hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-5-ol	UR-CT205	15.8
12	2-(4-Hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-6-ol	UR-CT206	26
13	4-(Trifluoromethyl)-2-(4-hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-6-ol	UR-CT207	4.9
14	1-Ethyl-3-methyl-2-phenyl-1H-indol-6-ol	UR-CT208	66
15	2-(4-Hydroxyphenyl)-3-methyl-1-(6-(pyrrolidin-1-yl)hexyl)-1H-indol-5-ol	UR-CT216	300
16	4-((E)-4-(4-Hydroxyphenyl)hex-3-en-3-yl)phenol	UR-CT220	30
17	1-Ethyl-2-(4-hydroxyphenyl)-1H-indol-6-ol	UR-CT223	320
18	1-Butyl-2-(4-hydroxyphenyl)-1H-indol-6-ol	UR-CT224	66
19	1-Heptyl-2-(4-hydroxyphenyl)-1H-indol-6-ol	UR-CT225	8.9
20	1-(4-Chlorobenzoyl)indol-3-acetic acid	[8]	290
21	1-(4-Methoxybenzoyl)indol-3-acetic acid	[9]	640
22	1-(4-Trifluormethylbenzoyl)indol-3-acetic acid	[10]	300
23	1-(Biphenyl-4-ylcarbonyl)indol-3-acetic acid	[11]	59
24	1-Decanoylindol-3-acetic acid	[12]	250
25	1-(4-Fluorbenzoyl)-2-methylindol-3-acetic acid	[26]	210
26	1-(4-Chlorbenzoyl)-2-methylindol-3-acetic acid	[27]	120
27	1-(4-Methoxybenzoyl)-2-methylindol-3-acetic acid	[28]	290
28	1-(4-Trifluormethylbenzoyl)-2-methylindol-3-acetic acid	[29]	180
29	1-(p-Biphenylcarbonyl)-2-methylindol-3-acetic acid	[30]	44 ± 1.1
30	1-Decanoyl-2-methylindol-3-acetic acid	[31]	inactive
31	1-Hexadecanoyl-2-methylindol-3-acetic acid	[32]	3.9 ± 0.1
32	1-[6-(Biphenyl-4-yloxy)hexanoyl]-2-methylindol-3-acetic acid	[33]	50
33	4-[1-(p-Biphenylcarbonyl)indol-3-yl]butanoic acid-5-(biphenyl-4-oxy)pentylester	[42]	320
34	1-(4-Fluorobenzyl)indol-3-acetic acid	[43]	inactive
35	2-[1-(Biphenyl-4-ylmethyl)indol-3-yl]acetic acid	[44]	1200

36	1-(4-Fluorbenzyl)-2-methylindol-3-acetic acid	[45]	inactive
37	2-[1-(Biphenyl-4-ylmethyl)-2-methylindol-3-yl]acetic acid	[46]	inactive
38	1-(Biphenyl-4-ylmethyl)-3-(carboxymethyl)-5-hydroxyindol-2-carboxylic acid	[52]	16 ± 0.3
39	Lauryl gallate	Chapter 5	13 ± 1
40	1,3-Diacetylbenzimidazole-2-thione	Chapter 5	160
41	3-phenyl-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)propan-1-one	Chapter 5	15 ± 1
42	tetradecane sulfonic acid	Chapter 5	12 ± 1
43	D-glucurono-6,3-lactone		> 20000
44	1-O-Benzyl-beta-D-glucofuranosidurono-6,3-lactone	6.1	> 4000
45	1-O-(3-Phenylpropyl)-beta-D-glucofuranosidurono-6,3-lactone	6.2	920 ± 103
46	1-O-(Octan-1-yl)-beta-D-glucofuranosidurono-6,3-lactone	6.3	171 ± 10
47	1-O-(8-Hydroxyoctan-1-yl)-beta-D-glucofuranosidurono-6,3-lactone	6.4	683 ± 62
48	1-O-(Dodecan-1-yl)-beta-D-glucofuranosidurono-6,3-lactone	6.5	29 ± 2
49	1-O-(Octan-1-yl)-beta-D-glucofuranosiduronic acid sodium salt	6.7	> 1200
50	1-O-(8-Hydroxyoctan-1-yl)-beta-D-glucofuranosiduronic acid sodium salt	6.8	1170 ± 70
51	1-O-(Dodecan-1-yl)-beta-D-glucofuranosiduronic acid sodium salt	6.9	36 ± 5
52	1-O-(Hexadecan-1-yl)-beta-D-glucofuranosiduronic acid	6.10	4.7 ± 0.1
53	1-O-(Dodecan-1-yl)-beta-D-glucofuranosiduronamide	6.11	82 ± 4
54	1-O-(Hexadecan-1-yl)-beta-D-glucofuranosiduronamide	6.12	> 50
55	1-O-(Hexadecan-1-yl)-N-benzyl-beta-D-glucofuranosiduronamide	6.13	> 50
56	1-O-(Hexadecan-1-yl)-N-(2-phenylethyl)-beta-D-glucofuranosiduronamide	6.14	> 40
57	1-O-(Hexadecan-1-yl)-N-butyl-beta-D-glucofuranosiduronamide	6.15	> 40
58	1-O-(Hexadecan-1-yl)-N-(decan-1-yl)-beta-D-glucofuranosiduronamide	6.16	> 20
59	1-O-(Hexadecan-1-yl)-N-(2-hydroxyethyl)-beta-D-glucofuranosiduronamide	6.17	> 50
60	1-O-(Hexadecan-1-yl)-N-(4-aminobutyl)-beta-D-glucofuranosiduronamide	6.18	60 ± 5
61	1-O-(Hexadecan-1-yl)-N-(12-aminododecan-1-yl)-beta-D-glucofuranosiduronamide	6.19	> 20
62	1-O-(Hexadecan-1-yl)-N-(2-carboxyethyl)-beta-D-glucofuranosiduronamide	6.23	2.1 ± 0.1
63	1-O-(Hexadecan-1-yl)-N-(3-carboxypropyl)-beta-D-glucofuranosiduronamide	6.24	4.2 ± 0.1
64	1-O-(Hexadecan-1-yl)-5-O-(2,2-dimethylpropanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.27	> 30
65	1-O-(Hexadecan-1-yl)-5-O-propanoyl-beta-D-glucofuranosidurono-6,3-lactone	6.28	> 30
66	1-O-(Dodecan-1-yl)-2-O-(2-carboxyethanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.41	22 ± 2
67	1-O-(Hexadecan-1-yl)-2-O-(2-carboxyethanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.42	2.9 ± 0.1
68	1-O-(Hexadecan-1-yl)-2-O-(3-carboxypropanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.43	3.7 ± 0.1
69	1-O-(Hexadecan-1-yl)-2-O-(2-dimethylaminoethanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.44	16 ± 2
70	1-O-(Hexadecan-1-yl)-5-O-(2-carboxyethanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.47	3.9 ± 0.1
71	1-O-(Hexadecan-1-yl)-5-O-(3-carboxypropanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.48	2.3 ± 0.2
72	1-O-(Hexadecan-1-yl)-2,5-di-O-(2-carboxyethanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.51	2.3 ± 0.1

73	1-O-(Hexadecan-1-yl)-2,5-di-O-(3-carboxypropanoyl)-beta-D-glucofuranosidurono-6,3-lactone	6.52	2.6 ± 0.1
74	1-O-(Hexadecan-1-yl)-beta-D-glucofuranosidurono-6,3-lactone-2-sulfate	6.54	3.5 ± 0.2
75	1-O-(Hexadecan-1-yl)-beta-D-glucofuranosidurono-6,3-lactone-2,5-disulfate	6.55	3.1 ± 0.1
76	6-O-Octanoyl-L-ascorbic acid	7.2a	772 ± 19
77	6-O-Decanoyl-L-ascorbic acid	7.2b	102 ± 5
78	6-O-Undecanoyl-L-ascorbic acid	7.2c	72 ± 2
79	6-O-Dodecanoyl-L-ascorbic acid	7.2d	47 ± 2
80	6-O-Tridecanoyl-L-ascorbic acid	7.2e	14 ± 1
81	6-O-Tetradecanoyl-L-ascorbic acid	7.2f	8.4 ± 0.2
82	6-O-(Biphenyl-4-ylacetyl)-L-ascorbic acid	7.2g	358 ± 15
83	6-O-[6-(Phenylthio)hexanoyl]ascorbic acid	7.48	607 ± 58
84	6-O-[6-(Benzylthio)hexanoyl]ascorbic acid	7.49	461 ± 19
85	6-O-(6-Phenoxyhexanoyl)ascorbic acid	7.50	759 ± 45
86	6-O-[6-(4-Ethylphenoxy)hexanoyl]ascorbic acid	7.51	280 ± 9
87	6-O-[6-(4-Phenylphenoxy)hexanoyl]ascorbic acid	7.52	61 ± 1
88	6-O-[6-(4-Benzyloxyphenoxy)hexanoyl]ascorbic acid	7.53	76 ± 1
89	6-O-(6-Benzyloxyhexanoyl)ascorbic acid	7.54	437 ± 77
90	6-O-[6-(4-Phenylphenoxy)hexanoyl]ascorbic acid	7.55	102 ± 2
91	6-O-{6-[4-(Thiophen-3-yl)phenoxy]hexanoyl}ascorbic acid	7.56	54 ± 2
92	6-O-{6-[4-(2-Methoxyphenyl)phenyloxy]hexanoyl}ascorbic acid	7.57	58 ± 6
93	6-O-{6-[4-(4-Methylphenyl)phenyloxy]hexanoyl}ascorbic acid	7.58	24 ± 1
94	6-O-{6-[4-(2-Methylphenyl)phenyloxy]hexanoyl}ascorbic acid	7.59	67 ± 2
95	6-O-{6-[4-(4-Hydroxyphenyl)phenyloxy]hexanoyl}ascorbic acid	7.60	66 ± 1
96	6-O-{6-[4-(3-Hydroxyphenyl)phenyloxy]hexanoyl}ascorbic acid	7.61	125 ± 3
97	6-O-(11-Phenoxyundecanoyl)ascorbic acid	7.62	31 ± 1
98	6-O-[11-(4-Phenylphenoxy)undecanoyl]ascorbic acid	7.63	7.5 ± 0.2
99	6-O-[3-(Decan-1-yloxy)benzoyl]-L-ascorbic acid	7.88	5.4 ± 0.2
100	6-O-[2-(Hexan-1-yl)dodecanoyl]-L-ascorbic acid	7.91	2.7 ± 0.1
101	6-O-[2-(2-Phenylethyl)dodecanoyl]-L-ascorbic acid	7.93	6.8 ± 0.2
102	6-O-[2-(4-Methoxybenzyl)dodecanoyl]-L-ascorbic acid	7.94	5.3 ± 0.3
103	6-O-[2-(Hexan-1-yl)octadecanoyl]-L-ascorbic acid	7.95	2.2 ± 0.2
104	6-O-{6-[4-(4-Methoxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid	7.96	37 ± 1
105	5-O-,6-O-Bis{6-[4-(4-methoxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid	7.96a	8.0 ± 0.6
106	6-O-{6-[4-(Naphthalen-1-yl)phenoxy]hexanoyl}-L-ascorbic acid	7.97	11 ± 1
107	5,6-O-Bis{6-[4-(naphthalen-1-yl)phenoxy]hexanoyl}-L-ascorbic acid	7.97a	9.8 ± 1.0
108	5,6-O-Bis[5-(heptan-1-yloxy)pentanoyl]-L-ascorbic acid	7.98a	17 ± 1
109	5,6-O-Bis[4-(decan-1-yloxy)benzoyl]-L-ascorbic acid	7.100a	15 ± 1

110	6-O-(11-Carboxyundecanoyl)-L-ascorbic acid	7.101	124 ± 6
111	6-O-{6-[4-(4-Carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid	7.102	60 ± 2
112	6-O-{6-[4-(3-Carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid	7.103	94 ± 8
113	5,6-O-Bis{6-[4-(3-carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid	7.103a	4.7 ± 0.4
114	3-{(1S)-1-[(5R)-3,4-Dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-(dodecanoyloxy)ethyloxy}-3-oxopropanoic acid	7.106	14 ± 1
115	Bis{(2S)-2-[(5R)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} dodecanedioate	7.110	22 ± 2
116	Bis{(2S)-2-[(5R)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} tetradecanedioate	7.111	22 ± 1
117	Bis{(2S)-2-[(5R)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} hexadecanedioate	7.112	7.4 ± 0.2
118	Bis{(2S)-2-[(5R)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} 13-oxo-12-azatricosanedioate	7.117	55 ± 2
119	6-O-Hexadecanoyl-2-O-(3-phenylpropanoyl)-L-ascorbic acid	7.118	4.9 ± 0.3
120	2-O-(3-Carboxypropanoyl)-6-O-hexadecanoyl-L-ascorbic acid	7.119	1.7 ± 0.1
121	6-O-palmitoylascorbic acid		5000
122	2-O-(4-Carboxybutanoyl)-6-O-hexadecanoyl-L-ascorbic acid	7.120	2.5 ± 0.2
123	2-O-(5-Carboxypentanoyl)-6-O-hexadecanoyl-L-ascorbic acid	7.121	2.8 ± 0.1
124	2,3-O-Bis(5-carboxypentanoyl)-6-O-hexadecanoyl-L-ascorbic acid	7.121a	4.9 ± 0.2
125	2,3-O-Bis(carboxymethyl)-6-O-dodecanoyl-L-ascorbic acid	7.132	12 ± 1
126	2-O-(Carboxymethyl)-6-O-dodecanoyl-L-ascorbic acid	7.146	15 ± 0.6
127	2-O-(Carboxymethyl)-6-O-[11-(4-phenylphenoxy)undecanoyl]-L-ascorbic acid	7.147	2.4 ± 0.1
128	3-O-(Carboxymethyl)-6-O-dodecanoyl-L-ascorbic acid	7.148	4.8 ± 0.3
129	indole-3-butanoic acid		2200
130	1-(3-Phenylpropanoyl)-1H-indole-3-butanoic acid	8.7	72 ± 4
131	1-Decanoyl-1H-indole-3-butanoic acid	8.8	> 100
132	N-[2-Deoxy-D-glucopyranose-2-yl]-1-decanoyl-1H-indole-3-butanoic acid amide	8.8a	> 200
133	1-(4-Chlorobenzoyl)-1H-indole-3-butanoic acid	8.9	125 ± 5
134	1,1'-(p-Phenylendiacetyl)diindol-3,3'-dibutanoic acid	8.19	17 ± 3
135	1,1'-Octanedioyldiindol-3,3'-dibutanoic acid	8.20	> 70
136	1,1'-Dodecanedioyldiindol-3,3'-dibutanoic acid	8.21	18 ± 1
137	1,1'-Hexadecanedioyldiindol-3,3'-dibutanoic acid	8.22	13 ± 1
138	11-[3-(3-Carboxypropyl)-1H-indol-1-yl]undecanoic acid	8.32	12 ± 1
139	16-[3-(3-Carboxypropyl)-1H-indol-1-yl]hexadecanoic acid	8.33	16.5 ± 0.4
140	1-[11-Oxo-11-(thiazol-2-ylamino)undecan-1yl]-1H-indole-3-butanoic acid	8.42	> 100
141	1-[11-(3-Hydroxyphenylamino)-11-oxoundecan-1yl]-1H-indole-3-butanoic acid	8.45	7.4 ± 3
142	1-{11-[(S)-2-((R)-3,4-Dihydroxy-2-oxo-2,5-dihydrofuran-5-yl)-2-hydroxyethoxy]-11-oxoundecan-1-yl}-1H-indole-3-butanoic acid	8.46	7.7 ± 0.5
143	1-[16-(3-Hydroxyphenylamino)-16-oxohexadecan-1-yl]-1H-indole-3-butanoic acid	8.48	> 30
144	1-{16-[(S)-2-((R)-3,4-Dihydroxy-2-oxo-2,5-dihydrofuran-5-yl)-2-hydroxyethoxy]-16-oxohexadecan-1-yl}-1H-indole-3-butanoic acid	8.49	8.1 ± 0.3
145	5-Benzyloxy-2-carboxy-1H-indole-3-butanoic acid	8.53	164 ± 1
146	5-Benzyloxy-1H-indole-3-butanoic acid	8.54	318 ± 9

147	5-Hydroxy-1H-indole-3-butanoic acid	8.55	inactive
148	2-Carboxy-5-(3-phenylpropoxy)-1H-indole-3-butanoic acid	8.62	53 ± 2
149	2-Carboxy-5-(hexan-1-yloxy)-1H-indole-3-butanoic acid	8.63	44 ± 3
150	2-Carboxy-5-(dodecan-1-yloxy)-1H-indole-3-butanoic acid	8.64	3.0 ± 0.1
151	2-Carboxy-5-(hexadecan-1-yloxy)-1H-indole-3-butanoic acid	8.65	2.4 ± 0.1
152	2-Carboxy-5-(octadecan-1-yloxy)-1H-indole-3-butanoic acid	8.66	6.3 ± 0.2
153	2-Carboxy-5-(hexadecan-1-yloxy)-1-propyl-1H-indole-3-butanoic acid	8.70	> 50
154	2-Carboxy-5-(hexadecan-1-yloxy)-1-(pyridin-3-ylmethyl)-1H-indole-3-butanoic acid hydrochloride	8.71	15 ± 1
155	1-(4-Bromobenzyl)-2-carboxy-5-(hexadecan-1yloxy)-1H-indole-3-butanoic acid	8.72	108 ± 10
156	5-Benzyloxy-1-(decan-1-yl)-2-ethoxycarbonyl-1H-indole-3-butanoic acid	8.74	> 40
157	5-Benzyloxy-3-(3-carboxypropyl)-1-(decan-1-yl)-1H-indole-2-carboxylic acid	8.75	20 ± 2
158	1-(Decan-1-yl)-2-ethoxycarbonyl-5-hydroxy-1H-indole-3-butanoic acid	8.76	7.5 ± 0.6
159	2-Carboxy-1-(decan-1-yl)-5-hydroxy-1H-indole-3-butanoic acid	8.77	9.3 ± 0.3
160	N-[2-Deoxy-D-glucopyranose-2-yl]-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide	8.78	189 ± 33
161	1,4-Phenylene-bis(beta-D-glucopyranoside)	9.8	1608 ± 60
162	Sodium 2-[((3R,8aR)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methylthio]acetate	9.5	> 3000
163	1-O-(Hexadecan-1-yl)-beta-d-glucofuranosidurono-6,3-lactone	6.6	9.5 ± 0.7
164	1-O-(Hexadecan-1-yl)-N-(trans-4-carboxycyclohexylmethy)-beta-d-glucofuranosiduronamide	6.25	> 50
165	1-O-(Hexadecan-1-yl)-beta-D-glucuronic acid	6.26	> 60
166	6-O-[2-(Decan-1-yloxy)benzoyl]-L-ascorbic acid	7.87	21 ± 1
167	6-O-[2-(Hexan-1-yl)decanoyl]-L-ascorbic acid	7.89	9.3 ± 0.7
168	6-O-[2-(Propan-1-yl)dodecanoyl]-L-ascorbic acid	7.90	13 ± 1
169	6-O-[2-(Decan-1-yl)dodecanoyl]-L-ascorbic acid	7.92	6.4 ± 0.2
170	6-O-[5-(Heptan-1-yloxy)pentanoyl]-L-ascorbic acid	7.98	257 ± 9
171	6-O-[6-(Hexan-1-yloxy)hexanoyl]-L-ascorbic acid	7.99	221 ± 20
172	5,6-O-Bis[6-(hexan-1-yloxy)hexanoyl]-L-ascorbic acid	7.99a	26 ± 3
173	6-O-[4-(Decan-1-yloxy)benzoyl]-L-ascorbic acid	7.100	8.3 ± 0.3
174	6-O-(Dodecan-1-yl)-L-ascorbic acid	7.127	26 ± 1
175	6-O-(Octadecan-1-yl)-L-ascorbic acid	7.128	1.6 ± 0.1
176	2-O-(Dodecan-1-yl)-L-ascorbic acid	7.139	40 ± 2
177	2-O-(Hexadecan-1-yl)-L-ascorbic acid	7.140	4.1 ± 0.2
178	3-O-(Dodecan-1-yl)-L-ascorbic acid	7.149	26 ± 1
179	3-O-(Tetradecan-1-yl)-L-ascorbic acid	7.150	6.0 ± 0.2
180	4-[1-(4-methoxybenzoyl)-1H-indol-3-yl]butanoic acid	8.10	> 400
181	4-[1-(Biphenyl-4-ylcarbonyl)-1H-indol-3-yl]butanoic acid	8.11	> 60
182	4-[1-(2-(4-methoxyphenyl)acetyl)-1H-indol-3-yl]butanoic acid	8.12	> 250
183	4-[1-(2-(4-hydroxyphenyl)acetyl)-1H-indol-3-yl]butanoic acid	8.23	490 ± 30

184	1-[11-Oxo-11-(4-phenylpiperazin-1-yl)undecan-1yl]-1H-indole-3-butanoic acid ammonium salt	8.43	> 50
185	1-[11-Oxo-11-(piperazin-1-yl)undecan-1yl]-1H-indole-3-butanoic ammonium salt	8.44	61 ± 1
186	1-[16-Oxo-16-(4-phenylpiperazin-1-yl)hexadecan-1yl]-1H-indole-3-butanoic acid trifluoroacetic acid salt	8.47	> 30
187	Hexadecyl 2-(trimethylammonio)ethyl phosphate	9.25	1.6 ± 0.3
188	Octadecyl 2-(trimethylammonio)ethyl phosphate	9.26	1.9 ± 0.1
189	2-(1-Methylpiperidinium-1-yl)ethyl octadecyl phosphate	9.27	0.88 ± 0.1
190	1,1-Dimethylpiperidinium-4-yl octadecyl phosphate	9.28	1.5 ± 0.2
191	(E)-3-(1-Hydroxyhexadecylidene)-1-methylpyrrolidine-2,4-dione	Melophlin A	6.6 ± 3
192	(5S,E)-3-(1-Hydroxy-4-methyldodecylidene)-1,5-dimethylpyrrolidine-2,4-dione	Melophlin B	75 ± 7
193	(E)-3-(1-Hydroxy-5-methyldodecylidene)-1,5-dimethylpyrrolidine-2,4-dione	Melophlin C	33 ± 2
194	(E)-3-(1-Hydroxytetradecylidene)-1-methylpyrrolidine-2,4-dione	Melophlin G	14 ± 1
195	(E)-3-(1-Hydroxyhexadecylidene)-1,5-dimethylpyrrolidine-2,4-dione	Melophlin P	9.0 ±1.0
196	(E)-3-(1-Hydroxy-13-methyltetradecylidene)-1,5-dimethylpyrrolidine-2,4-dione	Melophlin Q	> 250
197	(E)-3-(1-Hydroxy-12-methyltetradecylidene)-1,5-dimethylpyrrolidine-2,4-dione	Melophlin R	18 ± 1
198	Calcium bis[(E)-3-(1-hydroxyhexadecylidene)-1-methylpyrrolidine-2,4-dione]	Ca(Melophlin A)2	5.0 ± 0.2
199	(E)-3-Dodec-5-enoyl-4-hydroxy-5-methylenefuran-2(5H)-one	Agglomerin B	> 100
200	Dodecyl 3,4,5-trihydroxybenzoate	Chapter 5	13 ± 1
201	2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-inden-3-yl]acetic acid	5-Indomethacin	350
202	2-[2-(2,6-Dichlorophenylamino)phenyl]acetic acid	5-Diclofenac	161 ± 11
203	sodium 5,5'-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4-oxo-4H-chromene-2-carboxylate)	5-DSCG	1208 ± 81
204	3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one	5-Phloretin	213 ± 8
205	3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	5-Kaempferol	237 ± 15
206	(2R,3R)-2-((2R,3R)-3-(3,4-Dimethoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3,5,7-trihydroxychroman-4-one	5-Sylibinin	296 ± 18
207	(3β,18α)-30-Hydroxy-11,30-dioxoolean-12-en-3-yl 2-O-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid	5-Glycyrrhizic acid	46 ± 2
208	3β-Hydroxy-11-oxoolean-12-en-30-oic acid	5-Glycyrrhetinic acid	54 ± 3
209	(2S,4aS,6aS,6bR,8aR,10S,12aS,12bR,14bR)-10-(3-Carboxypropanoyloxy)-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid	5-Carbenoxolone	20 ± 1
210	1-(Pyridin-3-ylmethyl)indoline	1	37% @ 3 mM
211	1-(Pyridin-3-ylmethyl)-1H-indole	7	65% @ 2 mM
212	(1H-Indol-1-yl)(pyridin-3-yl)methanone	8	60% @ 1.5 mM
213	1-Nicotinoyl-1H-indole-2-carboxylic acid	19	45% @ 3.2 mM
214	1-Nicotinoyl-1H-indole-3-carboxylic acid	20	80% @ 3.3 mM
215	2-(1-Nicotinoyl-1H-indol-3-yl)acetic acid	21	50% @ 3.2 mM
216	4-(1-Nicotinoyl-1H-indol-3-yl)butanoic acid	22	80% @ 3.2 mM
217	1-Nicotinoyl-1H-indole-5-carboxylic acid	23	1850 ± 50
218	1-(4-Chlorobenzoyl)-1H-indole-2-carboxylic acid	34	80% @3.2 mM

219	1-(3-Chlorobenzoyl)-1H-indole-2-carboxylic acid	35	950 ± 10
220	1-(4-Chlorobenzoyl)-1H-indole-3-carboxylic acid	36	70% @ 3.2 mM
221	1-(3-Chlorobenzoyl)-1H-indole-3-carboxylic acid	37	50% @ 3.2 mM
222	2-[1-(4-Chlorobenzoyl)-1H-indol-3-yl]acetic acid	38	580 ± 40
223	2-[1-(3-Chlorobenzoyl)-1H-indol-3-yl]acetic acid	39	480 ± 30
224	4-[1-(4-Chlorobenzoyl)-1H-indol-3-yl]butanoic acid	40	125 ± 5
225	4-[1-(3-Chlorobenzoyl)-1H-indol-3-yl]butanoic acid	41	330 ± 10
226	1-(4-Chlorobenzoyl)-1H-indole-5-carboxylic acid	42	20% @ 600 µM
227	1-(3-Chlorobenzoyl)-1H-indole-5-carboxylic acid	43	45% @ 700 µM
228	4-[1-(4-Methoxybenzoyl)-1H-indol-3-yl]butanoic acid	45	70% @ 1.8 mM
229	4-[1-(Biphenyl-4-ylcarbonyl)-1H-indol-3-yl]butanoic acid	51	60% @120 µM
230	4-{1-[2-(Biphenyl-4-yl)acetyl]-1H-indol-3-yl}butanoic acid	52	30% @ 200 µM
231	4-[1-(4-Benzoylbenzoyl)-1H-indol-3-yl]butanoic acid	53	20% @ 170 µM
232	4-[1-(2-Naphthoyl)-1H-indol-3-yl]butanoic acid	54	80% @ 100 µM
233	4-{1-[2-(4-Methoxyphenyl)acetyl]-1H-indol-3-yl}butanoic acid	55	30% @ 270 µM
234	4-[1-(4-Hydroxybenzoyl)-1H-indol-3-yl]butanoic acid	58	490 ± 30
235	5-Methoxy-2-(4-methoxyphenyl)-3-methylindole	9.4b	880
236	5-Hydroxy-2-(4-hydroxyphenyl)indole	9.6a	75% @ 1000 µM
237	6-Hydroxy-2-(4-hydroxyphenyl)-3-methylindole	9.6b	330
238	5-Hydroxy-2-(4-hydroxyphenyl)-3-methylindole	9.6c	740
239	5-Hydroxy-2-(4-hydroxyphenyl)-1-methyl-3-methylindole	9.6d	480
240	5-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-propylindole	9.6e	220
241	5-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-pentylindole	9.6f	23
242	5-Hydroxy-2-(4-hydroxyphenyl)-1-heptyl-3-methylindole	9.6g	26
243	1-Decyl-2-(4-hydroxyphenyl)-1H-indol-6-ol	9.23	13
244	1-Decyl-2-(4-hydroxyphenyl)-1H-indol-6-yl sulfamate	9.24	8
245	2-O-,3-O-Dimethyl-L-ascorbic acid	4.6	inactive
246	2-O-,3-O-Dibenzyl-L-ascorbic acid	4.7	355 ± 59
247	2-O-,3-O-Ethan-1,2-diyl-L-ascorbic acid	4.8	24% @ 2000 µM
248	2-O-,3-O-Dimethyl-6-O-hexadecanoyl-L-ascorbic acid	4.9	5% @ 160 µM
249	2-O-,3-O-Dibenzyl-6-O-hexadecanoyl-L-ascorbic acid	4.10	inactive
250	2-O-,3-O-Ethan-1,2-diyl-6-O-hexadecanoyl-L-ascorbic acid	4.11	32% @ 190 µM
251	6-O-(2,2-Dimethylpropanoyl)-L-ascorbic acid	4.18	43% @ 1100 µM
252	6-O-Hexanoyl-L-ascorbic acid	4.19	475 ± 16
253	6-O-Dodecanoyl-L-ascorbic acid	4.20	6.71 ± 0.28
254	6-O-Hexadecanoyl-L-ascorbic acid	4.21	4.22 ± 0.13
255	6-O-Octadecanoyl-L-ascorbic acid	4.22	0.93 ± 0.11

256	6-O-Benzoyl-L-ascorbic acid	4.23	131.6 ± 5.9
257	1-(2-Thioxo-1H-benzo[d]imidazol-1-yl)ethanone	5.3	20% @ 460 μM
258	1-(2-Thioxo-1H-benzo[d]imidazol-1-yl)propan-1one	5.4	4% @ 180 μM
259	1-(2-Thioxo-1H-benzo[d]imidazol-1-yl)butan-1-one	5.5	7% @ 190 μM
260	1-(2-Thioxo-1H-benzo[d]imidazol-1-yl)hexan-1-one	5.6	14% @ 170 μM
261	1-(2-Thioxo-1H-benzo[d]imidazol-1-yl)-3-phenylpropan-1-one	5.7	5% @ 100 μM
262	(3-Chlorophenyl)(2-thioxo-1H-benzo[d]imidazol-1-yl)methanone	5.8	16% @ 400 μM
263	2-(Ethylsulfanyl)-1H-benzo[d]imidazole	5.9	18% @ 2000 μM
264	1-[2-(Ethylsulfanyl)-1H-benzo[d]imidazol-1-yl]ethanone	5.12	4% @ 420 μM
265	1-[2-(Heptylsulfanyl)-1H-benzo[d]imidazol-1-yl]ethanone	5.13	inactive
266	1-(2-(Benzylsulfanyl)-1H-benzo[d]imidazol-1-yl)ethanone	5.14	4% @ 100 μM
267	1-[2-(4-Methoxybenzylsulfanyl)-1H-benzo[d]imidazol-1-yl]ethanone	5.15	inactive
268	1-(3-Acetyl-1,2-dihydro-2-thioxobenzo[d]imidazol-1-yl)hexan-1-one	5.16	50% @ 49 μM
269	1,3-Diacetyl-1H-benzo[d]imidazol-2(3H)-one	5.18	10% @ 200 μM
270	1,3-Diethyl-1H-benzo[d]imidazol-2(3H)-one	5.19	40% @ 4900 μM
271	1,3-Diethyl-1H-benzo[d]imidazole-2(3H)-thione	5.20	inactive
272	1-Ethyl-1H-benzo[d]imidazole-2(3H)-thione	5.24	19% @ 4200 μM
273	1-(3-Ethyl-1,2-dihydro-2-thioxobenzo[d]imidazole-1-yl)ethanone	5.25	10% @ 380 μM
274	Methyl 2-thioxo-3H-benzo[d]imidazole-5-carboxylate	5.27	25% @ 4500 μM
275	1-(2-Methyl-1H-benzo[d]imidazol-1-yl)propan-1-one	5.29	12% @ 2000 μM
276	1-(2-Thioxobenzo[d]oxazol-3(2H)-yl)ethanone	6.5	125 ± 5
277	1-(2-Thioxobenzo[d]oxazol-3(2H)-yl)propan-1-one	6.6	128 ± 5
278	1-(2-Thioxobenzo[d]oxazol-3(2H)-yl)hexan-1-one	6.7	51% @ 130 μM
279	1-(2-Thioxobenzo[d]oxazol-3(2H)-yl)decan-1-one	6.8	41% @ 100 μM
280	1-(2-Thioxobenzo[d]oxazol-3(2H)-yl)hexadecan-1-one	6.9	23% @ 63 μM
281	2-Phenyl-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)ethanone	6.10	260 ± 41
282	3-Phenyl-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)propan-1-one	6.11	15 ± 1
283	4-Phenyl-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)butan-1-one	6.12	47% @ 100 μM
284	(E)-3-Phenyl-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)prop-2-en-1-one	6.13	9% @ 80 μM
285	3-Cyclohexyl-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)propan-1-one	6.14	227 ± 122
286	2-Phenoxy-1-(2-thioxobenzo[d]oxazol-3(2H)-yl)ethanone	6.15	213 ± 30
287	Benzyl 2-thioxobenzo[d]oxazol-3(2H)-carboxylate	6.16	inactive
288	3-Methanesulfonylbenzoxazole-2-thione	6.19	inactive
289	3-Acetylbenzo[d]oxazol-2(3H)-one	6.21	inactive
290	3-Hexanoylbenzo[d]oxazol-2(3H)-one	6.22	inactive
291	3-Ethylbenzo[d]oxazole-2(3H)-thione	6.24	46% @ 400 μM
292	Methyl 2-sulfanylbenzo[d]oxazole-5-carboxylate	6.28	1299 ± 223

293	Methyl 3-(3-phenylpropanoyl)-2,3-dihydro-2-thioxobenzo[d]oxazole-5-carboxylate	6.29	56% @ 200 μM
294	2-Sulfanylbenzo[d]oxazole-5-sulfonic acid	6.30	781 ± 100
295	N,N-Dimethylbenzo[d]oxazol-2-amine	6.33	20% @ 5700 μM
296	1-(2-Methylbenzofuran-3-yl)ethanone	6.35	43% @ 2000 μM
297	1-(2-Methylbenzofuran-3-yl)-3-phenylpropan-1-one	6.36	inactive
298	1-(5-Amino-2-methyl-1H-indol-1-yl)propan-1-one	7.20	20% @ 2000 μM
299	1-(5-Amino-2-methyl-1H-indol-1-yl)hexan-1-one	7.21	42% @ 600 μM
300	1-(5-Amino-2-methyl-1H-indol-1-yl)decan-1-one	7.22	12% @ 100 μM
301	1-(5-Amino-2-methyl-1H-indol-1-yl)-3-phenylpropan-1-one	7.23	3 % @ 200 μM
302	1-(5-Amino-2-methyl-1H-indol-1-yl)-4-phenylbutan-1-one	7.24	10% @ 200 μM
303	5-Amino-2-methyl-1H-indole	7.25	17% @ 5000 μM
304	5-Amino-2-methyl-1-propyl-1H-indole	7.34	15% @ 2000 μM
305	5-Amino-1-hexyl -2-methyl-1H-indole	7.35	334 ± 6
306	5-Amino-2-methyl-1-octyl-1H-indole	7.36	285 ± 12
307	5-Amino-1-decyl-2-methyl-1H-indole	7.37	47 ± 2
308	5-Amino-2-methyl-1-(3-phenylpropyl)-1H-indole	7.38	31% @ 420 μM
309	5-Amino-2-methyl-1-(4-phenylbenzyl)-1H-indole	7.39	13% @ 200 μM
310	Ethyl 5-(5-amino-2-methyl-1H-indol-1-yl)pentanoate	7.40	25% @ 1600 μM
311	N-(1-Decyl-2-methyl-1H-indol-5-yl)methanesulfonamide	7.47	16% @ 50 μM
312	Ethyl 5-(5-amino-2-methyl-3-m-chlorobenzoyl-1H-indol-1-yl)pentanoate	7.54	20% @ 100 μM
313	Ethyl 5-(5-amino-2-methyl-3-propanoyl-1H-indol-1-yl)pentanoate	7.55	52% @ 1600 μM
314	1-(5-Amino-1-hexyl-2-methyl-1H-indol-3-yl)propan-1-one	7.56	60% @ 400 μM
315	1-(1H-Indol-1-yl)-3-phenylpropan-1-one	7.60	inactive
316	1-(3-Acetyl-1H-indol-yl)-3-phenylpropan-1-one	7.61	inactive
317	1-(3-Phenylpropanoyl)-1H-indole-5-carboxylic acid	7.62	12% @ 200 μM
318	2-Methyl-1H-indole-5-sulfonic acid	7.65	2310 ± 66
319	1,3-Diacetylbenzimidazoline-2-thione	5.1	160
320	1H-Benzo[d]imidazole-2(3H)-thione	5.2	1148 ± 50
321	2-Methyl-1H-benzo[d]imidazole	5.28	inactive
322	Benzo[d]oxazole-2(3H)-thione	6.1	42% @ 3800 μM
323	Oxazolo[4,5-b]pyridine-2(3H)-thione	6.37	10% @ 2200 μM
324	Benzo[d]thiazole-2(3H)-thione	6.38	45% @ 3700 μM
325	(Z)-1-(3-Methylbenzo[d]thiazol-2(3H)-ylidene)hydrazine	6.39	12% @ 2000 μM
326	2-(4-Hydroxyphenyl)-3-methyl-1H-indol-5-ol	6.40	740
327	2-(4-hydroxyphenyl)-3-methyl-1-propyl-1H-indol-5-ol	6.41	220
328	1-Heptyl-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	6.42	26
329	5,6-Di-O-dodecanoyl-L-ascorbic acid	7a	no data

330	5,6-Di-O-hexadecanoyl-L-ascorbic acid	7b	no data
331	5,6-Di-O-{2-[2-(2-methoxyethoxy)ethoxy]acetyl}-L-ascorbic acid	7c	> 120
332	6-O-Dodecanoyl-L-ascorbic acid	8a	47 ± 2
333	6-O-{2-[2-(2-Methoxyethoxy)ethoxy]acetyl}-L-ascorbic acid	8c	no data
334	6-O-Dodecanoyl-5-O-ethanoyl-L-ascorbic acid	10d	27 ± 1
335	6-O-Dodecanoyl-5-O-propanoyl-L-ascorbic acid	10e	42 ± 2
336	5-O-Butanoyl-6-O-dodecanoyl-L-ascorbic acid	10f	23 ± 1
337	6-O-Dodecanoyl-5-O-pentanoyl-L-ascorbic acid	10g	22 ± 2
338	6-O-Dodecanoyl-5-O-octanoyl-L-ascorbic acid	10h	13.3 ± 0.3
339	5-O-Decanoyl-6-O-dodecanoyl-L-ascorbic acid	10i	9.2 ± 0.8
340	6-O-Dodecanoyl-5-O-hexadecanoyl-L-ascorbic acid	10j	1.9 ± 0.7
341	5-O-[5-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)pentanoyl]-6-O-dodecanoyl-L-ascorbic acid	10k	7 ± 1
342	6-O-Dodecanoyl-5-O-(3-phenylpropanoyl)-L-ascorbic acid	10l	7.9 ± 0.4
343	5-O-(3-Carboxypropanoyl)-6-O-dodecanoyl-L-ascorbic acid	10m	no data
344	5-O-(4-Acetamidobutanoyl)-6-O-dodecanoyl-L-ascorbic acid	10n	24 ± 2
345	6-O-Dodecanoyl-5-O-{2-[2-(2-methoxyethoxy)ethoxy]acetyl}-L-ascorbic acid	10o	11.6 ± 0.8
346	2-O-[5-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)pentanoyl]-6-O-hexadecanoyl-L-ascorbic acid	11	2.7 ± 0.2
347	6-O-Hexadecanoyl-2,3-dihydro-L-ascorbic acid	12	90 ± 10

The molecules of the present library (“library 1“) were selected from: compounds 1-19: cf. chapter 7; compounds 20-38: Textor, C., Hemmstoffe humaner und bakterieller Hyaluronidasen: Synthese und Struktur-Wirkungs-Beziehungen von N-Acylindolen, diploma thesis, University of Regensburg, Regensburg, 2008.; compounds 39-209: Spickenreither, M., Inhibitors of bacterial and mammalian hyaluronidases: design, synthesis and structure-activity relationships with focus on human enzymes, doctoral thesis, University of Regensburg, Regensburg, 2007.; compounds 210-234: Spickenreither, M. Hemmstoffe bakterieller Hyaluronat Lyasen: Synthese und Struktur-Wirkungs-Beziehungen von N-Acylindolen, diploma thesis, University of Regensburg, Regensburg, 2004.; compounds 235-244: Salmen, S. Inhibitors of bacterial and mammalian hyaluronidase, Synthesis and structure-activity relationships, doctoral thesis, University of Regensburg, Regensburg, 2003.; compounds 245-328: Braun, S., New inhibitors of bacterial hyaluronidase - synthesis and structure-activity relationships, doctoral thesis, University of Regensburg, Regensburg, 2005.; compounds 329-347: Binder, F., Hemmstoffe humaner Hyaluronidasen: Synthese und Untersuchung an rekombinanten Enzymen, diploma thesis, University of Regensburg, Regensburg, 2007.

B.1.2 Compound library of hyaluronidase inhibitors (library 2)

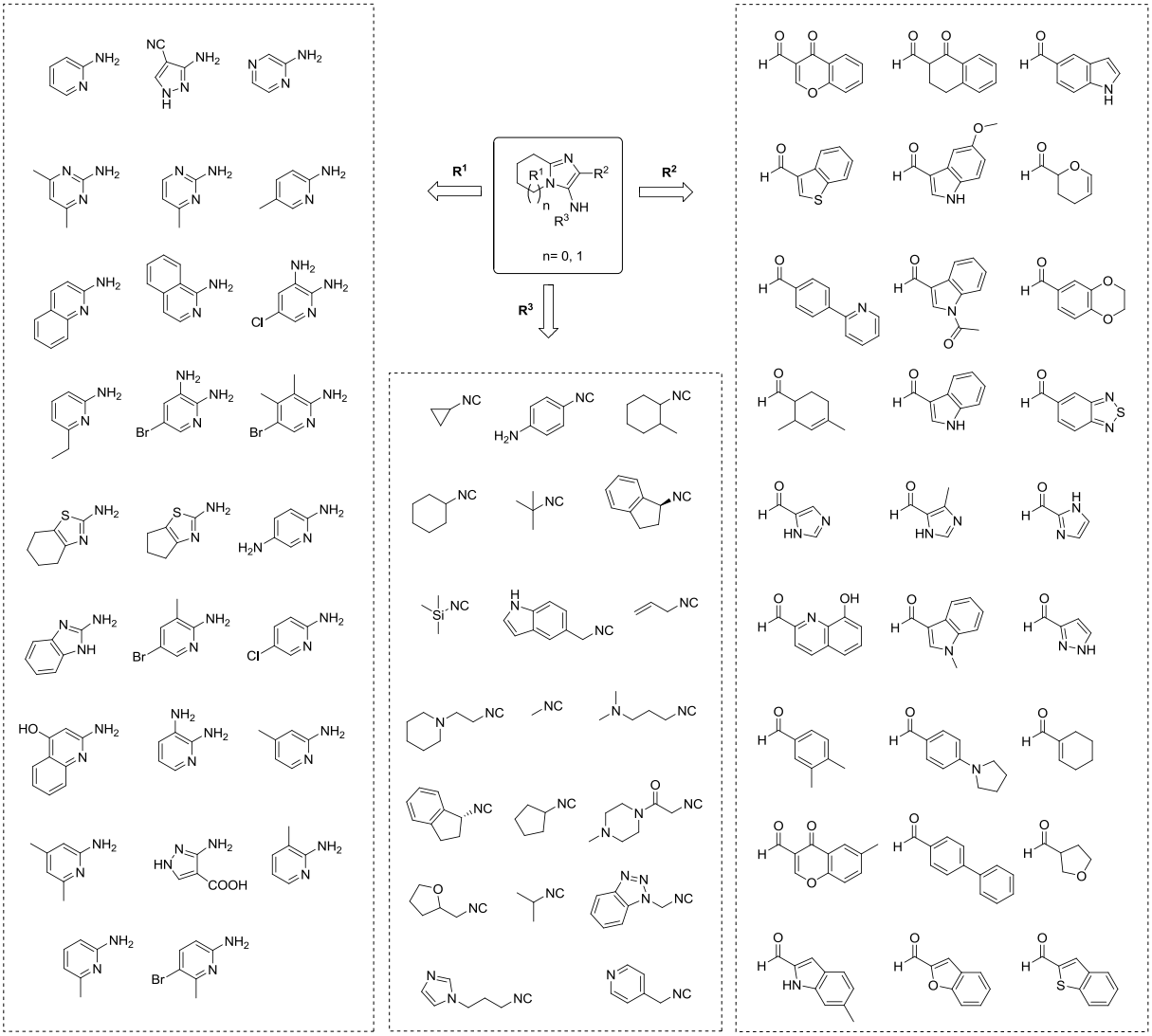
Compound library of hyaluronidase inhibitors (32 inhibitors, target molecule: *Sag*Hyal$_{4755}$). The substances served as templates for the exploration of bioisosteric molecules.

Index	Name	ID	IC_{50} (µM) *Sag*Hyal$_{4755}$
1	1-Decyl-2-(4-hydroxyphenyl)-3-methyl-1H-indol-6-ol	UR-CT201	19
2	2-(4-Hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-5-ol	UR-CT205	19
3	2-(4-Hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-1H-indol-6-ol	UR-CT206	20
4	2-(4-Hydroxyphenyl)-3-methyl-1-(perfluorobenzyl)-4-(trifluoromethyl)-1H-indol-6-ol	UR-CT207	6
5	2-(4-Hydroxyphenyl)-3-methyl-1-(6-(pyrrolidin-1-yl)hexyl)-1H-indol-5-ol	UR-CT216	19
6	2-(4-Hydroxyphenyl)-3-methyl-1-(4-(pyrrolidin-1-yl)butyl)-1H-indol-5-ol	UR-CT218	330
7	1-Butyl-2-(4-hydroxyphenyl)-1H-indol-6-ol	UR-CT224	66
8	1-Heptyl-2-(4-hydroxyphenyl)-1H-indol-6-ol	UR-CT225	18
9	1-(4-Aminobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT316	49
10	4-Chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-1H-indol-6-ol	UR-CT317	7
11	1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT318	120
12	2-(3-Hydroxyphenyl)-3-methyl-1-propyl-1H-indol-5-ol	UR-CT319	105
13	3-Ethyl-2-(4-hydroxyphenyl)-1-propyl-1H-indol-6-ol	UR-CT321	67
14	4-Chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-propyl-1H-indol-6-ol	UR-CT323	10

15	2-(4-Hydroxyphenyl)-1,3-dimethyl-1H-indol-5-ol	UR-CT324	370
16	1-Benzyl-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT340	31
17	4-Chloro-2-(4-hydroxyphenyl)-3-methylbenzo[b]thiophen-5-ol	UR-CT344	24
18	4-Chloro-3-ethyl-2-(4-hydroxyphenyl)benzo[b]thiophen-5-ol	UR-CT345	15
19	1-(4-Azidobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT348	22
20	2-(4-Hydroxyphenyl)-3-propylbenzo[b]thiophen-5-ol	UR-CT352	38
21	1-(4-Bromobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT355	19
22	1-(Biphenyl-4-ylmethyl)-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT356	21
23	1-Ethyl-3-methyl-2-phenyl-1H-indol-6-ol	UR-CT370	750
24	3-(4-Hydroxybenzyl)benzo[b]thiophen-5-ol	UR-CT372	150
25	3-(4-Hydroxybenzyl)-2-methylbenzo[b]thiophen-6-ol	UR-CT383	119
26	2-(3-Hydroxyphenyl)-1-methyl-1H-indol-5-ol	UR-CT385	460
27	4-(5-Ethyl-7-methyl-5H-[1,3]dioxolo[4,5-f]indol-6-yl)phenol	UR-CT397	95
28	2-(4-Hydroxyphenyl)-3-methyl-1-[4-(piperidin-1-ylmethyl)benzyl]-1H-indol-5-ol	UR-CT398	120
29	1-(4-Fluorobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT402	43
30	2-(4-Hydroxyphenyl)-3-methyl-1-(4-methylbenzyl)-1H-indol-5-ol	UR-CT403	46
31	4-Chloro-2-(4-hydroxyphenyl)-3-propylbenzo[b]thiophen-5-ol	UR-CT404	17
32	1-(4-Chlorobenzyl)-2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol	UR-CT423	19

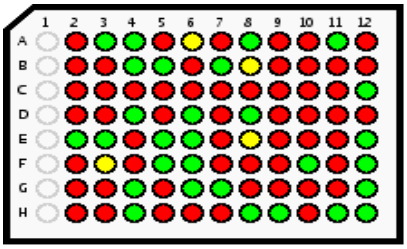
B.2 Screening plates ori.hya.1-9

B.2.1 Starting materials of plates ori.hya.1-9



B.2.2 Mass spectal analysis of plates ori.hya.1-9

ori.hya.1



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	398.23 g/mol	0.00 g/mol	0
B2	0	369.21 g/mol	0.00 g/mol	0
C2	0	388.16 g/mol	0.00 g/mol	0
D2	0	341.17 g/mol	0.00 g/mol	0
E2	0,14	400.30 g/mol	400.24 g/mol	7995,78
F2	0	383.22 g/mol	0.00 g/mol	0
G2	0	372.19 g/mol	0.00 g/mol	0
H2	0	387.19 g/mol	0.00 g/mol	0
A3	0,02	392.22 g/mol	392.19 g/mol	520,23
B3	0	369.21 g/mol	0.00 g/mol	0
C3	0	399.23 g/mol	0.00 g/mol	0
D3	0	339.19 g/mol	0.00 g/mol	0
E3	0,08	392.22 g/mol	392.18 g/mol	1860,13
F3	0	388.16 g/mol	388.14 g/mol	69,78
G3	0	384.24 g/mol	0.00 g/mol	0
H3	0	384.21 g/mol	0.00 g/mol	0
A4	0,04	368.22 g/mol	368.18 g/mol	1043,63
B4	0,04	347.27 g/mol	347.22 g/mol	5837,65
C4	0	393.24 g/mol	0.00 g/mol	0
D4	0,02	385.18 g/mol	385.15 g/mol	577,95
E4	0	385.25 g/mol	0.00 g/mol	0
F4	0	333.21 g/mol	0.00 g/mol	0
G4	0,02	379.19 g/mol	379.18 g/mol	442,79
H4	0	394.24 g/mol	394.20 g/mol	4391,94
A5	0	383.18 g/mol	0.00 g/mol	0
B5	0,04	371.16 g/mol	371.23 g/mol	928,86
C5	0	340.18 g/mol	0.00 g/mol	0
D5	0	332.22 g/mol	0.00 g/mol	0
E5	0,04	398.20 g/mol	398.17 g/mol	989,05
F5	0,02	369.21 g/mol	369.21 g/mol	435,95
G5	0	374.19 g/mol	0.00 g/mol	0
H5	0	397.24 g/mol	0.00 g/mol	0
A6	0	371.16 g/mol	371.14 g/mol	71,27
B6	0	388.30 g/mol	0.00 g/mol	0
C6	0	369.20 g/mol	0.00 g/mol	0
D6	0,04	396.19 g/mol	396.16 g/mol	918,62
E6	0	399.16 g/mol	399.12 g/mol	173,78
F6	0,08	371.16 g/mol	371.13 g/mol	1774,75
G6	0,02	393.21 g/mol	393.16 g/mol	462,78
H6	0	345.20 g/mol	0.00 g/mol	0

A7	0	375.27 g/mol	0.00 g/mol	0
B7	0,02	368.22 g/mol	368.19 g/mol	452,38
C7	0	361.25 g/mol	0.00 g/mol	0
D7	0	341.16 g/mol	0.00 g/mol	0
E7	0	398.23 g/mol	0.00 g/mol	0
F7	0	397.09 g/mol	0.00 g/mol	0
G7	0,02	348.26 g/mol	348.21 g/mol	516,15
H7	0	393.21 g/mol	0.00 g/mol	0
A8	0,1	354.20 g/mol	354.18 g/mol	2343,29
B8	0	390.20 g/mol	390.19 g/mol	31,07
C8	0	379.19 g/mol	0.00 g/mol	0
D8	0	247.11 g/mol	247.07 g/mol	701,69
E8	0	368.22 g/mol	368.19 g/mol	52,83
F8	0	383.22 g/mol	0.00 g/mol	0
G8	0	355.19 g/mol	355.18 g/mol	16,59
H8	0	379.17 g/mol	379.14 g/mol	3442,3
A9	0	375.13 g/mol	375.12 g/mol	13,81
B9	0	369.21 g/mol	0.00 g/mol	0
C9	0	376.25 g/mol	0.00 g/mol	0
D9	0	379.23 g/mol	0.00 g/mol	0
E9	0	385.21 g/mol	0.00 g/mol	0
F9	0	369.05 g/mol	0.00 g/mol	0
G9	0	371.16 g/mol	371.12 g/mol	16,46
H9	0,02	391.10 g/mol	391.17 g/mol	452,89
A10	0	392.16 g/mol	0.00 g/mol	0
B10	0	371.22 g/mol	0.00 g/mol	0
C10	0	399.23 g/mol	0.00 g/mol	0
D10	0	388.19 g/mol	0.00 g/mol	0
E10	0	370.24 g/mol	0.00 g/mol	0
F10	0,02	382.24 g/mol	382.20 g/mol	499,37
G10	0	347.27 g/mol	0.00 g/mol	0
H10	0	362.23 g/mol	0.00 g/mol	0
A11	0,04	323.16 g/mol	323.14 g/mol	1093,01
B11	0	389.22 g/mol	0.00 g/mol	0
C11	0	361.29 g/mol	0.00 g/mol	0
D11	0	362.23 g/mol	0.00 g/mol	0
E11	0	397.10 g/mol	0.00 g/mol	0
F11	0	360.26 g/mol	0.00 g/mol	0
G11	0	368.22 g/mol	368.19 g/mol	18,13
H11	0,04	354.20 g/mol	354.17 g/mol	1025,36
A12	0	387.27 g/mol	0.00 g/mol	0
B12	0	398.07 g/mol	0.00 g/mol	0
C12	0,04	384.21 g/mol	384.18 g/mol	918,28
D12	0	391.27 g/mol	0.00 g/mol	0
E12	0,04	397.24 g/mol	397.20 g/mol	1029,91
F12	0,02	368.22 g/mol	368.20 g/mol	425,76
G12	0,02	392.22 g/mol	392.19 g/mol	569,46
H12	0,02	393.13 g/mol	393.11 g/mol	631,62

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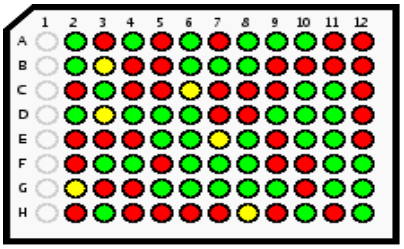
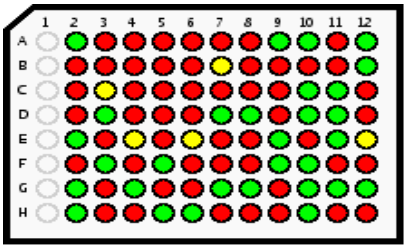


Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,02	306.16 g/mol	306.09 g/mol	404,1
B2	0,02	379.19 g/mol	379.17 g/mol	549,56
C2	0	397.24 g/mol	0.00 g/mol	0
D2	0,08	389.24 g/mol	389.19 g/mol	4105,58
E2	0	319.19 g/mol	0.00 g/mol	0
F2	0	366.19 g/mol	0.00 g/mol	0
G2	0	319.14 g/mol	319.13 g/mol	56,33
H2	0	383.12 g/mol	0.00 g/mol	0
A3	0	392.10 g/mol	0.00 g/mol	0
B3	0	373.14 g/mol	373.12 g/mol	73,3
C3	0	319.19 g/mol	319.20 g/mol	111,34
D3	0	391.12 g/mol	391.10 g/mol	87,52
E3	0	369.21 g/mol	369.19 g/mol	21,14
F3	0	386.16 g/mol	386.14 g/mol	2962,63
G3	0	379.23 g/mol	0.00 g/mol	0
H3	0,02	381.18 g/mol	381.14 g/mol	496,75
A4	0,02	343.23 g/mol	343.21 g/mol	427,46
B4	0	380.22 g/mol	0.00 g/mol	0
C4	0	315.15 g/mol	0.00 g/mol	0
D4	0,04	355.19 g/mol	355.19 g/mol	861,15
E4	0	369.10 g/mol	369.08 g/mol	16,07
F4	0,1	389.24 g/mol	389.19 g/mol	13800,3
G4	0	353.21 g/mol	0.00 g/mol	0
H4	0	366.16 g/mol	0.00 g/mol	0
A5	0	384.26 g/mol	0.00 g/mol	0
B5	0	393.10 g/mol	0.00 g/mol	0
C5	0	383.22 g/mol	0.00 g/mol	0
D5	0,06	373.20 g/mol	373.17 g/mol	1453,1
E5	0,12	394.22 g/mol	394.17 g/mol	10862,64
F5	0	377.25 g/mol	0.00 g/mol	0
G5	0,1	399.16 g/mol	399.13 g/mol	11790,12
H5	0	372.15 g/mol	0.00 g/mol	0
A6	0,1	354.20 g/mol	354.17 g/mol	2582,51
B6	0,02	368.22 g/mol	368.20 g/mol	460,69
C6	0	398.17 g/mol	398.13 g/mol	96,66
D6	0,02	309.14 g/mol	309.14 g/mol	470,67
E6	0	320.13 g/mol	320.12 g/mol	200,22
F6	0,04	375.22 g/mol	375.18 g/mol	17601,96
G6	0,08	379.23 g/mol	379.19 g/mol	10698,72
H6	0	397.19 g/mol	0.00 g/mol	0

A7	0	343.18 g/mol	0.00 g/mol	0
B7	0,02	383.22 g/mol	383.19 g/mol	489,74
C7	0	355.19 g/mol	0.00 g/mol	0
D7	0	314.16 g/mol	0.00 g/mol	0
E7	0	369.21 g/mol	369.19 g/mol	68,88
F7	0,1	347.27 g/mol	347.19 g/mol	7876,02
G7	0,04	375.22 g/mol	375.17 g/mol	16147,8
H7	0	397.10 g/mol	0.00 g/mol	0
A8	0,02	375.12 g/mol	375.11 g/mol	533,72
B8	0,06	378.20 g/mol	378.17 g/mol	8947,5
C8	0	369.25 g/mol	0.00 g/mol	0
D8	0	369.25 g/mol	0.00 g/mol	0
E8	0,02	378.23 g/mol	378.20 g/mol	654,95
F8	0,06	384.21 g/mol	384.18 g/mol	1702,71
G8	0,02	348.21 g/mol	348.19 g/mol	423,43
H8	0	348.21 g/mol	348.25 g/mol	42,33
A9	0	397.09 g/mol	397.06 g/mol	155,87
B9	0	386.24 g/mol	0.00 g/mol	0
C9	0	337.21 g/mol	0.00 g/mol	0
D9	0,06	370.27 g/mol	370.24 g/mol	8272,14
E9	0	395.16 g/mol	0.00 g/mol	0
F9	0	385.18 g/mol	0.00 g/mol	0
G9	0,16	389.24 g/mol	389.19 g/mol	11152,44
H9	0	305.17 g/mol	0.00 g/mol	0
A10	0	375.13 g/mol	375.11 g/mol	102,84
B10	0	380.18 g/mol	0.00 g/mol	0
C10	0	387.16 g/mol	387.14 g/mol	105,77
D10	0	358.24 g/mol	358.20 g/mol	260,49
E10	0,04	354.20 g/mol	354.25 g/mol	832,31
F10	0	398.07 g/mol	0.00 g/mol	0
G10	0	358.14 g/mol	0.00 g/mol	0
H10	0	374.14 g/mol	374.12 g/mol	166,16
A11	0	393.08 g/mol	0.00 g/mol	0
B11	0	293.17 g/mol	0.00 g/mol	0
C11	0,04	391.19 g/mol	391.20 g/mol	1056,94
D11	0	352.14 g/mol	352.11 g/mol	141,11
E11	0	390.31 g/mol	390.25 g/mol	451,84
F11	0,06	362.23 g/mol	362.20 g/mol	1434,29
G11	0,02	384.21 g/mol	384.17 g/mol	484,09
H11	0	383.22 g/mol	0.00 g/mol	0
A12	0	384.05 g/mol	0.00 g/mol	0
B12	0	371.22 g/mol	0.00 g/mol	0
C12	0	277.14 g/mol	0.00 g/mol	0
D12	0	325.11 g/mol	0.00 g/mol	0
E12	0	387.20 g/mol	0.00 g/mol	0
F12	0,04	343.23 g/mol	343.20 g/mol	910,9
G12	0,02	331.18 g/mol	331.16 g/mol	461,11
H12	0,08	368.22 g/mol	368.19 g/mol	2078,07

ori.hya.3



Well	Peak width	Expected mass	Found mass	Intensity
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B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,02	374.19 g/mol	374.19 g/mol	429,21
B2	0	357.16 g/mol	0.00 g/mol	0
C2	0	307.19 g/mol	0.00 g/mol	0
D2	0	393.21 g/mol	393.18 g/mol	18
E2	0,02	394.18 g/mol	394.14 g/mol	788,95
F2	0	355.18 g/mol	355.15 g/mol	13,43
G2	0,04	386.28 g/mol	386.24 g/mol	929,78
H2	0,04	385.21 g/mol	385.20 g/mol	878,54
A3	0	374.28 g/mol	0.00 g/mol	0
B3	0	319.15 g/mol	0.00 g/mol	0
C3	0	351.15 g/mol	351.13 g/mol	30,81
D3	0,02	354.24 g/mol	354.20 g/mol	425,32
E3	0	390.18 g/mol	0.00 g/mol	0
F3	0,04	377.19 g/mol	377.15 g/mol	996,94
G3	0	385.24 g/mol	0.00 g/mol	0
H3	0	377.28 g/mol	0.00 g/mol	0
A4	0	362.23 g/mol	0.00 g/mol	0
B4	0	355.19 g/mol	0.00 g/mol	0
C4	0	337.13 g/mol	0.00 g/mol	0
D4	0	378.23 g/mol	0.00 g/mol	0
E4	0	385.14 g/mol	385.09 g/mol	45,44
F4	0	397.14 g/mol	397.16 g/mol	29,06
G4	0,02	382.24 g/mol	382.21 g/mol	440,8
H4	0	329.16 g/mol	0.00 g/mol	0
A5	0	374.14 g/mol	0.00 g/mol	0
B5	0	324.14 g/mol	0.00 g/mol	0
C5	0	370.24 g/mol	0.00 g/mol	0
D5	0	390.24 g/mol	0.00 g/mol	0
E5	0	361.25 g/mol	0.00 g/mol	0
F5	0,12	375.26 g/mol	375.30 g/mol	12756,3
G5	0	355.18 g/mol	0.00 g/mol	0
H5	0,02	369.21 g/mol	369.19 g/mol	433,69
A6	0	400.17 g/mol	0.00 g/mol	0
B6	0	329.16 g/mol	0.00 g/mol	0
C6	0	358.17 g/mol	0.00 g/mol	0
D6	0	384.21 g/mol	0.00 g/mol	0
E6	0	368.18 g/mol	368.18 g/mol	53,05
F6	0	379.24 g/mol	0.00 g/mol	0
G6	0	382.18 g/mol	0.00 g/mol	0
H6	0,06	354.20 g/mol	354.18 g/mol	1721,65

A7	0	386.24 g/mol	0.00 g/mol	0
B7	0	385.05 g/mol	385.05 g/mol	57,3
C7	0	385.20 g/mol	0.00 g/mol	0
D7	0,02	372.19 g/mol	372.16 g/mol	5108,36
E7	0	372.15 g/mol	0.00 g/mol	0
F7	0	355.23 g/mol	0.00 g/mol	0
G7	0	358.13 g/mol	358.13 g/mol	118,48
H7	0	294.09 g/mol	0.00 g/mol	0
A8	0	351.19 g/mol	0.00 g/mol	0
B8	0	348.21 g/mol	0.00 g/mol	0
C8	0	344.22 g/mol	0.00 g/mol	0
D8	0,02	354.20 g/mol	354.18 g/mol	564,92
E8	0	399.07 g/mol	0.00 g/mol	0
F8	0	346.24 g/mol	346.22 g/mol	25,46
G8	0,12	354.20 g/mol	354.18 g/mol	2901,61
H8	0	359.22 g/mol	0.00 g/mol	0
A9	0,06	383.27 g/mol	383.22 g/mol	9005,34
B9	0	338.12 g/mol	0.00 g/mol	0
C9	0	350.19 g/mol	0.00 g/mol	0
D9	0	379.19 g/mol	0.00 g/mol	0
E9	0	388.15 g/mol	388.11 g/mol	164,8
F9	0,16	396.33 g/mol	396.28 g/mol	11961,66
G9	0	373.14 g/mol	373.14 g/mol	28,44
H9	0	345.16 g/mol	0.00 g/mol	0
A10	0,12	367.21 g/mol	367.17 g/mol	8674,74
B10	0	376.25 g/mol	0.00 g/mol	0
C10	0	370.18 g/mol	370.16 g/mol	364,9
D10	0,08	358.13 g/mol	358.10 g/mol	1676,42
E10	0	366.14 g/mol	0.00 g/mol	0
F10	0,02	383.07 g/mol	383.05 g/mol	565,56
G10	0,04	400.25 g/mol	400.21 g/mol	6838,44
H10	0,08	330.20 g/mol	330.24 g/mol	23109,78
A11	0	320.22 g/mol	0.00 g/mol	0
B11	0	324.13 g/mol	0.00 g/mol	0
C11	0,06	366.25 g/mol	366.20 g/mol	5925,85
D11	0,06	343.19 g/mol	343.18 g/mol	1357,06
E11	0	392.29 g/mol	392.25 g/mol	1385,38
F11	0	345.18 g/mol	0.00 g/mol	0
G11	0	377.11 g/mol	377.10 g/mol	2042,68
H11	0	384.21 g/mol	0.00 g/mol	0
A12	0,14	382.16 g/mol	382.14 g/mol	12029,22
B12	0,02	398.23 g/mol	398.21 g/mol	550,45
C12	0	334.24 g/mol	0.00 g/mol	0
D12	0	372.26 g/mol	0.00 g/mol	0
E12	0	343.19 g/mol	343.18 g/mol	56,92
F12	0	374.28 g/mol	0.00 g/mol	0
G12	0,08	330.20 g/mol	330.16 g/mol	5951,54
H12	0	393.28 g/mol	0.00 g/mol	0

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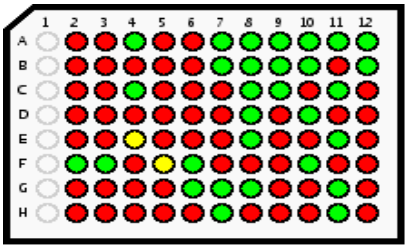

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	391.12 g/mol	0.00 g/mol	0
B2	0	389.15 g/mol	0.00 g/mol	0
C2	0	343.18 g/mol	0.00 g/mol	0
D2	0	385.24 g/mol	0.00 g/mol	0
E2	0	339.17 g/mol	0.00 g/mol	0
F2	0,06	380.22 g/mol	380.19 g/mol	9223,2
G2	0	375.31 g/mol	0.00 g/mol	0
H2	0	377.25 g/mol	0.00 g/mol	0
A3	0	334.15 g/mol	0.00 g/mol	0
B3	0	380.18 g/mol	0.00 g/mol	0
C3	0	323.12 g/mol	0.00 g/mol	0
D3	0	361.25 g/mol	0.00 g/mol	0
E3	0	393.20 g/mol	0.00 g/mol	0
F3	0,06	398.17 g/mol	398.14 g/mol	8703,72
G3	0	398.13 g/mol	0.00 g/mol	0
H3	0	368.20 g/mol	0.00 g/mol	0
A4	0	383.16 g/mol	383.13 g/mol	106,56
B4	0	347.20 g/mol	0.00 g/mol	0
C4	0,1	389.21 g/mol	389.18 g/mol	13188,96
D4	0	324.14 g/mol	0.00 g/mol	0
E4	0	380.22 g/mol	380.20 g/mol	82,09
F4	0	313.17 g/mol	0.00 g/mol	0
G4	0	379.19 g/mol	379.19 g/mol	22,68
H4	0	395.12 g/mol	0.00 g/mol	0
A5	0	383.07 g/mol	0.00 g/mol	0
B5	0	383.07 g/mol	0.00 g/mol	0
C5	0	363.27 g/mol	0.00 g/mol	0
D5	0	377.24 g/mol	0.00 g/mol	0
E5	0	354.24 g/mol	0.00 g/mol	0
F5	0	378.18 g/mol	378.15 g/mol	38,43
G5	0	374.14 g/mol	0.00 g/mol	0
H5	0	341.17 g/mol	0.00 g/mol	0
A6	0	369.16 g/mol	0.00 g/mol	0
B6	0	372.15 g/mol	0.00 g/mol	0
C6	0	377.17 g/mol	0.00 g/mol	0
D6	0	309.14 g/mol	0.00 g/mol	0
E6	0	327.19 g/mol	0.00 g/mol	0
F6	0,02	393.10 g/mol	393.08 g/mol	923,14
G6	0,02	383.23 g/mol	383.21 g/mol	521,88
H6	0	382.19 g/mol	382.18 g/mol	19,24

A7	0	379.23 g/mol	379.20 g/mol	19127,16
B7	0	384.06 g/mol	384.05 g/mol	130,36
C7	0	384.08 g/mol	0.00 g/mol	0
D7	0	352.17 g/mol	0.00 g/mol	0
E7	0	348.16 g/mol	0.00 g/mol	0
F7	0	398.14 g/mol	0.00 g/mol	0
G7	0,06	345.21 g/mol	345.19 g/mol	1408,82
H7	0	373.14 g/mol	373.13 g/mol	151,63
A8	0,1	398.17 g/mol	398.14 g/mol	9860,94
B8	0,12	366.20 g/mol	366.17 g/mol	7363,26
C8	0	371.12 g/mol	371.12 g/mol	134,76
D8	0	387.20 g/mol	387.17 g/mol	359,84
E8	0,02	319.19 g/mol	319.20 g/mol	482,29
F8	0	305.17 g/mol	0.00 g/mol	0
G8	0,04	393.10 g/mol	393.08 g/mol	1028,01
H8	0	355.12 g/mol	0.00 g/mol	0
A9	0,02	341.10 g/mol	341.09 g/mol	495,4
B9	0	342.14 g/mol	342.13 g/mol	114,13
C9	0,02	360.17 g/mol	360.16 g/mol	519,57
D9	0	277.14 g/mol	0.00 g/mol	0
E9	0	363.15 g/mol	0.00 g/mol	0
F9	0	353.21 g/mol	0.00 g/mol	0
G9	0	397.09 g/mol	0.00 g/mol	0
H9	0	319.23 g/mol	319.21 g/mol	23,04
A10	0,14	385.24 g/mol	385.21 g/mol	12127,2
B10	0,02	363.13 g/mol	363.07 g/mol	471,75
C10	0	341.16 g/mol	0.00 g/mol	0
D10	0,12	392.22 g/mol	392.19 g/mol	12760,2
E10	0	327.14 g/mol	0.00 g/mol	0
F10	0	386.06 g/mol	386.04 g/mol	167,06
G10	0	384.05 g/mol	0.00 g/mol	0
H10	0	380.15 g/mol	0.00 g/mol	0
A11	0,06	370.22 g/mol	370.18 g/mol	6602,22
B11	0	369.20 g/mol	0.00 g/mol	0
C11	0,02	374.14 g/mol	374.07 g/mol	440,83
D11	0	372.19 g/mol	372.17 g/mol	13
E11	0,02	355.19 g/mol	355.19 g/mol	408,59
F11	0	383.19 g/mol	0.00 g/mol	0
G11	0	233.09 g/mol	233.09 g/mol	322,66
H11	0,08	397.14 g/mol	397.10 g/mol	2951,55
A12	0,06	325.10 g/mol	325.09 g/mol	6790,32
B12	0,02	348.21 g/mol	348.20 g/mol	526,73
C12	0	354.21 g/mol	0.00 g/mol	0
D12	0	371.27 g/mol	0.00 g/mol	0
E12	0	395.12 g/mol	0.00 g/mol	0
F12	0	348.26 g/mol	0.00 g/mol	0
G12	0	399.23 g/mol	0.00 g/mol	0
H12	0	398.08 g/mol	0.00 g/mol	0

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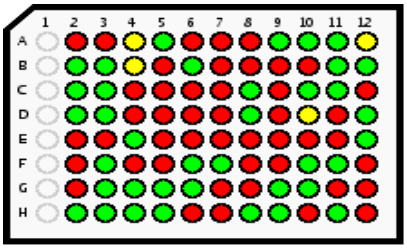

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	299.07 g/mol	0.00 g/mol	0
B2	0	385.18 g/mol	385.15 g/mol	148,51
C2	0	364.18 g/mol	364.16 g/mol	298,84
D2	0,02	368.22 g/mol	368.24 g/mol	444,15
E2	0	384.21 g/mol	0.00 g/mol	0
F2	0	395.12 g/mol	0.00 g/mol	0
G2	0	397.14 g/mol	0.00 g/mol	0
H2	0	394.09 g/mol	394.08 g/mol	182,05
A3	0	368.20 g/mol	0.00 g/mol	0
B3	0,08	350.14 g/mol	350.12 g/mol	24200,7
C3	0,1	382.16 g/mol	382.14 g/mol	24800,52
D3	0,02	380.16 g/mol	380.14 g/mol	517,01
E3	0	319.12 g/mol	0.00 g/mol	0
F3	0,1	343.19 g/mol	343.17 g/mol	2197,03
G3	0,04	383.23 g/mol	383.21 g/mol	1142,89
H3	0	344.22 g/mol	344.19 g/mol	3876,95
A4	0	340.11 g/mol	340.17 g/mol	50,07
B4	0	365.17 g/mol	365.15 g/mol	78,47
C4	0	261.13 g/mol	0.00 g/mol	0
D4	0	373.16 g/mol	0.00 g/mol	0
E4	0,02	319.23 g/mol	319.21 g/mol	566,65
F4	0	319.23 g/mol	319.22 g/mol	27,41
G4	0,02	397.33 g/mol	397.29 g/mol	515,46
H4	0,02	393.24 g/mol	393.22 g/mol	640,21
A5	0,06	330.20 g/mol	330.19 g/mol	6257,76
B5	0	379.19 g/mol	0.00 g/mol	0
C5	0	318.20 g/mol	0.00 g/mol	0
D5	0	342.14 g/mol	0.00 g/mol	0
E5	0	343.23 g/mol	0.00 g/mol	0
F5	0	383.18 g/mol	0.00 g/mol	0
G5	0,02	370.27 g/mol	370.26 g/mol	475,11
H5	0,02	355.18 g/mol	355.10 g/mol	453,52
A6	0	329.16 g/mol	0.00 g/mol	0
B6	0,02	360.12 g/mol	360.11 g/mol	560,13
C6	0	313.17 g/mol	0.00 g/mol	0
D6	0	390.20 g/mol	0.00 g/mol	0
E6	0	396.10 g/mol	0.00 g/mol	0
F6	0	385.14 g/mol	385.11 g/mol	320,19
G6	0,12	330.20 g/mol	330.18 g/mol	11878,32
H6	0	345.20 g/mol	0.00 g/mol	0

A7	0	388.30 g/mol	0.00 g/mol	0
B7	0	360.26 g/mol	0.00 g/mol	0
C7	0	354.13 g/mol	0.00 g/mol	0
D7	0	382.17 g/mol	0.00 g/mol	0
E7	0	364.23 g/mol	0.00 g/mol	0
F7	0,02	377.21 g/mol	377.19 g/mol	696,65
G7	0	291.15 g/mol	0.00 g/mol	0
H7	0	383.26 g/mol	0.00 g/mol	0
A8	0	355.18 g/mol	0.00 g/mol	0
B8	0	377.21 g/mol	0.00 g/mol	0
C8	0	355.08 g/mol	355.06 g/mol	104,23
D8	0	383.23 g/mol	383.21 g/mol	114,35
E8	0	383.22 g/mol	0.00 g/mol	0
F8	0	388.18 g/mol	388.18 g/mol	17,27
G8	0	365.25 g/mol	0.00 g/mol	0
H8	0	332.18 g/mol	332.16 g/mol	136,45
A9	0	376.25 g/mol	376.21 g/mol	4672,25
B9	0	365.17 g/mol	0.00 g/mol	0
C9	0	337.13 g/mol	0.00 g/mol	0
D9	0	281.21 g/mol	0.00 g/mol	0
E9	0	330.16 g/mol	0.00 g/mol	0
F9	0	390.24 g/mol	0.00 g/mol	0
G9	0,02	333.25 g/mol	333.16 g/mol	411,64
H9	0,04	368.22 g/mol	368.20 g/mol	858,02
A10	0,06	393.24 g/mol	393.21 g/mol	11696,16
B10	0	374.28 g/mol	0.00 g/mol	0
C10	0,1	330.20 g/mol	330.17 g/mol	10599,18
D10	0	396.11 g/mol	396.07 g/mol	38,59
E10	0	355.18 g/mol	0.00 g/mol	0
F10	0	374.18 g/mol	374.15 g/mol	4030,68
G10	0,02	347.20 g/mol	347.18 g/mol	482,2
H10	0	358.17 g/mol	358.16 g/mol	20,39
A11	0,02	394.24 g/mol	394.21 g/mol	628,91
B11	0	365.22 g/mol	365.18 g/mol	734,28
C11	0,04	338.12 g/mol	338.10 g/mol	964,26
D11	0	352.14 g/mol	0.00 g/mol	0
E11	0	327.19 g/mol	0.00 g/mol	0
F11	0,02	383.22 g/mol	383.19 g/mol	464,26
G11	0	313.17 g/mol	0.00 g/mol	0
H11	0,08	372.26 g/mol	372.22 g/mol	6564,66
A12	0	368.29 g/mol	368.25 g/mol	84,72
B12	0,02	368.29 g/mol	368.26 g/mol	647,74
C12	0	371.23 g/mol	0.00 g/mol	0
D12	0,04	398.13 g/mol	398.09 g/mol	6243,24
E12	0,06	339.17 g/mol	339.14 g/mol	1262,63
F12	0	355.23 g/mol	0.00 g/mol	0
G12	0	377.28 g/mol	0.00 g/mol	0
H12	0	340.17 g/mol	0.00 g/mol	0

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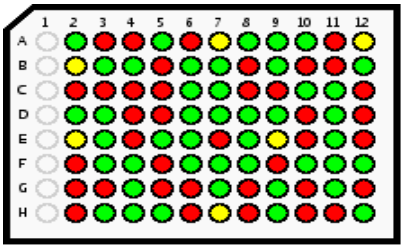

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,22	368.25 g/mol	368.21 g/mol	8607,48
B2	0	345.18 g/mol	345.17 g/mol	43,56
C2	0	370.04 g/mol	0.00 g/mol	0
D2	0	368.20 g/mol	368.16 g/mol	3989
E2	0	349.15 g/mol	349.13 g/mol	83,89
F2	0	315.14 g/mol	0.00 g/mol	0
G2	0	341.17 g/mol	0.00 g/mol	0
H2	0	379.26 g/mol	0.00 g/mol	0
A3	0	370.19 g/mol	0.00 g/mol	0
B3	0,02	368.16 g/mol	368.13 g/mol	652,93
C3	0	344.19 g/mol	0.00 g/mol	0
D3	0,14	347.27 g/mol	347.23 g/mol	12027,9
E3	0,02	319.23 g/mol	319.18 g/mol	368,4
F3	0,08	330.20 g/mol	330.17 g/mol	8446,44
G3	0	361.25 g/mol	0.00 g/mol	0
H3	0	331.17 g/mol	331.14 g/mol	1445,24
A4	0	329.16 g/mol	0.00 g/mol	0
B4	0,2	365.19 g/mol	365.15 g/mol	9418,08
C4	0	263.12 g/mol	0.00 g/mol	0
D4	0	233.09 g/mol	0.00 g/mol	0
E4	0	350.16 g/mol	0.00 g/mol	0
F4	0,02	339.20 g/mol	339.15 g/mol	585,44
G4	0,02	399.11 g/mol	399.08 g/mol	586,14
H4	0	395.12 g/mol	395.08 g/mol	2895,94
A5	0,04	335.22 g/mol	335.19 g/mol	1221,89
B5	0	371.23 g/mol	0.00 g/mol	0
C5	0	397.09 g/mol	0.00 g/mol	0
D5	0	313.17 g/mol	0.00 g/mol	0
E5	0,02	373.11 g/mol	373.12 g/mol	524,1
F5	0	313.09 g/mol	0.00 g/mol	0
G5	0	341.17 g/mol	0.00 g/mol	0
H5	0,02	381.25 g/mol	381.20 g/mol	13255,26
A6	0	348.26 g/mol	0.00 g/mol	0
B6	0,02	372.15 g/mol	372.13 g/mol	574,9
C6	0,14	397.14 g/mol	397.10 g/mol	11880,78
D6	0,08	372.22 g/mol	372.19 g/mol	25773,78
E6	0,02	359.15 g/mol	359.14 g/mol	483,22
F6	0,16	374.31 g/mol	374.26 g/mol	8266,2
G6	0	390.24 g/mol	390.21 g/mol	16,67
H6	0	376.16 g/mol	0.00 g/mol	0

A7	0	370.27 g/mol	370.24 g/mol	98,47
B7	0,02	332.22 g/mol	332.19 g/mol	526,06
C7	0	387.22 g/mol	387.18 g/mol	110,46
D7	0,06	393.24 g/mol	393.21 g/mol	21829,92
E7	0	370.27 g/mol	0.00 g/mol	0
F7	0,04	343.23 g/mol	343.20 g/mol	1062,83
G7	0,02	394.20 g/mol	394.18 g/mol	550,02
H7	0	398.06 g/mol	398.04 g/mol	95,42
A8	0,06	396.19 g/mol	396.16 g/mol	9958,98
B8	0	280.14 g/mol	0.00 g/mol	0
C8	0	364.21 g/mol	0.00 g/mol	0
D8	0	341.17 g/mol	341.15 g/mol	202,27
E8	0,14	384.15 g/mol	384.13 g/mol	9423,9
F8	0,02	355.19 g/mol	355.18 g/mol	484,56
G8	0	291.15 g/mol	0.00 g/mol	0
H8	0	247.11 g/mol	0.00 g/mol	0
A9	0,02	309.14 g/mol	309.12 g/mol	4133,54
B9	0,06	346.17 g/mol	346.14 g/mol	6684,42
C9	0	360.11 g/mol	0.00 g/mol	0
D9	0,04	362.28 g/mol	362.24 g/mol	6804,66
E9	0	354.24 g/mol	354.22 g/mol	51,84
F9	0	305.17 g/mol	0.00 g/mol	0
G9	0,12	373.20 g/mol	373.17 g/mol	3014,77
H9	0,04	369.10 g/mol	369.07 g/mol	4745,91
A10	0,02	356.07 g/mol	356.05 g/mol	476,34
B10	0	384.08 g/mol	0.00 g/mol	0
C10	0,04	327.08 g/mol	327.08 g/mol	806,62
D10	0	346.12 g/mol	0.00 g/mol	0
E10	0	338.15 g/mol	0.00 g/mol	0
F10	0	347.16 g/mol	347.13 g/mol	3968,69
G10	0	395.26 g/mol	0.00 g/mol	0
H10	0	357.20 g/mol	0.00 g/mol	0
A11	0	376.21 g/mol	0.00 g/mol	0
B11	0	341.17 g/mol	0.00 g/mol	0
C11	0	396.10 g/mol	396.07 g/mol	9106,08
D11	0	369.10 g/mol	369.07 g/mol	944,45
E11	0,08	393.24 g/mol	393.21 g/mol	24539,22
F11	0,08	359.27 g/mol	359.23 g/mol	8821,08
G11	0,1	385.14 g/mol	385.11 g/mol	8261,46
H11	0	398.13 g/mol	0.00 g/mol	0
A12	0	380.15 g/mol	380.13 g/mol	81,05
B12	0,04	386.27 g/mol	386.22 g/mol	8640,6
C12	0	375.16 g/mol	0.00 g/mol	0
D12	0	349.21 g/mol	0.00 g/mol	0
E12	0	312.17 g/mol	0.00 g/mol	0
F12	0,04	309.14 g/mol	309.05 g/mol	884,53
G12	0	341.17 g/mol	0.00 g/mol	0
H12	0,02	333.25 g/mol	333.23 g/mol	411,49

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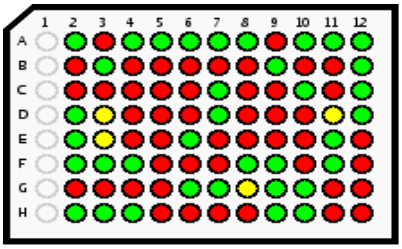

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,02	387.11 g/mol	387.09 g/mol	763,53
B2	0	379.08 g/mol	0.00 g/mol	0
C2	0	348.26 g/mol	0.00 g/mol	0
D2	0,14	375.26 g/mol	375.25 g/mol	13359,06
E2	0,08	357.25 g/mol	357.21 g/mol	7184,88
F2	0,14	398.17 g/mol	398.15 g/mol	11983,92
G2	0	348.26 g/mol	0.00 g/mol	0
H2	0	380.19 g/mol	380.15 g/mol	5157,56
A3	0	370.09 g/mol	0.00 g/mol	0
B3	0,02	386.13 g/mol	386.07 g/mol	525,1
C3	0	397.23 g/mol	0.00 g/mol	0
D3	0	388.23 g/mol	388.19 g/mol	76,59
E3	0	325.11 g/mol	325.13 g/mol	98,15
F3	0,12	369.25 g/mol	369.21 g/mol	3679,13
G3	0	360.23 g/mol	0.00 g/mol	0
H3	0	400.25 g/mol	400.23 g/mol	127,35
A4	0,02	356.17 g/mol	356.16 g/mol	545,58
B4	0	390.23 g/mol	390.21 g/mol	19,23
C4	0	396.15 g/mol	0.00 g/mol	0
D4	0	320.13 g/mol	0.00 g/mol	0
E4	0	344.22 g/mol	0.00 g/mol	0
F4	0,04	358.12 g/mol	358.09 g/mol	2905,31
G4	0	355.08 g/mol	0.00 g/mol	0
H4	0,06	349.18 g/mol	349.15 g/mol	6241,86
A5	0	357.20 g/mol	357.18 g/mol	115,27
B5	0	363.23 g/mol	0.00 g/mol	0
C5	0	358.21 g/mol	0.00 g/mol	0
D5	0	326.19 g/mol	0.00 g/mol	0
E5	0	372.21 g/mol	0.00 g/mol	0
F5	0	332.25 g/mol	0.00 g/mol	0
G5	0	386.22 g/mol	0.00 g/mol	0
H5	0	362.28 g/mol	0.00 g/mol	0
A6	0,14	343.23 g/mol	343.20 g/mol	4007,83
B6	0	341.21 g/mol	0.00 g/mol	0
C6	0	391.24 g/mol	0.00 g/mol	0
D6	0	383.07 g/mol	0.00 g/mol	0
E6	0,14	397.14 g/mol	397.11 g/mol	6614,7
F6	0	395.12 g/mol	0.00 g/mol	0
G6	0,02	350.14 g/mol	350.12 g/mol	753,85
H6	0	247.11 g/mol	0.00 g/mol	0

A7	0,02	323.16 g/mol	323.14 g/mol	724,94
B7	0	302.16 g/mol	0.00 g/mol	0
C7	0,02	318.20 g/mol	318.17 g/mol	568,87
D7	0,02	369.10 g/mol	369.08 g/mol	1756,58
E7	0	345.18 g/mol	0.00 g/mol	0
F7	0	391.24 g/mol	0.00 g/mol	0
G7	0,06	369.10 g/mol	369.07 g/mol	6287,22
H7	0	356.07 g/mol	356.05 g/mol	12,29
A8	0,06	354.18 g/mol	354.15 g/mol	12314,1
B8	0	352.16 g/mol	0.00 g/mol	0
C8	0	394.27 g/mol	0.00 g/mol	0
D8	0	333.16 g/mol	0.00 g/mol	0
E8	0	341.16 g/mol	0.00 g/mol	0
F8	0,02	366.20 g/mol	366.18 g/mol	642,55
G8	0	369.10 g/mol	369.08 g/mol	58,17
H8	0	372.04 g/mol	0.00 g/mol	0
A9	0	394.18 g/mol	0.00 g/mol	0
B9	0,04	360.21 g/mol	360.19 g/mol	1043,65
C9	0	369.25 g/mol	0.00 g/mol	0
D9	0	333.16 g/mol	0.00 g/mol	0
E9	0	309.14 g/mol	0.00 g/mol	0
F9	0,1	318.20 g/mol	318.19 g/mol	1882,3
G9	0,04	388.04 g/mol	388.03 g/mol	1282,42
H9	0,04	388.15 g/mol	388.12 g/mol	13353,18
A10	0	352.16 g/mol	352.15 g/mol	112,85
B10	0	294.16 g/mol	0.00 g/mol	0
C10	0,12	369.25 g/mol	369.21 g/mol	5360,92
D10	0	321.16 g/mol	0.00 g/mol	0
E10	0	329.21 g/mol	0.00 g/mol	0
F10	0	377.21 g/mol	0.00 g/mol	0
G10	0,02	357.25 g/mol	357.21 g/mol	3981,85
H10	0,02	379.08 g/mol	379.07 g/mol	481,52
A11	0	342.18 g/mol	342.16 g/mol	490,57
B11	0	318.19 g/mol	0.00 g/mol	0
C11	0	329.21 g/mol	0.00 g/mol	0
D11	0	315.19 g/mol	315.19 g/mol	51,45
E11	0,1	397.14 g/mol	397.11 g/mol	3118,5
F11	0,02	397.10 g/mol	397.07 g/mol	476,19
G11	0	398.06 g/mol	0.00 g/mol	0
H11	0	361.24 g/mol	0.00 g/mol	0
A12	0	368.29 g/mol	368.26 g/mol	211,57
B12	0,06	374.22 g/mol	374.19 g/mol	10251,24
C12	0,04	331.18 g/mol	331.17 g/mol	1122,12
D12	0,08	369.29 g/mol	369.26 g/mol	1889,83
E12	0	355.08 g/mol	0.00 g/mol	0
F12	0	383.07 g/mol	383.05 g/mol	20,82
G12	0	380.07 g/mol	0.00 g/mol	0
H12	0	355.10 g/mol	0.00 g/mol	0

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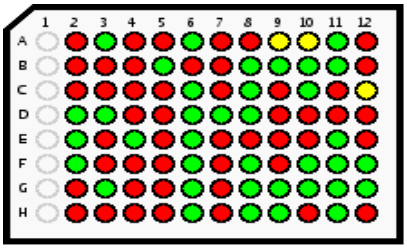

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	337.17 g/mol	0.00 g/mol	0
B2	0	337.18 g/mol	0.00 g/mol	0
C2	0	384.05 g/mol	0.00 g/mol	0
D2	0,04	397.25 g/mol	397.23 g/mol	1115,96
E2	0,02	387.27 g/mol	387.24 g/mol	972,19
F2	0,02	319.23 g/mol	319.28 g/mol	411,01
G2	0	292.14 g/mol	0.00 g/mol	0
H2	0	385.05 g/mol	0.00 g/mol	0
A3	0,04	326.14 g/mol	326.04 g/mol	1234,6
B3	0	326.08 g/mol	0.00 g/mol	0
C3	0	325.18 g/mol	0.00 g/mol	0
D3	0,02	332.22 g/mol	332.20 g/mol	476,13
E3	0	346.09 g/mol	0.00 g/mol	0
F3	0	362.28 g/mol	0.00 g/mol	0
G3	0,02	359.26 g/mol	359.23 g/mol	587,54
H3	0	312.12 g/mol	0.00 g/mol	0
A4	0	326.10 g/mol	0.00 g/mol	0
B4	0	308.21 g/mol	0.00 g/mol	0
C4	0	395.20 g/mol	0.00 g/mol	0
D4	0	313.17 g/mol	0.00 g/mol	0
E4	0,04	332.22 g/mol	332.19 g/mol	14859,96
F4	0	336.26 g/mol	0.00 g/mol	0
G4	0	277.14 g/mol	0.00 g/mol	0
H4	0	296.13 g/mol	0.00 g/mol	0
A5	0	321.20 g/mol	0.00 g/mol	0
B5	0	370.09 g/mol	370.07 g/mol	508,66
C5	0	341.10 g/mol	0.00 g/mol	0
D5	0	346.19 g/mol	0.00 g/mol	0
E5	0	362.23 g/mol	0.00 g/mol	0
F5	0	365.25 g/mol	0.00 g/mol	0
G5	0	339.20 g/mol	0.00 g/mol	0
H5	0	347.16 g/mol	0.00 g/mol	0
A6	0,02	388.09 g/mol	388.06 g/mol	451,46
B6	0	393.28 g/mol	0.00 g/mol	0
C6	0,04	392.29 g/mol	392.26 g/mol	1055,01
D6	0,02	397.10 g/mol	397.07 g/mol	470,53
E6	0	355.08 g/mol	355.07 g/mol	146,83
F6	0,02	398.13 g/mol	398.10 g/mol	5646,61
G6	0,06	396.19 g/mol	396.16 g/mol	8970,24
H6	0	369.14 g/mol	369.12 g/mol	100,17

A7	0	348.25 g/mol	0.00 g/mol	0
B7	0	334.14 g/mol	0.00 g/mol	0
C7	0	311.08 g/mol	0.00 g/mol	0
D7	0	358.17 g/mol	358.15 g/mol	104,35
E7	0	311.09 g/mol	0.00 g/mol	0
F7	0	351.15 g/mol	0.00 g/mol	0
G7	0	381.20 g/mol	0.00 g/mol	0
H7	0	304.18 g/mol	0.00 g/mol	0
A8	0	369.25 g/mol	0.00 g/mol	0
B8	0,12	370.08 g/mol	370.06 g/mol	5849,79
C8	0,02	372.04 g/mol	372.04 g/mol	531,43
D8	0,1	347.22 g/mol	347.19 g/mol	9676,92
E8	0	380.13 g/mol	0.00 g/mol	0
F8	0,08	383.14 g/mol	383.11 g/mol	8261,88
G8	0,06	399.13 g/mol	399.11 g/mol	9367,98
H8	0,02	344.22 g/mol	344.20 g/mol	812,81
A9	0	306.20 g/mol	306.19 g/mol	51,89
B9	0,02	319.19 g/mol	319.14 g/mol	453,2
C9	0	393.28 g/mol	0.00 g/mol	0
D9	0	397.21 g/mol	0.00 g/mol	0
E9	0	368.26 g/mol	368.23 g/mol	15,39
F9	0	335.22 g/mol	0.00 g/mol	0
G9	0,06	343.23 g/mol	343.20 g/mol	6096,12
H9	0,14	393.24 g/mol	393.21 g/mol	13191,48
A10	0	354.24 g/mol	354.21 g/mol	31,73
B10	0	333.15 g/mol	333.13 g/mol	1154,08
C10	0,06	397.18 g/mol	397.15 g/mol	9199,98
D10	0	395.22 g/mol	0.00 g/mol	0
E10	0	382.31 g/mol	382.27 g/mol	22,17
F10	0,02	368.26 g/mol	368.22 g/mol	444,9
G10	0,08	379.23 g/mol	379.19 g/mol	12279,9
H10	0	374.26 g/mol	0.00 g/mol	0
A11	0,02	382.28 g/mol	382.24 g/mol	946,6
B11	0,12	330.20 g/mol	330.18 g/mol	9550,98
C11	0	361.24 g/mol	0.00 g/mol	0
D11	0	378.14 g/mol	0.00 g/mol	0
E11	0,04	395.23 g/mol	395.21 g/mol	951,45
F11	0,02	379.23 g/mol	379.20 g/mol	24139,14
G11	0	384.29 g/mol	384.25 g/mol	217,1
H11	0,12	364.24 g/mol	364.22 g/mol	18772,08
A12	0	352.19 g/mol	0.00 g/mol	0
B12	0	304.18 g/mol	0.00 g/mol	0
C12	0	372.29 g/mol	372.25 g/mol	33,54
D12	0	323.22 g/mol	0.00 g/mol	0
E12	0	324.22 g/mol	0.00 g/mol	0
F12	0,12	348.20 g/mol	348.18 g/mol	6946,8
G12	0,02	341.21 g/mol	341.18 g/mol	1372,16
H12	0	365.28 g/mol	0.00 g/mol	0

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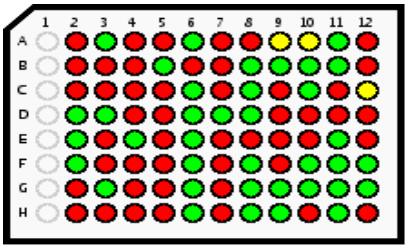
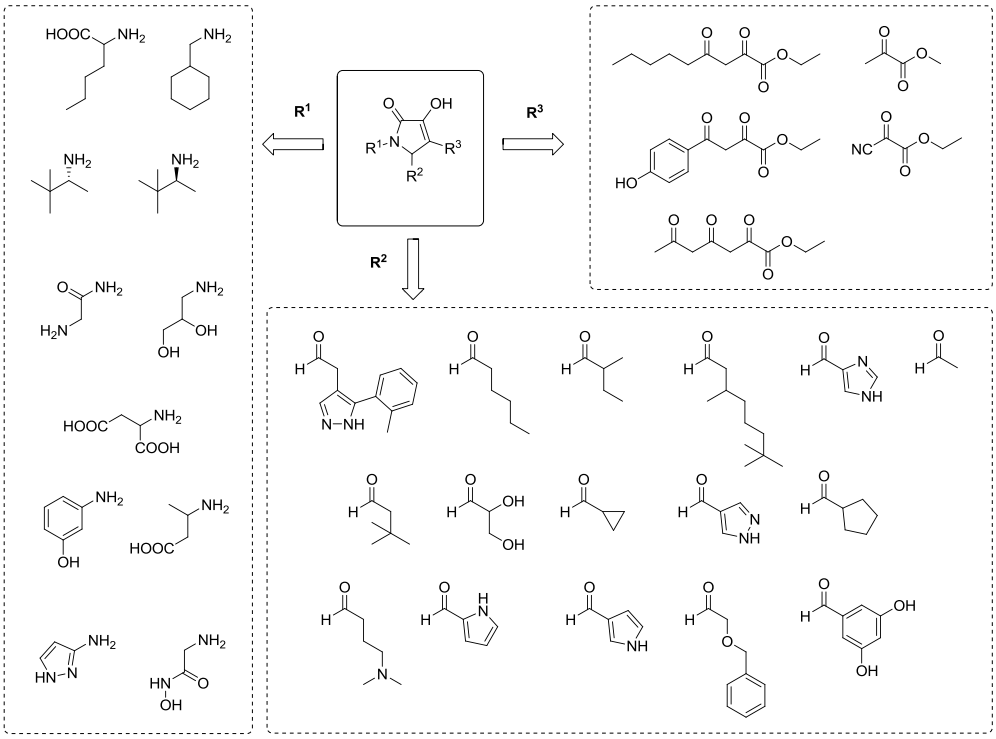


Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	374.28 g/mol	0.00 g/mol	0
B2	0,1	357.25 g/mol	357.20 g/mol	10336,74
C2	0	380.13 g/mol	0.00 g/mol	0
D2	0	318.20 g/mol	0.00 g/mol	0
E2	0	382.23 g/mol	0.00 g/mol	0
F2	0	384.21 g/mol	384.18 g/mol	84,18
G2	0,12	359.27 g/mol	359.22 g/mol	9745,68
H2	0,1	376.25 g/mol	376.20 g/mol	7211,04
A3	0,02	348.21 g/mol	348.19 g/mol	475,13
B3	0	387.11 g/mol	0.00 g/mol	0
C3	0	375.31 g/mol	0.00 g/mol	0
D3	0,02	370.27 g/mol	370.33 g/mol	3811,88
E3	0	341.21 g/mol	0.00 g/mol	0
F3	0,16	396.18 g/mol	396.15 g/mol	11425,14
G3	0,02	348.20 g/mol	348.18 g/mol	619,45
H3	0	368.26 g/mol	368.21 g/mol	98,8
A4	0	365.29 g/mol	0.00 g/mol	0
B4	0	381.18 g/mol	381.16 g/mol	63,94
C4	0,06	366.20 g/mol	366.16 g/mol	5132,2
D4	0	345.18 g/mol	0.00 g/mol	0
E4	0,06	312.22 g/mol	312.18 g/mol	13229,7
F4	0,16	333.25 g/mol	333.21 g/mol	6607,98
G4	0	332.25 g/mol	0.00 g/mol	0
H4	0,06	361.24 g/mol	361.20 g/mol	16786,8
A5	0,04	375.26 g/mol	375.21 g/mol	8996,28
B5	0	375.12 g/mol	375.10 g/mol	181,09
C5	0	306.17 g/mol	0.00 g/mol	0
D5	0	306.17 g/mol	0.00 g/mol	0
E5	0,18	376.23 g/mol	376.19 g/mol	9520,44
F5	0	362.23 g/mol	362.19 g/mol	5765,39
G5	0,1	378.14 g/mol	378.13 g/mol	2414,66
H5	0	398.28 g/mol	0.00 g/mol	0
A6	0	356.15 g/mol	0.00 g/mol	0
B6	0	381.18 g/mol	0.00 g/mol	0
C6	0	345.25 g/mol	0.00 g/mol	0
D6	0	309.20 g/mol	0.00 g/mol	0
E6	0,14	357.25 g/mol	357.21 g/mol	9835,86
F6	0,12	363.20 g/mol	363.16 g/mol	7259,04
G6	0,04	331.17 g/mol	331.14 g/mol	8715,18
H6	0,04	351.17 g/mol	351.15 g/mol	1038,32

A7	0	344.25 g/mol	0.00 g/mol	0
B7	0,08	357.25 g/mol	357.20 g/mol	2588,68
C7	0	343.19 g/mol	343.16 g/mol	70,64
D7	0,16	361.21 g/mol	361.18 g/mol	11929,38
E7	0	355.19 g/mol	0.00 g/mol	0
F7	0	332.16 g/mol	0.00 g/mol	0
G7	0,04	333.25 g/mol	333.21 g/mol	4794,53
H7	0	391.24 g/mol	0.00 g/mol	0
A8	0	368.15 g/mol	368.13 g/mol	31,75
B8	0	362.20 g/mol	0.00 g/mol	0
C8	0	305.17 g/mol	0.00 g/mol	0
D8	0	393.24 g/mol	0.00 g/mol	0
E8	0,04	381.25 g/mol	381.21 g/mol	15722,76
F8	0	383.08 g/mol	0.00 g/mol	0
G8	0	329.14 g/mol	0.00 g/mol	0
H8	0	353.21 g/mol	353.18 g/mol	273,39
A9	0	320.22 g/mol	0.00 g/mol	0
B9	0	362.23 g/mol	362.20 g/mol	3587,63
C9	0,04	336.19 g/mol	336.16 g/mol	16503,12
D9	0	375.22 g/mol	375.19 g/mol	558,69
E9	0,02	360.11 g/mol	360.08 g/mol	14597,46
F9	0	333.06 g/mol	0.00 g/mol	0
G9	0	370.16 g/mol	0.00 g/mol	0
H9	0	320.22 g/mol	0.00 g/mol	0
A10	0	332.22 g/mol	0.00 g/mol	0
B10	0,06	329.17 g/mol	329.17 g/mol	1210,58
C10	0	352.12 g/mol	0.00 g/mol	0
D10	0	348.11 g/mol	0.00 g/mol	0
E10	0	319.19 g/mol	0.00 g/mol	0
F10	0	345.18 g/mol	0.00 g/mol	0
G10	0	367.35 g/mol	0.00 g/mol	0
H10	0,02	365.17 g/mol	365.15 g/mol	564,95
A11	0	291.15 g/mol	0.00 g/mol	0
B11	0	353.29 g/mol	0.00 g/mol	0
C11	0	368.26 g/mol	0.00 g/mol	0
D11	0,12	319.23 g/mol	319.30 g/mol	19916,1
E11	0,12	319.23 g/mol	319.20 g/mol	7350,42
F11	0,02	345.25 g/mol	345.21 g/mol	5792,11
G11	0	305.17 g/mol	0.00 g/mol	0
H11	0	347.08 g/mol	347.06 g/mol	68,11
A12	0,06	332.25 g/mol	332.23 g/mol	1348,32
B12	0,16	361.24 g/mol	361.21 g/mol	8633,4
C12	0	379.19 g/mol	0.00 g/mol	0
D12	0	337.15 g/mol	0.00 g/mol	0
E12	0	385.21 g/mol	0.00 g/mol	0
F12	0,16	331.23 g/mol	331.19 g/mol	10343,58
G12	0,08	298.20 g/mol	298.17 g/mol	15521,58
H12	0	332.22 g/mol	332.21 g/mol	60,37

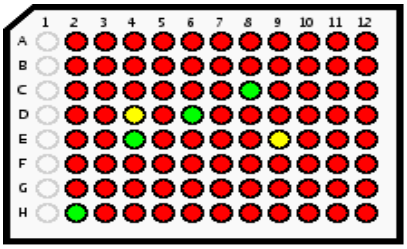
B.3 Screening plates ori.hya.10-19

B.3.1 Starting materials of plates ori.hya.10-19



B.3.2 Mass spectral analysis of plates ori.hya.10-19

ori.hya.10



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	326.33 g/mol	0.00 g/mol	0
B2	0	240.24 g/mol	0.00 g/mol	0
C2	0	198.18 g/mol	0.00 g/mol	0
D2	0	254.26 g/mol	0.00 g/mol	0
E2	0	254.26 g/mol	0.00 g/mol	0
F2	0	360.18 g/mol	0.00 g/mol	0
G2	0	224.20 g/mol	0.00 g/mol	0
H2	0,04	250.19 g/mol	250.18 g/mol	595,68
A3	0	356.30 g/mol	0.00 g/mol	0
B3	0	270.21 g/mol	0.00 g/mol	0
C3	0	228.15 g/mol	0.00 g/mol	0
D3	0	284.23 g/mol	0.00 g/mol	0
E3	0	284.23 g/mol	0.00 g/mol	0
F3	0	390.15 g/mol	0.00 g/mol	0
G3	0	254.17 g/mol	0.00 g/mol	0
H3	0	280.16 g/mol	0.00 g/mol	0
A4	0	338.33 g/mol	0.00 g/mol	0
B4	0	252.24 g/mol	0.00 g/mol	0
C4	0	210.18 g/mol	0.00 g/mol	0
D4	0	266.26 g/mol	266.19 g/mol	72,97
E4	0,02	266.26 g/mol	266.24 g/mol	616,27
F4	0	372.18 g/mol	0.00 g/mol	0
G4	0	236.20 g/mol	0.00 g/mol	0
H4	0	262.19 g/mol	0.00 g/mol	0
A5	0	299.24 g/mol	0.00 g/mol	0
B5	0	213.15 g/mol	0.00 g/mol	0
C5	0	171.09 g/mol	0.00 g/mol	0
D5	0	227.17 g/mol	0.00 g/mol	0
E5	0	227.17 g/mol	0.00 g/mol	0
F5	0	333.09 g/mol	0.00 g/mol	0
G5	0	197.11 g/mol	0.00 g/mol	0
H5	0	223.10 g/mol	0.00 g/mol	0
A6	0	316.26 g/mol	0.00 g/mol	0
B6	0	230.17 g/mol	0.00 g/mol	0
C6	0	188.11 g/mol	0.00 g/mol	0
D6	0	244.19 g/mol	244.19 g/mol	833,15
E6	0	244.19 g/mol	0.00 g/mol	0
F6	0	350.11 g/mol	0.00 g/mol	0
G6	0	214.13 g/mol	0.00 g/mol	0
H6	0	240.12 g/mol	0.00 g/mol	0

A7	0	308.24 g/mol	0.00 g/mol	0
B7	0	222.15 g/mol	0.00 g/mol	0
C7	0	180.09 g/mol	0.00 g/mol	0
D7	0	236.17 g/mol	0.00 g/mol	0
E7	0	236.17 g/mol	0.00 g/mol	0
F7	0	342.09 g/mol	0.00 g/mol	0
G7	0	206.11 g/mol	0.00 g/mol	0
H7	0	232.09 g/mol	0.00 g/mol	0
A8	0	328.26 g/mol	0.00 g/mol	0
B8	0	242.17 g/mol	0.00 g/mol	0
C8	0,02	200.11 g/mol	200.05 g/mol	310,39
D8	0	256.19 g/mol	0.00 g/mol	0
E8	0	256.19 g/mol	0.00 g/mol	0
F8	0	362.11 g/mol	0.00 g/mol	0
G8	0	226.13 g/mol	0.00 g/mol	0
H8	0	252.12 g/mol	0.00 g/mol	0
A9	0	358.23 g/mol	0.00 g/mol	0
B9	0	272.14 g/mol	0.00 g/mol	0
C9	0	230.08 g/mol	0.00 g/mol	0
D9	0	286.16 g/mol	0.00 g/mol	0
E9	0	286.16 g/mol	286.19 g/mol	77,03
F9	0	392.08 g/mol	0.00 g/mol	0
G9	0	256.10 g/mol	0.00 g/mol	0
H9	0	282.09 g/mol	0.00 g/mol	0
A10	0	330.24 g/mol	0.00 g/mol	0
B10	0	244.15 g/mol	0.00 g/mol	0
C10	0	202.09 g/mol	0.00 g/mol	0
D10	0	258.17 g/mol	0.00 g/mol	0
E10	0	258.17 g/mol	0.00 g/mol	0
F10	0	364.09 g/mol	0.00 g/mol	0
G10	0	228.11 g/mol	0.00 g/mol	0
H10	0	254.09 g/mol	0.00 g/mol	0
A11	0	315.24 g/mol	0.00 g/mol	0
B11	0	229.15 g/mol	0.00 g/mol	0
C11	0	187.09 g/mol	0.00 g/mol	0
D11	0	243.16 g/mol	0.00 g/mol	0
E11	0	243.16 g/mol	0.00 g/mol	0
F11	0	349.09 g/mol	0.00 g/mol	0
G11	0	213.11 g/mol	0.00 g/mol	0
H11	0	239.09 g/mol	0.00 g/mol	0
A12	0	334.25 g/mol	0.00 g/mol	0
B12	0	248.16 g/mol	0.00 g/mol	0
C12	0	206.10 g/mol	0.00 g/mol	0
D12	0	262.18 g/mol	0.00 g/mol	0
E12	0	262.18 g/mol	0.00 g/mol	0
F12	0	368.10 g/mol	0.00 g/mol	0
G12	0	232.12 g/mol	0.00 g/mol	0
H12	0	258.10 g/mol	0.00 g/mol	0

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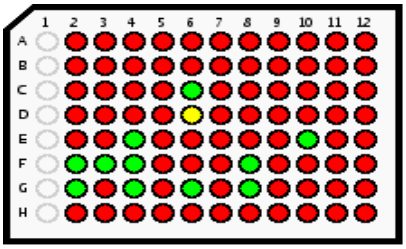

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	249.19 g/mol	0.00 g/mol	0
B2	0	249.19 g/mol	0.00 g/mol	0
C2	0	252.24 g/mol	0.00 g/mol	0
D2	0	250.19 g/mol	0.00 g/mol	0
E2	0	269.27 g/mol	0.00 g/mol	0
F2	0	292.19 g/mol	292.12 g/mol	766,22
G2	0,02	304.23 g/mol	304.25 g/mol	695
H2	0	244.19 g/mol	0.00 g/mol	0
A3	0	279.16 g/mol	0.00 g/mol	0
B3	0	279.16 g/mol	0.00 g/mol	0
C3	0	282.21 g/mol	0.00 g/mol	0
D3	0	280.16 g/mol	0.00 g/mol	0
E3	0	299.24 g/mol	0.00 g/mol	0
F3	0	322.16 g/mol	322.19 g/mol	127,14
G3	0	334.20 g/mol	0.00 g/mol	0
H3	0	274.16 g/mol	0.00 g/mol	0
A4	0	261.19 g/mol	0.00 g/mol	0
B4	0	261.19 g/mol	0.00 g/mol	0
C4	0	264.24 g/mol	0.00 g/mol	0
D4	0	262.19 g/mol	0.00 g/mol	0
E4	0,04	281.27 g/mol	281.29 g/mol	680,12
F4	0	304.19 g/mol	304.22 g/mol	458,78
G4	0,02	316.23 g/mol	316.26 g/mol	661,53
H4	0	256.19 g/mol	0.00 g/mol	0
A5	0	222.10 g/mol	0.00 g/mol	0
B5	0	222.10 g/mol	0.00 g/mol	0
C5	0	225.15 g/mol	0.00 g/mol	0
D5	0	223.10 g/mol	0.00 g/mol	0
E5	0	242.18 g/mol	0.00 g/mol	0
F5	0	265.10 g/mol	0.00 g/mol	0
G5	0	277.14 g/mol	0.00 g/mol	0
H5	0	217.10 g/mol	0.00 g/mol	0
A6	0	239.12 g/mol	0.00 g/mol	0
B6	0	239.12 g/mol	0.00 g/mol	0
C6	0,02	242.17 g/mol	242.19 g/mol	296,91
D6	0	240.12 g/mol	240.16 g/mol	44,98
E6	0	259.20 g/mol	0.00 g/mol	0
F6	0	282.12 g/mol	0.00 g/mol	0
G6	0,02	294.16 g/mol	294.20 g/mol	531,12
H6	0	234.12 g/mol	0.00 g/mol	0

A7	0	231.10 g/mol	0.00 g/mol	0
B7	0	231.10 g/mol	0.00 g/mol	0
C7	0	234.15 g/mol	0.00 g/mol	0
D7	0	232.09 g/mol	0.00 g/mol	0
E7	0	251.18 g/mol	0.00 g/mol	0
F7	0	274.10 g/mol	0.00 g/mol	0
G7	0	286.14 g/mol	0.00 g/mol	0
H7	0	226.10 g/mol	0.00 g/mol	0
A8	0	251.12 g/mol	0.00 g/mol	0
B8	0	251.12 g/mol	0.00 g/mol	0
C8	0	254.17 g/mol	0.00 g/mol	0
D8	0	252.12 g/mol	0.00 g/mol	0
E8	0	271.20 g/mol	0.00 g/mol	0
F8	0	294.12 g/mol	294.17 g/mol	144,91
G8	0,02	306.16 g/mol	306.20 g/mol	474,73
H8	0	246.12 g/mol	0.00 g/mol	0
A9	0	281.09 g/mol	0.00 g/mol	0
B9	0	281.09 g/mol	0.00 g/mol	0
C9	0	284.14 g/mol	0.00 g/mol	0
D9	0	282.09 g/mol	0.00 g/mol	0
E9	0	301.17 g/mol	0.00 g/mol	0
F9	0	324.09 g/mol	0.00 g/mol	0
G9	0	336.13 g/mol	0.00 g/mol	0
H9	0	276.09 g/mol	0.00 g/mol	0
A10	0	253.10 g/mol	0.00 g/mol	0
B10	0	253.10 g/mol	0.00 g/mol	0
C10	0	256.15 g/mol	0.00 g/mol	0
D10	0	254.09 g/mol	0.00 g/mol	0
E10	0,06	273.18 g/mol	273.09 g/mol	992,71
F10	0	296.09 g/mol	0.00 g/mol	0
G10	0	308.14 g/mol	0.00 g/mol	0
H10	0	248.10 g/mol	0.00 g/mol	0
A11	0	238.10 g/mol	0.00 g/mol	0
B11	0	238.10 g/mol	0.00 g/mol	0
C11	0	241.14 g/mol	0.00 g/mol	0
D11	0	239.09 g/mol	0.00 g/mol	0
E11	0	258.18 g/mol	0.00 g/mol	0
F11	0	281.09 g/mol	0.00 g/mol	0
G11	0	293.14 g/mol	0.00 g/mol	0
H11	0	233.10 g/mol	0.00 g/mol	0
A12	0	257.11 g/mol	0.00 g/mol	0
B12	0	257.11 g/mol	0.00 g/mol	0
C12	0	260.16 g/mol	0.00 g/mol	0
D12	0	258.10 g/mol	0.00 g/mol	0
E12	0	277.19 g/mol	0.00 g/mol	0
F12	0	300.10 g/mol	0.00 g/mol	0
G12	0	312.15 g/mol	0.00 g/mol	0
H12	0	252.11 g/mol	0.00 g/mol	0

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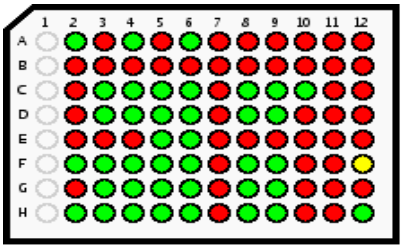

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,02	424.43 g/mol	424.44 g/mol	685,28
B2	0	338.33 g/mol	0.00 g/mol	0
C2	0	296.28 g/mol	0.00 g/mol	0
D2	0	352.35 g/mol	352.37 g/mol	15,57
E2	0	352.35 g/mol	0.00 g/mol	0
F2	0,04	458.27 g/mol	458.32 g/mol	1508,92
G2	0	322.29 g/mol	0.00 g/mol	0
H2	0	348.28 g/mol	348.31 g/mol	253,39
A3	0	454.39 g/mol	0.00 g/mol	0
B3	0	368.30 g/mol	0.00 g/mol	0
C3	0	326.24 g/mol	326.27 g/mol	858,98
D3	0	382.32 g/mol	382.35 g/mol	503,88
E3	0	382.32 g/mol	0.00 g/mol	0
F3	0,02	488.24 g/mol	488.30 g/mol	809,32
G3	0	352.26 g/mol	352.30 g/mol	239,62
H3	0,04	378.25 g/mol	378.29 g/mol	5187,38
A4	0,02	436.43 g/mol	436.45 g/mol	712,84
B4	0	350.33 g/mol	0.00 g/mol	0
C4	0	308.27 g/mol	308.30 g/mol	258,85
D4	0,02	364.35 g/mol	364.36 g/mol	1302,88
E4	0	364.35 g/mol	0.00 g/mol	0
F4	0,04	470.27 g/mol	470.32 g/mol	2114,48
G4	0,02	334.29 g/mol	334.31 g/mol	4360,56
H4	0,08	360.28 g/mol	360.31 g/mol	7503,66
A5	0	397.33 g/mol	0.00 g/mol	0
B5	0	311.24 g/mol	0.00 g/mol	0
C5	0	269.18 g/mol	269.21 g/mol	756,2
D5	0	325.26 g/mol	325.30 g/mol	362,23
E5	0,02	325.26 g/mol	325.29 g/mol	598,47
F5	0,06	431.18 g/mol	431.25 g/mol	2000,59
G5	0,02	295.20 g/mol	295.25 g/mol	2595,72
H5	0	321.19 g/mol	321.24 g/mol	271,83
A6	0	414.36 g/mol	414.41 g/mol	170,41
B6	0	328.26 g/mol	0.00 g/mol	0
C6	0,08	286.21 g/mol	286.23 g/mol	4047,16
D6	0	342.28 g/mol	342.31 g/mol	819,31
E6	0	342.28 g/mol	342.31 g/mol	278,25
F6	0,06	448.20 g/mol	448.28 g/mol	1934,16
G6	0,04	312.22 g/mol	312.29 g/mol	1552,42
H6	0,02	338.21 g/mol	338.25 g/mol	4248,56

A7	0	406.33 g/mol	0.00 g/mol	0
B7	0	320.24 g/mol	0.00 g/mol	0
C7	0	278.18 g/mol	0.00 g/mol	0
D7	0	334.26 g/mol	0.00 g/mol	0
E7	0	334.26 g/mol	0.00 g/mol	0
F7	0	440.18 g/mol	0.00 g/mol	0
G7	0	304.20 g/mol	0.00 g/mol	0
H7	0	330.19 g/mol	0.00 g/mol	0
A8	0	426.36 g/mol	0.00 g/mol	0
B8	0	340.26 g/mol	0.00 g/mol	0
C8	0,02	298.20 g/mol	298.25 g/mol	476,57
D8	0,02	354.28 g/mol	354.32 g/mol	743,26
E8	0	354.28 g/mol	0.00 g/mol	0
F8	0,02	460.20 g/mol	460.28 g/mol	800,76
G8	0,02	324.22 g/mol	324.26 g/mol	887,66
H8	0,06	350.21 g/mol	350.25 g/mol	5803,76
A9	0	456.32 g/mol	0.00 g/mol	0
B9	0	370.23 g/mol	0.00 g/mol	0
C9	0	328.17 g/mol	328.22 g/mol	331,89
D9	0,02	384.25 g/mol	384.30 g/mol	565,79
E9	0	384.25 g/mol	0.00 g/mol	0
F9	0,02	490.17 g/mol	490.26 g/mol	674,71
G9	0,02	354.19 g/mol	354.25 g/mol	890,53
H9	0,02	380.18 g/mol	380.24 g/mol	1043,03
A10	0	428.33 g/mol	0.00 g/mol	0
B10	0	342.24 g/mol	0.00 g/mol	0
C10	0,06	300.18 g/mol	300.22 g/mol	1344
D10	0	356.26 g/mol	0.00 g/mol	0
E10	0	356.26 g/mol	0.00 g/mol	0
F10	0	462.18 g/mol	0.00 g/mol	0
G10	0	326.20 g/mol	0.00 g/mol	0
H10	0	352.18 g/mol	0.00 g/mol	0
A11	0	413.33 g/mol	0.00 g/mol	0
B11	0	327.24 g/mol	0.00 g/mol	0
C11	0	285.18 g/mol	0.00 g/mol	0
D11	0	341.26 g/mol	0.00 g/mol	0
E11	0	341.26 g/mol	0.00 g/mol	0
F11	0	447.18 g/mol	0.00 g/mol	0
G11	0	311.20 g/mol	0.00 g/mol	0
H11	0	337.18 g/mol	0.00 g/mol	0
A12	0	432.34 g/mol	0.00 g/mol	0
B12	0	346.25 g/mol	0.00 g/mol	0
C12	0	304.19 g/mol	0.00 g/mol	0
D12	0	360.27 g/mol	0.00 g/mol	0
E12	0	360.27 g/mol	0.00 g/mol	0
F12	0	466.19 g/mol	466.27 g/mol	86,47
G12	0	330.21 g/mol	0.00 g/mol	0
H12	0,02	356.19 g/mol	356.25 g/mol	658,3

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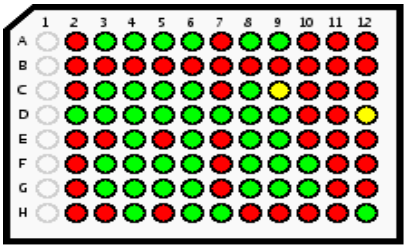

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	347.29 g/mol	0.00 g/mol	0
B2	0	347.29 g/mol	0.00 g/mol	0
C2	0	350.33 g/mol	0.00 g/mol	0
D2	0,04	348.28 g/mol	348.31 g/mol	1094,69
E2	0	367.37 g/mol	0.00 g/mol	0
F2	0	390.28 g/mol	0.00 g/mol	0
G2	0	402.33 g/mol	0.00 g/mol	0
H2	0	342.28 g/mol	0.00 g/mol	0
A3	0	377.25 g/mol	377.31 g/mol	159,5
B3	0	377.25 g/mol	0.00 g/mol	0
C3	0,02	380.30 g/mol	380.34 g/mol	1186,01
D3	0	378.25 g/mol	378.30 g/mol	2846,47
E3	0	397.33 g/mol	0.00 g/mol	0
F3	0,02	420.25 g/mol	420.31 g/mol	977,35
G3	0	432.29 g/mol	432.34 g/mol	1503,2
H3	0	372.25 g/mol	0.00 g/mol	0
A4	0	359.29 g/mol	359.33 g/mol	404,9
B4	0	359.29 g/mol	0.00 g/mol	0
C4	0,02	362.33 g/mol	362.36 g/mol	1812,37
D4	0,1	360.28 g/mol	360.32 g/mol	13390,62
E4	0	379.37 g/mol	379.39 g/mol	152,65
F4	0,04	402.28 g/mol	402.33 g/mol	1243,05
G4	0	414.33 g/mol	414.37 g/mol	646,28
H4	0,04	354.28 g/mol	354.33 g/mol	1137,45
A5	0,1	320.20 g/mol	320.26 g/mol	2045,48
B5	0	320.20 g/mol	0.00 g/mol	0
C5	0,02	323.24 g/mol	323.31 g/mol	1068,37
D5	0,14	321.19 g/mol	321.24 g/mol	7852,2
E5	0	340.27 g/mol	0.00 g/mol	0
F5	0,04	363.19 g/mol	363.25 g/mol	1286,92
G5	0	375.23 g/mol	375.28 g/mol	5694,58
H5	0	315.19 g/mol	0.00 g/mol	0
A6	0	337.22 g/mol	337.27 g/mol	316,51
B6	0	337.22 g/mol	0.00 g/mol	0
C6	0,02	340.26 g/mol	340.30 g/mol	838,66
D6	0,16	338.21 g/mol	338.26 g/mol	6119,1
E6	0	357.30 g/mol	357.34 g/mol	115,8
F6	0	380.21 g/mol	380.27 g/mol	793,06
G6	0,04	392.26 g/mol	392.30 g/mol	7377,3
H6	0,04	332.21 g/mol	332.24 g/mol	886,63

A7	0	329.19 g/mol	0.00 g/mol	0
B7	0	329.19 g/mol	0.00 g/mol	0
C7	0	332.24 g/mol	0.00 g/mol	0
D7	0	330.19 g/mol	330.25 g/mol	132,13
E7	0	349.27 g/mol	0.00 g/mol	0
F7	0	372.19 g/mol	0.00 g/mol	0
G7	0	384.23 g/mol	0.00 g/mol	0
H7	0	324.19 g/mol	324.25 g/mol	189,89
A8	0	349.22 g/mol	349.27 g/mol	605,29
B8	0	349.22 g/mol	0.00 g/mol	0
C8	0	352.26 g/mol	352.31 g/mol	261,8
D8	0,12	350.21 g/mol	350.26 g/mol	6900,78
E8	0,02	369.30 g/mol	369.26 g/mol	591,81
F8	0	392.21 g/mol	392.27 g/mol	294,98
G8	0,04	404.26 g/mol	404.31 g/mol	1244,5
H8	0	344.21 g/mol	0.00 g/mol	0
A9	0	379.18 g/mol	379.25 g/mol	126,95
B9	0	379.18 g/mol	0.00 g/mol	0
C9	0	382.23 g/mol	382.29 g/mol	50,84
D9	0,02	380.18 g/mol	380.24 g/mol	5404,8
E9	0,02	399.26 g/mol	399.32 g/mol	648,16
F9	0,02	422.18 g/mol	422.25 g/mol	884,15
G9	0,02	434.22 g/mol	434.30 g/mol	743,71
H9	0	374.18 g/mol	0.00 g/mol	0
A10	0	351.19 g/mol	0.00 g/mol	0
B10	0	351.19 g/mol	0.00 g/mol	0
C10	0	354.24 g/mol	0.00 g/mol	0
D10	0	352.18 g/mol	0.00 g/mol	0
E10	0	371.27 g/mol	0.00 g/mol	0
F10	0	394.19 g/mol	394.25 g/mol	385,35
G10	0	406.23 g/mol	406.29 g/mol	166,47
H10	0	346.19 g/mol	0.00 g/mol	0
A11	0	336.19 g/mol	0.00 g/mol	0
B11	0	336.19 g/mol	0.00 g/mol	0
C11	0	339.24 g/mol	0.00 g/mol	0
D11	0	337.18 g/mol	0.00 g/mol	0
E11	0	356.27 g/mol	0.00 g/mol	0
F11	0	379.18 g/mol	0.00 g/mol	0
G11	0	391.23 g/mol	0.00 g/mol	0
H11	0	331.19 g/mol	0.00 g/mol	0
A12	0	355.20 g/mol	0.00 g/mol	0
B12	0	355.20 g/mol	0.00 g/mol	0
C12	0	358.25 g/mol	0.00 g/mol	0
D12	0	356.19 g/mol	356.21 g/mol	33,08
E12	0	375.28 g/mol	0.00 g/mol	0
F12	0	398.20 g/mol	0.00 g/mol	0
G12	0	410.24 g/mol	0.00 g/mol	0
H12	0	350.20 g/mol	350.26 g/mol	137,74

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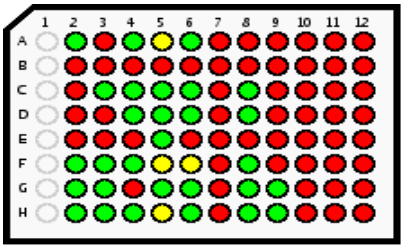

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	446.36 g/mol	446.42 g/mol	159,67
B2	0	360.27 g/mol	0.00 g/mol	0
C2	0	318.21 g/mol	0.00 g/mol	0
D2	0	374.29 g/mol	0.00 g/mol	0
E2	0	374.29 g/mol	0.00 g/mol	0
F2	0,02	480.21 g/mol	480.29 g/mol	880,97
G2	0,02	344.23 g/mol	344.28 g/mol	602,41
H2	0	370.21 g/mol	370.27 g/mol	198,58
A3	0	476.33 g/mol	0.00 g/mol	0
B3	0	390.24 g/mol	0.00 g/mol	0
C3	0,02	348.18 g/mol	348.24 g/mol	629,69
D3	0	404.26 g/mol	0.00 g/mol	0
E3	0	404.26 g/mol	0.00 g/mol	0
F3	0	510.18 g/mol	510.27 g/mol	244,33
G3	0,04	374.20 g/mol	374.26 g/mol	1015,55
H3	0,02	400.18 g/mol	400.26 g/mol	517,01
A4	0,02	458.36 g/mol	458.41 g/mol	849,14
B4	0	372.27 g/mol	0.00 g/mol	0
C4	0	330.21 g/mol	330.26 g/mol	267,67
D4	0,02	386.29 g/mol	386.34 g/mol	606,11
E4	0	386.29 g/mol	0.00 g/mol	0
F4	0,02	492.21 g/mol	492.30 g/mol	680,27
G4	0	356.23 g/mol	0.00 g/mol	0
H4	0	382.21 g/mol	382.28 g/mol	187,96
A5	0	419.27 g/mol	419.33 g/mol	78
B5	0	333.18 g/mol	0.00 g/mol	0
C5	0,04	291.12 g/mol	291.18 g/mol	756,54
D5	0,04	347.20 g/mol	347.25 g/mol	1327,46
E5	0	347.20 g/mol	347.25 g/mol	142,33
F5	0	453.12 g/mol	453.22 g/mol	87,63
G5	0	317.14 g/mol	317.12 g/mol	210,41
H5	0	343.12 g/mol	343.14 g/mol	52,59
A6	0	436.29 g/mol	436.35 g/mol	269,78
B6	0	350.20 g/mol	0.00 g/mol	0
C6	0,04	308.14 g/mol	308.19 g/mol	1122,01
D6	0,02	364.22 g/mol	364.27 g/mol	820,15
E6	0	364.22 g/mol	0.00 g/mol	0
F6	0	470.14 g/mol	470.23 g/mol	78,66
G6	0	334.16 g/mol	334.22 g/mol	398,65
H6	0,02	360.14 g/mol	360.21 g/mol	493,26

A7	0	428.27 g/mol	0.00 g/mol	0
B7	0	342.17 g/mol	0.00 g/mol	0
C7	0	300.12 g/mol	0.00 g/mol	0
D7	0	356.19 g/mol	0.00 g/mol	0
E7	0	356.19 g/mol	0.00 g/mol	0
F7	0	462.11 g/mol	0.00 g/mol	0
G7	0	326.13 g/mol	0.00 g/mol	0
H7	0	352.12 g/mol	0.00 g/mol	0
A8	0	448.29 g/mol	0.00 g/mol	0
B8	0	362.20 g/mol	0.00 g/mol	0
C8	0,02	320.14 g/mol	320.19 g/mol	573,39
D8	0	376.22 g/mol	376.28 g/mol	127,06
E8	0	376.22 g/mol	0.00 g/mol	0
F8	0	482.14 g/mol	482.23 g/mol	338,17
G8	0,06	346.16 g/mol	346.22 g/mol	1557,32
H8	0	372.14 g/mol	372.22 g/mol	211,52
A9	0	478.26 g/mol	0.00 g/mol	0
B9	0	392.17 g/mol	0.00 g/mol	0
C9	0	350.11 g/mol	0.00 g/mol	0
D9	0	406.19 g/mol	0.00 g/mol	0
E9	0	406.19 g/mol	0.00 g/mol	0
F9	0	512.11 g/mol	0.00 g/mol	0
G9	0,02	376.13 g/mol	376.20 g/mol	660,02
H9	0,04	402.11 g/mol	402.21 g/mol	982,41
A10	0	450.26 g/mol	0.00 g/mol	0
B10	0	364.17 g/mol	0.00 g/mol	0
C10	0	322.11 g/mol	0.00 g/mol	0
D10	0	378.19 g/mol	0.00 g/mol	0
E10	0	378.19 g/mol	0.00 g/mol	0
F10	0	484.11 g/mol	0.00 g/mol	0
G10	0	348.13 g/mol	0.00 g/mol	0
H10	0	374.12 g/mol	0.00 g/mol	0
A11	0	435.26 g/mol	0.00 g/mol	0
B11	0	349.17 g/mol	0.00 g/mol	0
C11	0	307.11 g/mol	0.00 g/mol	0
D11	0	363.19 g/mol	0.00 g/mol	0
E11	0	363.19 g/mol	0.00 g/mol	0
F11	0	469.11 g/mol	0.00 g/mol	0
G11	0	333.13 g/mol	0.00 g/mol	0
H11	0	359.12 g/mol	0.00 g/mol	0
A12	0	454.27 g/mol	0.00 g/mol	0
B12	0	368.18 g/mol	0.00 g/mol	0
C12	0	326.12 g/mol	0.00 g/mol	0
D12	0	382.20 g/mol	0.00 g/mol	0
E12	0	382.20 g/mol	0.00 g/mol	0
F12	0	488.12 g/mol	0.00 g/mol	0
G12	0	352.14 g/mol	0.00 g/mol	0
H12	0	378.13 g/mol	0.00 g/mol	0

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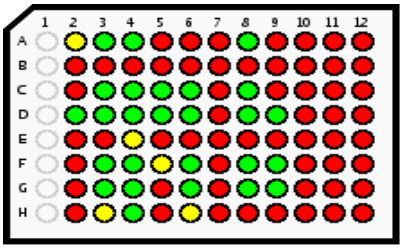

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	369.22 g/mol	369.28 g/mol	47,88
B2	0	369.22 g/mol	0.00 g/mol	0
C2	0	372.27 g/mol	0.00 g/mol	0
D2	0	370.21 g/mol	370.27 g/mol	416,83
E2	0	389.30 g/mol	0.00 g/mol	0
F2	0	412.22 g/mol	0.00 g/mol	0
G2	0	424.26 g/mol	0.00 g/mol	0
H2	0	364.22 g/mol	0.00 g/mol	0
A3	0	399.19 g/mol	399.25 g/mol	288,21
B3	0	399.19 g/mol	0.00 g/mol	0
C3	0	402.24 g/mol	402.30 g/mol	116,53
D3	0,04	400.18 g/mol	400.25 g/mol	1162,33
E3	0	419.27 g/mol	0.00 g/mol	0
F3	0	442.18 g/mol	442.26 g/mol	108,93
G3	0,04	454.23 g/mol	454.29 g/mol	1394,39
H3	0	394.19 g/mol	394.25 g/mol	97,33
A4	0,02	381.22 g/mol	381.27 g/mol	621,63
B4	0	381.22 g/mol	0.00 g/mol	0
C4	0	384.27 g/mol	384.31 g/mol	272,4
D4	0	382.21 g/mol	382.26 g/mol	4861,51
E4	0	401.30 g/mol	401.35 g/mol	67,18
F4	0,06	424.21 g/mol	424.28 g/mol	1788,38
G4	0,04	436.26 g/mol	436.32 g/mol	1167,04
H4	0,02	376.22 g/mol	376.27 g/mol	645,94
A5	0	342.13 g/mol	0.00 g/mol	0
B5	0	342.13 g/mol	0.00 g/mol	0
C5	0,02	345.18 g/mol	345.23 g/mol	693,43
D5	0,02	343.12 g/mol	343.19 g/mol	685,86
E5	0	362.21 g/mol	0.00 g/mol	0
F5	0	385.12 g/mol	385.20 g/mol	56,87
G5	0	397.17 g/mol	0.00 g/mol	0
H5	0	337.13 g/mol	0.00 g/mol	0
A6	0	359.15 g/mol	0.00 g/mol	0
B6	0	359.15 g/mol	0.00 g/mol	0
C6	0	362.20 g/mol	362.25 g/mol	485,1
D6	0	360.14 g/mol	360.20 g/mol	1080,24
E6	0	379.23 g/mol	0.00 g/mol	0
F6	0	402.15 g/mol	402.22 g/mol	108,91
G6	0	414.19 g/mol	414.25 g/mol	1559,75
H6	0	354.15 g/mol	354.21 g/mol	36,5

A7	0	351.13 g/mol	0.00 g/mol	0
B7	0	351.13 g/mol	0.00 g/mol	0
C7	0	354.17 g/mol	0.00 g/mol	0
D7	0	352.12 g/mol	0.00 g/mol	0
E7	0	371.21 g/mol	0.00 g/mol	0
F7	0	394.12 g/mol	0.00 g/mol	0
G7	0	406.17 g/mol	0.00 g/mol	0
H7	0	346.12 g/mol	0.00 g/mol	0
A8	0	371.15 g/mol	371.22 g/mol	128,18
B8	0	371.15 g/mol	0.00 g/mol	0
C8	0,02	374.20 g/mol	374.26 g/mol	628,49
D8	0,02	372.14 g/mol	372.22 g/mol	640
E8	0	391.23 g/mol	0.00 g/mol	0
F8	0,04	414.15 g/mol	414.23 g/mol	1204,52
G8	0	426.19 g/mol	426.26 g/mol	225,38
H8	0	366.15 g/mol	0.00 g/mol	0
A9	0	401.12 g/mol	401.21 g/mol	25,64
B9	0	401.12 g/mol	0.00 g/mol	0
C9	0	404.17 g/mol	0.00 g/mol	0
D9	0,04	402.11 g/mol	402.20 g/mol	1040,92
E9	0	421.20 g/mol	0.00 g/mol	0
F9	0,02	444.11 g/mol	444.20 g/mol	624,82
G9	0,04	456.16 g/mol	456.24 g/mol	1188,47
H9	0	396.12 g/mol	0.00 g/mol	0
A10	0	373.12 g/mol	0.00 g/mol	0
B10	0	373.12 g/mol	0.00 g/mol	0
C10	0	376.17 g/mol	0.00 g/mol	0
D10	0	374.12 g/mol	0.00 g/mol	0
E10	0	393.20 g/mol	0.00 g/mol	0
F10	0	416.12 g/mol	0.00 g/mol	0
G10	0	428.16 g/mol	0.00 g/mol	0
H10	0	368.12 g/mol	0.00 g/mol	0
A11	0	358.12 g/mol	0.00 g/mol	0
B11	0	358.12 g/mol	0.00 g/mol	0
C11	0	361.17 g/mol	0.00 g/mol	0
D11	0	359.12 g/mol	0.00 g/mol	0
E11	0	378.20 g/mol	0.00 g/mol	0
F11	0	401.12 g/mol	0.00 g/mol	0
G11	0	413.16 g/mol	0.00 g/mol	0
H11	0	353.12 g/mol	0.00 g/mol	0
A12	0	377.13 g/mol	0.00 g/mol	0
B12	0	377.13 g/mol	0.00 g/mol	0
C12	0	380.18 g/mol	0.00 g/mol	0
D12	0	378.13 g/mol	0.00 g/mol	0
E12	0	397.21 g/mol	0.00 g/mol	0
F12	0	420.13 g/mol	0.00 g/mol	0
G12	0	432.17 g/mol	0.00 g/mol	0
H12	0	372.13 g/mol	0.00 g/mol	0

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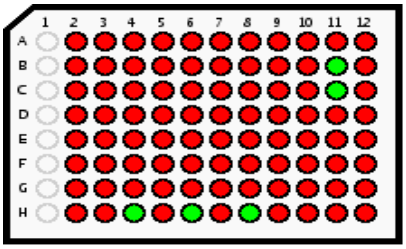

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	368.35 g/mol	0.00 g/mol	0
B2	0	282.26 g/mol	0.00 g/mol	0
C2	0	240.20 g/mol	0.00 g/mol	0
D2	0	296.28 g/mol	0.00 g/mol	0
E2	0	296.28 g/mol	0.00 g/mol	0
F2	0	402.20 g/mol	0.00 g/mol	0
G2	0	266.22 g/mol	0.00 g/mol	0
H2	0	292.20 g/mol	0.00 g/mol	0
A3	0	398.32 g/mol	0.00 g/mol	0
B3	0	312.22 g/mol	0.00 g/mol	0
C3	0	270.17 g/mol	0.00 g/mol	0
D3	0	326.24 g/mol	0.00 g/mol	0
E3	0	326.24 g/mol	0.00 g/mol	0
F3	0	432.17 g/mol	0.00 g/mol	0
G3	0	296.18 g/mol	0.00 g/mol	0
H3	0	322.17 g/mol	0.00 g/mol	0
A4	0	380.35 g/mol	0.00 g/mol	0
B4	0	294.26 g/mol	0.00 g/mol	0
C4	0	252.20 g/mol	0.00 g/mol	0
D4	0	308.27 g/mol	0.00 g/mol	0
E4	0	308.27 g/mol	0.00 g/mol	0
F4	0	414.20 g/mol	0.00 g/mol	0
G4	0	278.22 g/mol	0.00 g/mol	0
H4	0,02	304.20 g/mol	304.28 g/mol	367,45
A5	0	341.26 g/mol	0.00 g/mol	0
B5	0	255.16 g/mol	0.00 g/mol	0
C5	0	213.11 g/mol	0.00 g/mol	0
D5	0	269.18 g/mol	0.00 g/mol	0
E5	0	269.18 g/mol	0.00 g/mol	0
F5	0	375.11 g/mol	0.00 g/mol	0
G5	0	239.12 g/mol	0.00 g/mol	0
H5	0	265.11 g/mol	0.00 g/mol	0
A6	0	358.28 g/mol	0.00 g/mol	0
B6	0	272.19 g/mol	0.00 g/mol	0
C6	0	230.13 g/mol	0.00 g/mol	0
D6	0	286.21 g/mol	0.00 g/mol	0
E6	0	286.21 g/mol	0.00 g/mol	0
F6	0	392.13 g/mol	0.00 g/mol	0
G6	0	256.15 g/mol	0.00 g/mol	0
H6	0	282.13 g/mol	282.22 g/mol	327,3

A7	0	350.25 g/mol	0.00 g/mol	0
B7	0	264.16 g/mol	0.00 g/mol	0
C7	0	222.10 g/mol	0.00 g/mol	0
D7	0	278.18 g/mol	0.00 g/mol	0
E7	0	278.18 g/mol	0.00 g/mol	0
F7	0	384.10 g/mol	0.00 g/mol	0
G7	0	248.12 g/mol	0.00 g/mol	0
H7	0	274.11 g/mol	0.00 g/mol	0
A8	0	370.28 g/mol	0.00 g/mol	0
B8	0	284.19 g/mol	0.00 g/mol	0
C8	0	242.13 g/mol	0.00 g/mol	0
D8	0	298.20 g/mol	0.00 g/mol	0
E8	0	298.20 g/mol	0.00 g/mol	0
F8	0	404.13 g/mol	0.00 g/mol	0
G8	0	268.15 g/mol	0.00 g/mol	0
H8	0	294.13 g/mol	294.21 g/mol	617,65
A9	0	400.25 g/mol	0.00 g/mol	0
B9	0	314.15 g/mol	0.00 g/mol	0
C9	0	272.10 g/mol	0.00 g/mol	0
D9	0	328.17 g/mol	0.00 g/mol	0
E9	0	328.17 g/mol	0.00 g/mol	0
F9	0	434.10 g/mol	0.00 g/mol	0
G9	0	298.11 g/mol	0.00 g/mol	0
H9	0	324.10 g/mol	0.00 g/mol	0
A10	0	372.25 g/mol	0.00 g/mol	0
B10	0	286.16 g/mol	0.00 g/mol	0
C10	0	244.10 g/mol	0.00 g/mol	0
D10	0	300.18 g/mol	0.00 g/mol	0
E10	0	300.18 g/mol	0.00 g/mol	0
F10	0	406.10 g/mol	0.00 g/mol	0
G10	0	270.12 g/mol	0.00 g/mol	0
H10	0	296.11 g/mol	0.00 g/mol	0
A11	0	357.25 g/mol	0.00 g/mol	0
B11	0	271.16 g/mol	271.25 g/mol	857,77
C11	0,18	229.10 g/mol	229.14 g/mol	28144,44
D11	0	285.18 g/mol	0.00 g/mol	0
E11	0	285.18 g/mol	0.00 g/mol	0
F11	0	391.10 g/mol	0.00 g/mol	0
G11	0	255.12 g/mol	0.00 g/mol	0
H11	0	281.10 g/mol	0.00 g/mol	0
A12	0	376.26 g/mol	0.00 g/mol	0
B12	0	290.17 g/mol	0.00 g/mol	0
C12	0	248.11 g/mol	0.00 g/mol	0
D12	0	304.19 g/mol	0.00 g/mol	0
E12	0	304.19 g/mol	0.00 g/mol	0
F12	0	410.11 g/mol	0.00 g/mol	0
G12	0	274.13 g/mol	0.00 g/mol	0
H12	0	300.12 g/mol	0.00 g/mol	0

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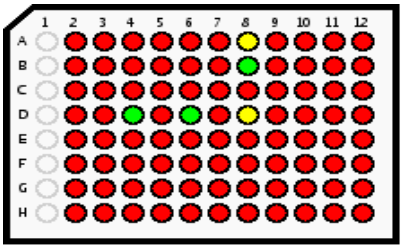
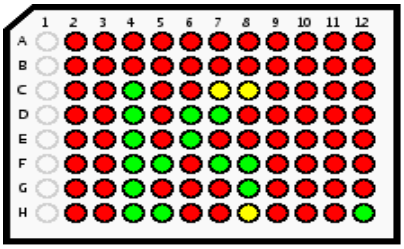


Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	291.21 g/mol	0.00 g/mol	0
B2	0	291.21 g/mol	0.00 g/mol	0
C2	0	294.26 g/mol	0.00 g/mol	0
D2	0	292.20 g/mol	0.00 g/mol	0
E2	0	311.29 g/mol	0.00 g/mol	0
F2	0	334.20 g/mol	0.00 g/mol	0
G2	0	346.25 g/mol	0.00 g/mol	0
H2	0	286.21 g/mol	0.00 g/mol	0
A3	0	321.18 g/mol	0.00 g/mol	0
B3	0	321.18 g/mol	0.00 g/mol	0
C3	0	324.22 g/mol	0.00 g/mol	0
D3	0	322.17 g/mol	0.00 g/mol	0
E3	0	341.26 g/mol	0.00 g/mol	0
F3	0	364.17 g/mol	0.00 g/mol	0
G3	0	376.22 g/mol	0.00 g/mol	0
H3	0	316.17 g/mol	0.00 g/mol	0
A4	0	303.21 g/mol	0.00 g/mol	0
B4	0	303.21 g/mol	0.00 g/mol	0
C4	0	306.25 g/mol	0.00 g/mol	0
D4	0,02	304.20 g/mol	304.28 g/mol	537,62
E4	0	323.29 g/mol	0.00 g/mol	0
F4	0	346.20 g/mol	0.00 g/mol	0
G4	0	358.25 g/mol	0.00 g/mol	0
H4	0	298.20 g/mol	0.00 g/mol	0
A5	0	264.12 g/mol	0.00 g/mol	0
B5	0	264.12 g/mol	0.00 g/mol	0
C5	0	267.16 g/mol	0.00 g/mol	0
D5	0	265.11 g/mol	0.00 g/mol	0
E5	0	284.20 g/mol	0.00 g/mol	0
F5	0	307.11 g/mol	0.00 g/mol	0
G5	0	319.16 g/mol	0.00 g/mol	0
H5	0	259.11 g/mol	0.00 g/mol	0
A6	0	281.14 g/mol	0.00 g/mol	0
B6	0	281.14 g/mol	0.00 g/mol	0
C6	0	284.19 g/mol	0.00 g/mol	0
D6	0,02	282.13 g/mol	282.20 g/mol	401,37
E6	0	301.22 g/mol	0.00 g/mol	0
F6	0	324.13 g/mol	0.00 g/mol	0
G6	0	336.18 g/mol	0.00 g/mol	0
H6	0	276.14 g/mol	0.00 g/mol	0

A7	0	273.11 g/mol	0.00 g/mol	0
B7	0	273.11 g/mol	0.00 g/mol	0
C7	0	276.16 g/mol	0.00 g/mol	0
D7	0	274.11 g/mol	0.00 g/mol	0
E7	0	293.19 g/mol	0.00 g/mol	0
F7	0	316.11 g/mol	0.00 g/mol	0
G7	0	328.15 g/mol	0.00 g/mol	0
H7	0	268.11 g/mol	0.00 g/mol	0
A8	0	293.14 g/mol	293.14 g/mol	33,45
B8	0,04	293.14 g/mol	293.13 g/mol	678,44
C8	0	296.18 g/mol	0.00 g/mol	0
D8	0	294.13 g/mol	294.22 g/mol	72,88
E8	0	313.22 g/mol	0.00 g/mol	0
F8	0	336.13 g/mol	0.00 g/mol	0
G8	0	348.18 g/mol	0.00 g/mol	0
H8	0	288.14 g/mol	0.00 g/mol	0
A9	0	323.11 g/mol	0.00 g/mol	0
B9	0	323.11 g/mol	0.00 g/mol	0
C9	0	326.15 g/mol	0.00 g/mol	0
D9	0	324.10 g/mol	0.00 g/mol	0
E9	0	343.19 g/mol	0.00 g/mol	0
F9	0	366.10 g/mol	0.00 g/mol	0
G9	0	378.15 g/mol	0.00 g/mol	0
H9	0	318.10 g/mol	0.00 g/mol	0
A10	0	295.11 g/mol	0.00 g/mol	0
B10	0	295.11 g/mol	0.00 g/mol	0
C10	0	298.16 g/mol	0.00 g/mol	0
D10	0	296.11 g/mol	0.00 g/mol	0
E10	0	315.19 g/mol	0.00 g/mol	0
F10	0	338.11 g/mol	0.00 g/mol	0
G10	0	350.15 g/mol	0.00 g/mol	0
H10	0	290.11 g/mol	0.00 g/mol	0
A11	0	280.11 g/mol	0.00 g/mol	0
B11	0	280.11 g/mol	0.00 g/mol	0
C11	0	283.16 g/mol	0.00 g/mol	0
D11	0	281.10 g/mol	0.00 g/mol	0
E11	0	300.19 g/mol	0.00 g/mol	0
F11	0	323.11 g/mol	0.00 g/mol	0
G11	0	335.15 g/mol	0.00 g/mol	0
H11	0	275.11 g/mol	0.00 g/mol	0
A12	0	299.12 g/mol	0.00 g/mol	0
B12	0	299.12 g/mol	0.00 g/mol	0
C12	0	302.17 g/mol	0.00 g/mol	0
D12	0	300.12 g/mol	0.00 g/mol	0
E12	0	319.20 g/mol	0.00 g/mol	0
F12	0	342.12 g/mol	0.00 g/mol	0
G12	0	354.16 g/mol	0.00 g/mol	0
H12	0	294.12 g/mol	0.00 g/mol	0

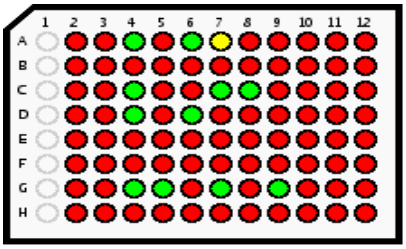
ori.hya.18



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	351.33 g/mol	0.00 g/mol	0
B2	0	265.23 g/mol	0.00 g/mol	0
C2	0	223.18 g/mol	0.00 g/mol	0
D2	0	279.25 g/mol	0.00 g/mol	0
E2	0	279.25 g/mol	0.00 g/mol	0
F2	0	385.17 g/mol	0.00 g/mol	0
G2	0	249.19 g/mol	0.00 g/mol	0
H2	0	275.18 g/mol	0.00 g/mol	0
A3	0	381.29 g/mol	0.00 g/mol	0
B3	0	295.20 g/mol	0.00 g/mol	0
C3	0	253.14 g/mol	0.00 g/mol	0
D3	0	309.22 g/mol	0.00 g/mol	0
E3	0	309.22 g/mol	0.00 g/mol	0
F3	0	415.14 g/mol	0.00 g/mol	0
G3	0	279.16 g/mol	0.00 g/mol	0
H3	0	305.15 g/mol	0.00 g/mol	0
A4	0	363.33 g/mol	0.00 g/mol	0
B4	0	277.23 g/mol	0.00 g/mol	0
C4	0	235.18 g/mol	235.12 g/mol	2092,15
D4	0,04	291.25 g/mol	291.27 g/mol	3520,17
E4	0,02	291.25 g/mol	291.28 g/mol	618,88
F4	0	397.17 g/mol	397.12 g/mol	1689,06
G4	0	261.19 g/mol	261.22 g/mol	2079,64
H4	0,08	287.18 g/mol	287.22 g/mol	4718,07
A5	0	324.23 g/mol	0.00 g/mol	0
B5	0	238.14 g/mol	0.00 g/mol	0
C5	0	196.08 g/mol	0.00 g/mol	0
D5	0	252.16 g/mol	0.00 g/mol	0
E5	0	252.16 g/mol	0.00 g/mol	0
F5	0,02	358.08 g/mol	358.16 g/mol	465,98
G5	0	222.10 g/mol	0.00 g/mol	0
H5	0	248.09 g/mol	248.15 g/mol	188,84
A6	0	341.26 g/mol	0.00 g/mol	0
B6	0	255.16 g/mol	0.00 g/mol	0
C6	0	213.11 g/mol	0.00 g/mol	0
D6	0	269.18 g/mol	269.22 g/mol	1435,49
E6	0,02	269.18 g/mol	269.22 g/mol	655,95
F6	0	375.11 g/mol	0.00 g/mol	0
G6	0	239.12 g/mol	0.00 g/mol	0
H6	0	265.11 g/mol	0.00 g/mol	0

A7	0	333.23 g/mol	0.00 g/mol	0
B7	0	247.14 g/mol	0.00 g/mol	0
C7	0	205.08 g/mol	205.13 g/mol	55,89
D7	0	261.16 g/mol	261.20 g/mol	186,64
E7	0	261.16 g/mol	0.00 g/mol	0
F7	0,02	367.08 g/mol	367.16 g/mol	613,28
G7	0	231.10 g/mol	0.00 g/mol	0
H7	0	257.09 g/mol	0.00 g/mol	0
A8	0	353.26 g/mol	0.00 g/mol	0
B8	0	267.16 g/mol	0.00 g/mol	0
C8	0	225.11 g/mol	225.16 g/mol	50,92
D8	0	281.18 g/mol	0.00 g/mol	0
E8	0	281.18 g/mol	0.00 g/mol	0
F8	0	387.10 g/mol	387.19 g/mol	157,65
G8	0,02	251.12 g/mol	251.16 g/mol	545,1
H8	0	277.11 g/mol	277.17 g/mol	53,86
A9	0	383.22 g/mol	0.00 g/mol	0
B9	0	297.13 g/mol	0.00 g/mol	0
C9	0	255.07 g/mol	0.00 g/mol	0
D9	0	311.15 g/mol	0.00 g/mol	0
E9	0	311.15 g/mol	0.00 g/mol	0
F9	0	417.07 g/mol	0.00 g/mol	0
G9	0	281.09 g/mol	0.00 g/mol	0
H9	0	307.08 g/mol	0.00 g/mol	0
A10	0	355.23 g/mol	0.00 g/mol	0
B10	0	269.14 g/mol	0.00 g/mol	0
C10	0	227.08 g/mol	0.00 g/mol	0
D10	0	283.16 g/mol	0.00 g/mol	0
E10	0	283.16 g/mol	0.00 g/mol	0
F10	0	389.08 g/mol	0.00 g/mol	0
G10	0	253.10 g/mol	0.00 g/mol	0
H10	0	279.08 g/mol	0.00 g/mol	0
A11	0	340.23 g/mol	0.00 g/mol	0
B11	0	254.14 g/mol	0.00 g/mol	0
C11	0	212.08 g/mol	0.00 g/mol	0
D11	0	268.16 g/mol	0.00 g/mol	0
E11	0	268.16 g/mol	0.00 g/mol	0
F11	0	374.08 g/mol	0.00 g/mol	0
G11	0	238.10 g/mol	0.00 g/mol	0
H11	0	264.08 g/mol	0.00 g/mol	0
A12	0	359.24 g/mol	0.00 g/mol	0
B12	0	273.15 g/mol	0.00 g/mol	0
C12	0	231.09 g/mol	0.00 g/mol	0
D12	0	287.17 g/mol	0.00 g/mol	0
E12	0	287.17 g/mol	0.00 g/mol	0
F12	0	393.09 g/mol	0.00 g/mol	0
G12	0	257.11 g/mol	0.00 g/mol	0
H12	0,02	283.09 g/mol	283.15 g/mol	459,6

ori.hya.19

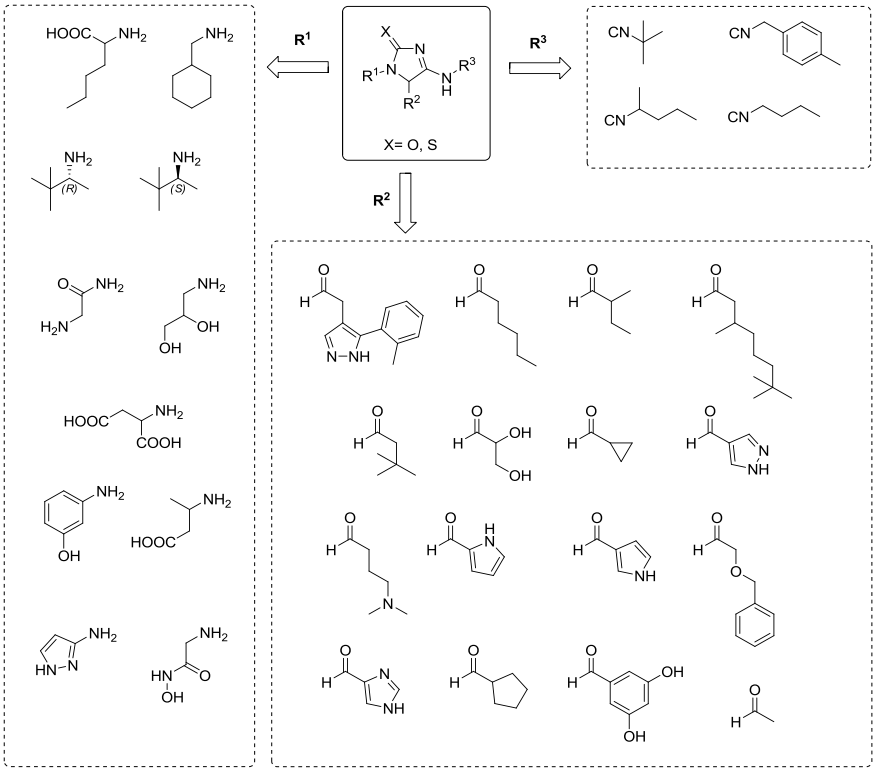


Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	274.19 g/mol	0.00 g/mol	0
B2	0	274.19 g/mol	0.00 g/mol	0
C2	0	277.23 g/mol	0.00 g/mol	0
D2	0	275.18 g/mol	0.00 g/mol	0
E2	0	294.27 g/mol	0.00 g/mol	0
F2	0	317.18 g/mol	0.00 g/mol	0
G2	0	329.23 g/mol	0.00 g/mol	0
H2	0	269.18 g/mol	0.00 g/mol	0
A3	0	304.16 g/mol	0.00 g/mol	0
B3	0	304.16 g/mol	0.00 g/mol	0
C3	0	307.20 g/mol	0.00 g/mol	0
D3	0	305.15 g/mol	0.00 g/mol	0
E3	0	324.23 g/mol	0.00 g/mol	0
F3	0	347.15 g/mol	0.00 g/mol	0
G3	0	359.20 g/mol	0.00 g/mol	0
H3	0	299.15 g/mol	0.00 g/mol	0
A4	0	286.19 g/mol	286.24 g/mol	457,09
B4	0	286.19 g/mol	0.00 g/mol	0
C4	0	289.23 g/mol	289.29 g/mol	223,92
D4	0	287.18 g/mol	287.23 g/mol	4371,62
E4	0	306.27 g/mol	0.00 g/mol	0
F4	0	329.18 g/mol	0.00 g/mol	0
G4	0,08	341.23 g/mol	341.27 g/mol	5304,19
H4	0	281.18 g/mol	0.00 g/mol	0
A5	0	247.10 g/mol	0.00 g/mol	0
B5	0	247.10 g/mol	0.00 g/mol	0
C5	0	250.14 g/mol	0.00 g/mol	0
D5	0	248.09 g/mol	0.00 g/mol	0
E5	0	267.18 g/mol	0.00 g/mol	0
F5	0	290.09 g/mol	0.00 g/mol	0
G5	0	302.14 g/mol	302.19 g/mol	1872,48
H5	0	242.09 g/mol	0.00 g/mol	0
A6	0,02	264.12 g/mol	264.11 g/mol	375,81
B6	0	264.12 g/mol	0.00 g/mol	0
C6	0	267.16 g/mol	0.00 g/mol	0
D6	0	265.11 g/mol	265.17 g/mol	429,03
E6	0	284.20 g/mol	0.00 g/mol	0
F6	0	307.11 g/mol	307.16 g/mol	26,76
G6	0	319.16 g/mol	0.00 g/mol	0
H6	0	259.11 g/mol	0.00 g/mol	0

A7	0	256.09 g/mol	256.16 g/mol	77,46
B7	0	256.09 g/mol	0.00 g/mol	0
C7	0	259.14 g/mol	259.18 g/mol	359,82
D7	0	257.09 g/mol	0.00 g/mol	0
E7	0	276.17 g/mol	0.00 g/mol	0
F7	0	299.09 g/mol	0.00 g/mol	0
G7	0,02	311.13 g/mol	311.20 g/mol	617,77
H7	0	251.09 g/mol	0.00 g/mol	0
A8	0	276.12 g/mol	0.00 g/mol	0
B8	0	276.12 g/mol	0.00 g/mol	0
C8	0,06	279.16 g/mol	279.21 g/mol	1165,51
D8	0	277.11 g/mol	0.00 g/mol	0
E8	0	296.20 g/mol	0.00 g/mol	0
F8	0	319.11 g/mol	0.00 g/mol	0
G8	0	331.16 g/mol	0.00 g/mol	0
H8	0	271.11 g/mol	0.00 g/mol	0
A9	0	306.09 g/mol	0.00 g/mol	0
B9	0	306.09 g/mol	0.00 g/mol	0
C9	0	309.13 g/mol	0.00 g/mol	0
D9	0	307.08 g/mol	0.00 g/mol	0
E9	0	326.16 g/mol	0.00 g/mol	0
F9	0	349.08 g/mol	0.00 g/mol	0
G9	0	361.13 g/mol	361.20 g/mol	198,37
H9	0	301.08 g/mol	0.00 g/mol	0
A10	0	278.09 g/mol	0.00 g/mol	0
B10	0	278.09 g/mol	0.00 g/mol	0
C10	0	281.14 g/mol	0.00 g/mol	0
D10	0	279.08 g/mol	0.00 g/mol	0
E10	0	298.17 g/mol	0.00 g/mol	0
F10	0	321.09 g/mol	0.00 g/mol	0
G10	0	333.13 g/mol	0.00 g/mol	0
H10	0	273.09 g/mol	0.00 g/mol	0
A11	0	263.09 g/mol	0.00 g/mol	0
B11	0	263.09 g/mol	0.00 g/mol	0
C11	0	266.14 g/mol	0.00 g/mol	0
D11	0	264.08 g/mol	0.00 g/mol	0
E11	0	283.17 g/mol	0.00 g/mol	0
F11	0	306.09 g/mol	0.00 g/mol	0
G11	0	318.13 g/mol	0.00 g/mol	0
H11	0	258.09 g/mol	0.00 g/mol	0
A12	0	282.10 g/mol	0.00 g/mol	0
B12	0	282.10 g/mol	0.00 g/mol	0
C12	0	285.15 g/mol	0.00 g/mol	0
D12	0	283.09 g/mol	283.17 g/mol	18,56
E12	0	302.18 g/mol	0.00 g/mol	0
F12	0	325.10 g/mol	0.00 g/mol	0
G12	0	337.14 g/mol	0.00 g/mol	0
H12	0	277.10 g/mol	0.00 g/mol	0

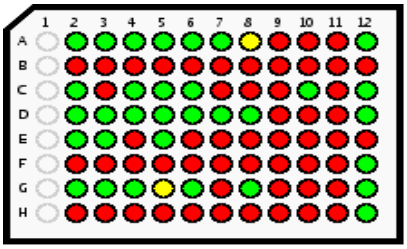
B.4 Screening plates ori.hya.20-35

B.4.1 Starting materials of plates ori.hya.20-35



B.4.2 Mass spectral analysis of plates ori.hya.20-35

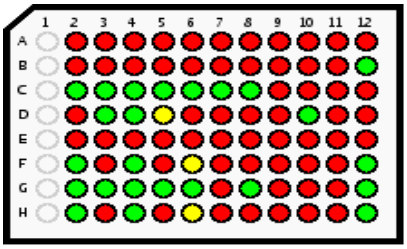
ori.hya.20



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,1	382.42 g/mol	382.44 g/mol	11084,64
B2	0	296.33 g/mol	0.00 g/mol	0
C2	0,1	254.27 g/mol	254.29 g/mol	7765,74
D2	0,12	310.35 g/mol	310.37 g/mol	7343,7
E2	0	310.35 g/mol	310.37 g/mol	438,98
F2	0	416.27 g/mol	0.00 g/mol	0
G2	0	280.29 g/mol	280.32 g/mol	551,19
H2	0	306.28 g/mol	0.00 g/mol	0
A3	0	412.39 g/mol	412.43 g/mol	203,38
B3	0	326.30 g/mol	0.00 g/mol	0
C3	0	284.24 g/mol	0.00 g/mol	0
D3	0	340.32 g/mol	340.35 g/mol	184,03
E3	0	340.32 g/mol	340.35 g/mol	194,14
F3	0	446.24 g/mol	0.00 g/mol	0
G3	0,04	310.26 g/mol	310.22 g/mol	1581,98
H3	0	336.25 g/mol	0.00 g/mol	0
A4	0,12	394.42 g/mol	394.44 g/mol	16202,64
B4	0	308.33 g/mol	0.00 g/mol	0
C4	0,1	266.27 g/mol	266.19 g/mol	6322,5
D4	0,12	322.35 g/mol	322.37 g/mol	6463,62
E4	0	322.35 g/mol	0.00 g/mol	0
F4	0	428.27 g/mol	0.00 g/mol	0
G4	0,06	292.29 g/mol	292.32 g/mol	1402,27
H4	0	318.28 g/mol	0.00 g/mol	0
A5	0,12	355.33 g/mol	355.36 g/mol	6249,72
B5	0	269.24 g/mol	0.00 g/mol	0
C5	0,02	227.18 g/mol	227.22 g/mol	295,68
D5	0,04	283.26 g/mol	283.29 g/mol	3091,91
E5	0,02	283.26 g/mol	283.29 g/mol	2389,84
F5	0	389.18 g/mol	0.00 g/mol	0
G5	0	253.20 g/mol	253.24 g/mol	54,74
H5	0	279.19 g/mol	0.00 g/mol	0
A6	0,08	372.35 g/mol	372.38 g/mol	8201,52
B6	0	286.26 g/mol	0.00 g/mol	0
C6	0,04	244.20 g/mol	244.16 g/mol	990,71
D6	0,08	300.28 g/mol	300.34 g/mol	9002,04
E6	0	300.28 g/mol	0.00 g/mol	0
F6	0	406.20 g/mol	0.00 g/mol	0
G6	0,02	270.22 g/mol	270.26 g/mol	355,9
H6	0	296.21 g/mol	0.00 g/mol	0

A7	0,02	364.33 g/mol	364.34 g/mol	625,38
B7	0	278.24 g/mol	0.00 g/mol	0
C7	0	236.18 g/mol	0.00 g/mol	0
D7	0	292.26 g/mol	292.28 g/mol	232,54
E7	0	292.26 g/mol	0.00 g/mol	0
F7	0	398.18 g/mol	0.00 g/mol	0
G7	0	262.20 g/mol	262.29 g/mol	12,51
H7	0	288.18 g/mol	0.00 g/mol	0
A8	0	384.35 g/mol	384.38 g/mol	53,6
B8	0	298.26 g/mol	0.00 g/mol	0
C8	0	256.20 g/mol	256.24 g/mol	12,32
D8	0,02	312.28 g/mol	312.31 g/mol	685,68
E8	0	312.28 g/mol	0.00 g/mol	0
F8	0	418.20 g/mol	0.00 g/mol	0
G8	0	282.22 g/mol	282.26 g/mol	310,11
H8	0	308.21 g/mol	0.00 g/mol	0
A9	0	414.32 g/mol	0.00 g/mol	0
B9	0	328.23 g/mol	0.00 g/mol	0
C9	0	286.17 g/mol	0.00 g/mol	0
D9	0	342.25 g/mol	0.00 g/mol	0
E9	0	342.25 g/mol	0.00 g/mol	0
F9	0	448.17 g/mol	0.00 g/mol	0
G9	0	312.19 g/mol	0.00 g/mol	0
H9	0	338.18 g/mol	0.00 g/mol	0
A10	0	386.33 g/mol	0.00 g/mol	0
B10	0	300.24 g/mol	0.00 g/mol	0
C10	0,02	258.18 g/mol	258.22 g/mol	347,63
D10	0	314.26 g/mol	0.00 g/mol	0
E10	0	314.26 g/mol	0.00 g/mol	0
F10	0	420.18 g/mol	0.00 g/mol	0
G10	0	284.20 g/mol	0.00 g/mol	0
H10	0	310.18 g/mol	0.00 g/mol	0
A11	0	371.33 g/mol	0.00 g/mol	0
B11	0	285.23 g/mol	0.00 g/mol	0
C11	0	243.18 g/mol	0.00 g/mol	0
D11	0	299.25 g/mol	0.00 g/mol	0
E11	0	299.25 g/mol	0.00 g/mol	0
F11	0	405.18 g/mol	0.00 g/mol	0
G11	0	269.20 g/mol	0.00 g/mol	0
H11	0	295.18 g/mol	0.00 g/mol	0
A12	0	390.34 g/mol	390.38 g/mol	212,13
B12	0	304.25 g/mol	0.00 g/mol	0
C12	0,02	262.19 g/mol	262.23 g/mol	2266,35
D12	0,1	318.27 g/mol	318.30 g/mol	8497,68
E12	0	318.27 g/mol	0.00 g/mol	0
F12	0	424.19 g/mol	424.26 g/mol	5447,18
G12	0,02	288.21 g/mol	288.25 g/mol	26593,02
H12	0	314.19 g/mol	314.24 g/mol	2666,64

ori.hya.21



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	305.28 g/mol	0.00 g/mol	0
B2	0	305.28 g/mol	0.00 g/mol	0
C2	0,21	308.33 g/mol	308.34 g/mol	6444,96
D2	0	306.28 g/mol	0.00 g/mol	0
E2	0	325.36 g/mol	0.00 g/mol	0
F2	0,04	348.28 g/mol	348.30 g/mol	2870,14
G2	0,16	360.32 g/mol	360.34 g/mol	8271,12
H2	0,1	300.28 g/mol	300.29 g/mol	11570,82
A3	0	335.25 g/mol	0.00 g/mol	0
B3	0	335.25 g/mol	0.00 g/mol	0
C3	0	338.30 g/mol	338.32 g/mol	356,43
D3	0,08	336.25 g/mol	336.27 g/mol	5556,1
E3	0	355.33 g/mol	0.00 g/mol	0
F3	0	378.25 g/mol	0.00 g/mol	0
G3	0	390.29 g/mol	390.32 g/mol	442,03
H3	0	330.25 g/mol	0.00 g/mol	0
A4	0	317.28 g/mol	0.00 g/mol	0
B4	0	317.28 g/mol	0.00 g/mol	0
C4	0,1	320.33 g/mol	320.34 g/mol	22660,74
D4	0	318.28 g/mol	318.31 g/mol	186,99
E4	0	337.36 g/mol	0.00 g/mol	0
F4	0,06	360.28 g/mol	360.31 g/mol	4412,08
G4	0,12	372.32 g/mol	372.35 g/mol	13107,84
H4	0,04	312.28 g/mol	312.30 g/mol	3566,95
A5	0	278.19 g/mol	0.00 g/mol	0
B5	0	278.19 g/mol	0.00 g/mol	0
C5	0,1	281.24 g/mol	281.25 g/mol	6343,86
D5	0	279.19 g/mol	279.22 g/mol	33,72
E5	0	298.27 g/mol	0.00 g/mol	0
F5	0	321.19 g/mol	0.00 g/mol	0
G5	0	333.23 g/mol	333.27 g/mol	4363,86
H5	0	273.19 g/mol	0.00 g/mol	0
A6	0	295.21 g/mol	0.00 g/mol	0
B6	0	295.21 g/mol	0.00 g/mol	0
C6	0,12	298.26 g/mol	298.28 g/mol	5646,16
D6	0	296.21 g/mol	0.00 g/mol	0
E6	0	315.29 g/mol	0.00 g/mol	0
F6	0	338.21 g/mol	338.26 g/mol	56,11
G6	0,08	350.25 g/mol	350.29 g/mol	17088,96
H6	0	290.21 g/mol	290.24 g/mol	49,03

A7	0	287.19 g/mol	0.00 g/mol	0
B7	0	287.19 g/mol	0.00 g/mol	0
C7	0,02	290.24 g/mol	290.27 g/mol	442,31
D7	0	288.18 g/mol	0.00 g/mol	0
E7	0	307.27 g/mol	0.00 g/mol	0
F7	0	330.19 g/mol	0.00 g/mol	0
G7	0	342.23 g/mol	0.00 g/mol	0
H7	0	282.19 g/mol	0.00 g/mol	0
A8	0	307.21 g/mol	0.00 g/mol	0
B8	0	307.21 g/mol	0.00 g/mol	0
C8	0,02	310.26 g/mol	310.29 g/mol	527,6
D8	0	308.21 g/mol	0.00 g/mol	0
E8	0	327.29 g/mol	0.00 g/mol	0
F8	0	350.21 g/mol	0.00 g/mol	0
G8	0,02	362.25 g/mol	362.29 g/mol	4408,94
H8	0	302.21 g/mol	0.00 g/mol	0
A9	0	337.18 g/mol	0.00 g/mol	0
B9	0	337.18 g/mol	0.00 g/mol	0
C9	0	340.23 g/mol	0.00 g/mol	0
D9	0	338.18 g/mol	0.00 g/mol	0
E9	0	357.26 g/mol	0.00 g/mol	0
F9	0	380.18 g/mol	0.00 g/mol	0
G9	0	392.22 g/mol	0.00 g/mol	0
H9	0	332.18 g/mol	0.00 g/mol	0
A10	0	309.19 g/mol	0.00 g/mol	0
B10	0	309.19 g/mol	0.00 g/mol	0
C10	0	312.24 g/mol	0.00 g/mol	0
D10	0,02	310.18 g/mol	310.25 g/mol	369,57
E10	0	329.27 g/mol	0.00 g/mol	0
F10	0	352.18 g/mol	0.00 g/mol	0
G10	0	364.23 g/mol	0.00 g/mol	0
H10	0	304.19 g/mol	0.00 g/mol	0
A11	0	294.19 g/mol	0.00 g/mol	0
B11	0	294.19 g/mol	0.00 g/mol	0
C11	0	297.23 g/mol	0.00 g/mol	0
D11	0	295.18 g/mol	0.00 g/mol	0
E11	0	314.27 g/mol	0.00 g/mol	0
F11	0	337.18 g/mol	0.00 g/mol	0
G11	0	349.23 g/mol	0.00 g/mol	0
H11	0	289.18 g/mol	0.00 g/mol	0
A12	0	313.20 g/mol	0.00 g/mol	0
B12	0	313.20 g/mol	313.25 g/mol	207,65
C12	0	316.25 g/mol	0.00 g/mol	0
D12	0	314.19 g/mol	314.25 g/mol	14,35
E12	0	333.28 g/mol	0.00 g/mol	0
F12	0,08	356.19 g/mol	356.25 g/mol	5108,36
G12	0,16	368.24 g/mol	368.29 g/mol	16250,04
H12	0,02	308.20 g/mol	308.23 g/mol	389,73

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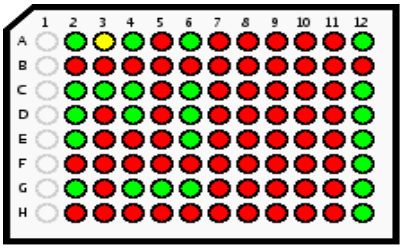

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,08	450.36 g/mol	450.39 g/mol	5300,26
B2	0	364.27 g/mol	0.00 g/mol	0
C2	0,02	322.21 g/mol	322.25 g/mol	3691,85
D2	0,08	378.29 g/mol	378.32 g/mol	6337,5
E2	0,02	378.29 g/mol	378.36 g/mol	636,86
F2	0	484.21 g/mol	0.00 g/mol	0
G2	0	348.23 g/mol	348.28 g/mol	123,36
H2	0	374.21 g/mol	0.00 g/mol	0
A3	0	480.33 g/mol	480.39 g/mol	73,9
B3	0	394.24 g/mol	0.00 g/mol	0
C3	0,02	352.18 g/mol	352.23 g/mol	626,03
D3	0	408.26 g/mol	0.00 g/mol	0
E3	0	408.26 g/mol	0.00 g/mol	0
F3	0	514.18 g/mol	0.00 g/mol	0
G3	0	378.20 g/mol	0.00 g/mol	0
H3	0	404.18 g/mol	0.00 g/mol	0
A4	0,04	462.36 g/mol	462.40 g/mol	8047,62
B4	0	376.27 g/mol	0.00 g/mol	0
C4	0	334.21 g/mol	334.26 g/mol	347,01
D4	0,02	390.29 g/mol	390.32 g/mol	7740,06
E4	0	390.29 g/mol	0.00 g/mol	0
F4	0	496.21 g/mol	0.00 g/mol	0
G4	0,06	360.23 g/mol	360.27 g/mol	1489,34
H4	0	386.21 g/mol	0.00 g/mol	0
A5	0	423.27 g/mol	0.00 g/mol	0
B5	0	337.18 g/mol	0.00 g/mol	0
C5	0	295.12 g/mol	0.00 g/mol	0
D5	0	351.20 g/mol	0.00 g/mol	0
E5	0	351.20 g/mol	0.00 g/mol	0
F5	0	457.12 g/mol	0.00 g/mol	0
G5	0,02	321.14 g/mol	321.21 g/mol	541,35
H5	0	347.12 g/mol	0.00 g/mol	0
A6	0	440.29 g/mol	440.34 g/mol	234,31
B6	0	354.20 g/mol	0.00 g/mol	0
C6	0	312.14 g/mol	312.19 g/mol	362,97
D6	0	368.22 g/mol	368.26 g/mol	3896,12
E6	0	368.22 g/mol	368.27 g/mol	213,02
F6	0	474.14 g/mol	0.00 g/mol	0
G6	0,02	338.16 g/mol	338.21 g/mol	853,99
H6	0	364.14 g/mol	0.00 g/mol	0

A7	0	432.27 g/mol	0.00 g/mol	0
B7	0	346.17 g/mol	0.00 g/mol	0
C7	0	304.12 g/mol	0.00 g/mol	0
D7	0	360.19 g/mol	0.00 g/mol	0
E7	0	360.19 g/mol	0.00 g/mol	0
F7	0	466.12 g/mol	0.00 g/mol	0
G7	0	330.14 g/mol	0.00 g/mol	0
H7	0	356.12 g/mol	0.00 g/mol	0
A8	0	452.29 g/mol	0.00 g/mol	0
B8	0	366.20 g/mol	0.00 g/mol	0
C8	0	324.14 g/mol	0.00 g/mol	0
D8	0	380.22 g/mol	0.00 g/mol	0
E8	0	380.22 g/mol	0.00 g/mol	0
F8	0	486.14 g/mol	0.00 g/mol	0
G8	0	350.16 g/mol	0.00 g/mol	0
H8	0	376.14 g/mol	0.00 g/mol	0
A9	0	482.26 g/mol	0.00 g/mol	0
B9	0	396.17 g/mol	0.00 g/mol	0
C9	0	354.11 g/mol	0.00 g/mol	0
D9	0	410.19 g/mol	0.00 g/mol	0
E9	0	410.19 g/mol	0.00 g/mol	0
F9	0	516.11 g/mol	0.00 g/mol	0
G9	0	380.13 g/mol	0.00 g/mol	0
H9	0	406.11 g/mol	0.00 g/mol	0
A10	0	454.26 g/mol	0.00 g/mol	0
B10	0	368.17 g/mol	0.00 g/mol	0
C10	0	326.11 g/mol	0.00 g/mol	0
D10	0	382.19 g/mol	0.00 g/mol	0
E10	0	382.19 g/mol	0.00 g/mol	0
F10	0	488.11 g/mol	0.00 g/mol	0
G10	0	352.13 g/mol	0.00 g/mol	0
H10	0	378.12 g/mol	0.00 g/mol	0
A11	0	439.26 g/mol	0.00 g/mol	0
B11	0	353.17 g/mol	0.00 g/mol	0
C11	0	311.11 g/mol	0.00 g/mol	0
D11	0	367.19 g/mol	0.00 g/mol	0
E11	0	367.19 g/mol	0.00 g/mol	0
F11	0	473.11 g/mol	0.00 g/mol	0
G11	0	337.13 g/mol	0.00 g/mol	0
H11	0	363.12 g/mol	0.00 g/mol	0
A12	0,04	458.27 g/mol	458.34 g/mol	1460,27
B12	0	372.18 g/mol	0.00 g/mol	0
C12	0,02	330.12 g/mol	330.19 g/mol	671,69
D12	0,02	386.20 g/mol	386.27 g/mol	726,51
E12	0,02	386.20 g/mol	386.27 g/mol	564,88
F12	0	492.12 g/mol	492.22 g/mol	375,7
G12	0,02	356.14 g/mol	356.20 g/mol	12720,12
H12	0,02	382.13 g/mol	382.21 g/mol	695,15

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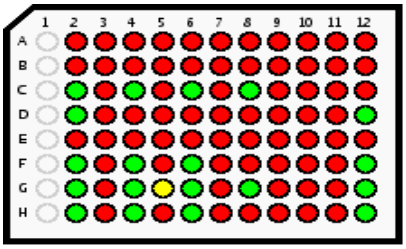

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	373.22 g/mol	0.00 g/mol	0
B2	0	373.22 g/mol	0.00 g/mol	0
C2	0,16	376.27 g/mol	376.31 g/mol	4122,18
D2	0,02	374.21 g/mol	374.27 g/mol	575,96
E2	0	393.30 g/mol	0.00 g/mol	0
F2	0,02	416.22 g/mol	416.28 g/mol	825,06
G2	0	428.26 g/mol	428.31 g/mol	1776,89
H2	0,1	368.22 g/mol	368.27 g/mol	11345,64
A3	0	403.19 g/mol	0.00 g/mol	0
B3	0	403.19 g/mol	0.00 g/mol	0
C3	0	406.24 g/mol	0.00 g/mol	0
D3	0	404.18 g/mol	0.00 g/mol	0
E3	0	423.27 g/mol	0.00 g/mol	0
F3	0	446.18 g/mol	0.00 g/mol	0
G3	0	458.23 g/mol	0.00 g/mol	0
H3	0	398.19 g/mol	0.00 g/mol	0
A4	0	385.22 g/mol	0.00 g/mol	0
B4	0	385.22 g/mol	0.00 g/mol	0
C4	0,1	388.27 g/mol	388.31 g/mol	7059,42
D4	0	386.21 g/mol	0.00 g/mol	0
E4	0	405.30 g/mol	0.00 g/mol	0
F4	0	428.22 g/mol	428.29 g/mol	732,49
G4	0	440.26 g/mol	440.32 g/mol	511,25
H4	0,02	380.22 g/mol	380.27 g/mol	921,13
A5	0	346.13 g/mol	0.00 g/mol	0
B5	0	346.13 g/mol	0.00 g/mol	0
C5	0	349.18 g/mol	0.00 g/mol	0
D5	0	347.12 g/mol	0.00 g/mol	0
E5	0	366.21 g/mol	0.00 g/mol	0
F5	0	389.12 g/mol	0.00 g/mol	0
G5	0	401.17 g/mol	401.25 g/mol	62,47
H5	0	341.13 g/mol	0.00 g/mol	0
A6	0	363.15 g/mol	0.00 g/mol	0
B6	0	363.15 g/mol	0.00 g/mol	0
C6	0,02	366.20 g/mol	366.25 g/mol	4441,91
D6	0	364.14 g/mol	0.00 g/mol	0
E6	0	383.23 g/mol	0.00 g/mol	0
F6	0,04	406.15 g/mol	406.20 g/mol	1054,93
G6	0,02	418.19 g/mol	418.26 g/mol	753,58
H6	0	358.15 g/mol	358.21 g/mol	154,74

A7	0	355.13 g/mol	0.00 g/mol	0
B7	0	355.13 g/mol	0.00 g/mol	0
C7	0	358.17 g/mol	0.00 g/mol	0
D7	0	356.12 g/mol	0.00 g/mol	0
E7	0	375.21 g/mol	0.00 g/mol	0
F7	0	398.12 g/mol	0.00 g/mol	0
G7	0	410.17 g/mol	0.00 g/mol	0
H7	0	350.12 g/mol	0.00 g/mol	0
A8	0	375.15 g/mol	0.00 g/mol	0
B8	0	375.15 g/mol	0.00 g/mol	0
C8	0,02	378.20 g/mol	378.26 g/mol	616,67
D8	0	376.14 g/mol	0.00 g/mol	0
E8	0	395.23 g/mol	0.00 g/mol	0
F8	0	418.15 g/mol	0.00 g/mol	0
G8	0,02	430.19 g/mol	430.27 g/mol	667,03
H8	0	370.15 g/mol	0.00 g/mol	0
A9	0	405.12 g/mol	0.00 g/mol	0
B9	0	405.12 g/mol	0.00 g/mol	0
C9	0	408.17 g/mol	0.00 g/mol	0
D9	0	406.11 g/mol	0.00 g/mol	0
E9	0	425.20 g/mol	0.00 g/mol	0
F9	0	448.11 g/mol	0.00 g/mol	0
G9	0	460.16 g/mol	0.00 g/mol	0
H9	0	400.12 g/mol	0.00 g/mol	0
A10	0	377.13 g/mol	0.00 g/mol	0
B10	0	377.13 g/mol	0.00 g/mol	0
C10	0	380.17 g/mol	0.00 g/mol	0
D10	0	378.12 g/mol	0.00 g/mol	0
E10	0	397.20 g/mol	0.00 g/mol	0
F10	0	420.12 g/mol	0.00 g/mol	0
G10	0	432.17 g/mol	0.00 g/mol	0
H10	0	372.12 g/mol	0.00 g/mol	0
A11	0	362.12 g/mol	0.00 g/mol	0
B11	0	362.12 g/mol	0.00 g/mol	0
C11	0	365.17 g/mol	0.00 g/mol	0
D11	0	363.12 g/mol	0.00 g/mol	0
E11	0	382.20 g/mol	0.00 g/mol	0
F11	0	405.12 g/mol	0.00 g/mol	0
G11	0	417.16 g/mol	0.00 g/mol	0
H11	0	357.12 g/mol	0.00 g/mol	0
A12	0	381.14 g/mol	0.00 g/mol	0
B12	0	381.14 g/mol	0.00 g/mol	0
C12	0	384.18 g/mol	0.00 g/mol	0
D12	0,02	382.13 g/mol	382.21 g/mol	752,86
E12	0	401.21 g/mol	0.00 g/mol	0
F12	0	424.13 g/mol	424.23 g/mol	183,51
G12	0,06	436.17 g/mol	436.25 g/mol	9209,34
H12	0	376.13 g/mol	376.21 g/mol	134,95

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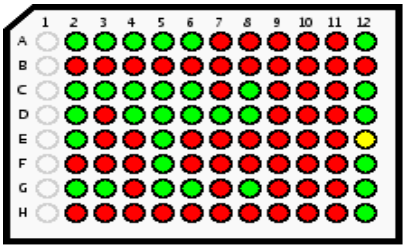

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,12	382.42 g/mol	382.43 g/mol	8462,28
B2	0	296.33 g/mol	0.00 g/mol	0
C2	0,06	254.27 g/mol	254.28 g/mol	8992,44
D2	0,08	310.35 g/mol	310.36 g/mol	6986,1
E2	0,06	310.35 g/mol	310.36 g/mol	1303,33
F2	0	416.27 g/mol	0.00 g/mol	0
G2	0	280.29 g/mol	280.32 g/mol	109,06
H2	0	306.28 g/mol	0.00 g/mol	0
A3	0,02	412.39 g/mol	412.42 g/mol	746,15
B3	0	326.30 g/mol	0.00 g/mol	0
C3	0	284.24 g/mol	284.27 g/mol	496,71
D3	0	340.32 g/mol	0.00 g/mol	0
E3	0	340.32 g/mol	0.00 g/mol	0
F3	0	446.24 g/mol	0.00 g/mol	0
G3	0,02	310.26 g/mol	310.29 g/mol	823,96
H3	0	336.25 g/mol	0.00 g/mol	0
A4	0,08	394.42 g/mol	394.44 g/mol	12839,28
B4	0	308.33 g/mol	0.00 g/mol	0
C4	0,1	266.27 g/mol	266.29 g/mol	2163
D4	0,1	322.35 g/mol	322.37 g/mol	2220,32
E4	0	322.35 g/mol	0.00 g/mol	0
F4	0	428.27 g/mol	0.00 g/mol	0
G4	0	292.29 g/mol	0.00 g/mol	0
H4	0	318.28 g/mol	0.00 g/mol	0
A5	0,04	355.33 g/mol	355.37 g/mol	1176,63
B5	0	269.24 g/mol	0.00 g/mol	0
C5	0,04	227.18 g/mol	227.23 g/mol	771,11
D5	0,04	283.26 g/mol	283.29 g/mol	1168,93
E5	0	283.26 g/mol	283.29 g/mol	353,15
F5	0,06	389.18 g/mol	389.25 g/mol	1572,05
G5	0	253.20 g/mol	253.25 g/mol	284,68
H5	0	279.19 g/mol	279.24 g/mol	23,77
A6	0,1	372.35 g/mol	372.38 g/mol	8002,26
B6	0	286.26 g/mol	0.00 g/mol	0
C6	0	244.20 g/mol	244.24 g/mol	135,22
D6	0,1	300.28 g/mol	300.31 g/mol	5835,16
E6	0	300.28 g/mol	0.00 g/mol	0
F6	0	406.20 g/mol	0.00 g/mol	0
G6	0,02	270.22 g/mol	270.27 g/mol	497,46
H6	0	296.21 g/mol	0.00 g/mol	0

A7	0	364.33 g/mol	0.00 g/mol	0
B7	0	278.24 g/mol	0.00 g/mol	0
C7	0	236.18 g/mol	0.00 g/mol	0
D7	0	292.26 g/mol	292.27 g/mol	154,57
E7	0	292.26 g/mol	0.00 g/mol	0
F7	0	398.18 g/mol	0.00 g/mol	0
G7	0	262.20 g/mol	0.00 g/mol	0
H7	0	288.18 g/mol	0.00 g/mol	0
A8	0	384.35 g/mol	0.00 g/mol	0
B8	0	298.26 g/mol	0.00 g/mol	0
C8	0,02	256.20 g/mol	256.24 g/mol	346,62
D8	0	312.28 g/mol	312.31 g/mol	420,43
E8	0	312.28 g/mol	0.00 g/mol	0
F8	0	418.20 g/mol	0.00 g/mol	0
G8	0	282.22 g/mol	282.26 g/mol	206,61
H8	0	308.21 g/mol	0.00 g/mol	0
A9	0	414.32 g/mol	0.00 g/mol	0
B9	0	328.23 g/mol	0.00 g/mol	0
C9	0	286.17 g/mol	0.00 g/mol	0
D9	0	342.25 g/mol	0.00 g/mol	0
E9	0	342.25 g/mol	0.00 g/mol	0
F9	0	448.17 g/mol	0.00 g/mol	0
G9	0	312.19 g/mol	0.00 g/mol	0
H9	0	338.18 g/mol	0.00 g/mol	0
A10	0	386.33 g/mol	0.00 g/mol	0
B10	0	300.24 g/mol	0.00 g/mol	0
C10	0	258.18 g/mol	0.00 g/mol	0
D10	0	314.26 g/mol	0.00 g/mol	0
E10	0	314.26 g/mol	0.00 g/mol	0
F10	0	420.18 g/mol	0.00 g/mol	0
G10	0	284.20 g/mol	0.00 g/mol	0
H10	0	310.18 g/mol	0.00 g/mol	0
A11	0	371.33 g/mol	0.00 g/mol	0
B11	0	285.23 g/mol	0.00 g/mol	0
C11	0	243.18 g/mol	0.00 g/mol	0
D11	0	299.25 g/mol	0.00 g/mol	0
E11	0	299.25 g/mol	0.00 g/mol	0
F11	0	405.18 g/mol	0.00 g/mol	0
G11	0	269.20 g/mol	0.00 g/mol	0
H11	0	295.18 g/mol	0.00 g/mol	0
A12	0,02	390.34 g/mol	390.30 g/mol	4383,26
B12	0	304.25 g/mol	0.00 g/mol	0
C12	0	262.19 g/mol	262.25 g/mol	8839,14
D12	0,02	318.27 g/mol	318.31 g/mol	813,52
E12	0	318.27 g/mol	318.30 g/mol	66,95
F12	0,02	424.19 g/mol	424.27 g/mol	4230,76
G12	0,16	288.21 g/mol	288.25 g/mol	6028,74
H12	0	314.19 g/mol	314.17 g/mol	178,47

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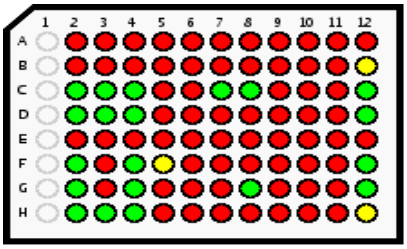

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	305.28 g/mol	0.00 g/mol	0
B2	0	305.28 g/mol	0.00 g/mol	0
C2	0,06	308.33 g/mol	308.35 g/mol	13529,52
D2	0,02	306.28 g/mol	306.32 g/mol	395,66
E2	0	325.36 g/mol	0.00 g/mol	0
F2	0	348.28 g/mol	348.32 g/mol	389,16
G2	0,1	360.32 g/mol	360.36 g/mol	7058,82
H2	0,16	300.28 g/mol	300.31 g/mol	6746,76
A3	0	335.25 g/mol	0.00 g/mol	0
B3	0	335.25 g/mol	0.00 g/mol	0
C3	0,02	338.30 g/mol	338.34 g/mol	708,49
D3	0,04	336.25 g/mol	336.29 g/mol	5113,58
E3	0	355.33 g/mol	0.00 g/mol	0
F3	0	378.25 g/mol	0.00 g/mol	0
G3	0	390.29 g/mol	0.00 g/mol	0
H3	0	330.25 g/mol	330.30 g/mol	453,17
A4	0	317.28 g/mol	0.00 g/mol	0
B4	0	317.28 g/mol	0.00 g/mol	0
C4	0,16	320.33 g/mol	320.43 g/mol	3676,97
D4	0,02	318.28 g/mol	318.32 g/mol	507,99
E4	0	337.36 g/mol	0.00 g/mol	0
F4	0	360.28 g/mol	360.33 g/mol	117,39
G4	0,08	372.32 g/mol	372.36 g/mol	7244,16
H4	0	312.28 g/mol	312.31 g/mol	2679,1
A5	0	278.19 g/mol	0.00 g/mol	0
B5	0	278.19 g/mol	0.00 g/mol	0
C5	0	281.24 g/mol	0.00 g/mol	0
D5	0	279.19 g/mol	0.00 g/mol	0
E5	0	298.27 g/mol	0.00 g/mol	0
F5	0	321.19 g/mol	321.28 g/mol	32,33
G5	0	333.23 g/mol	0.00 g/mol	0
H5	0	273.19 g/mol	0.00 g/mol	0
A6	0	295.21 g/mol	0.00 g/mol	0
B6	0	295.21 g/mol	0.00 g/mol	0
C6	0	298.26 g/mol	0.00 g/mol	0
D6	0	296.21 g/mol	0.00 g/mol	0
E6	0	315.29 g/mol	0.00 g/mol	0
F6	0	338.21 g/mol	0.00 g/mol	0
G6	0	350.25 g/mol	0.00 g/mol	0
H6	0	290.21 g/mol	0.00 g/mol	0

A7	0	287.19 g/mol	287.27 g/mol	13,96
B7	0	287.19 g/mol	0.00 g/mol	0
C7	0,02	290.24 g/mol	290.30 g/mol	512,51
D7	0	288.18 g/mol	0.00 g/mol	0
E7	0	307.27 g/mol	0.00 g/mol	0
F7	0	330.19 g/mol	0.00 g/mol	0
G7	0	342.23 g/mol	0.00 g/mol	0
H7	0	282.19 g/mol	0.00 g/mol	0
A8	0	307.21 g/mol	0.00 g/mol	0
B8	0	307.21 g/mol	0.00 g/mol	0
C8	0,02	310.26 g/mol	310.30 g/mol	528,78
D8	0	308.21 g/mol	0.00 g/mol	0
E8	0	327.29 g/mol	0.00 g/mol	0
F8	0	350.21 g/mol	0.00 g/mol	0
G8	0	362.25 g/mol	362.31 g/mol	1827,91
H8	0	302.21 g/mol	0.00 g/mol	0
A9	0	337.18 g/mol	0.00 g/mol	0
B9	0	337.18 g/mol	0.00 g/mol	0
C9	0	340.23 g/mol	0.00 g/mol	0
D9	0	338.18 g/mol	0.00 g/mol	0
E9	0	357.26 g/mol	0.00 g/mol	0
F9	0	380.18 g/mol	0.00 g/mol	0
G9	0	392.22 g/mol	0.00 g/mol	0
H9	0	332.18 g/mol	0.00 g/mol	0
A10	0	309.19 g/mol	0.00 g/mol	0
B10	0	309.19 g/mol	0.00 g/mol	0
C10	0	312.24 g/mol	0.00 g/mol	0
D10	0	310.18 g/mol	0.00 g/mol	0
E10	0	329.27 g/mol	0.00 g/mol	0
F10	0	352.18 g/mol	0.00 g/mol	0
G10	0	364.23 g/mol	0.00 g/mol	0
H10	0	304.19 g/mol	0.00 g/mol	0
A11	0	294.19 g/mol	0.00 g/mol	0
B11	0	294.19 g/mol	0.00 g/mol	0
C11	0	297.23 g/mol	0.00 g/mol	0
D11	0	295.18 g/mol	0.00 g/mol	0
E11	0	314.27 g/mol	0.00 g/mol	0
F11	0	337.18 g/mol	0.00 g/mol	0
G11	0	349.23 g/mol	0.00 g/mol	0
H11	0	289.18 g/mol	0.00 g/mol	0
A12	0	313.20 g/mol	0.00 g/mol	0
B12	0	313.20 g/mol	313.25 g/mol	62,88
C12	0,02	316.25 g/mol	316.29 g/mol	906,68
D12	0	314.19 g/mol	314.25 g/mol	1576,97
E12	0	333.28 g/mol	0.00 g/mol	0
F12	0,12	356.19 g/mol	356.25 g/mol	5585,7
G12	0,08	368.24 g/mol	368.30 g/mol	22000,14
H12	0	308.20 g/mol	308.25 g/mol	70,75

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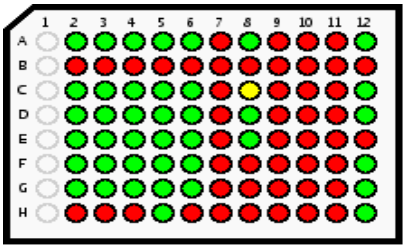

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,1	396.44 g/mol	396.47 g/mol	14312,88
B2	0	310.35 g/mol	0.00 g/mol	0
C2	0,04	268.29 g/mol	268.31 g/mol	11044,98
D2	0,14	324.37 g/mol	324.39 g/mol	5554,61
E2	0	324.37 g/mol	324.39 g/mol	4998,91
F2	0	430.29 g/mol	430.36 g/mol	125,66
G2	0	294.31 g/mol	294.33 g/mol	2120,55
H2	0	320.30 g/mol	0.00 g/mol	0
A3	0	426.41 g/mol	426.44 g/mol	1109,15
B3	0	340.32 g/mol	0.00 g/mol	0
C3	0	298.26 g/mol	298.29 g/mol	420,03
D3	0	354.34 g/mol	354.37 g/mol	117,31
E3	0	354.34 g/mol	354.37 g/mol	405,49
F3	0,02	460.26 g/mol	460.34 g/mol	735,34
G3	0	324.28 g/mol	324.31 g/mol	1498,82
H3	0	350.27 g/mol	0.00 g/mol	0
A4	0,12	408.44 g/mol	408.47 g/mol	9370,5
B4	0	322.35 g/mol	0.00 g/mol	0
C4	0,14	280.29 g/mol	280.32 g/mol	2775,21
D4	0,14	336.37 g/mol	336.40 g/mol	3312,02
E4	0,12	336.37 g/mol	336.39 g/mol	5498,54
F4	0	442.29 g/mol	442.35 g/mol	3009,38
G4	0,12	306.31 g/mol	306.34 g/mol	6811,86
H4	0	332.30 g/mol	0.00 g/mol	0
A5	0,06	369.35 g/mol	369.38 g/mol	1991,94
B5	0	283.26 g/mol	0.00 g/mol	0
C5	0,02	241.20 g/mol	241.23 g/mol	582,54
D5	0,1	297.28 g/mol	297.31 g/mol	2308,29
E5	0	297.28 g/mol	297.32 g/mol	1249,71
F5	0,04	403.20 g/mol	403.27 g/mol	1051,1
G5	0	267.22 g/mol	267.26 g/mol	465,6
H5	0,02	293.21 g/mol	293.24 g/mol	437,34
A6	0,16	386.37 g/mol	386.40 g/mol	7192,44
B6	0	300.28 g/mol	0.00 g/mol	0
C6	0	258.22 g/mol	258.25 g/mol	937,21
D6	0,17	314.30 g/mol	314.33 g/mol	5510,68
E6	0,04	314.30 g/mol	314.33 g/mol	2576,78
F6	0	420.22 g/mol	420.29 g/mol	330,59
G6	0	284.24 g/mol	284.27 g/mol	961,34
H6	0	310.23 g/mol	0.00 g/mol	0

A7	0	378.35 g/mol	0.00 g/mol	0
B7	0	292.26 g/mol	0.00 g/mol	0
C7	0	250.20 g/mol	0.00 g/mol	0
D7	0	306.28 g/mol	0.00 g/mol	0
E7	0	306.28 g/mol	0.00 g/mol	0
F7	0	412.20 g/mol	0.00 g/mol	0
G7	0	276.22 g/mol	0.00 g/mol	0
H7	0	302.20 g/mol	0.00 g/mol	0
A8	0	398.37 g/mol	398.40 g/mol	504,08
B8	0	312.28 g/mol	0.00 g/mol	0
C8	0	270.22 g/mol	270.26 g/mol	95,45
D8	0,02	326.30 g/mol	326.26 g/mol	664,08
E8	0,02	326.30 g/mol	326.33 g/mol	485,59
F8	0	432.22 g/mol	0.00 g/mol	0
G8	0	296.24 g/mol	296.28 g/mol	23,71
H8	0	322.23 g/mol	0.00 g/mol	0
A9	0	428.34 g/mol	0.00 g/mol	0
B9	0	342.25 g/mol	0.00 g/mol	0
C9	0	300.19 g/mol	0.00 g/mol	0
D9	0	356.27 g/mol	0.00 g/mol	0
E9	0	356.27 g/mol	0.00 g/mol	0
F9	0	462.19 g/mol	0.00 g/mol	0
G9	0	326.21 g/mol	0.00 g/mol	0
H9	0	352.19 g/mol	0.00 g/mol	0
A10	0	400.35 g/mol	0.00 g/mol	0
B10	0	314.26 g/mol	0.00 g/mol	0
C10	0	272.20 g/mol	0.00 g/mol	0
D10	0	328.27 g/mol	0.00 g/mol	0
E10	0	328.27 g/mol	0.00 g/mol	0
F10	0	434.20 g/mol	0.00 g/mol	0
G10	0	298.22 g/mol	0.00 g/mol	0
H10	0	324.20 g/mol	0.00 g/mol	0
A11	0	385.35 g/mol	0.00 g/mol	0
B11	0	299.25 g/mol	0.00 g/mol	0
C11	0	257.20 g/mol	0.00 g/mol	0
D11	0	313.27 g/mol	0.00 g/mol	0
E11	0	313.27 g/mol	0.00 g/mol	0
F11	0	419.19 g/mol	0.00 g/mol	0
G11	0	283.21 g/mol	0.00 g/mol	0
H11	0	309.20 g/mol	0.00 g/mol	0
A12	0	404.36 g/mol	404.40 g/mol	154,81
B12	0	318.27 g/mol	0.00 g/mol	0
C12	0,14	276.21 g/mol	276.24 g/mol	5485,86
D12	0	332.28 g/mol	332.32 g/mol	3304,46
E12	0	332.28 g/mol	0.00 g/mol	0
F12	0,06	438.21 g/mol	438.28 g/mol	10231,74
G12	0,04	302.23 g/mol	302.26 g/mol	9700,8
H12	0	328.21 g/mol	328.26 g/mol	2015,84

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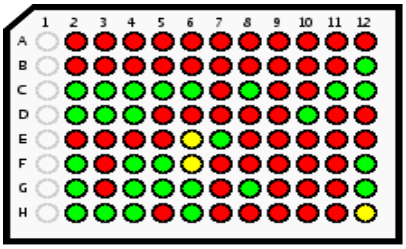

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	319.30 g/mol	0.00 g/mol	0
B2	0	319.30 g/mol	0.00 g/mol	0
C2	0,17	322.35 g/mol	322.36 g/mol	7113,54
D2	0,04	320.30 g/mol	320.30 g/mol	838,15
E2	0	339.38 g/mol	0.00 g/mol	0
F2	0	362.30 g/mol	362.33 g/mol	478,14
G2	0,14	374.34 g/mol	374.37 g/mol	4027,25
H2	0,21	314.30 g/mol	314.31 g/mol	7022,04
A3	0	349.27 g/mol	0.00 g/mol	0
B3	0	349.27 g/mol	0.00 g/mol	0
C3	0,02	352.32 g/mol	352.35 g/mol	690,24
D3	0	350.27 g/mol	350.17 g/mol	4826,46
E3	0	369.35 g/mol	0.00 g/mol	0
F3	0	392.27 g/mol	0.00 g/mol	0
G3	0	404.31 g/mol	0.00 g/mol	0
H3	0,02	344.27 g/mol	344.30 g/mol	562,61
A4	0	331.30 g/mol	0.00 g/mol	0
B4	0	331.30 g/mol	0.00 g/mol	0
C4	0,12	334.35 g/mol	334.37 g/mol	23682,6
D4	0,04	332.30 g/mol	332.32 g/mol	998,11
E4	0	351.38 g/mol	0.00 g/mol	0
F4	0,02	374.30 g/mol	374.33 g/mol	3095,36
G4	0,12	386.34 g/mol	386.37 g/mol	8536,14
H4	0	326.30 g/mol	326.33 g/mol	321,84
A5	0	292.21 g/mol	0.00 g/mol	0
B5	0	292.21 g/mol	0.00 g/mol	0
C5	0	295.26 g/mol	295.31 g/mol	281,79
D5	0	293.21 g/mol	0.00 g/mol	0
E5	0	312.29 g/mol	0.00 g/mol	0
F5	0,02	335.21 g/mol	335.25 g/mol	521,96
G5	0,02	347.25 g/mol	347.29 g/mol	587,78
H5	0	287.21 g/mol	0.00 g/mol	0
A6	0	309.23 g/mol	0.00 g/mol	0
B6	0	309.23 g/mol	0.00 g/mol	0
C6	0,12	312.28 g/mol	312.31 g/mol	5503,82
D6	0	310.23 g/mol	0.00 g/mol	0
E6	0	329.31 g/mol	329.34 g/mol	33,84
F6	0	352.23 g/mol	352.28 g/mol	56,85
G6	0,16	364.27 g/mol	364.29 g/mol	7383,18
H6	0	304.23 g/mol	304.26 g/mol	251,55

A7	0	301.21 g/mol	0.00 g/mol	0
B7	0	301.21 g/mol	0.00 g/mol	0
C7	0	304.26 g/mol	0.00 g/mol	0
D7	0	302.20 g/mol	0.00 g/mol	0
E7	0,04	321.29 g/mol	321.34 g/mol	829,73
F7	0	344.21 g/mol	0.00 g/mol	0
G7	0	356.25 g/mol	0.00 g/mol	0
H7	0	296.21 g/mol	0.00 g/mol	0
A8	0	321.23 g/mol	0.00 g/mol	0
B8	0	321.23 g/mol	0.00 g/mol	0
C8	0,02	324.28 g/mol	324.31 g/mol	398,63
D8	0	322.23 g/mol	0.00 g/mol	0
E8	0	341.31 g/mol	0.00 g/mol	0
F8	0	364.23 g/mol	0.00 g/mol	0
G8	0,06	376.27 g/mol	376.31 g/mol	3717,88
H8	0	316.23 g/mol	0.00 g/mol	0
A9	0	351.20 g/mol	0.00 g/mol	0
B9	0	351.20 g/mol	0.00 g/mol	0
C9	0	354.25 g/mol	0.00 g/mol	0
D9	0	352.19 g/mol	0.00 g/mol	0
E9	0	371.28 g/mol	0.00 g/mol	0
F9	0	394.20 g/mol	0.00 g/mol	0
G9	0	406.24 g/mol	0.00 g/mol	0
H9	0	346.20 g/mol	0.00 g/mol	0
A10	0	323.21 g/mol	0.00 g/mol	0
B10	0	323.21 g/mol	0.00 g/mol	0
C10	0	326.25 g/mol	0.00 g/mol	0
D10	0,02	324.20 g/mol	324.24 g/mol	389,65
E10	0	343.29 g/mol	0.00 g/mol	0
F10	0	366.20 g/mol	0.00 g/mol	0
G10	0	378.25 g/mol	0.00 g/mol	0
H10	0	318.21 g/mol	0.00 g/mol	0
A11	0	308.21 g/mol	0.00 g/mol	0
B11	0	308.21 g/mol	0.00 g/mol	0
C11	0	311.25 g/mol	311.20 g/mol	347,92
D11	0	309.20 g/mol	0.00 g/mol	0
E11	0	328.29 g/mol	0.00 g/mol	0
F11	0	351.20 g/mol	0.00 g/mol	0
G11	0	363.25 g/mol	0.00 g/mol	0
H11	0	303.20 g/mol	0.00 g/mol	0
A12	0	327.22 g/mol	0.00 g/mol	0
B12	0	327.22 g/mol	327.27 g/mol	143,19
C12	0	330.26 g/mol	330.30 g/mol	268,7
D12	0	328.21 g/mol	0.00 g/mol	0
E12	0	347.30 g/mol	0.00 g/mol	0
F12	0	370.21 g/mol	370.27 g/mol	487,87
G12	0,04	382.26 g/mol	382.31 g/mol	16097,64
H12	0	322.21 g/mol	322.27 g/mol	62,04

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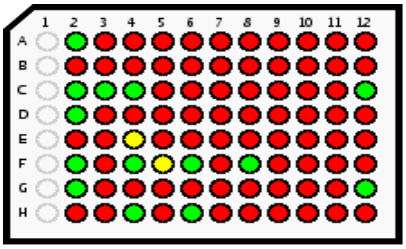
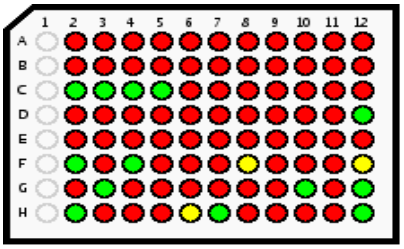


Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,08	398.40 g/mol	398.42 g/mol	6675,6
B2	0	312.31 g/mol	0.00 g/mol	0
C2	0,14	270.25 g/mol	270.27 g/mol	4350,84
D2	0,06	326.33 g/mol	326.35 g/mol	14933,04
E2	0	326.33 g/mol	0.00 g/mol	0
F2	0,06	432.25 g/mol	432.31 g/mol	4821,4
G2	0,08	296.27 g/mol	296.30 g/mol	5253,32
H2	0	322.25 g/mol	0.00 g/mol	0
A3	0	428.37 g/mol	0.00 g/mol	0
B3	0	342.28 g/mol	0.00 g/mol	0
C3	0,04	300.22 g/mol	300.25 g/mol	752,42
D3	0	356.30 g/mol	0.00 g/mol	0
E3	0	356.30 g/mol	0.00 g/mol	0
F3	0	462.22 g/mol	0.00 g/mol	0
G3	0	326.24 g/mol	0.00 g/mol	0
H3	0	352.22 g/mol	0.00 g/mol	0
A4	0	410.40 g/mol	0.00 g/mol	0
B4	0	324.31 g/mol	0.00 g/mol	0
C4	0	282.25 g/mol	282.25 g/mol	262,57
D4	0	338.33 g/mol	0.00 g/mol	0
E4	0	338.33 g/mol	338.38 g/mol	93,52
F4	0	444.25 g/mol	444.31 g/mol	1602,21
G4	0	308.27 g/mol	0.00 g/mol	0
H4	0,02	334.25 g/mol	334.31 g/mol	768,26
A5	0	371.31 g/mol	0.00 g/mol	0
B5	0	285.22 g/mol	0.00 g/mol	0
C5	0	243.16 g/mol	0.00 g/mol	0
D5	0	299.24 g/mol	0.00 g/mol	0
E5	0	299.24 g/mol	0.00 g/mol	0
F5	0	405.16 g/mol	405.24 g/mol	77,25
G5	0	269.18 g/mol	0.00 g/mol	0
H5	0	295.16 g/mol	0.00 g/mol	0
A6	0	388.33 g/mol	0.00 g/mol	0
B6	0	302.24 g/mol	0.00 g/mol	0
C6	0	260.18 g/mol	0.00 g/mol	0
D6	0	316.26 g/mol	0.00 g/mol	0
E6	0	316.26 g/mol	0.00 g/mol	0
F6	0,02	422.18 g/mol	422.25 g/mol	764,17
G6	0	286.20 g/mol	0.00 g/mol	0
H6	0,02	312.18 g/mol	312.25 g/mol	359,15

A7	0	380.31 g/mol	0.00 g/mol	0
B7	0	294.21 g/mol	0.00 g/mol	0
C7	0	252.16 g/mol	0.00 g/mol	0
D7	0	308.23 g/mol	0.00 g/mol	0
E7	0	308.23 g/mol	0.00 g/mol	0
F7	0	414.16 g/mol	0.00 g/mol	0
G7	0	278.18 g/mol	0.00 g/mol	0
H7	0	304.16 g/mol	0.00 g/mol	0
A8	0	400.33 g/mol	0.00 g/mol	0
B8	0	314.24 g/mol	0.00 g/mol	0
C8	0	272.18 g/mol	0.00 g/mol	0
D8	0	328.26 g/mol	0.00 g/mol	0
E8	0	328.26 g/mol	0.00 g/mol	0
F8	0,04	434.18 g/mol	434.26 g/mol	1234,76
G8	0	298.20 g/mol	0.00 g/mol	0
H8	0	324.18 g/mol	0.00 g/mol	0
A9	0	430.30 g/mol	0.00 g/mol	0
B9	0	344.21 g/mol	0.00 g/mol	0
C9	0	302.15 g/mol	0.00 g/mol	0
D9	0	358.23 g/mol	0.00 g/mol	0
E9	0	358.23 g/mol	0.00 g/mol	0
F9	0	464.15 g/mol	0.00 g/mol	0
G9	0	328.17 g/mol	0.00 g/mol	0
H9	0	354.15 g/mol	0.00 g/mol	0
A10	0	402.30 g/mol	0.00 g/mol	0
B10	0	316.21 g/mol	0.00 g/mol	0
C10	0	274.15 g/mol	0.00 g/mol	0
D10	0	330.23 g/mol	0.00 g/mol	0
E10	0	330.23 g/mol	0.00 g/mol	0
F10	0	436.15 g/mol	0.00 g/mol	0
G10	0	300.17 g/mol	0.00 g/mol	0
H10	0	326.16 g/mol	0.00 g/mol	0
A11	0	387.30 g/mol	0.00 g/mol	0
B11	0	301.21 g/mol	0.00 g/mol	0
C11	0	259.15 g/mol	0.00 g/mol	0
D11	0	315.23 g/mol	0.00 g/mol	0
E11	0	315.23 g/mol	0.00 g/mol	0
F11	0	421.15 g/mol	0.00 g/mol	0
G11	0	285.17 g/mol	0.00 g/mol	0
H11	0	311.16 g/mol	0.00 g/mol	0
A12	0	406.31 g/mol	0.00 g/mol	0
B12	0	320.22 g/mol	0.00 g/mol	0
C12	0,02	278.16 g/mol	278.23 g/mol	1311,05
D12	0	334.24 g/mol	0.00 g/mol	0
E12	0	334.24 g/mol	0.00 g/mol	0
F12	0	440.16 g/mol	0.00 g/mol	0
G12	0,14	304.18 g/mol	304.23 g/mol	2622,53
H12	0	330.17 g/mol	0.00 g/mol	0

ori.hya.29



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	321.26 g/mol	0.00 g/mol	0
B2	0	321.26 g/mol	0.00 g/mol	0
C2	0,2	324.31 g/mol	324.33 g/mol	7922,64
D2	0	322.25 g/mol	0.00 g/mol	0
E2	0	341.34 g/mol	0.00 g/mol	0
F2	0	364.26 g/mol	364.30 g/mol	5591,75
G2	0	376.30 g/mol	0.00 g/mol	0
H2	0	316.26 g/mol	316.29 g/mol	3543,11
A3	0	351.23 g/mol	0.00 g/mol	0
B3	0	351.23 g/mol	0.00 g/mol	0
C3	0,1	354.28 g/mol	354.32 g/mol	2265,93
D3	0	352.22 g/mol	0.00 g/mol	0
E3	0	371.31 g/mol	0.00 g/mol	0
F3	0	394.22 g/mol	0.00 g/mol	0
G3	0,02	406.27 g/mol	406.32 g/mol	1540,16
H3	0	346.23 g/mol	0.00 g/mol	0
A4	0	333.26 g/mol	0.00 g/mol	0
B4	0	333.26 g/mol	0.00 g/mol	0
C4	0	336.31 g/mol	336.36 g/mol	151,89
D4	0	334.25 g/mol	0.00 g/mol	0
E4	0	353.34 g/mol	0.00 g/mol	0
F4	0	376.26 g/mol	376.32 g/mol	554,4
G4	0	388.30 g/mol	0.00 g/mol	0
H4	0	328.26 g/mol	0.00 g/mol	0
A5	0	294.17 g/mol	0.00 g/mol	0
B5	0	294.17 g/mol	0.00 g/mol	0
C5	0,08	297.22 g/mol	297.25 g/mol	3614,03
D5	0	295.16 g/mol	0.00 g/mol	0
E5	0	314.25 g/mol	0.00 g/mol	0
F5	0	337.17 g/mol	0.00 g/mol	0
G5	0	349.21 g/mol	0.00 g/mol	0
H5	0	289.17 g/mol	289.23 g/mol	24,6
A6	0	311.19 g/mol	0.00 g/mol	0
B6	0	311.19 g/mol	0.00 g/mol	0
C6	0	314.24 g/mol	0.00 g/mol	0
D6	0	312.18 g/mol	0.00 g/mol	0
E6	0	331.27 g/mol	0.00 g/mol	0
F6	0	354.19 g/mol	0.00 g/mol	0
G6	0	366.23 g/mol	0.00 g/mol	0
H6	0	306.19 g/mol	306.25 g/mol	85,64

A7	0	303.17 g/mol	0.00 g/mol	0
B7	0	303.17 g/mol	0.00 g/mol	0
C7	0	306.21 g/mol	0.00 g/mol	0
D7	0	304.16 g/mol	0.00 g/mol	0
E7	0	323.25 g/mol	0.00 g/mol	0
F7	0	346.16 g/mol	0.00 g/mol	0
G7	0	358.21 g/mol	0.00 g/mol	0
H7	0	298.16 g/mol	298.22 g/mol	270,74
A8	0	323.19 g/mol	0.00 g/mol	0
B8	0	323.19 g/mol	0.00 g/mol	0
C8	0	326.24 g/mol	0.00 g/mol	0
D8	0	324.18 g/mol	0.00 g/mol	0
E8	0	343.27 g/mol	0.00 g/mol	0
F8	0	366.19 g/mol	366.25 g/mol	73,06
G8	0	378.23 g/mol	0.00 g/mol	0
H8	0	318.19 g/mol	318.24 g/mol	29,42
A9	0	353.16 g/mol	0.00 g/mol	0
B9	0	353.16 g/mol	0.00 g/mol	0
C9	0	356.21 g/mol	0.00 g/mol	0
D9	0	354.15 g/mol	0.00 g/mol	0
E9	0	373.24 g/mol	0.00 g/mol	0
F9	0	396.15 g/mol	0.00 g/mol	0
G9	0	408.20 g/mol	0.00 g/mol	0
H9	0	348.16 g/mol	0.00 g/mol	0
A10	0	325.17 g/mol	0.00 g/mol	0
B10	0	325.17 g/mol	0.00 g/mol	0
C10	0	328.21 g/mol	0.00 g/mol	0
D10	0	326.16 g/mol	0.00 g/mol	0
E10	0	345.25 g/mol	0.00 g/mol	0
F10	0	368.16 g/mol	0.00 g/mol	0
G10	0,14	380.21 g/mol	380.30 g/mol	3240,79
H10	0	320.16 g/mol	0.00 g/mol	0
A11	0	310.16 g/mol	0.00 g/mol	0
B11	0	310.16 g/mol	0.00 g/mol	0
C11	0	313.21 g/mol	0.00 g/mol	0
D11	0	311.16 g/mol	0.00 g/mol	0
E11	0	330.24 g/mol	0.00 g/mol	0
F11	0	353.16 g/mol	0.00 g/mol	0
G11	0	365.20 g/mol	0.00 g/mol	0
H11	0	305.16 g/mol	0.00 g/mol	0
A12	0	329.18 g/mol	0.00 g/mol	0
B12	0	329.18 g/mol	0.00 g/mol	0
C12	0	332.22 g/mol	0.00 g/mol	0
D12	0,02	330.17 g/mol	330.23 g/mol	935,06
E12	0	349.25 g/mol	0.00 g/mol	0
F12	0	372.17 g/mol	372.26 g/mol	84,09
G12	0,04	384.22 g/mol	384.29 g/mol	1537,56
H12	0,06	324.17 g/mol	324.14 g/mol	1393,9

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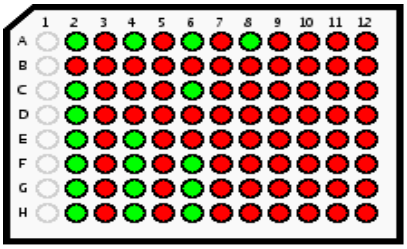

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,16	466.34 g/mol	466.37 g/mol	8102,64
B2	0	380.25 g/mol	0.00 g/mol	0
C2	0,08	338.19 g/mol	338.22 g/mol	4184,05
D2	0,1	394.26 g/mol	394.30 g/mol	6140,34
E2	0,06	394.26 g/mol	394.30 g/mol	1766,76
F2	0,04	500.19 g/mol	500.26 g/mol	1267,05
G2	0,06	364.21 g/mol	364.24 g/mol	4133,19
H2	0,06	390.19 g/mol	390.24 g/mol	1511,39
A3	0	496.31 g/mol	0.00 g/mol	0
B3	0	410.21 g/mol	0.00 g/mol	0
C3	0	368.16 g/mol	0.00 g/mol	0
D3	0	424.23 g/mol	0.00 g/mol	0
E3	0	424.23 g/mol	0.00 g/mol	0
F3	0	530.15 g/mol	0.00 g/mol	0
G3	0	394.17 g/mol	0.00 g/mol	0
H3	0	420.16 g/mol	0.00 g/mol	0
A4	0,02	478.34 g/mol	478.38 g/mol	790,63
B4	0	392.24 g/mol	0.00 g/mol	0
C4	0	350.19 g/mol	0.00 g/mol	0
D4	0	406.26 g/mol	0.00 g/mol	0
E4	0,02	406.26 g/mol	406.32 g/mol	553,11
F4	0,04	512.19 g/mol	512.26 g/mol	1936,72
G4	0	376.21 g/mol	376.25 g/mol	2288,96
H4	0,04	402.19 g/mol	402.25 g/mol	3544,96
A5	0	439.25 g/mol	0.00 g/mol	0
B5	0	353.15 g/mol	0.00 g/mol	0
C5	0	311.10 g/mol	0.00 g/mol	0
D5	0	367.17 g/mol	0.00 g/mol	0
E5	0	367.17 g/mol	0.00 g/mol	0
F5	0	473.09 g/mol	0.00 g/mol	0
G5	0	337.11 g/mol	0.00 g/mol	0
H5	0	363.10 g/mol	0.00 g/mol	0
A6	0,02	456.27 g/mol	456.33 g/mol	766,64
B6	0	370.18 g/mol	0.00 g/mol	0
C6	0,04	328.12 g/mol	328.17 g/mol	1097,09
D6	0	384.20 g/mol	0.00 g/mol	0
E6	0	384.20 g/mol	0.00 g/mol	0
F6	0	490.12 g/mol	490.20 g/mol	722,83
G6	0	354.14 g/mol	354.19 g/mol	388,68
H6	0	380.12 g/mol	380.19 g/mol	278,62

A7	0	448.24 g/mol	0.00 g/mol	0
B7	0	362.15 g/mol	0.00 g/mol	0
C7	0	320.09 g/mol	0.00 g/mol	0
D7	0	376.17 g/mol	0.00 g/mol	0
E7	0	376.17 g/mol	0.00 g/mol	0
F7	0	482.09 g/mol	0.00 g/mol	0
G7	0	346.11 g/mol	0.00 g/mol	0
H7	0	372.10 g/mol	0.00 g/mol	0
A8	0,02	468.27 g/mol	468.34 g/mol	745,53
B8	0	382.18 g/mol	0.00 g/mol	0
C8	0	340.12 g/mol	0.00 g/mol	0
D8	0	396.19 g/mol	0.00 g/mol	0
E8	0	396.19 g/mol	0.00 g/mol	0
F8	0	502.12 g/mol	0.00 g/mol	0
G8	0	366.14 g/mol	0.00 g/mol	0
H8	0	392.12 g/mol	0.00 g/mol	0
A9	0	498.24 g/mol	0.00 g/mol	0
B9	0	412.14 g/mol	0.00 g/mol	0
C9	0	370.09 g/mol	0.00 g/mol	0
D9	0	426.16 g/mol	0.00 g/mol	0
E9	0	426.16 g/mol	0.00 g/mol	0
F9	0	532.08 g/mol	0.00 g/mol	0
G9	0	396.10 g/mol	0.00 g/mol	0
H9	0	422.09 g/mol	0.00 g/mol	0
A10	0	470.24 g/mol	0.00 g/mol	0
B10	0	384.15 g/mol	0.00 g/mol	0
C10	0	342.09 g/mol	0.00 g/mol	0
D10	0	398.17 g/mol	0.00 g/mol	0
E10	0	398.17 g/mol	0.00 g/mol	0
F10	0	504.09 g/mol	0.00 g/mol	0
G10	0	368.11 g/mol	0.00 g/mol	0
H10	0	394.10 g/mol	0.00 g/mol	0
A11	0	455.24 g/mol	0.00 g/mol	0
B11	0	369.15 g/mol	0.00 g/mol	0
C11	0	327.09 g/mol	0.00 g/mol	0
D11	0	383.17 g/mol	0.00 g/mol	0
E11	0	383.17 g/mol	0.00 g/mol	0
F11	0	489.09 g/mol	0.00 g/mol	0
G11	0	353.11 g/mol	0.00 g/mol	0
H11	0	379.09 g/mol	0.00 g/mol	0
A12	0	474.25 g/mol	0.00 g/mol	0
B12	0	388.16 g/mol	0.00 g/mol	0
C12	0	346.10 g/mol	0.00 g/mol	0
D12	0	402.18 g/mol	0.00 g/mol	0
E12	0	402.18 g/mol	0.00 g/mol	0
F12	0	508.10 g/mol	0.00 g/mol	0
G12	0	372.12 g/mol	0.00 g/mol	0
H12	0	398.11 g/mol	0.00 g/mol	0

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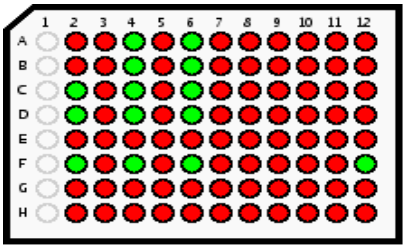
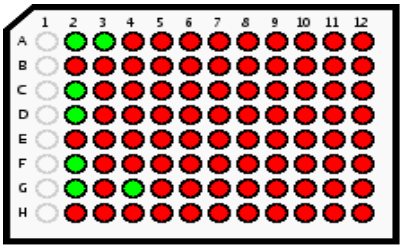


Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	389.20 g/mol	0.00 g/mol	0
B2	0	389.20 g/mol	0.00 g/mol	0
C2	0,08	392.24 g/mol	392.29 g/mol	7376,88
D2	0	390.19 g/mol	390.25 g/mol	335,58
E2	0	409.28 g/mol	0.00 g/mol	0
F2	0,04	432.19 g/mol	432.26 g/mol	4004,96
G2	0	444.24 g/mol	0.00 g/mol	0
H2	0	384.19 g/mol	0.00 g/mol	0
A3	0	419.17 g/mol	0.00 g/mol	0
B3	0	419.17 g/mol	0.00 g/mol	0
C3	0	422.21 g/mol	0.00 g/mol	0
D3	0	420.16 g/mol	0.00 g/mol	0
E3	0	439.25 g/mol	0.00 g/mol	0
F3	0	462.16 g/mol	0.00 g/mol	0
G3	0	474.21 g/mol	0.00 g/mol	0
H3	0	414.16 g/mol	0.00 g/mol	0
A4	0	401.20 g/mol	401.26 g/mol	157,46
B4	0,02	401.20 g/mol	401.26 g/mol	651,61
C4	0,02	404.24 g/mol	404.31 g/mol	924,31
D4	0,06	402.19 g/mol	402.25 g/mol	4699,29
E4	0	421.28 g/mol	0.00 g/mol	0
F4	0	444.19 g/mol	444.26 g/mol	2232,7
G4	0	456.24 g/mol	0.00 g/mol	0
H4	0	396.19 g/mol	0.00 g/mol	0
A5	0	362.11 g/mol	0.00 g/mol	0
B5	0	362.11 g/mol	0.00 g/mol	0
C5	0	365.15 g/mol	0.00 g/mol	0
D5	0	363.10 g/mol	0.00 g/mol	0
E5	0	382.19 g/mol	0.00 g/mol	0
F5	0	405.10 g/mol	0.00 g/mol	0
G5	0	417.15 g/mol	0.00 g/mol	0
H5	0	357.10 g/mol	0.00 g/mol	0
A6	0	379.13 g/mol	379.20 g/mol	130,79
B6	0	379.13 g/mol	379.16 g/mol	105,58
C6	0,02	382.17 g/mol	382.24 g/mol	651,01
D6	0	380.12 g/mol	380.21 g/mol	113,23
E6	0	399.21 g/mol	0.00 g/mol	0
F6	0	422.12 g/mol	422.21 g/mol	237,14
G6	0	434.17 g/mol	0.00 g/mol	0
H6	0	374.13 g/mol	0.00 g/mol	0

A7	0	371.10 g/mol	0.00 g/mol	0
B7	0	371.10 g/mol	0.00 g/mol	0
C7	0	374.15 g/mol	0.00 g/mol	0
D7	0	372.10 g/mol	0.00 g/mol	0
E7	0	391.18 g/mol	0.00 g/mol	0
F7	0	414.10 g/mol	0.00 g/mol	0
G7	0	426.14 g/mol	0.00 g/mol	0
H7	0	366.10 g/mol	0.00 g/mol	0
A8	0	391.13 g/mol	0.00 g/mol	0
B8	0	391.13 g/mol	0.00 g/mol	0
C8	0	394.17 g/mol	0.00 g/mol	0
D8	0	392.12 g/mol	0.00 g/mol	0
E8	0	411.21 g/mol	0.00 g/mol	0
F8	0	434.12 g/mol	0.00 g/mol	0
G8	0	446.17 g/mol	0.00 g/mol	0
H8	0	386.12 g/mol	0.00 g/mol	0
A9	0	421.10 g/mol	0.00 g/mol	0
B9	0	421.10 g/mol	0.00 g/mol	0
C9	0	424.14 g/mol	0.00 g/mol	0
D9	0	422.09 g/mol	0.00 g/mol	0
E9	0	441.18 g/mol	0.00 g/mol	0
F9	0	464.09 g/mol	0.00 g/mol	0
G9	0	476.14 g/mol	0.00 g/mol	0
H9	0	416.09 g/mol	0.00 g/mol	0
A10	0	393.10 g/mol	0.00 g/mol	0
B10	0	393.10 g/mol	0.00 g/mol	0
C10	0	396.15 g/mol	0.00 g/mol	0
D10	0	394.10 g/mol	0.00 g/mol	0
E10	0	413.18 g/mol	0.00 g/mol	0
F10	0	436.10 g/mol	0.00 g/mol	0
G10	0	448.14 g/mol	0.00 g/mol	0
H10	0	388.10 g/mol	0.00 g/mol	0
A11	0	378.10 g/mol	0.00 g/mol	0
B11	0	378.10 g/mol	0.00 g/mol	0
C11	0	381.15 g/mol	0.00 g/mol	0
D11	0	379.09 g/mol	0.00 g/mol	0
E11	0	398.18 g/mol	0.00 g/mol	0
F11	0	421.10 g/mol	0.00 g/mol	0
G11	0	433.14 g/mol	0.00 g/mol	0
H11	0	373.10 g/mol	0.00 g/mol	0
A12	0	397.11 g/mol	0.00 g/mol	0
B12	0	397.11 g/mol	0.00 g/mol	0
C12	0	400.16 g/mol	0.00 g/mol	0
D12	0	398.11 g/mol	0.00 g/mol	0
E12	0	417.19 g/mol	0.00 g/mol	0
F12	0	440.11 g/mol	440.20 g/mol	163,76
G12	0	452.15 g/mol	0.00 g/mol	0
H12	0	392.11 g/mol	0.00 g/mol	0

ori.hya.32



Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,08	398.40 g/mol	398.42 g/mol	25868,04
B2	0	312.31 g/mol	0.00 g/mol	0
C2	0,02	270.25 g/mol	270.27 g/mol	3223,4
D2	0,1	326.33 g/mol	326.35 g/mol	5853,22
E2	0	326.33 g/mol	0.00 g/mol	0
F2	0,02	432.25 g/mol	432.31 g/mol	936,19
G2	0,06	296.27 g/mol	296.29 g/mol	1652,24
H2	0	322.25 g/mol	0.00 g/mol	0
A3	0,04	428.37 g/mol	428.40 g/mol	1133,98
B3	0	342.28 g/mol	0.00 g/mol	0
C3	0	300.22 g/mol	0.00 g/mol	0
D3	0	356.30 g/mol	0.00 g/mol	0
E3	0	356.30 g/mol	0.00 g/mol	0
F3	0	462.22 g/mol	0.00 g/mol	0
G3	0	326.24 g/mol	0.00 g/mol	0
H3	0	352.22 g/mol	0.00 g/mol	0
A4	0	410.40 g/mol	0.00 g/mol	0
B4	0	324.31 g/mol	0.00 g/mol	0
C4	0	282.25 g/mol	0.00 g/mol	0
D4	0	338.33 g/mol	0.00 g/mol	0
E4	0	338.33 g/mol	0.00 g/mol	0
F4	0	444.25 g/mol	0.00 g/mol	0
G4	0,04	308.27 g/mol	308.32 g/mol	743,41
H4	0	334.25 g/mol	0.00 g/mol	0
A5	0	371.31 g/mol	0.00 g/mol	0
B5	0	285.22 g/mol	0.00 g/mol	0
C5	0	243.16 g/mol	0.00 g/mol	0
D5	0	299.24 g/mol	0.00 g/mol	0
E5	0	299.24 g/mol	0.00 g/mol	0
F5	0	405.16 g/mol	0.00 g/mol	0
G5	0	269.18 g/mol	0.00 g/mol	0
H5	0	295.16 g/mol	0.00 g/mol	0
A6	0	388.33 g/mol	0.00 g/mol	0
B6	0	302.24 g/mol	0.00 g/mol	0
C6	0	260.18 g/mol	0.00 g/mol	0
D6	0	316.26 g/mol	0.00 g/mol	0
E6	0	316.26 g/mol	0.00 g/mol	0
F6	0	422.18 g/mol	0.00 g/mol	0
G6	0	286.20 g/mol	0.00 g/mol	0
H6	0	312.18 g/mol	0.00 g/mol	0

A7	0	380.31 g/mol	0.00 g/mol	0
B7	0	294.21 g/mol	0.00 g/mol	0
C7	0	252.16 g/mol	0.00 g/mol	0
D7	0	308.23 g/mol	0.00 g/mol	0
E7	0	308.23 g/mol	0.00 g/mol	0
F7	0	414.16 g/mol	0.00 g/mol	0
G7	0	278.18 g/mol	0.00 g/mol	0
H7	0	304.16 g/mol	0.00 g/mol	0
A8	0	400.33 g/mol	0.00 g/mol	0
B8	0	314.24 g/mol	0.00 g/mol	0
C8	0	272.18 g/mol	0.00 g/mol	0
D8	0	328.26 g/mol	0.00 g/mol	0
E8	0	328.26 g/mol	0.00 g/mol	0
F8	0	434.18 g/mol	0.00 g/mol	0
G8	0	298.20 g/mol	0.00 g/mol	0
H8	0	324.18 g/mol	0.00 g/mol	0
A9	0	430.30 g/mol	0.00 g/mol	0
B9	0	344.21 g/mol	0.00 g/mol	0
C9	0	302.15 g/mol	0.00 g/mol	0
D9	0	358.23 g/mol	0.00 g/mol	0
E9	0	358.23 g/mol	0.00 g/mol	0
F9	0	464.15 g/mol	0.00 g/mol	0
G9	0	328.17 g/mol	0.00 g/mol	0
H9	0	354.15 g/mol	0.00 g/mol	0
A10	0	402.30 g/mol	402.32 g/mol	13,7
B10	0	316.21 g/mol	0.00 g/mol	0
C10	0	274.15 g/mol	0.00 g/mol	0
D10	0	330.23 g/mol	0.00 g/mol	0
E10	0	330.23 g/mol	0.00 g/mol	0
F10	0	436.15 g/mol	0.00 g/mol	0
G10	0	300.17 g/mol	0.00 g/mol	0
H10	0	326.16 g/mol	0.00 g/mol	0
A11	0	387.30 g/mol	0.00 g/mol	0
B11	0	301.21 g/mol	0.00 g/mol	0
C11	0	259.15 g/mol	0.00 g/mol	0
D11	0	315.23 g/mol	0.00 g/mol	0
E11	0	315.23 g/mol	0.00 g/mol	0
F11	0	421.15 g/mol	0.00 g/mol	0
G11	0	285.17 g/mol	0.00 g/mol	0
H11	0	311.16 g/mol	0.00 g/mol	0
A12	0	406.31 g/mol	0.00 g/mol	0
B12	0	320.22 g/mol	0.00 g/mol	0
C12	0	278.16 g/mol	0.00 g/mol	0
D12	0	334.24 g/mol	0.00 g/mol	0
E12	0	334.24 g/mol	0.00 g/mol	0
F12	0	440.16 g/mol	0.00 g/mol	0
G12	0	304.18 g/mol	0.00 g/mol	0
H12	0	330.17 g/mol	0.00 g/mol	0

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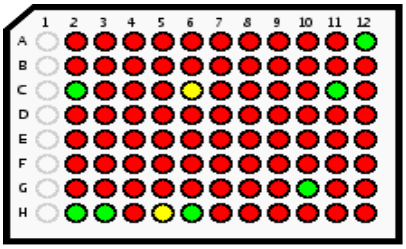

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	321.26 g/mol	0.00 g/mol	0
B2	0	321.26 g/mol	0.00 g/mol	0
C2	0,14	324.31 g/mol	324.32 g/mol	22138,86
D2	0	322.25 g/mol	0.00 g/mol	0
E2	0	341.34 g/mol	0.00 g/mol	0
F2	0	364.26 g/mol	0.00 g/mol	0
G2	0	376.30 g/mol	0.00 g/mol	0
H2	0,1	316.26 g/mol	316.28 g/mol	4309,88
A3	0	351.23 g/mol	0.00 g/mol	0
B3	0	351.23 g/mol	0.00 g/mol	0
C3	0	354.28 g/mol	0.00 g/mol	0
D3	0	352.22 g/mol	0.00 g/mol	0
E3	0	371.31 g/mol	0.00 g/mol	0
F3	0	394.23 g/mol	0.00 g/mol	0
G3	0	406.27 g/mol	0.00 g/mol	0
H3	0,02	346.23 g/mol	346.26 g/mol	1249,46
A4	0	333.26 g/mol	0.00 g/mol	0
B4	0	333.26 g/mol	0.00 g/mol	0
C4	0	336.31 g/mol	0.00 g/mol	0
D4	0	334.25 g/mol	0.00 g/mol	0
E4	0	353.34 g/mol	0.00 g/mol	0
F4	0	376.26 g/mol	0.00 g/mol	0
G4	0	388.30 g/mol	0.00 g/mol	0
H4	0	328.26 g/mol	0.00 g/mol	0
A5	0	294.17 g/mol	0.00 g/mol	0
B5	0	294.17 g/mol	0.00 g/mol	0
C5	0	297.22 g/mol	0.00 g/mol	0
D5	0	295.16 g/mol	0.00 g/mol	0
E5	0	314.25 g/mol	0.00 g/mol	0
F5	0	337.17 g/mol	0.00 g/mol	0
G5	0	349.21 g/mol	0.00 g/mol	0
H5	0	289.17 g/mol	289.19 g/mol	45,92
A6	0	311.19 g/mol	0.00 g/mol	0
B6	0	311.19 g/mol	0.00 g/mol	0
C6	0	314.24 g/mol	314.29 g/mol	31,93
D6	0	312.18 g/mol	0.00 g/mol	0
E6	0	331.27 g/mol	0.00 g/mol	0
F6	0	354.19 g/mol	0.00 g/mol	0
G6	0	366.23 g/mol	0.00 g/mol	0
H6	0	306.19 g/mol	306.24 g/mol	128,02

A7	0	303.17 g/mol	0.00 g/mol	0
B7	0	303.17 g/mol	0.00 g/mol	0
C7	0	306.21 g/mol	0.00 g/mol	0
D7	0	304.16 g/mol	0.00 g/mol	0
E7	0	323.25 g/mol	0.00 g/mol	0
F7	0	346.16 g/mol	0.00 g/mol	0
G7	0	358.21 g/mol	0.00 g/mol	0
H7	0	298.16 g/mol	0.00 g/mol	0
A8	0	323.19 g/mol	0.00 g/mol	0
B8	0	323.19 g/mol	0.00 g/mol	0
C8	0	326.24 g/mol	0.00 g/mol	0
D8	0	324.18 g/mol	0.00 g/mol	0
E8	0	343.27 g/mol	0.00 g/mol	0
F8	0	366.19 g/mol	0.00 g/mol	0
G8	0	378.23 g/mol	0.00 g/mol	0
H8	0	318.19 g/mol	0.00 g/mol	0
A9	0	353.16 g/mol	0.00 g/mol	0
B9	0	353.16 g/mol	0.00 g/mol	0
C9	0	356.21 g/mol	0.00 g/mol	0
D9	0	354.15 g/mol	0.00 g/mol	0
E9	0	373.24 g/mol	0.00 g/mol	0
F9	0	396.15 g/mol	0.00 g/mol	0
G9	0	408.20 g/mol	0.00 g/mol	0
H9	0	348.16 g/mol	0.00 g/mol	0
A10	0	325.17 g/mol	0.00 g/mol	0
B10	0	325.17 g/mol	0.00 g/mol	0
C10	0	328.21 g/mol	0.00 g/mol	0
D10	0	326.16 g/mol	0.00 g/mol	0
E10	0	345.25 g/mol	0.00 g/mol	0
F10	0	368.16 g/mol	0.00 g/mol	0
G10	0,02	380.21 g/mol	380.26 g/mol	654,41
H10	0	320.16 g/mol	0.00 g/mol	0
A11	0	310.16 g/mol	0.00 g/mol	0
B11	0	310.16 g/mol	0.00 g/mol	0
C11	0,02	313.21 g/mol	313.11 g/mol	1376,53
D11	0	311.16 g/mol	0.00 g/mol	0
E11	0	330.24 g/mol	0.00 g/mol	0
F11	0	353.16 g/mol	0.00 g/mol	0
G11	0	365.20 g/mol	0.00 g/mol	0
H11	0	305.16 g/mol	0.00 g/mol	0
A12	0	329.18 g/mol	329.24 g/mol	213,49
B12	0	329.18 g/mol	0.00 g/mol	0
C12	0	332.22 g/mol	0.00 g/mol	0
D12	0	330.17 g/mol	0.00 g/mol	0
E12	0	349.25 g/mol	0.00 g/mol	0
F12	0	372.17 g/mol	0.00 g/mol	0
G12	0	384.22 g/mol	0.00 g/mol	0
H12	0	324.17 g/mol	0.00 g/mol	0

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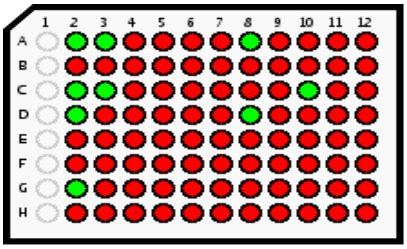

Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0,1	412.42 g/mol	412.44 g/mol	26194,74
B2	0	326.33 g/mol	0.00 g/mol	0
C2	0,22	284.27 g/mol	284.30 g/mol	4309,21
D2	0,04	340.35 g/mol	340.36 g/mol	10253,4
E2	0	340.35 g/mol	0.00 g/mol	0
F2	0	446.27 g/mol	0.00 g/mol	0
G2	0,14	310.29 g/mol	310.31 g/mol	7787,22
H2	0	336.27 g/mol	0.00 g/mol	0
A3	0	442.39 g/mol	442.42 g/mol	189,67
B3	0	356.30 g/mol	0.00 g/mol	0
C3	0,02	314.24 g/mol	314.28 g/mol	457,59
D3	0	370.32 g/mol	0.00 g/mol	0
E3	0	370.32 g/mol	0.00 g/mol	0
F3	0	476.24 g/mol	0.00 g/mol	0
G3	0	340.26 g/mol	0.00 g/mol	0
H3	0	366.24 g/mol	0.00 g/mol	0
A4	0	424.42 g/mol	0.00 g/mol	0
B4	0	338.33 g/mol	0.00 g/mol	0
C4	0	296.27 g/mol	0.00 g/mol	0
D4	0	352.35 g/mol	0.00 g/mol	0
E4	0	352.35 g/mol	0.00 g/mol	0
F4	0	458.27 g/mol	0.00 g/mol	0
G4	0	322.29 g/mol	0.00 g/mol	0
H4	0	348.27 g/mol	0.00 g/mol	0
A5	0	385.33 g/mol	0.00 g/mol	0
B5	0	299.24 g/mol	0.00 g/mol	0
C5	0	257.18 g/mol	0.00 g/mol	0
D5	0	313.26 g/mol	0.00 g/mol	0
E5	0	313.26 g/mol	0.00 g/mol	0
F5	0	419.18 g/mol	0.00 g/mol	0
G5	0	283.20 g/mol	0.00 g/mol	0
H5	0	309.18 g/mol	0.00 g/mol	0
A6	0	402.35 g/mol	0.00 g/mol	0
B6	0	316.26 g/mol	0.00 g/mol	0
C6	0	274.20 g/mol	0.00 g/mol	0
D6	0	330.28 g/mol	0.00 g/mol	0
E6	0	330.28 g/mol	0.00 g/mol	0
F6	0	436.20 g/mol	0.00 g/mol	0
G6	0	300.22 g/mol	0.00 g/mol	0
H6	0	326.20 g/mol	0.00 g/mol	0

A7	0	394.33 g/mol	0.00 g/mol	0
B7	0	308.23 g/mol	0.00 g/mol	0
C7	0	266.18 g/mol	0.00 g/mol	0
D7	0	322.25 g/mol	0.00 g/mol	0
E7	0	322.25 g/mol	0.00 g/mol	0
F7	0	428.18 g/mol	0.00 g/mol	0
G7	0	292.19 g/mol	0.00 g/mol	0
H7	0	318.18 g/mol	0.00 g/mol	0
A8	0	414.35 g/mol	414.43 g/mol	244,1
B8	0	328.26 g/mol	0.00 g/mol	0
C8	0	286.20 g/mol	0.00 g/mol	0
D8	0,02	342.28 g/mol	342.35 g/mol	475,75
E8	0	342.28 g/mol	0.00 g/mol	0
F8	0	448.20 g/mol	0.00 g/mol	0
G8	0	312.22 g/mol	0.00 g/mol	0
H8	0	338.20 g/mol	0.00 g/mol	0
A9	0	444.32 g/mol	0.00 g/mol	0
B9	0	358.23 g/mol	0.00 g/mol	0
C9	0	316.17 g/mol	0.00 g/mol	0
D9	0	372.25 g/mol	0.00 g/mol	0
E9	0	372.25 g/mol	0.00 g/mol	0
F9	0	478.17 g/mol	0.00 g/mol	0
G9	0	342.19 g/mol	0.00 g/mol	0
H9	0	368.17 g/mol	0.00 g/mol	0
A10	0	416.32 g/mol	0.00 g/mol	0
B10	0	330.23 g/mol	0.00 g/mol	0
C10	0,06	288.17 g/mol	288.22 g/mol	1046,54
D10	0	344.25 g/mol	0.00 g/mol	0
E10	0	344.25 g/mol	0.00 g/mol	0
F10	0	450.17 g/mol	0.00 g/mol	0
G10	0	314.19 g/mol	0.00 g/mol	0
H10	0	340.18 g/mol	0.00 g/mol	0
A11	0	401.32 g/mol	0.00 g/mol	0
B11	0	315.23 g/mol	0.00 g/mol	0
C11	0	273.17 g/mol	0.00 g/mol	0
D11	0	329.25 g/mol	0.00 g/mol	0
E11	0	329.25 g/mol	0.00 g/mol	0
F11	0	435.17 g/mol	0.00 g/mol	0
G11	0	299.19 g/mol	0.00 g/mol	0
H11	0	325.18 g/mol	0.00 g/mol	0
A12	0	420.33 g/mol	0.00 g/mol	0
B12	0	334.24 g/mol	0.00 g/mol	0
C12	0	292.18 g/mol	0.00 g/mol	0
D12	0	348.26 g/mol	0.00 g/mol	0
E12	0	348.26 g/mol	0.00 g/mol	0
F12	0	454.18 g/mol	0.00 g/mol	0
G12	0	318.20 g/mol	0.00 g/mol	0
H12	0	344.19 g/mol	0.00 g/mol	0

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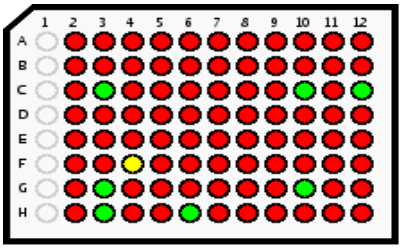


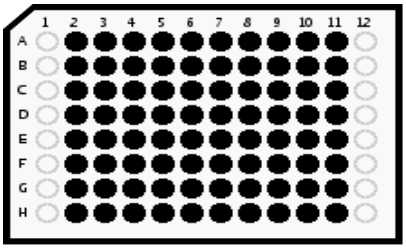
Well	Peak width	Expected mass	Found mass	Intensity
A1				
B1				
C1				
D1				
E1				
F1				
G1				
H1				
A2	0	335.28 g/mol	0.00 g/mol	0
B2	0	335.28 g/mol	0.00 g/mol	0
C2	0	338.33 g/mol	0.00 g/mol	0
D2	0	336.27 g/mol	0.00 g/mol	0
E2	0	355.36 g/mol	0.00 g/mol	0
F2	0	378.28 g/mol	0.00 g/mol	0
G2	0	390.32 g/mol	0.00 g/mol	0
H2	0	330.28 g/mol	0.00 g/mol	0
A3	0	365.25 g/mol	0.00 g/mol	0
B3	0	365.25 g/mol	0.00 g/mol	0
C3	0	368.30 g/mol	368.33 g/mol	276,72
D3	0	366.24 g/mol	0.00 g/mol	0
E3	0	385.33 g/mol	0.00 g/mol	0
F3	0	408.24 g/mol	0.00 g/mol	0
G3	0	420.29 g/mol	420.36 g/mol	125,5
H3	0	360.25 g/mol	360.20 g/mol	290,42
A4	0	347.28 g/mol	0.00 g/mol	0
B4	0	347.28 g/mol	0.00 g/mol	0
C4	0	350.33 g/mol	0.00 g/mol	0
D4	0	348.27 g/mol	0.00 g/mol	0
E4	0	367.36 g/mol	0.00 g/mol	0
F4	0	390.28 g/mol	390.35 g/mol	71,37
G4	0	402.32 g/mol	0.00 g/mol	0
H4	0	342.28 g/mol	0.00 g/mol	0
A5	0	308.19 g/mol	0.00 g/mol	0
B5	0	308.19 g/mol	0.00 g/mol	0
C5	0	311.24 g/mol	0.00 g/mol	0
D5	0	309.18 g/mol	0.00 g/mol	0
E5	0	328.27 g/mol	0.00 g/mol	0
F5	0	351.18 g/mol	0.00 g/mol	0
G5	0	363.23 g/mol	0.00 g/mol	0
H5	0	303.19 g/mol	0.00 g/mol	0
A6	0	325.21 g/mol	0.00 g/mol	0
B6	0	325.21 g/mol	0.00 g/mol	0
C6	0	328.26 g/mol	0.00 g/mol	0
D6	0	326.20 g/mol	0.00 g/mol	0
E6	0	345.29 g/mol	0.00 g/mol	0
F6	0	368.21 g/mol	0.00 g/mol	0
G6	0	380.25 g/mol	0.00 g/mol	0
H6	0	320.21 g/mol	320.25 g/mol	131,77

A7	0	317.19 g/mol	0.00 g/mol	0
B7	0	317.19 g/mol	0.00 g/mol	0
C7	0	320.23 g/mol	0.00 g/mol	0
D7	0	318.18 g/mol	0.00 g/mol	0
E7	0	337.27 g/mol	0.00 g/mol	0
F7	0	360.18 g/mol	0.00 g/mol	0
G7	0	372.23 g/mol	0.00 g/mol	0
H7	0	312.18 g/mol	0.00 g/mol	0
A8	0	337.21 g/mol	0.00 g/mol	0
B8	0	337.21 g/mol	0.00 g/mol	0
C8	0	340.26 g/mol	0.00 g/mol	0
D8	0	338.20 g/mol	0.00 g/mol	0
E8	0	357.29 g/mol	0.00 g/mol	0
F8	0	380.21 g/mol	0.00 g/mol	0
G8	0	392.25 g/mol	0.00 g/mol	0
H8	0	332.21 g/mol	0.00 g/mol	0
A9	0	367.18 g/mol	0.00 g/mol	0
B9	0	367.18 g/mol	0.00 g/mol	0
C9	0	370.23 g/mol	0.00 g/mol	0
D9	0	368.17 g/mol	0.00 g/mol	0
E9	0	387.26 g/mol	0.00 g/mol	0
F9	0	410.17 g/mol	0.00 g/mol	0
G9	0	422.22 g/mol	0.00 g/mol	0
H9	0	362.18 g/mol	0.00 g/mol	0
A10	0	339.19 g/mol	0.00 g/mol	0
B10	0	339.19 g/mol	0.00 g/mol	0
C10	0,06	342.23 g/mol	342.29 g/mol	1220,74
D10	0	340.18 g/mol	0.00 g/mol	0
E10	0	359.26 g/mol	0.00 g/mol	0
F10	0	382.18 g/mol	0.00 g/mol	0
G10	0,04	394.23 g/mol	394.29 g/mol	1068,83
H10	0	334.18 g/mol	0.00 g/mol	0
A11	0	324.18 g/mol	0.00 g/mol	0
B11	0	324.18 g/mol	0.00 g/mol	0
C11	0	327.23 g/mol	0.00 g/mol	0
D11	0	325.18 g/mol	0.00 g/mol	0
E11	0	344.26 g/mol	0.00 g/mol	0
F11	0	367.18 g/mol	0.00 g/mol	0
G11	0	379.22 g/mol	0.00 g/mol	0
H11	0	319.18 g/mol	0.00 g/mol	0
A12	0	343.19 g/mol	0.00 g/mol	0
B12	0	343.19 g/mol	0.00 g/mol	0
C12	0,02	346.24 g/mol	346.29 g/mol	449,15
D12	0	344.19 g/mol	0.00 g/mol	0
E12	0	363.27 g/mol	0.00 g/mol	0
F12	0	386.19 g/mol	0.00 g/mol	0
G12	0	398.23 g/mol	0.00 g/mol	0
H12	0	338.19 g/mol	0.00 g/mol	0

B.5 Screening plates ori.hya.scr.1-7

Generated compounds from plates **ori.hya.10**-**35** were combined to plates **ori.hya.scr.1**-**7**.

ori.hya.scr.1



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E2	199.1056 g/mol	ori.hya.10_C8
F2	285.1524 g/mol	ori.hya.10_E9
G2	291.1821 g/mol	ori.hya.11_F2
H2	303.2267 g/mol	ori.hya.11_G2
A3	321.1508 g/mol	ori.hya.11_F3
B3	280.2658 g/mol	ori.hya.11_E4
C3	303.1816 g/mol	ori.hya.11_F4
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E3	241.1641 g/mol	ori.hya.11_C6
F3	239.1101 g/mol	ori.hya.11_D6
G3	293.1569 g/mol	ori.hya.11_G6
H3	293.1119 g/mol	ori.hya.11_F8
A4	305.1564 g/mol	ori.hya.11_G8
B4	272.1710 g/mol	ori.hya.11_E10
C4	423.4176 g/mol	ori.hya.12_A2
D4	351.3452 g/mol	ori.hya.12_D2
E4	457.2666 g/mol	ori.hya.12_F2
F4	347.2712 g/mol	ori.hya.12_H2
G4	325.2360 g/mol	ori.hya.12_C3
H4	381.3140 g/mol	ori.hya.12_D3
A5	487.2354 g/mol	ori.hya.12_F3
B5	351.2550 g/mol	ori.hya.12_G3
C5	377.2401 g/mol	ori.hya.12_H3
D5	435.4172 g/mol	ori.hya.12_A4
E5	307.2667 g/mol	ori.hya.12_C4
F5	363.3447 g/mol	ori.hya.12_D4
G5	469.2662 g/mol	ori.hya.12_F4
H5	333.2857 g/mol	ori.hya.12_G4
A6	359.2708 g/mol	ori.hya.12_H4
B6	268.1761 g/mol	ori.hya.12_C5
C6	324.2541 g/mol	ori.hya.12_D5
D6	324.2541 g/mol	ori.hya.12_E5
E6	430.1755 g/mol	ori.hya.12_F5
F6	294.1951 g/mol	ori.hya.12_G5
G6	320.1801 g/mol	ori.hya.12_H5
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E7	311.2165 g/mol	ori.hya.12_G6
F7	337.2015 g/mol	ori.hya.12_H6
G7	297.1970 g/mol	ori.hya.12_C8
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H8	379.1698 g/mol	ori.hya.12_H9
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B9	465.1811 g/mol	ori.hya.12_F12
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G9	377.2401 g/mol	ori.hya.13_D3
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A10	431.2870 g/mol	ori.hya.13_G3
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C10	361.3247 g/mol	ori.hya.13_C4
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E10	378.3572 g/mol	ori.hya.13_E4
F10	401.2731 g/mol	ori.hya.13_F4
G10	413.3177 g/mol	ori.hya.13_G4
H10	353.2750 g/mol	ori.hya.13_H4
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C11	320.1801 g/mol	ori.hya.13_D5
D11	362.1825 g/mol	ori.hya.13_F5
E11	374.2271 g/mol	ori.hya.13_G5
F11	336.2086 g/mol	ori.hya.13_A6
G11	339.2555 g/mol	ori.hya.13_C6
H11	337.2015 g/mol	ori.hya.13_D6
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F12		
G12		
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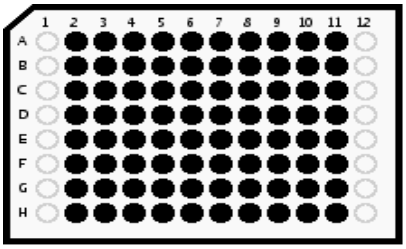

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E2	329.1777 g/mol	ori.hya.13_D7
F2	323.1820 g/mol	ori.hya.13_H7
G2	348.2081 g/mol	ori.hya.13_A8
H2	351.2550 g/mol	ori.hya.13_C8
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G3	379.1698 g/mol	ori.hya.13_D9
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C4	393.1783 g/mol	ori.hya.13_F10
D4	405.2229 g/mol	ori.hya.13_G10
E4	355.1856 g/mol	ori.hya.13_D12
F4	349.1899 g/mol	ori.hya.13_H12
G4	445.3516 g/mol	ori.hya.14_A2
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A5	343.2201 g/mol	ori.hya.14_G2
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C5	347.1699 g/mol	ori.hya.14_C3
D5	509.1693 g/mol	ori.hya.14_F3
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F5	399.1740 g/mol	ori.hya.14_H3
G5	457.3511 g/mol	ori.hya.14_A4
H5	329.2006 g/mol	ori.hya.14_C4
A6	385.2787 g/mol	ori.hya.14_D4
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D7	307.1314 g/mol	ori.hya.14_C6
E7	363.2095 g/mol	ori.hya.14_D6
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G7	333.1505 g/mol	ori.hya.14_G6
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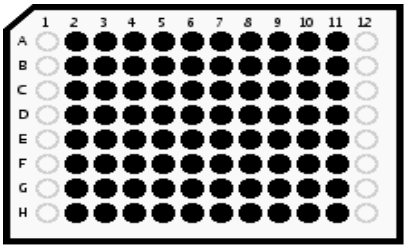

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E2	401.1038 g/mol	ori.hya.15_D9
F2	443.1061 g/mol	ori.hya.15_F9
G2	455.1506 g/mol	ori.hya.15_G9
H2	303.1927 g/mol	ori.hya.16_H4
A3	281.1235 g/mol	ori.hya.16_H6
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G3	292.1300 g/mol	ori.hya.17_A8
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E4	396.1667 g/mol	ori.hya.18_F4
F4	260.1863 g/mol	ori.hya.18_G4
G4	286.1713 g/mol	ori.hya.18_H4
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H5	386.0970 g/mol	ori.hya.18_F8
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F7	310.1252 g/mol	ori.hya.19_G7
G7	278.1556 g/mol	ori.hya.19_C8
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F8	279.2839 g/mol	ori.hya.20_G2
G8	411.3841 g/mol	ori.hya.20_A3
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C9	393.4149 g/mol	ori.hya.20_A4
D9	265.2643 g/mol	ori.hya.20_C4
E9	321.3424 g/mol	ori.hya.20_D4
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E10	243.1952 g/mol	ori.hya.20_C6
F10	299.2732 g/mol	ori.hya.20_D6
G10	269.2142 g/mol	ori.hya.20_G6
H10	363.3219 g/mol	ori.hya.20_A7
A11	291.2494 g/mol	ori.hya.20_D7
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E11	311.2727 g/mol	ori.hya.20_D8
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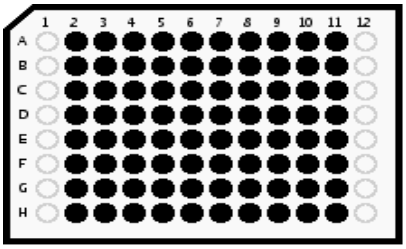

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F2	307.3229 g/mol	ori.hya.21_C2
G2	347.2712 g/mol	ori.hya.21_F2
H2	359.3158 g/mol	ori.hya.21_G2
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C3	335.2377 g/mol	ori.hya.21_D3
D3	389.2846 g/mol	ori.hya.21_G3
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F3	317.2684 g/mol	ori.hya.21_D4
G3	359.2708 g/mol	ori.hya.21_F4
H3	371.3154 g/mol	ori.hya.21_G4
A4	311.2727 g/mol	ori.hya.21_H4
B4	280.2318 g/mol	ori.hya.21_C5
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D4	332.2248 g/mol	ori.hya.21_G5
E4	297.2532 g/mol	ori.hya.21_C6
F4	337.2015 g/mol	ori.hya.21_F6
G4	349.2462 g/mol	ori.hya.21_G6
H4	289.2034 g/mol	ori.hya.21_H6
A5	289.2294 g/mol	ori.hya.21_C7
B5	309.2527 g/mol	ori.hya.21_C8
C5	361.2456 g/mol	ori.hya.21_G8
D5	309.1736 g/mol	ori.hya.21_D10
E5	312.1903 g/mol	ori.hya.21_B12
F5	313.1833 g/mol	ori.hya.21_D12
G5	355.1856 g/mol	ori.hya.21_F12
H5	367.2303 g/mol	ori.hya.21_G12
A6	307.1876 g/mol	ori.hya.21_H12
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C6	321.2017 g/mol	ori.hya.22_C2
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F6	347.2207 g/mol	ori.hya.22_G2
G6	479.3210 g/mol	ori.hya.22_A3
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D7	359.2203 g/mol	ori.hya.22_G4
E7	320.1296 g/mol	ori.hya.22_G5
F7	439.2825 g/mol	ori.hya.22_A6
G7	311.1320 g/mol	ori.hya.22_C6
H7	367.2101 g/mol	ori.hya.22_D6
A8	367.2101 g/mol	ori.hya.22_E6
B8	337.1510 g/mol	ori.hya.22_G6
C8	457.2667 g/mol	ori.hya.22_A12
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E8	385.1942 g/mol	ori.hya.22_D12
F8	385.1942 g/mol	ori.hya.22_E12
G8	491.1156 g/mol	ori.hya.22_F12
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D9	415.2081 g/mol	ori.hya.23_F2
E9	427.2526 g/mol	ori.hya.23_G2
F9	367.2100 g/mol	ori.hya.23_H2
G9	387.2593 g/mol	ori.hya.23_C4
H9	427.2076 g/mol	ori.hya.23_F4
A10	439.2523 g/mol	ori.hya.23_G4
B10	379.2096 g/mol	ori.hya.23_H4
C10	400.1615 g/mol	ori.hya.23_G5
D10	365.1900 g/mol	ori.hya.23_C6
E10	405.1383 g/mol	ori.hya.23_F6
F10	417.1830 g/mol	ori.hya.23_G6
G10	357.1403 g/mol	ori.hya.23_H6
H10	377.1895 g/mol	ori.hya.23_C8
A11	429.1825 g/mol	ori.hya.23_G8
B11	381.1202 g/mol	ori.hya.23_D12
C11	423.1225 g/mol	ori.hya.23_F12
D11	435.1671 g/mol	ori.hya.23_G12
E11	375.1245 g/mol	ori.hya.23_H12
F11	381.4153 g/mol	ori.hya.24_A2
G11	253.2648 g/mol	ori.hya.24_C2
H11	309.3429 g/mol	ori.hya.24_D2

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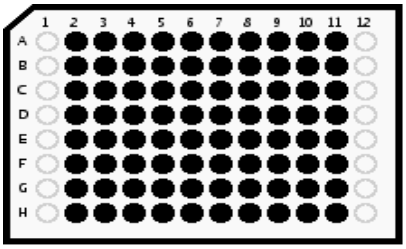

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C2	411.3842 g/mol	ori.hya.24_A3
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E2	309.2527 g/mol	ori.hya.24_G3
F2	393.4149 g/mol	ori.hya.24_A4
G2	265.2644 g/mol	ori.hya.24_C4
H2	321.3424 g/mol	ori.hya.24_D4
A3	354.3242 g/mol	ori.hya.24_A5
B3	226.1738 g/mol	ori.hya.24_C5
C3	282.2518 g/mol	ori.hya.24_D5
D3	282.2518 g/mol	ori.hya.24_E5
E3	388.1732 g/mol	ori.hya.24_F5
F3	252.1928 g/mol	ori.hya.24_G5
G3	278.1778 g/mol	ori.hya.24_H5
H3	371.3456 g/mol	ori.hya.24_A6
A4	243.1952 g/mol	ori.hya.24_C6
B4	299.2732 g/mol	ori.hya.24_D6
C4	269.2142 g/mol	ori.hya.24_G6
D4	291.2494 g/mol	ori.hya.24_D7
E4	255.1947 g/mol	ori.hya.24_C8
F4	311.2727 g/mol	ori.hya.24_D8
G4	281.2137 g/mol	ori.hya.24_G8
H4	389.3298 g/mol	ori.hya.24_A12
A5	261.1792 g/mol	ori.hya.24_C12
B5	317.2573 g/mol	ori.hya.24_D12
C5	317.2573 g/mol	ori.hya.24_E12
D5	423.1787 g/mol	ori.hya.24_F12
E5	287.1983 g/mol	ori.hya.24_G12
F5	313.1833 g/mol	ori.hya.24_H12
G5	307.3228 g/mol	ori.hya.25_C2
H5	305.2689 g/mol	ori.hya.25_D2
A6	347.2712 g/mol	ori.hya.25_F2
B6	359.3159 g/mol	ori.hya.25_G2
C6	299.2731 g/mol	ori.hya.25_H2
D6	337.2917 g/mol	ori.hya.25_C3
E6	335.2378 g/mol	ori.hya.25_D3
F6	329.2420 g/mol	ori.hya.25_H3
G6	319.3224 g/mol	ori.hya.25_C4
H6	317.2684 g/mol	ori.hya.25_D4

A7	359.2708 g/mol	ori.hya.25_F4
B7	371.3154 g/mol	ori.hya.25_G4
C7	311.2727 g/mol	ori.hya.25_H4
D7	320.1801 g/mol	ori.hya.25_F5
E7	286.1823 g/mol	ori.hya.25_A7
F7	289.2294 g/mol	ori.hya.25_C7
G7	309.2527 g/mol	ori.hya.25_C8
H7	361.2457 g/mol	ori.hya.25_G8
A8	312.1903 g/mol	ori.hya.25_B12
B8	315.2373 g/mol	ori.hya.25_C12
C8	313.1833 g/mol	ori.hya.25_D12
D8	355.1856 g/mol	ori.hya.25_F12
E8	367.2303 g/mol	ori.hya.25_G12
F8	307.1876 g/mol	ori.hya.25_H12
G8	395.4348 g/mol	ori.hya.26_A2
H8	267.2844 g/mol	ori.hya.26_C2
A9	323.3624 g/mol	ori.hya.26_D2
B9	323.3624 g/mol	ori.hya.26_E2
C9	429.2838 g/mol	ori.hya.26_F2
D9	293.3033 g/mol	ori.hya.26_G2
E9	425.4036 g/mol	ori.hya.26_A3
F9	297.2531 g/mol	ori.hya.26_C3
G9	353.3312 g/mol	ori.hya.26_D3
H9	353.3312 g/mol	ori.hya.26_E3
A10	459.2526 g/mol	ori.hya.26_F3
B10	323.2722 g/mol	ori.hya.26_G3
C10	407.4344 g/mol	ori.hya.26_A4
D10	279.2839 g/mol	ori.hya.26_C4
E10	335.3619 g/mol	ori.hya.26_D4
F10	335.3619 g/mol	ori.hya.26_E4
G10	441.2834 g/mol	ori.hya.26_F4
H10	305.3029 g/mol	ori.hya.26_G4
A11	368.3437 g/mol	ori.hya.26_A5
B11	240.1933 g/mol	ori.hya.26_C5
C11	296.2713 g/mol	ori.hya.26_D5
D11	296.2713 g/mol	ori.hya.26_E5
E11	402.1927 g/mol	ori.hya.26_F5
F11	266.2123 g/mol	ori.hya.26_G5
G11	292.1973 g/mol	ori.hya.26_H5
H11	385.3651 g/mol	ori.hya.26_A6
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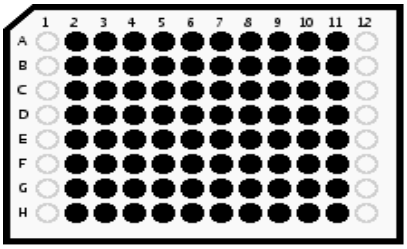
B12		
C12		
D12		
E12		
F12		
G12		
H12		

ori.hya.scr.6



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C2	313.2927 g/mol	ori.hya.26_E6
D2	419.2141 g/mol	ori.hya.26_F6
E2	283.2337 g/mol	ori.hya.26_G6
F2	397.3646 g/mol	ori.hya.26_A8
G2	269.2142 g/mol	ori.hya.26_C8
H2	325.2922 g/mol	ori.hya.26_D8
A3	325.2922 g/mol	ori.hya.26_E8
B3	295.2332 g/mol	ori.hya.26_G8
C3	403.3493 g/mol	ori.hya.26_A12
D3	275.1988 g/mol	ori.hya.26_C12
E3	331.2769 g/mol	ori.hya.26_D12
F3	437.1982 g/mol	ori.hya.26_F12
G3	301.2178 g/mol	ori.hya.26_G12
H3	327.2028 g/mol	ori.hya.26_H12
A4	321.3423 g/mol	ori.hya.27_C2
B4	319.2884 g/mol	ori.hya.27_D2
C4	361.2907 g/mol	ori.hya.27_F2
D4	373.3354 g/mol	ori.hya.27_G2
E4	313.2926 g/mol	ori.hya.27_H2
F4	351.3112 g/mol	ori.hya.27_C3
G4	349.2572 g/mol	ori.hya.27_D3
H4	343.2615 g/mol	ori.hya.27_H3
A5	333.3419 g/mol	ori.hya.27_C4
B5	331.2880 g/mol	ori.hya.27_D4
C5	373.2903 g/mol	ori.hya.27_F4
D5	385.3349 g/mol	ori.hya.27_G4
E5	325.2922 g/mol	ori.hya.27_H4
F5	294.2513 g/mol	ori.hya.27_C5
G5	334.1996 g/mol	ori.hya.27_F5
H5	346.2443 g/mol	ori.hya.27_G5
A6	311.2727 g/mol	ori.hya.27_C6
B6	328.3052 g/mol	ori.hya.27_E6
C6	351.2210 g/mol	ori.hya.27_F6
D6	363.2657 g/mol	ori.hya.27_G6
E6	303.2229 g/mol	ori.hya.27_H6
F6	320.2814 g/mol	ori.hya.27_E7
G6	323.2722 g/mol	ori.hya.27_C8
H6	375.2652 g/mol	ori.hya.27_G8

A7	323.1931 g/mol	ori.hya.27_D10
B7	310.2457 g/mol	ori.hya.27_C11
C7	326.2098 g/mol	ori.hya.27_B12
D7	329.2568 g/mol	ori.hya.27_C12
E7	369.2052 g/mol	ori.hya.27_F12
F7	381.2498 g/mol	ori.hya.27_G12
G7	321.2071 g/mol	ori.hya.27_H12
H7	397.3924 g/mol	ori.hya.28_A2
A8	269.2420 g/mol	ori.hya.28_C2
B8	325.3200 g/mol	ori.hya.28_D2
C8	431.2414 g/mol	ori.hya.28_F2
D8	295.2610 g/mol	ori.hya.28_G2
E8	299.2108 g/mol	ori.hya.28_C3
F8	281.2415 g/mol	ori.hya.28_C4
G8	337.3195 g/mol	ori.hya.28_E4
H8	443.2410 g/mol	ori.hya.28_F4
A9	333.2456 g/mol	ori.hya.28_H4
B9	404.1503 g/mol	ori.hya.28_F5
C9	421.1717 g/mol	ori.hya.28_F6
D9	311.1763 g/mol	ori.hya.28_H6
E9	433.1712 g/mol	ori.hya.28_F8
F9	277.1564 g/mol	ori.hya.28_C12
G9	303.1754 g/mol	ori.hya.28_G12
H9	323.3000 g/mol	ori.hya.29_C2
A10	363.2483 g/mol	ori.hya.29_F2
B10	315.2503 g/mol	ori.hya.29_H2
C10	353.2688 g/mol	ori.hya.29_C3
D10	405.2617 g/mol	ori.hya.29_G3
E10	335.2995 g/mol	ori.hya.29_C4
F10	375.2479 g/mol	ori.hya.29_F4
G10	296.2089 g/mol	ori.hya.29_C5
H10	288.1592 g/mol	ori.hya.29_H5
A11	305.1806 g/mol	ori.hya.29_H6
B11	297.1568 g/mol	ori.hya.29_H7
C11	365.1782 g/mol	ori.hya.29_F8
D11	317.1801 g/mol	ori.hya.29_H8
E11	379.1977 g/mol	ori.hya.29_G10
F11	329.1604 g/mol	ori.hya.29_D12
G11	371.1628 g/mol	ori.hya.29_F12
H11	383.2074 g/mol	ori.hya.29_G12
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C12		
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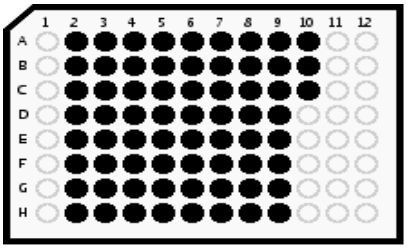
E12		
F12		
G12		
H12		

ori.hya.scr.7



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C2	337.1788 g/mol	ori.hya.30_C2
D2	393.2569 g/mol	ori.hya.30_D2
E2	393.2568 g/mol	ori.hya.30_E2
F2	499.1783 g/mol	ori.hya.30_F2
G2	363.1978 g/mol	ori.hya.30_G2
H2	389.1829 g/mol	ori.hya.30_H2
A3	477.3288 g/mol	ori.hya.30_A4
B3	405.2564 g/mol	ori.hya.30_E4
C3	511.1778 g/mol	ori.hya.30_F4
D3	375.1974 g/mol	ori.hya.30_G4
E3	401.1824 g/mol	ori.hya.30_H4
F3	455.2596 g/mol	ori.hya.30_A6
G3	327.1091 g/mol	ori.hya.30_C6
H3	489.1085 g/mol	ori.hya.30_F6
A4	353.1281 g/mol	ori.hya.30_G6
B4	379.1131 g/mol	ori.hya.30_H6
C4	467.2591 g/mol	ori.hya.30_A8
D4	391.2368 g/mol	ori.hya.31_C2
E4	389.1829 g/mol	ori.hya.31_D2
F4	431.1852 g/mol	ori.hya.31_F2
G4	400.1894 g/mol	ori.hya.31_A4
H4	400.1894 g/mol	ori.hya.31_B4
A5	403.2364 g/mol	ori.hya.31_C4
B5	401.1824 g/mol	ori.hya.31_D4
C5	443.1847 g/mol	ori.hya.31_F4
D5	378.1201 g/mol	ori.hya.31_A6
E5	378.1201 g/mol	ori.hya.31_B6
F5	381.1671 g/mol	ori.hya.31_C6
G5	379.1131 g/mol	ori.hya.31_D6
H5	421.1154 g/mol	ori.hya.31_F6
A6	439.0996 g/mol	ori.hya.31_F12
B6	397.3924 g/mol	ori.hya.32_A2
C6	269.2420 g/mol	ori.hya.32_C2
D6	325.3200 g/mol	ori.hya.32_D2
E6	431.2414 g/mol	ori.hya.32_F2
F6	295.2610 g/mol	ori.hya.32_G2
G6	427.3613 g/mol	ori.hya.32_A3
H6	307.2605 g/mol	ori.hya.32_G4

A7	401.2971 g/mol	ori.hya.32_A10
B7	323.3000 g/mol	ori.hya.33_C2
C7	315.2502 g/mol	ori.hya.33_H2
D7	345.2191 g/mol	ori.hya.33_H3
E7	288.1592 g/mol	ori.hya.33_H5
F7	313.2303 g/mol	ori.hya.33_C6
G7	305.1806 g/mol	ori.hya.33_H6
H7	379.1977 g/mol	ori.hya.33_G10
A8	312.2033 g/mol	ori.hya.33_C11
B8	328.1674 g/mol	ori.hya.33_A12
C8	411.4120 g/mol	ori.hya.34_A2
D8	283.2615 g/mol	ori.hya.34_C2
E8	339.3395 g/mol	ori.hya.34_D2
F8	309.2805 g/mol	ori.hya.34_G2
G8	441.3807 g/mol	ori.hya.34_A3
H8	313.2303 g/mol	ori.hya.34_C3
A9	413.3418 g/mol	ori.hya.34_A8
B9	341.2693 g/mol	ori.hya.34_D8
C9	287.1662 g/mol	ori.hya.34_C10
D9	367.2883 g/mol	ori.hya.35_C3
E9	419.2812 g/mol	ori.hya.35_G3
F9	359.2386 g/mol	ori.hya.35_H3
G9	389.2674 g/mol	ori.hya.35_F4
H9	319.2001 g/mol	ori.hya.35_H6
A10	341.2242 g/mol	ori.hya.35_C10
B10	393.2172 g/mol	ori.hya.35_G10
C10	345.2340 g/mol	ori.hya.35_C12
D10		
E10		
F10		
G10		
H10		
A11		
B11		
C11		
D11		
E11		
F11		
G11		
H11		
A12		
B12		
C12		
D12		
E12		
F12		
G12		
H12		

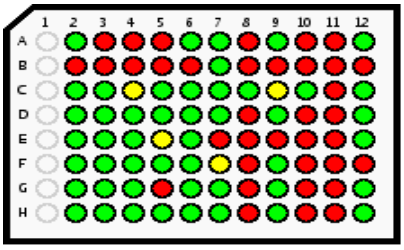
B.6 Screening plates ori.hya.44-47

B.6.1 Starting materials of plates ori.hya.44-47

Cf. section 5.4.1; chemsets (see Figure 5.12) are indicated for each well

B.6.2 Mass spectral analysis of plates ori.hya.44-47

ori.hya.44



Well	Chemset	Peak width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**9** {*1,1,1*}	0,04	490.13 g/mol	490.13 g/mol	1269,61
B2	**9** {*2,1,1*}	0	506.13 g/mol	506.06 g/mol	29,69
C2	**9** {*3,1,1*}	0,02	490.13 g/mol	490.12 g/mol	2134,32
D2	**9** {*4,1,1*}	0	490.13 g/mol	490.13 g/mol	111,63
E2	**9** {*5,1,1*}	0,02	558.05 g/mol	558.05 g/mol	687,43
F2	**9** {*6,1,1*}	0	526.11 g/mol	526.11 g/mol	230,33
G2	**9** {*7,1,1*}	0	520.15 g/mol	520.13 g/mol	301,08
H2	**9** {*8,1,1*}	0,02	474.14 g/mol	474.13 g/mol	596,6
A3	**9** {*1,2,1*}	0	474.14 g/mol	0.00 g/mol	0
B3	**9** {*2,2,1*}	0	490.13 g/mol	0.00 g/mol	0
C3	**9** {*3,2,1*}	0	474.14 g/mol	474.13 g/mol	1228,17
D3	**9** {*4,2,1*}	0,02	474.14 g/mol	474.13 g/mol	2725,56
E3	**9** {*5,2,1*}	0	542.05 g/mol	542.09 g/mol	268,73
F3	**9** {*6,2,1*}	0,02	510.11 g/mol	510.11 g/mol	761,93
G3	**9** {*7,2,1*}	0	504.15 g/mol	504.14 g/mol	261,5
H3	**9** {*8,2,1*}	0,02	458.14 g/mol	458.15 g/mol	587,98
A4	**9** {*1,3,1*}	0	474.14 g/mol	0.00 g/mol	0
B4	**9** {*2,3,1*}	0	490.13 g/mol	0.00 g/mol	0
C4	**9** {*3,3,1*}	0	474.14 g/mol	474.14 g/mol	67,84
D4	**9** {*4,3,1*}	0,04	474.14 g/mol	474.13 g/mol	4088,77
E4	**9** {*5,3,1*}	0,02	542.05 g/mol	542.06 g/mol	706,63
F4	**9** {*6,3,1*}	0,02	510.11 g/mol	510.11 g/mol	2104,57
G4	**9** {*7,3,1*}	0	504.15 g/mol	504.14 g/mol	526,36
H4	**9** {*8,3,1*}	0,02	458.14 g/mol	458.14 g/mol	714,5
A5	**9** {*1,4,1*}	0	490.13 g/mol	0.00 g/mol	0
B5	**9** {*2,4,1*}	0	506.13 g/mol	0.00 g/mol	0
C5	**9** {*3,4,1*}	0	490.13 g/mol	490.13 g/mol	864,02
D5	**9** {*4,4,1*}	0	490.13 g/mol	490.13 g/mol	271,59
E5	**9** {*5,4,1*}	0	558.05 g/mol	558.05 g/mol	80,96
F5	**9** {*6,4,1*}	0,04	526.11 g/mol	526.12 g/mol	1407,7
G5	**9** {*7,4,1*}	0	520.15 g/mol	0.00 g/mol	0
H5	**9** {*8,4,1*}	0,02	474.14 g/mol	474.14 g/mol	689,74

A6	**9**	{*1,5,1*}	0	490.13 g/mol	490.13 g/mol	331,48
B6	**9**	{*2,5,1*}	0	506.13 g/mol	0.00 g/mol	0
C6	**9**	{*3,5,1*}	0	490.13 g/mol	490.13 g/mol	1469,48
D6	**9**	{*4,5,1*}	0	490.13 g/mol	490.12 g/mol	2527,78
E6	**9**	{*5,5,1*}	0	558.05 g/mol	558.07 g/mol	100,13
F6	**9**	{*6,5,1*}	0	526.11 g/mol	526.11 g/mol	1252,35
G6	**9**	{*7,5,1*}	0	520.15 g/mol	520.14 g/mol	395,82
H6	**9**	{*8,5,1*}	0,04	474.14 g/mol	474.14 g/mol	1208,87
A7	**9**	{*1,6,1*}	0	490.13 g/mol	490.13 g/mol	743,6
B7	**9**	{*2,6,1*}	0	506.13 g/mol	506.13 g/mol	115,56
C7	**9**	{*3,6,1*}	0	490.13 g/mol	490.13 g/mol	544,75
D7	**9**	{*4,6,1*}	0	490.13 g/mol	490.13 g/mol	1198,17
E7	**9**	{*5,6,1*}	0	558.05 g/mol	0.00 g/mol	0
F7	**9**	{*6,6,1*}	0	526.11 g/mol	526.12 g/mol	71,66
G7	**9**	{*7,6,1*}	0	520.15 g/mol	520.14 g/mol	368,34
H7	**9**	{*8,6,1*}	0,02	474.14 g/mol	474.14 g/mol	715,47
A8	**9**	{*1,7,1*}	0	506.13 g/mol	0.00 g/mol	0
B8	**9**	{*2,7,1*}	0	522.12 g/mol	0.00 g/mol	0
C8	**9**	{*3,7,1*}	0,04	506.13 g/mol	506.13 g/mol	1337,1
D8	**9**	{*4,7,1*}	0	506.13 g/mol	0.00 g/mol	0
E8	**9**	{*5,7,1*}	0	574.04 g/mol	0.00 g/mol	0
F8	**9**	{*6,7,1*}	0	542.10 g/mol	542.12 g/mol	20,07
G8	**9**	{*7,7,1*}	0	536.14 g/mol	0.00 g/mol	0
H8	**9**	{*8,7,1*}	0	490.13 g/mol	0.00 g/mol	0
A9	**9**	{*1,8,1*}	0,02	490.13 g/mol	490.14 g/mol	861,34
B9	**9**	{*2,8,1*}	0	506.13 g/mol	0.00 g/mol	0
C9	**9**	{*3,8,1*}	0	490.13 g/mol	490.13 g/mol	97,14
D9	**9**	{*4,8,1*}	0	490.13 g/mol	490.13 g/mol	2535,46
E9	**9**	{*5,8,1*}	0	558.05 g/mol	0.00 g/mol	0
F9	**9**	{*6,8,1*}	0	526.11 g/mol	526.11 g/mol	1207,41
G9	**9**	{*7,8,1*}	0,06	520.15 g/mol	520.14 g/mol	2109,19
H9	**9**	{*8,8,1*}	0,02	474.14 g/mol	474.14 g/mol	723,85
A10	**9**	{*1,9,1*}	0	506.13 g/mol	0.00 g/mol	0
B10	**9**	{*2,9,1*}	0	522.12 g/mol	0.00 g/mol	0
C10	**9**	{*3,9,1*}	0	506.13 g/mol	506.14 g/mol	124,36
D10	**9**	{*4,9,1*}	0	506.13 g/mol	0.00 g/mol	0
E10	**9**	{*5,9,1*}	0	574.04 g/mol	0.00 g/mol	0
F10	**9**	{*6,9,1*}	0	542.10 g/mol	0.00 g/mol	0
G10	**9**	{*7,9,1*}	0	536.14 g/mol	0.00 g/mol	0
H10	**9**	{*8,9,1*}	0	490.13 g/mol	0.00 g/mol	0
A11	**9**	{*1,10,1*}	0	506.13 g/mol	0.00 g/mol	0
B11	**9**	{*2,10,1*}	0	522.12 g/mol	0.00 g/mol	0
C11	**9**	{*3,10,1*}	0	506.13 g/mol	0.00 g/mol	0
D11	**9**	{*4,10,1*}	0	506.13 g/mol	0.00 g/mol	0
E11	**9**	{*5,10,1*}	0	574.04 g/mol	0.00 g/mol	0
F11	**9**	{*6,10,1*}	0	542.10 g/mol	0.00 g/mol	0
G11	**9**	{*7,10,1*}	0	536.14 g/mol	0.00 g/mol	0
H11	**9**	{*8,10,1*}	0	490.13 g/mol	0.00 g/mol	0
A12	**9**	{*1,11,1*}	0,02	506.13 g/mol	506.13 g/mol	992,11
B12	**9**	{*2,11,1*}	0	522.12 g/mol	0.00 g/mol	0
C12	**9**	{*3,11,1*}	0	506.13 g/mol	506.13 g/mol	181,16
D12	**9**	{*4,11,1*}	0	506.13 g/mol	506.13 g/mol	784,38
E12	**9**	{*5,11,1*}	0,04	574.04 g/mol	574.05 g/mol	1548,62
F12	**9**	{*6,11,1*}	0	542.10 g/mol	0.00 g/mol	0
G12	**9**	{*7,11,1*}	0	536.14 g/mol	536.14 g/mol	219,51
H12	**9**	{*8,11,1*}	0,04	490.13 g/mol	490.13 g/mol	1284,58

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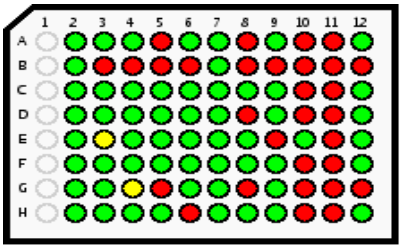

Well	Chemset	Peak width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**9** {*1,1,2*}	0	478.23 g/mol	478.21 g/mol	688,61
B2	**9** {*2,1,2*}	0,04	494.22 g/mol	494.21 g/mol	1429,33
C2	**9** {*3,1,2*}	0,08	478.23 g/mol	478.20 g/mol	6519,36
D2	**9** {*4,1,2*}	0	478.23 g/mol	478.21 g/mol	347,4
E2	**9** {*5,1,2*}	0	546.14 g/mol	546.13 g/mol	1060,2
F2	**9** {*6,1,2*}	0	514.20 g/mol	514.19 g/mol	785,32
G2	**9** {*7,1,2*}	0	508.24 g/mol	508.22 g/mol	628,4
H2	**9** {*8,1,2*}	0,06	462.23 g/mol	462.22 g/mol	1745,75
A3	**9** {*1,2,2*}	0	462.23 g/mol	462.22 g/mol	520,23
B3	**9** {*2,2,2*}	0	478.23 g/mol	0.00 g/mol	0
C3	**9** {*3,2,2*}	0,06	462.23 g/mol	462.21 g/mol	5856,08
D3	**9** {*4,2,2*}	0,06	462.23 g/mol	462.21 g/mol	6174,66
E3	**9** {*5,2,2*}	0	530.15 g/mol	530.15 g/mol	75,63
F3	**9** {*6,2,2*}	0,06	498.21 g/mol	498.19 g/mol	5900,78
G3	**9** {*7,2,2*}	0	492.25 g/mol	492.23 g/mol	456,83
H3	**9** {*8,2,2*}	0,02	446.24 g/mol	446.22 g/mol	663,45
A4	**9** {*1,3,2*}	0	462.23 g/mol	462.20 g/mol	2891,31
B4	**9** {*2,3,2*}	0	478.23 g/mol	0.00 g/mol	0
C4	**9** {*3,3,2*}	0	462.23 g/mol	462.21 g/mol	2074,49
D4	**9** {*4,3,2*}	0,12	462.23 g/mol	462.21 g/mol	7535,1
E4	**9** {*5,3,2*}	0	530.15 g/mol	530.14 g/mol	121,38
F4	**9** {*6,3,2*}	0,02	498.21 g/mol	498.19 g/mol	6180,84
G4	**9** {*7,3,2*}	0	492.25 g/mol	492.22 g/mol	97,64
H4	**9** {*8,3,2*}	0,1	446.24 g/mol	446.23 g/mol	2774,07
A5	**9** {*1,4,2*}	0	478.23 g/mol	0.00 g/mol	0
B5	**9** {*2,4,2*}	0	494.22 g/mol	0.00 g/mol	0
C5	**9** {*3,4,2*}	0	478.23 g/mol	478.21 g/mol	1550,28
D5	**9** {*4,4,2*}	0,02	478.23 g/mol	478.21 g/mol	960,07
E5	**9** {*5,4,2*}	0	546.14 g/mol	546.13 g/mol	370,69
F5	**9** {*6,4,2*}	0,04	514.20 g/mol	514.19 g/mol	1745,02
G5	**9** {*7,4,2*}	0	508.24 g/mol	0.00 g/mol	0
H5	**9** {*8,4,2*}	0,02	462.23 g/mol	462.21 g/mol	2225,79
A6	**9** {*1,5,2*}	0	478.23 g/mol	478.21 g/mol	1128,28
B6	**9** {*2,5,2*}	0	494.22 g/mol	0.00 g/mol	0
C6	**9** {*3,5,2*}	0	478.23 g/mol	478.21 g/mol	3193,98
D6	**9** {*4,5,2*}	0,04	478.23 g/mol	478.21 g/mol	4675,63
E6	**9** {*5,5,2*}	0,04	546.14 g/mol	546.14 g/mol	1433,71
F6	**9** {*6,5,2*}	0,04	514.20 g/mol	514.19 g/mol	4367,79
G6	**9** {*7,5,2*}	0,02	508.24 g/mol	508.23 g/mol	750,82
H6	**9** {*8,5,2*}	0	462.23 g/mol	0.00 g/mol	0

A7	**9**	{*1,6,2*}	0,06	478.23 g/mol	478.21 g/mol	2492,31
B7	**9**	{*2,6,2*}	0,02	494.22 g/mol	494.21 g/mol	1020,77
C7	**9**	{*3,6,2*}	0	478.23 g/mol	478.21 g/mol	3111,04
D7	**9**	{*4,6,2*}	0	478.23 g/mol	478.21 g/mol	726,02
E7	**9**	{*5,6,2*}	0	546.14 g/mol	546.13 g/mol	1423,57
F7	**9**	{*6,6,2*}	0	514.20 g/mol	514.19 g/mol	1314,5
G7	**9**	{*7,6,2*}	0,06	508.24 g/mol	508.22 g/mol	2630,82
H7	**9**	{*8,6,2*}	0,02	462.23 g/mol	462.21 g/mol	972,34
A8	**9**	{*1,7,2*}	0	494.22 g/mol	0.00 g/mol	0
B8	**9**	{*2,7,2*}	0	510.22 g/mol	0.00 g/mol	0
C8	**9**	{*3,7,2*}	0,02	494.22 g/mol	494.22 g/mol	650,54
D8	**9**	{*4,7,2*}	0	494.22 g/mol	0.00 g/mol	0
E8	**9**	{*5,7,2*}	0,08	562.14 g/mol	562.13 g/mol	2739,47
F8	**9**	{*6,7,2*}	0,04	530.20 g/mol	530.19 g/mol	1358,93
G8	**9**	{*7,7,2*}	0	524.24 g/mol	0.00 g/mol	0
H8	**9**	{*8,7,2*}	0	478.23 g/mol	478.22 g/mol	200,89
A9	**9**	{*1,8,2*}	0,02	478.23 g/mol	478.21 g/mol	1233,26
B9	**9**	{*2,8,2*}	0	494.22 g/mol	0.00 g/mol	0
C9	**9**	{*3,8,2*}	0	478.23 g/mol	478.21 g/mol	3295,52
D9	**9**	{*4,8,2*}	0,06	478.23 g/mol	478.18 g/mol	7372,38
E9	**9**	{*5,8,2*}	0	546.14 g/mol	0.00 g/mol	0
F9	**9**	{*6,8,2*}	0,04	514.20 g/mol	514.19 g/mol	4990,58
G9	**9**	{*7,8,2*}	0	508.24 g/mol	508.22 g/mol	862,87
H9	**9**	{*8,8,2*}	0	462.23 g/mol	462.22 g/mol	500,94
A10	**9**	{*1,9,2*}	0	494.22 g/mol	0.00 g/mol	0
B10	**9**	{*2,9,2*}	0	510.22 g/mol	0.00 g/mol	0
C10	**9**	{*3,9,2*}	0	494.22 g/mol	0.00 g/mol	0
D10	**9**	{*4,9,2*}	0	494.22 g/mol	0.00 g/mol	0
E10	**9**	{*5,9,2*}	0,02	562.14 g/mol	562.14 g/mol	742,74
F10	**9**	{*6,9,2*}	0	530.20 g/mol	0.00 g/mol	0
G10	**9**	{*7,9,2*}	0	524.24 g/mol	0.00 g/mol	0
H10	**9**	{*8,9,2*}	0	478.23 g/mol	0.00 g/mol	0
A11	**9**	{*1,10,2*}	0	494.22 g/mol	0.00 g/mol	0
B11	**9**	{*2,10,2*}	0	510.22 g/mol	0.00 g/mol	0
C11	**9**	{*3,10,2*}	0	494.22 g/mol	0.00 g/mol	0
D11	**9**	{*4,10,2*}	0	494.22 g/mol	0.00 g/mol	0
E11	**9**	{*5,10,2*}	0	562.14 g/mol	0.00 g/mol	0
F11	**9**	{*6,10,2*}	0	530.20 g/mol	0.00 g/mol	0
G11	**9**	{*7,10,2*}	0	524.24 g/mol	0.00 g/mol	0
H11	**9**	{*8,10,2*}	0	478.23 g/mol	0.00 g/mol	0
A12	**9**	{*1,11,2*}	0,02	494.22 g/mol	494.21 g/mol	1065,45
B12	**9**	{*2,11,2*}	0	510.22 g/mol	0.00 g/mol	0
C12	**9**	{*3,11,2*}	0,02	494.22 g/mol	494.22 g/mol	663,12
D12	**9**	{*4,11,2*}	0	494.22 g/mol	494.20 g/mol	2585,34
E12	**9**	{*5,11,2*}	0	562.14 g/mol	562.13 g/mol	133,31
F12	**9**	{*6,11,2*}	0,06	530.20 g/mol	530.19 g/mol	2039,68
G12	**9**	{*7,11,2*}	0	524.24 g/mol	0.00 g/mol	0
H12	**9**	{*8,11,2*}	0,14	478.23 g/mol	478.21 g/mol	4455,74

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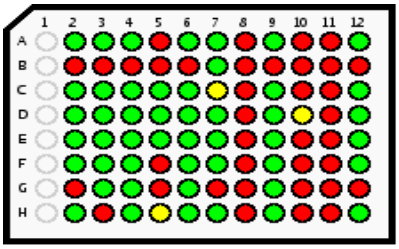

Well	Chemset	Peak width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**9** {*1,1,3*}	0	436.17 g/mol	436.14 g/mol	363,92
B2	**9** {*2,1,3*}	0	452.16 g/mol	0.00 g/mol	0
C2	**9** {*3,1,3*}	0,1	436.17 g/mol	436.13 g/mol	3705,87
D2	**9** {*4,1,3*}	0	436.17 g/mol	436.15 g/mol	353,43
E2	**9** {*5,1,3*}	0	504.08 g/mol	504.08 g/mol	418,88
F2	**9** {*6,1,3*}	0,08	472.14 g/mol	472.13 g/mol	2477,72
G2	**9** {*7,1,3*}	0	466.18 g/mol	0.00 g/mol	0
H2	**9** {*8,1,3*}	0,02	420.17 g/mol	420.16 g/mol	739,36
A3	**9** {*1,2,3*}	0,04	420.17 g/mol	420.15 g/mol	1561,49
B3	**9** {*2,2,3*}	0	436.17 g/mol	0.00 g/mol	0
C3	**9** {*3,2,3*}	0,04	420.17 g/mol	420.15 g/mol	1981,07
D3	**9** {*4,2,3*}	0,04	420.17 g/mol	420.15 g/mol	4241,53
E3	**9** {*5,2,3*}	0,02	488.09 g/mol	488.08 g/mol	735,95
F3	**9** {*6,2,3*}	0	456.15 g/mol	456.13 g/mol	854,77
G3	**9** {*7,2,3*}	0,04	450.19 g/mol	450.17 g/mol	1941,79
H3	**9** {*8,2,3*}	0	404.18 g/mol	0.00 g/mol	0
A4	**9** {*1,3,3*}	0,02	420.17 g/mol	420.15 g/mol	940,92
B4	**9** {*2,3,3*}	0	436.17 g/mol	0.00 g/mol	0
C4	**9** {*3,3,3*}	0	420.17 g/mol	420.15 g/mol	884,69
D4	**9** {*4,3,3*}	0,1	420.17 g/mol	420.15 g/mol	3419,79
E4	**9** {*5,3,3*}	0	488.09 g/mol	488.07 g/mol	1886,36
F4	**9** {*6,3,3*}	0	456.15 g/mol	456.12 g/mol	4482,14
G4	**9** {*7,3,3*}	0	450.19 g/mol	450.16 g/mol	1854,41
H4	**9** {*8,3,3*}	0	404.18 g/mol	404.17 g/mol	119,29
A5	**9** {*1,4,3*}	0	436.17 g/mol	0.00 g/mol	0
B5	**9** {*2,4,3*}	0	452.16 g/mol	0.00 g/mol	0
C5	**9** {*3,4,3*}	0,02	436.17 g/mol	436.15 g/mol	943,77
D5	**9** {*4,4,3*}	0	436.17 g/mol	436.15 g/mol	191,47
E5	**9** {*5,4,3*}	0	504.08 g/mol	504.09 g/mol	215,84
F5	**9** {*6,4,3*}	0	472.14 g/mol	0.00 g/mol	0
G5	**9** {*7,4,3*}	0	466.18 g/mol	0.00 g/mol	0
H5	**9** {*8,4,3*}	0	420.17 g/mol	420.27 g/mol	83,6
A6	**9** {*1,5,3*}	0	436.17 g/mol	436.16 g/mol	414,08
B6	**9** {*2,5,3*}	0	452.16 g/mol	0.00 g/mol	0
C6	**9** {*3,5,3*}	0	436.17 g/mol	436.15 g/mol	942,19
D6	**9** {*4,5,3*}	0,06	436.17 g/mol	436.15 g/mol	3935,73
E6	**9** {*5,5,3*}	0,02	504.08 g/mol	504.08 g/mol	1187,17
F6	**9** {*6,5,3*}	0	472.14 g/mol	472.13 g/mol	1185,1
G6	**9** {*7,5,3*}	0	466.18 g/mol	466.17 g/mol	452,27
H6	**9** {*8,5,3*}	0	420.17 g/mol	420.27 g/mol	128,98

A7	**9**	{*1,6,3*}	0	436.17 g/mol	436.16 g/mol	422,96
B7	**9**	{*2,6,3*}	0	452.16 g/mol	452.09 g/mol	104,81
C7	**9**	{*3,6,3*}	0	436.17 g/mol	436.17 g/mol	61,82
D7	**9**	{*4,6,3*}	0	436.17 g/mol	436.17 g/mol	110,85
E7	**9**	{*5,6,3*}	0	504.08 g/mol	504.08 g/mol	251,89
F7	**9**	{*6,6,3*}	0,04	472.14 g/mol	472.14 g/mol	1120,08
G7	**9**	{*7,6,3*}	0	466.18 g/mol	0.00 g/mol	0
H7	**9**	{*8,6,3*}	0,02	420.17 g/mol	420.16 g/mol	692,05
A8	**9**	{*1,7,3*}	0	452.16 g/mol	0.00 g/mol	0
B8	**9**	{*2,7,3*}	0	468.16 g/mol	0.00 g/mol	0
C8	**9**	{*3,7,3*}	0	452.16 g/mol	0.00 g/mol	0
D8	**9**	{*4,7,3*}	0	452.16 g/mol	0.00 g/mol	0
E8	**9**	{*5,7,3*}	0	520.08 g/mol	0.00 g/mol	0
F8	**9**	{*6,7,3*}	0	488.14 g/mol	0.00 g/mol	0
G8	**9**	{*7,7,3*}	0	482.18 g/mol	0.00 g/mol	0
H8	**9**	{*8,7,3*}	0	436.17 g/mol	0.00 g/mol	0
A9	**9**	{*1,8,3*}	0	436.17 g/mol	436.16 g/mol	363,48
B9	**9**	{*2,8,3*}	0	452.16 g/mol	0.00 g/mol	0
C9	**9**	{*3,8,3*}	0,02	436.17 g/mol	436.19 g/mol	2389,5
D9	**9**	{*4,8,3*}	0,02	436.17 g/mol	436.16 g/mol	4119,15
E9	**9**	{*5,8,3*}	0,02	504.08 g/mol	504.08 g/mol	1349,29
F9	**9**	{*6,8,3*}	0,12	472.14 g/mol	472.13 g/mol	5094,17
G9	**9**	{*7,8,3*}	0	466.18 g/mol	466.17 g/mol	307,94
H9	**9**	{*8,8,3*}	0,04	420.17 g/mol	420.26 g/mol	5228,38
A10	**9**	{*1,9,3*}	0	452.16 g/mol	0.00 g/mol	0
B10	**9**	{*2,9,3*}	0	468.16 g/mol	0.00 g/mol	0
C10	**9**	{*3,9,3*}	0	452.16 g/mol	0.00 g/mol	0
D10	**9**	{*4,9,3*}	0	452.16 g/mol	452.13 g/mol	36,23
E10	**9**	{*5,9,3*}	0	520.08 g/mol	0.00 g/mol	0
F10	**9**	{*6,9,3*}	0	488.14 g/mol	0.00 g/mol	0
G10	**9**	{*7,9,3*}	0	482.18 g/mol	0.00 g/mol	0
H10	**9**	{*8,9,3*}	0	436.17 g/mol	0.00 g/mol	0
A11	**9**	{*1,10,3*}	0	452.16 g/mol	0.00 g/mol	0
B11	**9**	{*2,10,3*}	0	468.16 g/mol	0.00 g/mol	0
C11	**9**	{*3,10,3*}	0	452.16 g/mol	0.00 g/mol	0
D11	**9**	{*4,10,3*}	0	452.16 g/mol	0.00 g/mol	0
E11	**9**	{*5,10,3*}	0	520.08 g/mol	0.00 g/mol	0
F11	**9**	{*6,10,3*}	0	488.14 g/mol	0.00 g/mol	0
G11	**9**	{*7,10,3*}	0	482.18 g/mol	0.00 g/mol	0
H11	**9**	{*8,10,3*}	0	436.17 g/mol	0.00 g/mol	0
A12	**9**	{*1,11,3*}	0,02	452.16 g/mol	452.16 g/mol	735,5
B12	**9**	{*2,11,3*}	0	468.16 g/mol	0.00 g/mol	0
C12	**9**	{*3,11,3*}	0	452.16 g/mol	452.15 g/mol	688,1
D12	**9**	{*4,11,3*}	0	452.16 g/mol	452.16 g/mol	261,02
E12	**9**	{*5,11,3*}	0,02	520.08 g/mol	520.08 g/mol	1340,93
F12	**9**	{*6,11,3*}	0	488.14 g/mol	488.14 g/mol	212,36
G12	**9**	{*7,11,3*}	0	482.18 g/mol	0.00 g/mol	0
H12	**9**	{*8,11,3*}	0	436.17 g/mol	436.16 g/mol	146,93

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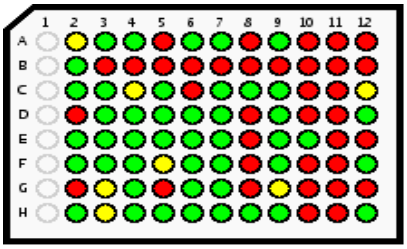

Well	Chemset	Peak width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**9** {*1,1,4*}	0	456.11 g/mol	456.11 g/mol	83,59
B2	**9** {*2,1,4*}	0,02	472.10 g/mol	472.11 g/mol	878,76
C2	**9** {*3,1,4*}	0,06	456.11 g/mol	456.11 g/mol	5477,36
D2	**9** {*4,1,4*}	0	456.11 g/mol	0.00 g/mol	0
E2	**9** {*5,1,4*}	0	524.02 g/mol	524.03 g/mol	271,76
F2	**9** {*6,1,4*}	0	492.08 g/mol	492.09 g/mol	754,01
G2	**9** {*7,1,4*}	0	486.12 g/mol	0.00 g/mol	0
H2	**9** {*8,1,4*}	0,02	440.11 g/mol	440.12 g/mol	804,62
A3	**9** {*1,2,4*}	0	440.11 g/mol	440.12 g/mol	160,23
B3	**9** {*2,2,4*}	0	456.11 g/mol	0.00 g/mol	0
C3	**9** {*3,2,4*}	0	440.11 g/mol	440.13 g/mol	153,95
D3	**9** {*4,2,4*}	0	440.11 g/mol	440.12 g/mol	348,75
E3	**9** {*5,2,4*}	0	508.03 g/mol	508.04 g/mol	169,05
F3	**9** {*6,2,4*}	0,02	476.09 g/mol	476.11 g/mol	758,61
G3	**9** {*7,2,4*}	0	470.13 g/mol	470.15 g/mol	71,23
H3	**9** {*8,2,4*}	0	424.12 g/mol	424.13 g/mol	59,59
A4	**9** {*1,3,4*}	0	440.11 g/mol	440.12 g/mol	185,9
B4	**9** {*2,3,4*}	0	456.11 g/mol	0.00 g/mol	0
C4	**9** {*3,3,4*}	0	440.11 g/mol	440.13 g/mol	75,12
D4	**9** {*4,3,4*}	0,04	440.11 g/mol	440.11 g/mol	5114,24
E4	**9** {*5,3,4*}	0	508.03 g/mol	508.04 g/mol	927,35
F4	**9** {*6,3,4*}	0	476.09 g/mol	476.09 g/mol	2376,22
G4	**9** {*7,3,4*}	0,02	470.13 g/mol	470.13 g/mol	800,88
H4	**9** {*8,3,4*}	0,02	424.12 g/mol	424.12 g/mol	635,15
A5	**9** {*1,4,4*}	0	456.11 g/mol	0.00 g/mol	0
B5	**9** {*2,4,4*}	0	472.10 g/mol	0.00 g/mol	0
C5	**9** {*3,4,4*}	0,02	456.11 g/mol	456.11 g/mol	955,58
D5	**9** {*4,4,4*}	0	456.11 g/mol	456.12 g/mol	244,93
E5	**9** {*5,4,4*}	0	524.02 g/mol	524.04 g/mol	246,4
F5	**9** {*6,4,4*}	0	492.08 g/mol	492.10 g/mol	85,49
G5	**9** {*7,4,4*}	0	486.12 g/mol	0.00 g/mol	0
H5	**9** {*8,4,4*}	0	440.11 g/mol	440.11 g/mol	3081,56
A6	**9** {*1,5,4*}	0	456.11 g/mol	456.12 g/mol	226,77
B6	**9** {*2,5,4*}	0	472.10 g/mol	0.00 g/mol	0
C6	**9** {*3,5,4*}	0	456.11 g/mol	0.00 g/mol	0
D6	**9** {*4,5,4*}	0	456.11 g/mol	456.11 g/mol	961,06
E6	**9** {*5,5,4*}	0,02	524.02 g/mol	524.05 g/mol	719,17
F6	**9** {*6,5,4*}	0,02	492.08 g/mol	492.10 g/mol	971,9
G6	**9** {*7,5,4*}	0,02	486.12 g/mol	486.15 g/mol	653,63
H6	**9** {*8,5,4*}	0	440.11 g/mol	440.12 g/mol	199,93

A7	**9**	{*1,6,4*}	0,02	456.11 g/mol	456.11 g/mol	1359,14
B7	**9**	{*2,6,4*}	0	472.10 g/mol	0.00 g/mol	0
C7	**9**	{*3,6,4*}	0,02	456.11 g/mol	456.11 g/mol	1858,84
D7	**9**	{*4,6,4*}	0	456.11 g/mol	456.11 g/mol	728,16
E7	**9**	{*5,6,4*}	0	524.02 g/mol	524.04 g/mol	330,5
F7	**9**	{*6,6,4*}	0	492.08 g/mol	492.10 g/mol	250,93
G7	**9**	{*7,6,4*}	0,02	486.12 g/mol	486.12 g/mol	947,54
H7	**9**	{*8,6,4*}	0,04	440.11 g/mol	440.12 g/mol	1289,39
A8	**9**	{*1,7,4*}	0	472.10 g/mol	0.00 g/mol	0
B8	**9**	{*2,7,4*}	0	488.10 g/mol	0.00 g/mol	0
C8	**9**	{*3,7,4*}	0,02	472.10 g/mol	472.09 g/mol	610,53
D8	**9**	{*4,7,4*}	0	472.10 g/mol	0.00 g/mol	0
E8	**9**	{*5,7,4*}	0	540.02 g/mol	0.00 g/mol	0
F8	**9**	{*6,7,4*}	0	508.08 g/mol	0.00 g/mol	0
G8	**9**	{*7,7,4*}	0	502.12 g/mol	0.00 g/mol	0
H8	**9**	{*8,7,4*}	0	456.11 g/mol	456.11 g/mol	547,28
A9	**9**	{*1,8,4*}	0	456.11 g/mol	456.11 g/mol	1209,43
B9	**9**	{*2,8,4*}	0	472.10 g/mol	0.00 g/mol	0
C9	**9**	{*3,8,4*}	0,02	456.11 g/mol	456.11 g/mol	602,53
D9	**9**	{*4,8,4*}	0	456.11 g/mol	456.11 g/mol	1296,05
E9	**9**	{*5,8,4*}	0	524.02 g/mol	524.04 g/mol	397,14
F9	**9**	{*6,8,4*}	0,02	492.08 g/mol	492.10 g/mol	1170,86
G9	**9**	{*7,8,4*}	0	486.12 g/mol	486.12 g/mol	83,29
H9	**9**	{*8,8,4*}	0	440.11 g/mol	440.12 g/mol	191,85
A10	**9**	{*1,9,4*}	0	472.10 g/mol	0.00 g/mol	0
B10	**9**	{*2,9,4*}	0	488.10 g/mol	0.00 g/mol	0
C10	**9**	{*3,9,4*}	0	472.10 g/mol	472.11 g/mol	27,54
D10	**9**	{*4,9,4*}	0	472.10 g/mol	0.00 g/mol	0
E10	**9**	{*5,9,4*}	0	540.02 g/mol	540.04 g/mol	102,46
F10	**9**	{*6,9,4*}	0	508.08 g/mol	0.00 g/mol	0
G10	**9**	{*7,9,4*}	0	502.12 g/mol	0.00 g/mol	0
H10	**9**	{*8,9,4*}	0	456.11 g/mol	0.00 g/mol	0
A11	**9**	{*1,10,4*}	0	472.10 g/mol	0.00 g/mol	0
B11	**9**	{*2,10,4*}	0	488.10 g/mol	0.00 g/mol	0
C11	**9**	{*3,10,4*}	0	472.10 g/mol	0.00 g/mol	0
D11	**9**	{*4,10,4*}	0	472.10 g/mol	0.00 g/mol	0
E11	**9**	{*5,10,4*}	0	540.02 g/mol	0.00 g/mol	0
F11	**9**	{*6,10,4*}	0	508.08 g/mol	0.00 g/mol	0
G11	**9**	{*7,10,4*}	0	502.12 g/mol	0.00 g/mol	0
H11	**9**	{*8,10,4*}	0	456.11 g/mol	0.00 g/mol	0
A12	**9**	{*1,11,4*}	0	472.10 g/mol	0.00 g/mol	0
B12	**9**	{*2,11,4*}	0	488.10 g/mol	0.00 g/mol	0
C12	**9**	{*3,11,4*}	0	472.10 g/mol	472.11 g/mol	59,66
D12	**9**	{*4,11,4*}	0,04	472.10 g/mol	472.11 g/mol	1403,23
E12	**9**	{*5,11,4*}	0	540.02 g/mol	0.00 g/mol	0
F12	**9**	{*6,11,4*}	0	508.08 g/mol	508.09 g/mol	105,65
G12	**9**	{*7,11,4*}	0	502.12 g/mol	0.00 g/mol	0
H12	**9**	{*8,11,4*}	0	456.11 g/mol	456.11 g/mol	959,16

B.7 Screening plates ori.hya.48-54

B.7.1 Starting materials ori.hya.48-54

Cf. section 6.2; chemsets (see Figure 6.5) are indicated for each well.

B.7.2 Mass spectral analysis of plates ori.hya.48-54

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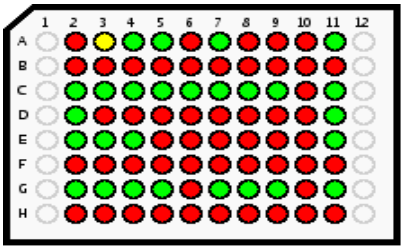

Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*1,1,1*}	0.0	298.19 g/mol	0.00 g/mol	0.00
B2	**13** {*2,1,1*}	0.0	298.19 g/mol	0.00 g/mol	0.00
C2	**13** {*3,1,1*}	0.10	298.19 g/mol	298.17 g/mol	26972.10
D2	**13** {*4,1,1*}	0.00	298.19 g/mol	298.15 g/mol	159.58
E2	**13** {*1,2,1*}	0.04	366.10 g/mol	366.08 g/mol	2210.30
F2	**13** {*2,2,1*}	0.0	366.10 g/mol	0.00 g/mol	0.00
G2	**13** {*3,2,1*}	0.18	366.10 g/mol	366.09 g/mol	9772.74
H2	**13** {*4,2,1*}	0.0	366.10 g/mol	0.00 g/mol	0.00
A3	**13** {*1,1,2*}	0.00	314.13 g/mol	314.13 g/mol	83.20
B3	**13** {*2,1,2*}	0.0	314.13 g/mol	0.00 g/mol	0.00
C3	**13** {*3,1,2*}	0.00	314.13 g/mol	314.12 g/mol	819.40
D3	**13** {*4,1,2*}	0.0	314.13 g/mol	0.00 g/mol	0.00
E3	**13** {*1,2,2*}	0.02	382.05 g/mol	382.05 g/mol	550.24
F3	**13** {*2,2,2*}	0.0	382.05 g/mol	0.00 g/mol	0.00
G3	**13** {*3,2,2*}	0.10	382.05 g/mol	382.03 g/mol	3067.49
H3	**13** {*4,2,2*}	0.0	382.05 g/mol	0.00 g/mol	0.00
A4	**13** {*1,1,3*}	0.00	282.15 g/mol	282.13 g/mol	332.72
B4	**13** {*2,1,3*}	0.0	282.15 g/mol	0.00 g/mol	0.00
C4	**13** {*3,1,3*}	0.10	282.15 g/mol	282.14 g/mol	25097.64
D4	**13** {*4,1,3*}	0.0	282.15 g/mol	0.00 g/mol	0.00
E4	**13** {*1,2,3*}	0.00	350.06 g/mol	350.06 g/mol	184.10
F4	**13** {*2,2,3*}	0.0	350.06 g/mol	0.00 g/mol	0.00
G4	**13** {*3,2,3*}	0.18	350.06 g/mol	350.06 g/mol	11722.50
H4	**13** {*4,2,3*}	0.0	350.06 g/mol	0.00 g/mol	0.00
A5	**13** {*1,1,4*}	0.02	300.16 g/mol	300.15 g/mol	372.41
B5	**13** {*2,1,4*}	0.0	300.16 g/mol	0.00 g/mol	0.00
C5	**13** {*3,1,4*}	0.22	300.16 g/mol	300.15 g/mol	5625.84
D5	**13** {*4,1,4*}	0.0	300.16 g/mol	0.00 g/mol	0.00
E5	**13** {*1,2,4*}	0.0	368.08 g/mol	0.00 g/mol	0.00
F5	**13** {*2,2,4*}	0.0	368.08 g/mol	0.00 g/mol	0.00
G5	**13** {*3,2,4*}	0.18	368.08 g/mol	368.07 g/mol	5040.71
H5	**13** {*4,2,4*}	0.0	368.08 g/mol	0.00 g/mol	0.00

A6	**13**	{*1,1,5*}	0.0	256.13 g/mol	0.00 g/mol	0.00
B6	**13**	{*2,1,5*}	0.0	256.13 g/mol	0.00 g/mol	0.00
C6	**13**	{*3,1,5*}	0.20	256.13 g/mol	256.12 g/mol	4821.45
D6	**13**	{*4,1,5*}	0.0	256.13 g/mol	0.00 g/mol	0.00
E6	**13**	{*1,2,5*}	0.0	324.04 g/mol	0.00 g/mol	0.00
F6	**13**	{*2,2,5*}	0.0	324.04 g/mol	0.00 g/mol	0.00
G6	**13**	{*3,2,5*}	0.0	324.04 g/mol	0.00 g/mol	0.00
H6	**13**	{*4,2,5*}	0.0	324.04 g/mol	0.00 g/mol	0.00
A7	**13**	{*1,1,6*}	0.02	298.19 g/mol	298.28 g/mol	613.06
B7	**13**	{*2,1,6*}	0.0	298.19 g/mol	0.00 g/mol	0.00
C7	**13**	{*3,1,6*}	0.08	298.19 g/mol	298.17 g/mol	25264.44
D7	**13**	{*4,1,6*}	0.0	298.19 g/mol	0.00 g/mol	0.00
E7	**13**	{*1,2,6*}	0.0	366.10 g/mol	0.00 g/mol	0.00
F7	**13**	{*2,2,6*}	0.0	366.10 g/mol	0.00 g/mol	0.00
G7	**13**	{*3,2,6*}	0.08	366.10 g/mol	366.07 g/mol	23434.20
H7	**13**	{*4,2,6*}	0.0	366.10 g/mol	0.00 g/mol	0.00
A8	**13**	{*1,1,7*}	0.0	318.14 g/mol	0.00 g/mol	0.00
B8	**13**	{*2,1,7*}	0.0	318.14 g/mol	0.00 g/mol	0.00
C8	**13**	{*3,1,7*}	0.02	318.14 g/mol	318.13 g/mol	4433.03
D8	**13**	{*4,1,7*}	0.0	318.14 g/mol	0.00 g/mol	0.00
E8	**13**	{*1,2,7*}	0.0	386.06 g/mol	0.00 g/mol	0.00
F8	**13**	{*2,2,7*}	0.0	386.06 g/mol	0.00 g/mol	0.00
G8	**13**	{*3,2,7*}	0.04	386.06 g/mol	386.06 g/mol	8377.62
H8	**13**	{*4,2,7*}	0.0	386.06 g/mol	0.00 g/mol	0.00
A9	**13**	{*1,1,8*}	0.0	282.15 g/mol	0.00 g/mol	0.00
B9	**13**	{*2,1,8*}	0.0	282.15 g/mol	0.00 g/mol	0.00
C9	**13**	{*3,1,8*}	0.06	282.15 g/mol	282.16 g/mol	1038.32
D9	**13**	{*4,1,8*}	0.0	282.15 g/mol	0.00 g/mol	0.00
E9	**13**	{*1,2,8*}	0.0	350.06 g/mol	0.00 g/mol	0.00
F9	**13**	{*2,2,8*}	0.0	350.06 g/mol	0.00 g/mol	0.00
G9	**13**	{*3,2,8*}	0.02	350.06 g/mol	350.06 g/mol	563.57
H9	**13**	{*4,2,8*}	0.0	350.06 g/mol	0.00 g/mol	0.00
A10	**13**	{*1,1,9*}	0.0	333.16 g/mol	0.00 g/mol	0.00
B10	**13**	{*2,1,9*}	0.0	333.16 g/mol	0.00 g/mol	0.00
C10	**13**	{*3,1,9*}	0.0	333.16 g/mol	0.00 g/mol	0.00
D10	**13**	{*4,1,9*}	0.0	333.16 g/mol	0.00 g/mol	0.00
E10	**13**	{*1,2,9*}	0.0	401.07 g/mol	0.00 g/mol	0.00
F10	**13**	{*2,2,9*}	0.0	401.07 g/mol	0.00 g/mol	0.00
G10	**13**	{*3,2,9*}	0.0	401.07 g/mol	0.00 g/mol	0.00
H10	**13**	{*4,2,9*}	0.0	401.07 g/mol	0.00 g/mol	0.00
A11	**13**	{*1,1,10*}	0.00	312.21 g/mol	312.20 g/mol	213.52
B11	**13**	{*2,1,10*}	0.0	312.21 g/mol	0.00 g/mol	0.00
C11	**13**	{*3,1,10*}	0.06	312.21 g/mol	312.21 g/mol	24369.72
D11	**13**	{*4,1,10*}	0.02	312.21 g/mol	312.17 g/mol	545.57
E11	**13**	{*1,2,10*}	0.00	380.12 g/mol	380.11 g/mol	1856.81
F11	**13**	{*2,2,10*}	0.0	380.12 g/mol	0.00 g/mol	0.00
G11	**13**	{*3,2,10*}	0.22	380.12 g/mol	380.11 g/mol	5362.93
H11	**13**	{*4,2,10*}	0.0	380.12 g/mol	0.00 g/mol	0.00
A12	*empty*					
B12	*empty*					
C12	*empty*					
D12	*empty*					
E12	*empty*					
F12	*empty*					
G12	*empty*					
H12	*empty*					

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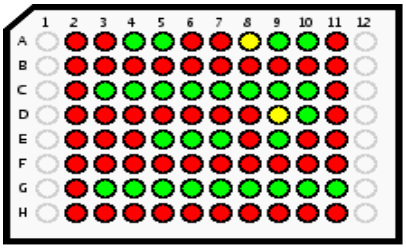

Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*1,1,11*}	0.0	313.20 g/mol	0.00 g/mol	0.00
B2	**13** {*2,1,11*}	0.0	313.20 g/mol	0.00 g/mol	0.00
C2	**13** {*3,1,11*}	0.0	313.20 g/mol	0.00 g/mol	0.00
D2	**13** {*4,1,11*}	0.0	313.20 g/mol	0.00 g/mol	0.00
E2	**13** {*1,2,11*}	0.0	381.11 g/mol	0.00 g/mol	0.00
F2	**13** {*2,2,11*}	0.0	381.11 g/mol	0.00 g/mol	0.00
G2	**13** {*3,2,11*}	0.0	381.11 g/mol	0.00 g/mol	0.00
H2	**13** {*4,2,11*}	0.0	381.11 g/mol	0.00 g/mol	0.00
A3	**13** {*1,1,12*}	0.0	314.18 g/mol	0.00 g/mol	0.00
B3	**13** {*2,1,12*}	0.0	314.18 g/mol	0.00 g/mol	0.00
C3	**13** {*3,1,12*}	0.14	314.18 g/mol	314.17 g/mol	26663.64
D3	**13** {*4,1,12*}	0.0	314.18 g/mol	0.00 g/mol	0.00
E3	**13** {*1,2,12*}	0.0	382.10 g/mol	0.00 g/mol	0.00
F3	**13** {*2,2,12*}	0.0	382.10 g/mol	0.00 g/mol	0.00
G3	**13** {*3,2,12*}	0.18	382.10 g/mol	382.09 g/mol	6680.52
H3	**13** {*4,2,12*}	0.0	382.10 g/mol	0.00 g/mol	0.00
A4	**13** {*1,1,13*}	0.02	312.21 g/mol	312.19 g/mol	679.95
B4	**13** {*2,1,13*}	0.0	312.21 g/mol	0.00 g/mol	0.00
C4	**13** {*3,1,13*}	0.06	312.21 g/mol	312.19 g/mol	23847.12
D4	**13** {*4,1,13*}	0.0	312.21 g/mol	0.00 g/mol	0.00
E4	**13** {*1,2,13*}	0.0	380.12 g/mol	0.00 g/mol	0.00
F4	**13** {*2,2,13*}	0.0	380.12 g/mol	0.00 g/mol	0.00
G4	**13** {*3,2,13*}	0.06	380.12 g/mol	380.11 g/mol	18585.48
H4	**13** {*4,2,13*}	0.0	380.12 g/mol	0.00 g/mol	0.00
A5	**13** {*1,1,14*}	0.02	310.19 g/mol	310.19 g/mol	693.83
B5	**13** {*2,1,14*}	0.0	310.19 g/mol	0.00 g/mol	0.00
C5	**13** {*3,1,14*}	0.12	310.19 g/mol	310.18 g/mol	28338.90
D5	**13** {*4,1,14*}	0.0	310.19 g/mol	0.00 g/mol	0.00
E5	**13** {*1,2,14*}	0.00	378.10 g/mol	378.04 g/mol	159.03
F5	**13** {*2,2,14*}	0.0	378.10 g/mol	0.00 g/mol	0.00
G5	**13** {*3,2,14*}	0.18	378.10 g/mol	378.10 g/mol	10638.06
H5	**13** {*4,2,14*}	0.0	378.10 g/mol	0.00 g/mol	0.00
A6	**13** {*1,1,15*}	0.0	284.17 g/mol	0.00 g/mol	0.00
B6	**13** {*2,1,15*}	0.0	284.17 g/mol	0.00 g/mol	0.00
C6	**13** {*3,1,15*}	0.10	284.17 g/mol	284.16 g/mol	26545.56
D6	**13** {*4,1,15*}	0.0	284.17 g/mol	0.00 g/mol	0.00
E6	**13** {*1,2,15*}	0.00	352.08 g/mol	352.08 g/mol	606.72
F6	**13** {*2,2,15*}	0.0	352.08 g/mol	0.00 g/mol	0.00
G6	**13** {*3,2,15*}	0.20	352.08 g/mol	352.08 g/mol	8563.62
H6	**13** {*4,2,15*}	0.0	352.08 g/mol	0.00 g/mol	0.00

A7	**13**	{*1,1,16*}	0.0	298.19 g/mol	0.00 g/mol	0.00
B7	**13**	{*2,1,16*}	0.0	298.19 g/mol	0.00 g/mol	0.00
C7	**13**	{*3,1,16*}	0.00	298.19 g/mol	298.18 g/mol	22064.82
D7	**13**	{*4,1,16*}	0.0	298.19 g/mol	0.00 g/mol	0.00
E7	**13**	{*1,2,16*}	0.00	366.10 g/mol	366.11 g/mol	153.76
F7	**13**	{*2,2,16*}	0.0	366.10 g/mol	0.00 g/mol	0.00
G7	**13**	{*3,2,16*}	0.00	366.10 g/mol	366.10 g/mol	14803.98
H7	**13**	{*4,2,16*}	0.0	366.10 g/mol	0.00 g/mol	0.00
A8	**13**	{*1,1,17*}	0.00	270.15 g/mol	270.15 g/mol	62.38
B8	**13**	{*2,1,17*}	0.0	270.15 g/mol	0.00 g/mol	0.00
C8	**13**	{*3,1,17*}	0.20	270.15 g/mol	270.15 g/mol	7492.92
D8	**13**	{*4,1,17*}	0.0	270.15 g/mol	0.00 g/mol	0.00
E8	**13**	{*1,2,17*}	0.0	338.06 g/mol	0.00 g/mol	0.00
F8	**13**	{*2,2,17*}	0.0	338.06 g/mol	0.00 g/mol	0.00
G8	**13**	{*3,2,17*}	0.16	338.06 g/mol	338.07 g/mol	6367.62
H8	**13**	{*4,2,17*}	0.0	338.06 g/mol	0.00 g/mol	0.00
A9	**13**	{*1,1,18*}	0.02	312.21 g/mol	312.19 g/mol	2271.17
B9	**13**	{*2,1,18*}	0.0	312.21 g/mol	0.00 g/mol	0.00
C9	**13**	{*3,1,18*}	0.02	312.21 g/mol	312.19 g/mol	23679.48
D9	**13**	{*4,1,18*}	0.00	312.21 g/mol	312.20 g/mol	54.58
E9	**13**	{*1,2,18*}	0.00	380.12 g/mol	380.12 g/mol	158.57
F9	**13**	{*2,2,18*}	0.0	380.12 g/mol	0.00 g/mol	0.00
G9	**13**	{*3,2,18*}	0.20	380.12 g/mol	380.15 g/mol	9630.60
H9	**13**	{*4,2,18*}	0.0	380.12 g/mol	0.00 g/mol	0.00
A10	**13**	{*1,1,19*}	0.00	312.21 g/mol	312.20 g/mol	265.49
B10	**13**	{*2,1,19*}	0.0	312.21 g/mol	0.00 g/mol	0.00
C10	**13**	{*3,1,19*}	0.08	312.21 g/mol	312.19 g/mol	23360.94
D10	**13**	{*4,1,19*}	0.02	312.21 g/mol	312.20 g/mol	448.60
E10	**13**	{*1,2,19*}	0.0	380.12 g/mol	0.00 g/mol	0.00
F10	**13**	{*2,2,19*}	0.0	380.12 g/mol	0.00 g/mol	0.00
G10	**13**	{*3,2,19*}	0.20	380.12 g/mol	380.12 g/mol	8438.94
H10	**13**	{*4,2,19*}	0.0	380.12 g/mol	0.00 g/mol	0.00
A11	**13**	{*1,1,20*}	0.0	314.18 g/mol	0.00 g/mol	0.00
B11	**13**	{*2,1,20*}	0.0	314.18 g/mol	0.00 g/mol	0.00
C11	**13**	{*3,1,20*}	0.0	314.18 g/mol	0.00 g/mol	0.00
D11	**13**	{*4,1,20*}	0.0	314.18 g/mol	0.00 g/mol	0.00
E11	**13**	{*1,2,20*}	0.0	382.10 g/mol	0.00 g/mol	0.00
F11	**13**	{*2,2,20*}	0.0	382.10 g/mol	0.00 g/mol	0.00
G11	**13**	{*3,2,20*}	0.00	382.10 g/mol	382.10 g/mol	12850.44
H11	**13**	{*4,2,20*}	0.0	382.10 g/mol	0.00 g/mol	0.00
A12		*empty*				
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C12		*empty*				
D12		*empty*				
E12		*empty*				
F12		*empty*				
G12		*empty*				
H12		*empty*				

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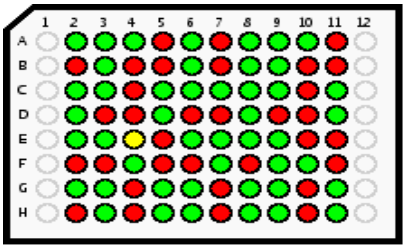

Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*5,3,1*}	0.02	328.20 g/mol	328.20 g/mol	3842.65
B2	**13** {*6,3,1*}	0.0	400.13 g/mol	0.00 g/mol	0.00
C2	**13** {*7,3,1*}	0.20	312.21 g/mol	312.19 g/mol	6345.72
D2	**13** {*8,3,1*}	0.06	316.17 g/mol	316.17 g/mol	21843.42
E2	**13** {*9,3,1*}	0.00	366.10 g/mol	366.10 g/mol	1586.96
F2	**13** {*10,3,1*}	0.0	313.20 g/mol	0.00 g/mol	0.00
G2	**13** {*5,4,1*}	0.08	328.20 g/mol	328.19 g/mol	22225.02
H2	**13** {*6,4,1*}	0.0	400.13 g/mol	0.00 g/mol	0.00
A3	**13** {*7,4,1*}	0.12	312.21 g/mol	312.23 g/mol	26459.58
B3	**13** {*8,4,1*}	0.10	316.17 g/mol	316.17 g/mol	24504.24
C3	**13** {*9,4,1*}	0.04	366.10 g/mol	366.10 g/mol	2808.07
D3	**13** {*10,4,1*}	0.0	313.20 g/mol	0.00 g/mol	0.00
E3	**13** {*5,5,1*}	0.08	344.19 g/mol	344.18 g/mol	17040.30
F3	**13** {*6,5,1*}	0.0	416.12 g/mol	0.00 g/mol	0.00
G3	**13** {*7,5,1*}	0.04	328.20 g/mol	328.19 g/mol	26148.78
H3	**13** {*8,5,1*}	0.08	332.17 g/mol	332.17 g/mol	25184.76
A4	**13** {*9,5,1*}	0.00	382.10 g/mol	382.09 g/mol	1090.46
B4	**13** {*10,5,1*}	0.0	329.19 g/mol	0.00 g/mol	0.00
C4	**13** {*5,6,1*}	0.0	344.19 g/mol	0.00 g/mol	0.00
D4	**13** {*6,6,1*}	0.0	416.12 g/mol	0.00 g/mol	0.00
E4	**13** {*7,6,1*}	0.00	328.20 g/mol	328.16 g/mol	91.62
F4	**13** {*8,6,1*}	0.10	332.17 g/mol	332.16 g/mol	4200.37
G4	**13** {*9,6,1*}	0.0	382.10 g/mol	0.00 g/mol	0.00
H4	**13** {*10,6,1*}	0.0	329.19 g/mol	0.00 g/mol	0.00
A5	**13** {*5,7,1*}	0.0	344.19 g/mol	0.00 g/mol	0.00
B5	**13** {*6,7,1*}	0.0	416.12 g/mol	0.00 g/mol	0.00
C5	**13** {*7,7,1*}	0.02	328.20 g/mol	328.17 g/mol	518.39
D5	**13** {*8,7,1*}	0.00	332.17 g/mol	332.16 g/mol	1526.43
E5	**13** {*9,7,1*}	0.0	382.10 g/mol	0.00 g/mol	0.00
F5	**13** {*10,7,1*}	0.0	329.19 g/mol	0.00 g/mol	0.00
G5	**13** {*5,8,1*}	0.10	340.24 g/mol	340.23 g/mol	25278.54
H5	**13** {*6,8,1*}	0.02	412.17 g/mol	412.17 g/mol	492.25
A6	**13** {*7,8,1*}	0.10	324.25 g/mol	324.23 g/mol	24151.14
B6	**13** {*8,8,1*}	0.16	328.22 g/mol	328.21 g/mol	28207.20
C6	**13** {*9,8,1*}	0.16	378.15 g/mol	378.13 g/mol	6088.38
D6	**13** {*10,8,1*}	0.0	325.24 g/mol	0.00 g/mol	0.00
E6	**13** {*5,9,1*}	0.00	468.02 g/mol	468.01 g/mol	671.45
F6	**13** {*6,9,1*}	0.0	539.95 g/mol	0.00 g/mol	0.00
G6	**13** {*7,9,1*}	0.02	452.03 g/mol	452.01 g/mol	4426.16
H6	**13** {*8,9,1*}	0.24	455.99 g/mol	455.99 g/mol	6698.22

A7	**13**	{*9,9,1*}	0.0	505.92 g/mol	0.00 g/mol	0.00
B7	**13**	{*10,9,1*}	0.0	453.02 g/mol	0.00 g/mol	0.00
C7	**13**	{*5,10,1*}	0.06	390.11 g/mol	390.09 g/mol	8152.38
D7	**13**	{*6,10,1*}	0.0	462.04 g/mol	0.00 g/mol	0.00
E7	**13**	{*7,10,1*}	0.20	374.12 g/mol	374.11 g/mol	7445.94
F7	**13**	{*8,10,1*}	0.04	378.09 g/mol	378.08 g/mol	23924.64
G7	**13**	{*9,10,1*}	0.0	428.02 g/mol	0.00 g/mol	0.00
H7	**13**	{*10,10,1*}	0.0	375.11 g/mol	0.00 g/mol	0.00
A8	**13**	{*5,3,2*}	0.06	344.15 g/mol	344.14 g/mol	4620.31
B8	**13**	{*6,3,2*}	0.06	416.08 g/mol	416.09 g/mol	1592.38
C8	**13**	{*7,3,2*}	0.18	328.15 g/mol	328.16 g/mol	7508.88
D8	**13**	{*8,3,2*}	0.14	332.12 g/mol	332.13 g/mol	7381.56
E8	**13**	{*9,3,2*}	0.18	382.05 g/mol	382.06 g/mol	5231.92
F8	**13**	{*10,3,2*}	0.0	329.15 g/mol	0.00 g/mol	0.00
G8	**13**	{*5,4,2*}	0.12	344.15 g/mol	344.15 g/mol	5099.78
H8	**13**	{*6,4,2*}	0.00	416.08 g/mol	416.09 g/mol	238.72
A9	**13**	{*7,4,2*}	0.06	328.15 g/mol	328.15 g/mol	9207.12
B9	**13**	{*8,4,2*}	0.21	332.12 g/mol	332.13 g/mol	6052.44
C9	**13**	{*9,4,2*}	0.02	382.05 g/mol	381.95 g/mol	1975.44
D9	**13**	{*10,4,2*}	0.0	329.15 g/mol	0.00 g/mol	0.00
E9	**13**	{*5,5,2*}	0.06	360.14 g/mol	360.14 g/mol	3280.38
F9	**13**	{*6,5,2*}	0.00	432.07 g/mol	432.08 g/mol	1548.80
G9	**13**	{*7,5,2*}	0.16	344.15 g/mol	344.15 g/mol	7523.10
H9	**13**	{*8,5,2*}	0.19	348.12 g/mol	348.12 g/mol	6739.26
A10	**13**	{*9,5,2*}	0.00	398.05 g/mol	398.05 g/mol	967.57
B10	**13**	{*10,5,2*}	0.0	345.14 g/mol	0.00 g/mol	0.00
C10	**13**	{*5,6,2*}	0.0	360.14 g/mol	0.00 g/mol	0.00
D10	**13**	{*6,6,2*}	0.0	432.07 g/mol	0.00 g/mol	0.00
E10	**13**	{*7,6,2*}	0.0	344.15 g/mol	0.00 g/mol	0.00
F10	**13**	{*8,6,2*}	0.02	348.12 g/mol	348.13 g/mol	400.91
G10	**13**	{*9,6,2*}	0.0	398.05 g/mol	0.00 g/mol	0.00
H10	**13**	{*10,6,2*}	0.0	345.14 g/mol	0.00 g/mol	0.00
A11	**13**	{*5,7,2*}	0.00	360.14 g/mol	360.14 g/mol	40812
B11	**13**	{*6,7,2*}	0.0	432.07 g/mol	0.00 g/mol	0.00
C11	**13**	{*7,7,2*}	0.00	344.15 g/mol	344.11 g/mol	134.28
D11	**13**	{*8,7,2*}	0.00	348.12 g/mol	348.13 g/mol	113.56
E11	**13**	{*9,7,2*}	0.00	398.05 g/mol	398.06 g/mol	29.52
F11	**13**	{*10,7,2*}	0.0	345.14 g/mol	0.00 g/mol	0.00
G11	**13**	{*5,8,2*}	0.10	356.19 g/mol	356.19 g/mol	20636.76
H11	**13**	{*6,8,2*}	0.06	428.12 g/mol	428.13 g/mol	1778.29
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C12		*empty*				
D12		*empty*				
E12		*empty*				
F12		*empty*				
G12		*empty*				
H12		*empty*				

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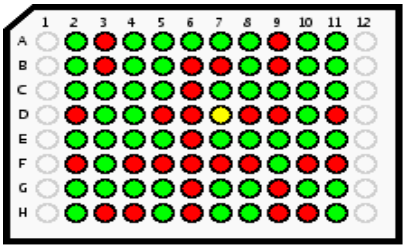

Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*7,8,2*}	0.02	340.20 g/mol	340.19 g/mol	24634.50
B2	**13** {*8,8,2*}	0.14	344.17 g/mol	344.17 g/mol	28045.86
C2	**13** {*9,8,2*}	0.10	394.10 g/mol	394.10 g/mol	5116.44
D2	**13** {*10,8,2*}	0.0	341.19 g/mol	0.00 g/mol	0.00
E2	**13** {*5,9,2*}	0.00	483.97 g/mol	483.97 g/mol	832.48
F2	**13** {*6,9,2*}	0.0	555.90 g/mol	0.00 g/mol	0.00
G2	**13** {*7,9,2*}	0.14	467.98 g/mol	467.98 g/mol	4849.32
H2	**13** {*8,9,2*}	0.06	471.94 g/mol	471.95 g/mol	5451.04
A3	**13** {*9,9,2*}	0.0	521.87 g/mol	0.00 g/mol	0.00
B3	**13** {*10,9,2*}	0.0	468.97 g/mol	0.00 g/mol	0.00
C3	**13** {*5,10,2*}	0.00	406.06 g/mol	406.06 g/mol	5196.89
D3	**13** {*6,10,2*}	0.00	477.99 g/mol	478.01 g/mol	231.51
E3	**13** {*7,10,2*}	0.14	390.07 g/mol	390.15 g/mol	6660.18
F3	**13** {*8,10,2*}	0.22	394.04 g/mol	394.05 g/mol	9598.98
G3	**13** {*9,10,2*}	0.06	443.97 g/mol	443.98 g/mol	1576.43
H3	**13** {*10,10,2*}	0.0	391.06 g/mol	0.00 g/mol	0.00
A4	**13** {*5,3,4*}	0.12	330.17 g/mol	330.17 g/mol	6977.70
B4	**13** {*6,3,4*}	0.04	402.10 g/mol	402.11 g/mol	1160.13
C4	**13** {*7,3,4*}	0.02	314.18 g/mol	314.17 g/mol	7932.42
D4	**13** {*8,3,4*}	0.08	318.15 g/mol	318.15 g/mol	15462.84
E4	**13** {*9,3,4*}	0.14	368.08 g/mol	368.09 g/mol	5596.55
F4	**13** {*10,3,4*}	0.0	315.17 g/mol	0.00 g/mol	0.00
G4	**13** {*5,4,4*}	0.12	330.17 g/mol	330.17 g/mol	6536.64
H4	**13** {*6,4,4*}	0.0	402.10 g/mol	0.00 g/mol	0.00
A5	**13** {*7,4,4*}	0.22	314.18 g/mol	314.18 g/mol	8740.32
B5	**13** {*8,4,4*}	0.20	318.15 g/mol	318.15 g/mol	14865.42
C5	**13** {*9,4,4*}	0.00	368.08 g/mol	368.08 g/mol	330.60
D5	**13** {*10,4,4*}	0.0	315.17 g/mol	0.00 g/mol	0.00
E5	**13** {*5,5,4*}	0.10	346.17 g/mol	346.16 g/mol	5736.05
F5	**13** {*6,5,4*}	0.0	418.10 g/mol	0.00 g/mol	0.00
G5	**13** {*7,5,4*}	0.18	330.17 g/mol	330.17 g/mol	9775.68
H5	**13** {*8,5,4*}	0.08	334.14 g/mol	334.15 g/mol	23892.00
A6	**13** {*9,5,4*}	0.00	384.07 g/mol	384.08 g/mol	220.85
B6	**13** {*10,5,4*}	0.0	331.17 g/mol	0.00 g/mol	0.00
C6	**13** {*5,6,4*}	0.0	346.17 g/mol	0.00 g/mol	0.00
D6	**13** {*6,6,4*}	0.0	418.10 g/mol	0.00 g/mol	0.00
E6	**13** {*7,6,4*}	0.0	330.17 g/mol	0.00 g/mol	0.00
F6	**13** {*8,6,4*}	0.0	334.14 g/mol	0.00 g/mol	0.00
G6	**13** {*9,6,4*}	0.0	384.07 g/mol	0.00 g/mol	0.00
H6	**13** {*10,6,4*}	0.0	331.17 g/mol	0.00 g/mol	0.00

A7	**13** {*5,7,4*}	0.02	346.17 g/mol	346.17 g/mol	412.90
B7	**13** {*6,7,4*}	0.0	418.10 g/mol	0.00 g/mol	0.00
C7	**13** {*7,7,4*}	0.00	330.17 g/mol	330.17 g/mol	4658.54
D7	**13** {*8,7,4*}	0.00	334.14 g/mol	334.16 g/mol	41.34
E7	**13** {*9,7,4*}	0.00	384.07 g/mol	384.09 g/mol	109.85
F7	**13** {*10,7,4*}	0.0	331.17 g/mol	0.00 g/mol	0.00
G7	**13** {*5,8,4*}	0.20	342.22 g/mol	342.21 g/mol	6006.78
H7	**13** {*6,8,4*}	0.00	414.15 g/mol	414.15 g/mol	120.40
A8	**13** {*7,8,4*}	0.04	326.22 g/mol	326.22 g/mol	15583.74
B8	**13** {*8,8,4*}	0.04	330.19 g/mol	330.20 g/mol	25294.14
C8	**13** {*9,8,4*}	0.00	380.12 g/mol	380.12 g/mol	1794.32
D8	**13** {*10,8,4*}	0.0	327.22 g/mol	0.00 g/mol	0.00
E8	**13** {*5,9,4*}	0.00	470.00 g/mol	469.99 g/mol	210.34
F8	**13** {*6,9,4*}	0.0	541.92 g/mol	0.00 g/mol	0.00
G8	**13** {*7,9,4*}	0.10	454.00 g/mol	454.00 g/mol	4324.68
H8	**13** {*8,9,4*}	0.02	457.97 g/mol	457.97 g/mol	7048.50
A9	**13** {*9,9,4*}	0.0	507.90 g/mol	0.00 g/mol	0.00
B9	**13** {*10,9,4*}	0.0	454.99 g/mol	0.00 g/mol	0.00
C9	**13** {*5,10,4*}	0.06	392.09 g/mol	392.08 g/mol	4181.36
D9	**13** {*6,10,4*}	0.0	464.02 g/mol	0.00 g/mol	0.00
E9	**13** {*7,10,4*}	0.00	376.09 g/mol	376.11 g/mol	9016.26
F9	**13** {*8,10,4*}	0.12	380.06 g/mol	380.07 g/mol	15810.72
G9	**13** {*9,10,4*}	0.0	429.99 g/mol	0.00 g/mol	0.00
H9	**13** {*10,10,4*}	0.0	377.09 g/mol	0.00 g/mol	0.00
A10	**13** {*5,3,6*}	0.20	328.20 g/mol	328.19 g/mol	9458.64
B10	**13** {*6,3,6*}	0.04	400.13 g/mol	400.13 g/mol	958.55
C10	**13** {*7,3,6*}	0.02	312.21 g/mol	312.20 g/mol	23969.22
D10	**13** {*8,3,6*}	0.08	316.17 g/mol	316.17 g/mol	27283.32
E10	**13** {*9,3,6*}	0.22	366.10 g/mol	366.10 g/mol	8975.28
F10	**13** {*10,3,6*}	0.0	313.20 g/mol	0.00 g/mol	0.00
G10	**13** {*5,4,6*}	0.06	328.20 g/mol	328.19 g/mol	15959.82
H10	**13** {*6,4,6*}	0.0	400.13 g/mol	0.00 g/mol	0.00
A11	**13** {*7,4,6*}	0.14	312.21 g/mol	312.20 g/mol	24103.86
B11	**13** {*8,4,6*}	0.12	316.17 g/mol	316.19 g/mol	25838.04
C11	**13** {*9,4,6*}	0.02	366.10 g/mol	366.10 g/mol	631.75
D11	**13** {*10,4,6*}	0.0	313.20 g/mol	0.00 g/mol	0.00
E11	**13** {*5,5,6*}	0.16	344.19 g/mol	344.29 g/mol	6593.76
F11	**13** {*6,5,6*}	0.0	416.12 g/mol	0.00 g/mol	0.00
G11	**13** {*7,5,6*}	0.14	328.20 g/mol	328.19 g/mol	26705.94
H11	**13** {*8,5,6*}	0.02	332.17 g/mol	332.17 g/mol	19495.92
A12	*empty*				
B12	*empty*				
C12	*empty*				
D12	*empty*				
E12	*empty*				
F12	*empty*				
G12	*empty*				
H12	*empty*				

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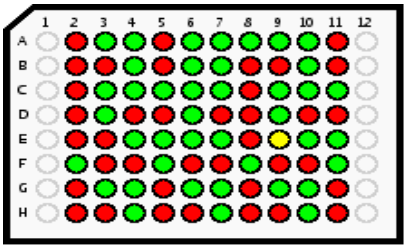

Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*9,5,6*}	0.0	382.10 g/mol	0.00 g/mol	0.00
B2	**13** {*10,5,6*}	0.0	329.19 g/mol	0.00 g/mol	0.00
C2	**13** {*5,6,6*}	0.0	344.19 g/mol	0.00 g/mol	0.00
D2	**13** {*6,6,6*}	0.0	416.12 g/mol	0.00 g/mol	0.00
E2	**13** {*7,6,6*}	0.0	328.20 g/mol	0.00 g/mol	0.00
F2	**13** {*8,6,6*}	0.00	332.17 g/mol	332.15 g/mol	645.72
G2	**13** {*9,6,6*}	0.0	382.10 g/mol	0.00 g/mol	0.00
H2	**13** {*10,6,6*}	0.0	329.19 g/mol	0.00 g/mol	0.00
A3	**13** {*5,7,6*}	0.04	344.19 g/mol	344.18 g/mol	3918.92
B3	**13** {*6,7,6*}	0.0	416.12 g/mol	0.00 g/mol	0.00
C3	**13** {*7,7,6*}	0.14	328.20 g/mol	328.18 g/mol	15947.04
D3	**13** {*8,7,6*}	0.10	332.17 g/mol	332.15 g/mol	6187.44
E3	**13** {*9,7,6*}	0.0	382.10 g/mol	0.00 g/mol	0.00
F3	**13** {*10,7,6*}	0.0	329.19 g/mol	0.00 g/mol	0.00
G3	**13** {*5,8,6*}	0.22	340.24 g/mol	340.21 g/mol	8771.64
H3	**13** {*6,8,6*}	0.0	412.17 g/mol	0.00 g/mol	0.00
A4	**13** {*7,8,6*}	0.22	324.25 g/mol	324.23 g/mol	30334.80
B4	**13** {*8,8,6*}	0.00	328.22 g/mol	328.21 g/mol	25420.50
C4	**13** {*9,8,6*}	0.00	378.15 g/mol	378.13 g/mol	1150.53
D4	**13** {*10,8,6*}	0.0	325.24 g/mol	0.00 g/mol	0.00
E4	**13** {*5,9,6*}	0.00	468.02 g/mol	468.00 g/mol	915.17
F4	**13** {*6,9,6*}	0.0	539.95 g/mol	0.00 g/mol	0.00
G4	**13** {*7,9,6*}	0.20	452.03 g/mol	452.01 g/mol	7009.20
H4	**13** {*8,9,6*}	0.02	455.99 g/mol	455.98 g/mol	7870.14
A5	**13** {*9,9,6*}	0.0	505.92 g/mol	0.00 g/mol	0.00
B5	**13** {*10,9,6*}	0.0	453.02 g/mol	0.00 g/mol	0.00
C5	**13** {*5,10,6*}	0.22	390.11 g/mol	390.10 g/mol	6585.30
D5	**13** {*6,10,6*}	0.0	462.04 g/mol	0.00 g/mol	0.00
E5	**13** {*7,10,6*}	0.22	374.12 g/mol	374.10 g/mol	7452.54
F5	**13** {*8,10,6*}	0.16	378.09 g/mol	378.08 g/mol	19545.24
G5	**13** {*9,10,6*}	0.0	428.02 g/mol	0.00 g/mol	0.00
H5	**13** {*10,10,6*}	0.0	375.11 g/mol	0.00 g/mol	0.00
A6	**13** {*5,3,15*}	0.22	314.18 g/mol	314.21 g/mol	6712.38
B6	**13** {*6,3,15*}	0.02	386.11 g/mol	386.11 g/mol	746.02
C6	**13** {*7,3,15*}	0.10	298.19 g/mol	298.18 g/mol	25164.00
D6	**13** {*8,3,15*}	0.14	302.15 g/mol	302.15 g/mol	27569.22
E6	**13** {*9,3,15*}	0.26	352.08 g/mol	352.08 g/mol	7914.12
F6	**13** {*10,3,15*}	0.0	299.18 g/mol	0.00 g/mol	0.00
G6	**13** {*5,4,15*}	0.16	314.18 g/mol	314.17 g/mol	21033.66
H6	**13** {*6,4,15*}	0.0	386.11 g/mol	0.00 g/mol	0.00

A7	**13** {*7,4,15*}	0.04	298.19 g/mol	298.18 g/mol	23566.62
B7	**13** {*8,4,15*}	0.06	302.15 g/mol	302.15 g/mol	6177.96
C7	**13** {*9,4,15*}	0.06	352.08 g/mol	352.08 g/mol	1820.34
D7	**13** {*10,4,15*}	0.0	299.18 g/mol	0.00 g/mol	0.00
E7	**13** {*5,5,15*}	0.14	330.17 g/mol	330.17 g/mol	7222.68
F7	**13** {*6,5,15*}	0.0	402.10 g/mol	0.00 g/mol	0.00
G7	**13** {*7,5,15*}	0.25	314.18 g/mol	314.18 g/mol	11725.86
H7	**13** {*8,5,15*}	0.26	318.15 g/mol	318.15 g/mol	7365.42
A8	**13** {*9,5,15*}	0.00	368.08 g/mol	368.08 g/mol	428.58
B8	**13** {*10,5,15*}	0.0	315.17 g/mol	0.00 g/mol	0.00
C8	**13** {*5,6,15*}	0.0	330.17 g/mol	0.00 g/mol	0.00
D8	**13** {*6,6,15*}	0.0	402.10 g/mol	0.00 g/mol	0.00
E8	**13** {*7,6,15*}	0.0	314.18 g/mol	0.00 g/mol	0.00
F8	**13** {*8,6,15*}	0.08	318.15 g/mol	318.14 g/mol	14245.56
G8	**13** {*9,6,15*}	0.0	368.08 g/mol	0.00 g/mol	0.00
H8	**13** {*10,6,15*}	0.0	315.17 g/mol	0.00 g/mol	0.00
A9	**13** {*5,7,15*}	0.02	330.17 g/mol	330.16 g/mol	2475.16
B9	**13** {*6,7,15*}	0.0	402.10 g/mol	0.00 g/mol	0.00
C9	**13** {*7,7,15*}	0.12	314.18 g/mol	314.17 g/mol	16917.60
D9	**13** {*8,7,15*}	0.12	318.15 g/mol	318.14 g/mol	4981.39
E9	**13** {*9,7,15*}	0.00	368.08 g/mol	368.09 g/mol	98.59
F9	**13** {*10,7,15*}	0.0	315.17 g/mol	0.00 g/mol	0.00
G9	**13** {*5,8,15*}	0.10	326.22 g/mol	326.21 g/mol	26247.48
H9	**13** {*6,8,15*}	0.0	398.15 g/mol	0.00 g/mol	0.00
A10	**13** {*7,8,15*}	0.14	310.23 g/mol	310.22 g/mol	27512.88
B10	**13** {*8,8,15*}	0.18	314.20 g/mol	314.19 g/mol	28619.88
C10	**13** {*9,8,15*}	0.06	364.13 g/mol	364.12 g/mol	3583.39
D10	**13** {*10,8,15*}	0.0	311.22 g/mol	0.00 g/mol	0.00
E10	**13** {*5,9,15*}	0.00	454.00 g/mol	454.00 g/mol	636.38
F10	**13** {*6,9,15*}	0.0	525.93 g/mol	0.00 g/mol	0.00
G10	**13** {*7,9,15*}	0.00	438.01 g/mol	438.00 g/mol	3621.29
H10	**13** {*8,9,15*}	0.02	441.98 g/mol	441.98 g/mol	9285.96
A11	**13** {*9,9,15*}	0.0	491.90 g/mol	0.00 g/mol	0.00
B11	**13** {*10,9,15*}	0.0	439.00 g/mol	0.00 g/mol	0.00
C11	**13** {*5,10,15*}	0.16	376.09 g/mol	376.09 g/mol	13129.98
D11	**13** {*6,10,15*}	0.0	448.02 g/mol	0.00 g/mol	0.00
E11	**13** {*7,10,15*}	0.24	360.10 g/mol	360.10 g/mol	7244.16
F11	**13** {*8,10,15*}	0.14	364.07 g/mol	364.07 g/mol	24588.48
G11	**13** {*9,10,15*}	0.0	414.00 g/mol	0.00 g/mol	0.00
H11	**13** {*10,10,15*}	0.0	361.09 g/mol	0.00 g/mol	0.00
A12	*empty*				
B12	*empty*				
C12	*empty*				
D12	*empty*				
E12	*empty*				
F12	*empty*				
G12	*empty*				
H12	*empty*				

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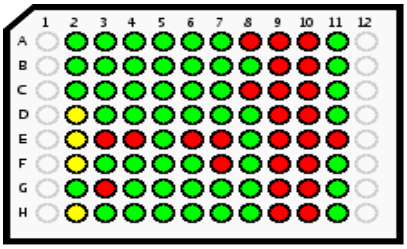
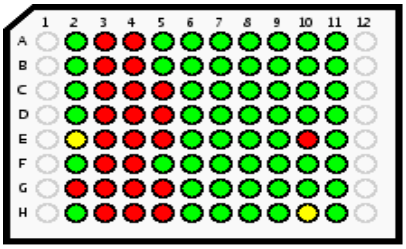


Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*11,11,21*}	0.00	437.07 g/mol	437.06 g/mol	153.73
B2	**13** {*3,11,21*}	0.00	385.15 g/mol	385.15 g/mol	1206.33
C2	**13** {*11,12,21*}	0.00	401.07 g/mol	401.08 g/mol	343.62
D2	**13** {*3,12,21*}	0.00	349.15 g/mol	349.16 g/mol	33.44
E2	**13** {*11,13,21*}	0.00	437.00 g/mol	437.02 g/mol	57.67
F2	**13** {*3,13,21*}	0.00	385.08 g/mol	385.17 g/mol	61.42
G2	**13** {*11,2,21*}	0.04	453.00 g/mol	453.01 g/mol	1093.16
H2	**13** {*3,2,21*}	0.00	401.07 g/mol	401.07 g/mol	37.82
A3	**13** {*11,11,22*}	0.04	440.07 g/mol	440.07 g/mol	7904.28
B3	**13** {*3,11,22*}	0.40	388.15 g/mol	388.15 g/mol	13486.68
C3	**13** {*11,12,22*}	0.08	404.08 g/mol	404.08 g/mol	15225.24
D3	**13** {*3,12,22*}	0.12	352.15 g/mol	352.15 g/mol	23614.38
E3	**13** {*11,13,22*}	0.0	440.00 g/mol	0.00 g/mol	0.00
F3	**13** {*3,13,22*}	0.44	388.08 g/mol	388.08 g/mol	10662.96
G3	**13** {*11,2,22*}	0.0	456.00 g/mol	0.00 g/mol	0.00
H3	**13** {*3,2,22*}	0.12	404.08 g/mol	404.07 g/mol	7670.22
A4	**13** {*11,11,23*}	0.06	467.04 g/mol	467.05 g/mol	4233.99
B4	**13** {*3,11,23*}	0.00	415.11 g/mol	415.14 g/mol	186.11
C4	**13** {*11,12,23*}	0.04	431.04 g/mol	431.05 g/mol	3706.48
D4	**13** {*3,12,23*}	0.00	379.12 g/mol	379.12 g/mol	1652.11
E4	**13** {*11,13,23*}	0.0	466.97 g/mol	0.00 g/mol	0.00
F4	**13** {*3,13,23*}	0.04	415.05 g/mol	415.06 g/mol	1178.56
G4	**13** {*11,2,23*}	0.00	482.96 g/mol	482.98 g/mol	350.58
H4	**13** {*3,2,23*}	0.06	431.04 g/mol	431.05 g/mol	4378.65
A5	**13** {*11,11,21*}	0.06	426.05 g/mol	426.06 g/mol	7564.74
B5	**13** {*3,11,24*}	0.18	374.13 g/mol	374.22 g/mol	27886.56
C5	**13** {*11,12,24*}	0.00	390.06 g/mol	390.06 g/mol	12667.20
D5	**13** {*3,12,24*}	0.20	338.13 g/mol	338.14 g/mol	10149.54
E5	**13** {*11,13,24*}	0.00	425.98 g/mol	426.00 g/mol	423.74
F5	**13** {*3,13,24*}	0.36	374.06 g/mol	374.07 g/mol	13504.02
G5	**13** {*11,2,24*}	0.00	441.98 g/mol	441.99 g/mol	7867.74
H5	**13** {*3,2,24*}	0.22	390.06 g/mol	390.13 g/mol	6788.16
A6	**13** {*11,11,25*}	0.00	458.03 g/mol	458.05 g/mol	280.48
B6	**13** {*3,11,25*}	0.22	406.11 g/mol	406.12 g/mol	8632.56
C6	**13** {*11,12,25*}	0.02	422.04 g/mol	422.06 g/mol	551.97
D6	**13** {*3,12,25*}	0.06	370.11 g/mol	370.12 g/mol	4785.12
E6	**13** {*11,13,25*}	0.0	457.96 g/mol	0.00 g/mol	0.00
F6	**13** {*3,13,25*}	0.00	406.04 g/mol	406.06 g/mol	1487.02
G6	**13** {*11,2,25*}	0.02	473.96 g/mol	473.99 g/mol	1161.52
H6	**13** {*3,2,25*}	0.12	422.04 g/mol	422.05 g/mol	5508.88

A7	**13**	{*11,11,26*}	0.12	440.04 g/mol	440.07 g/mol	3225.98
B7	**13**	{*3,11,26*}	0.04	388.12 g/mol	388.14 g/mol	1095.31
C7	**13**	{*11,12,26*}	0.06	404.05 g/mol	404.07 g/mol	1544.66
D7	**13**	{*3,12,26*}	0.00	352.13 g/mol	352.13 g/mol	317.52
E7	**13**	{*11,13,26*}	0.00	439.98 g/mol	439.99 g/mol	27.28
F7	**13**	{*3,13,26*}	0.0	388.05 g/mol	0.00 g/mol	0.00
G7	**13**	{*11,2,26*}	0.02	455.97 g/mol	455.99 g/mol	715.12
H7	**13**	{*3,2,26*}	0.02	404.05 g/mol	404.07 g/mol	601.31
A8	**13**	{*11,11,27*}	0.0	489.97 g/mol	0.00 g/mol	0.00
B8	**13**	{*3,11,27*}	0.02	438.05 g/mol	438.06 g/mol	2140.33
C8	**13**	{*11,12,27*}	0.0	453.98 g/mol	0.00 g/mol	0.00
D8	**13**	{*3,12,27*}	0.00	402.06 g/mol	402.07 g/mol	233.75
E8	**13**	{*11,13,27*}	0.00	489.91 g/mol	489.94 g/mol	129.30
F8	**13**	{*3,13,27*}	0.02	437.98 g/mol	438.01 g/mol	645.86
G8	**13**	{*11,2,27*}	0.00	505.90 g/mol	505.92 g/mol	1557.76
H8	**13**	{*3,2,27*}	0.00	453.98 g/mol	454.00 g/mol	2384.53
A9	**13**	{*11,11,28*}	0.0	496.10 g/mol	0.00 g/mol	0.00
B9	**13**	{*3,11,28*}	0.0	444.18 g/mol	0.00 g/mol	0.00
C9	**13**	{*11,12,28*}	0.0	460.11 g/mol	0.00 g/mol	0.00
D9	**13**	{*3,12,28*}	0.0	408.19 g/mol	0.00 g/mol	0.00
E9	**13**	{*11,13,28*}	0.0	496.04 g/mol	0.00 g/mol	0.00
F9	**13**	{*3,13,28*}	0.0	444.11 g/mol	0.00 g/mol	0.00
G9	**13**	{*11,2,28*}	0.0	512.03 g/mol	0.00 g/mol	0.00
H9	**13**	{*3,2,28*}	0.0	460.11 g/mol	0.00 g/mol	0.00
A10	**13**	{*11,11,29*}	0.0	443.13 g/mol	0.00 g/mol	0.00
B10	**13**	{*3,11,29*}	0.0	391.21 g/mol	0.00 g/mol	0.00
C10	**13**	{*11,12,29*}	0.0	407.13 g/mol	0.00 g/mol	0.00
D10	**13**	{*3,12,29*}	0.0	355.21 g/mol	0.00 g/mol	0.00
E10	**13**	{*11,13,29*}	0.0	443.06 g/mol	0.00 g/mol	0.00
F10	**13**	{*3,13,29*}	0.0	391.14 g/mol	0.00 g/mol	0.00
G10	**13**	{*11,2,29*}	0.0	459.06 g/mol	0.00 g/mol	0.00
H10	**13**	{*3,2,29*}	0.0	407.13 g/mol	0.00 g/mol	0.00
A11	**13**	{*11,11,30*}	0.00	423.05 g/mol	423.07 g/mol	620.09
B11	**13**	{*3,11,30*}	0.18	371.13 g/mol	371.16 g/mol	21238.74
C11	**13**	{*11,12,30*}	0.14	387.05 g/mol	387.06 g/mol	5620.52
D11	**13**	{*3,12,30*}	0.10	335.13 g/mol	335.14 g/mol	4406.42
E11	**13**	{*11,13,30*}	0.0	422.98 g/mol	0.00 g/mol	0.00
F11	**13**	{*3,13,30*}	0.00	371.06 g/mol	371.07 g/mol	1472.39
G11	**13**	{*11,2,30*}	0.00	438.98 g/mol	439.00 g/mol	933.28
H11	**13**	{*3,2,30*}	0.04	387.05 g/mol	387.07 g/mol	3431.95
A12		*empty*				
B12		*empty*				
C12		*empty*				
D12		*empty*				
E12		*empty*				
F12		*empty*				
G12		*empty*				
H12		*empty*				

ori.hya.54

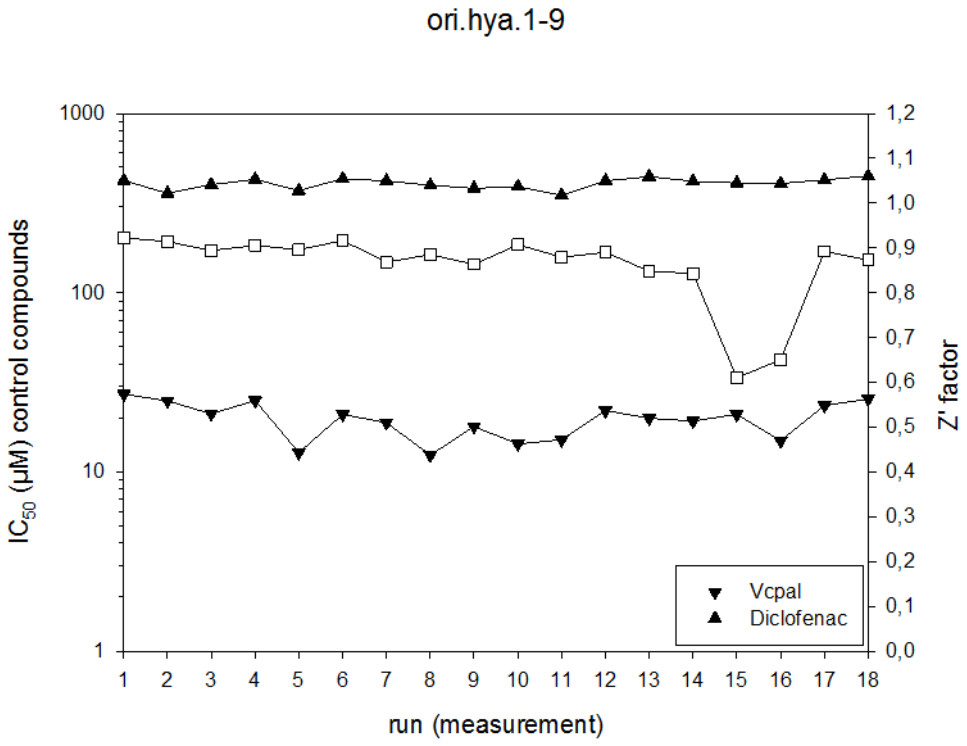


Well	Chemset	Peak Width	Expected mass	Found mass	Intensity
A1	*empty*				
B1	*empty*				
C1	*empty*				
D1	*empty*				
E1	*empty*				
F1	*empty*				
G1	*empty*				
H1	*empty*				
A2	**13** {*11,11,31*}	0.12	442.03 g/mol	442.05 g/mol	6292.44
B2	**13** {*3,11,31*}	0.24	390.11 g/mol	390.12 g/mol	29295.36
C2	**13** {*11,12,31*}	0.26	406.03 g/mol	406.04 g/mol	8643.72
D2	**13** {*3,12,31*}	0.20	354.11 g/mol	354.12 g/mol	10792.68
E2	**13** {*11,13,31*}	0.00	441.96 g/mol	441.99 g/mol	62.12
F2	**13** {*3,13,31*}	0.42	390.04 g/mol	390.05 g/mol	17343.42
G2	**13** {*11,2,31*}	0.0	457.96 g/mol	0.00 g/mol	0.00
H2	**13** {*3,2,31*}	0.22	406.03 g/mol	406.05 g/mol	9592.44
A3	**13** {*11,11,32*}	0.0	442.03 g/mol	0.00 g/mol	0.00
B3	**13** {*3,11,32*}	0.0	390.11 g/mol	0.00 g/mol	0.00
C3	**13** {*11,12,32*}	0.0	406.03 g/mol	0.00 g/mol	0.00
D3	**13** {*3,12,32*}	0.0	354.11 g/mol	0.00 g/mol	0.00
E3	**13** {*11,13,32*}	0.0	441.96 g/mol	0.00 g/mol	0.00
F3	**13** {*3,13,32*}	0.0	390.04 g/mol	0.00 g/mol	0.00
G3	**13** {*11,2,32*}	0.0	457.96 g/mol	0.00 g/mol	0.00
H3	**13** {*3,2,32*}	0.0	406.03 g/mol	0.00 g/mol	0.00
A4	**13** {*11,11,33*}	0.0	443.02 g/mol	0.00 g/mol	0.00
B4	**13** {*3,11,33*}	0.0	391.10 g/mol	0.00 g/mol	0.00
C4	**13** {*11,12,33*}	0.0	407.03 g/mol	0.00 g/mol	0.00
D4	**13** {*3,12,33*}	0.0	355.10 g/mol	0.00 g/mol	0.00
E4	**13** {*11,13,33*}	0.0	442.95 g/mol	0.00 g/mol	0.00
F4	**13** {*3,13,33*}	0.0	391.03 g/mol	0.00 g/mol	0.00
G4	**13** {*11,2,33*}	0.0	458.95 g/mol	0.00 g/mol	0.00
H4	**13** {*3,2,33*}	0.0	407.03 g/mol	0.00 g/mol	0.00
A5	**13** {*11,11,34*}	0.02	441.06 g/mol	441.06 g/mol	722.38
B5	**13** {*3,11,34*}	0.04	389.14 g/mol	389.15 g/mol	1020.68
C5	**13** {*11,12,34*}	0.0	405.07 g/mol	0.00 g/mol	0.00
D5	**13** {*3,12,34*}	0.0	353.15 g/mol	0.00 g/mol	0.00
E5	**13** {*11,13,34*}	0.0	441.00 g/mol	0.00 g/mol	0.00
F5	**13** {*3,13,34*}	0.00	389.07 g/mol	389.09 g/mol	297.69
G5	**13** {*11,2,34*}	0.0	456.99 g/mol	0.00 g/mol	0.00
H5	**13** {*3,2,34*}	0.0	405.07 g/mol	0.00 g/mol	0.00
A6	**13** {*11,11,35*}	0.14	440.08 g/mol	440.09 g/mol	8140.98
B6	**13** {*3,11,35*}	0.04	388.16 g/mol	388.23 g/mol	23523.66
C6	**13** {*11,12,35*}	0.16	404.09 g/mol	404.09 g/mol	6563.94
D6	**13** {*3,12,35*}	0.14	352.16 g/mol	352.17 g/mol	6735.72
E6	**13** {*11,13,35*}	0.04	440.01 g/mol	440.03 g/mol	1064.31
F6	**13** {*3,13,35*}	0.14	388.09 g/mol	388.10 g/mol	6375.42
G6	**13** {*11,2,35*}	0.00	456.01 g/mol	456.02 g/mol	237.79
H6	**13** {*3,2,35*}	0.00	404.09 g/mol	404.10 g/mol	199.78

A7	**13** {*11,11,1*}	0.12	402.10 g/mol	402.10 g/mol	7500.54
B7	**13** {*3,11,1*}	0.14	350.17 g/mol	350.17 g/mol	28180.98
C7	**13** {*11,12,1*}	0.22	366.10 g/mol	366.11 g/mol	9801.18
D7	**13** {*3,12,1*}	0.24	314.18 g/mol	314.18 g/mol	6114.78
E7	**13** {*11,13,1*}	0.00	402.03 g/mol	402.04 g/mol	1077.64
F7	**13** {*3,13,1*}	0.38	350.11 g/mol	350.11 g/mol	30374.22
G7	**13** {*11,2,1*}	0.16	418.02 g/mol	418.03 g/mol	13757.88
H7	**13** {*3,2,1*}	0.02	366.10 g/mol	366.11 g/mol	16382.34
A8	**13** {*11,11,2*}	0.18	418.05 g/mol	418.06 g/mol	19420.86
B8	**13** {*3,11,2*}	0.12	366.12 g/mol	366.14 g/mol	29501.52
C8	**13** {*11,12,2*}	0.20	382.05 g/mol	382.06 g/mol	7621.26
D8	**13** {*3,12,2*}	0.06	330.13 g/mol	330.14 g/mol	18568.44
E8	**13** {*11,13,2*}	0.02	417.98 g/mol	417.99 g/mol	3077.47
F8	**13** {*3,13,2*}	0.04	366.06 g/mol	366.07 g/mol	22446.54
G8	**13** {*11,2,2*}	0.02	433.97 g/mol	433.99 g/mol	6375.48
H8	**13** {*3,2,2*}	0.20	382.05 g/mol	382.06 g/mol	8296.98
A9	**13** {*11,11,4*}	0.36	404.07 g/mol	404.08 g/mol	14172.84
B9	**13** {*3,11,4*}	0.04	352.15 g/mol	352.16 g/mol	26174.82
C9	**13** {*11,12,4*}	0.18	368.08 g/mol	368.08 g/mol	12153.66
D9	**13** {*3,12,4*}	0.14	316.15 g/mol	316.16 g/mol	5829.50
E9	**13** {*11,13,4*}	0.00	404.01 g/mol	404.02 g/mol	570.38
F9	**13** {*3,13,4*}	0.20	352.08 g/mol	352.09 g/mol	24047.04
G9	**13** {*11,2,4*}	0.00	420.00 g/mol	420.01 g/mol	10388.70
H9	**13** {*3,2,4*}	0.16	368.08 g/mol	368.08 g/mol	6181.26
A10	**13** {*11,11,6*}	0.00	402.10 g/mol	402.10 g/mol	8936.82
B10	**13** {*3,11,6*}	0.20	350.17 g/mol	350.18 g/mol	28771.80
C10	**13** {*11,12,6*}	0.30	366.10 g/mol	366.11 g/mol	16596.60
D10	**13** {*3,12,6*}	0.18	314.18 g/mol	314.28 g/mol	15523.38
E10	**13** {*11,13,6*}	0.0	402.03 g/mol	0.00 g/mol	0.00
F10	**13** {*3,13,6*}	0.44	350.11 g/mol	350.11 g/mol	12862.56
G10	**13** {*11,2,6*}	0.12	418.02 g/mol	418.03 g/mol	10086.72
H10	**13** {*3,2,6*}	0.00	366.10 g/mol	366.10 g/mol	89.46
A11	**13** {*11,11,15*}	0.42	388.08 g/mol	388.08 g/mol	11918.94
B11	**13** {*3,11,15*}	0.10	336.15 g/mol	336.25 g/mol	26462.88
C11	**13** {*11,12,15*}	0.18	352.08 g/mol	352.09 g/mol	22371.12
D11	**13** {*3,12,15*}	0.10	300.16 g/mol	300.16 g/mol	19999.26
E11	**13** {*11,13,15*}	0.00	388.01 g/mol	388.02 g/mol	1039.55
F11	**13** {*3,13,15*}	0.10	336.09 g/mol	336.09 g/mol	9274.20
G11	**13** {*11,2,15*}	0.00	404.00 g/mol	404.01 g/mol	10842.30
H11	**13** {*3,2,15*}	0.18	352.08 g/mol	352.09 g/mol	7010.28
A12	*empty*				
B12	*empty*				
C12	*empty*				
D12	*empty*				
E12	*empty*				
F12	*empty*				
G12	*empty*				
H12	*empty*				

B.8 On-screen assay validation

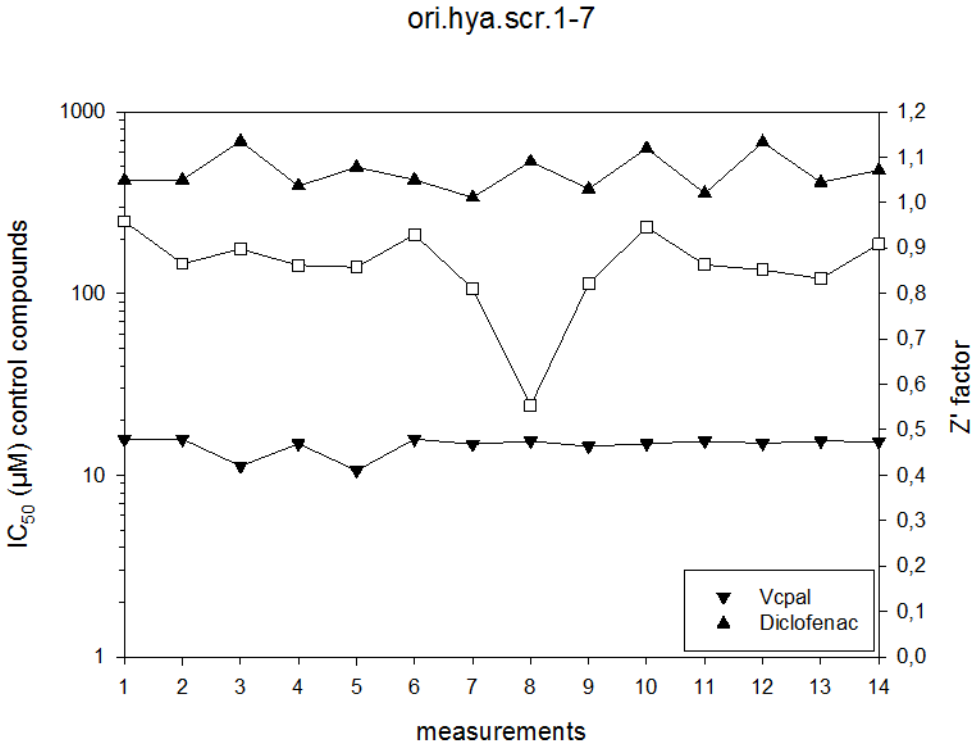
B.8.1 On-screen assay validation for plates ori.hya.1-9



Run (measurement)

No.	Description	SW	Z‘
1	ori.hya.1; plate 1	48.1	0.9220
2	ori.hya.1; plate 2	68.5	0.9140
3	ori.hya.2; plate 1	26.3	0.8940
4	ori.hya.2; plate 2	35.8	0.9050
5	ori.hya.3; plate 1	29.4	0.8960
6	ori.hya.3; plate 2	38.1	0.9160
7	ori.hya.4; plate 1	23.4	0.8680
8	ori.hya.4; plate 2	29.5	0.8850
9	ori.hya.5; plate 1	22.1	0.8630
10	ori.hya.5; plate 2	37.6	0.9070
11	ori.hya.6; plate 1	27.4	0.8790
12	ori.hya.6; plate 2	34.7	0.8900
13	ori.hya.7; plate 1	28.2	0.8480
14	ori.hya.7; plate 2	24.9	0.8430
15	ori.hya.8; plate 1	5.8	0.6100
16	ori.hya.8; plate 2	7.6	0.6500
17	ori.hya.9; plate 1	31.7	0.8920
18	ori.hya.9; plate 2	27.9	0.8730

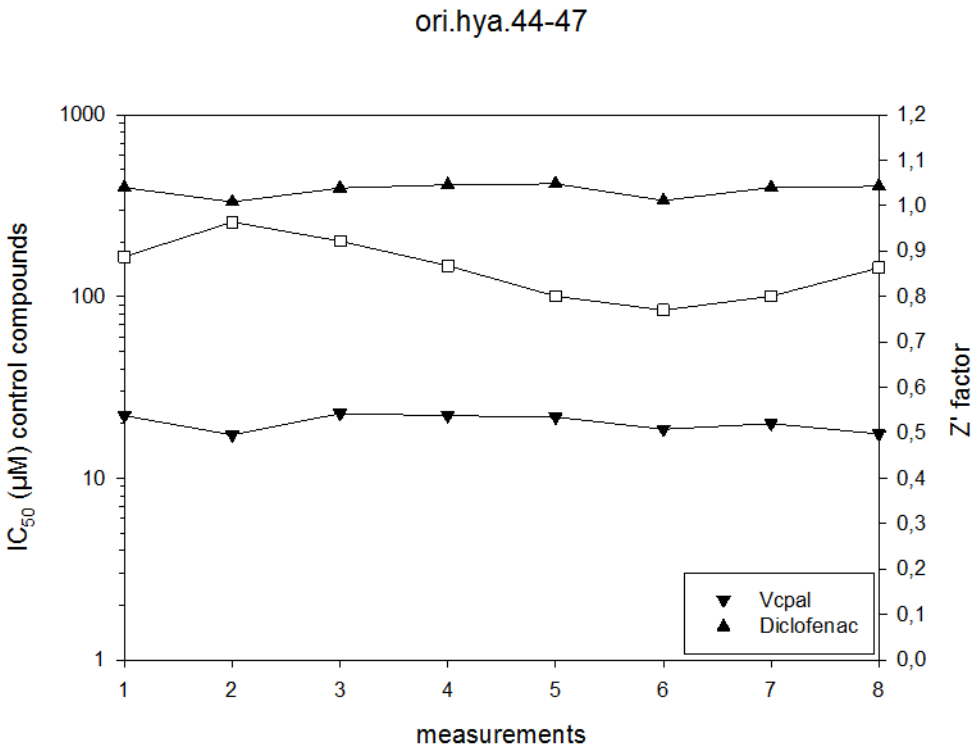
B.8.2 On-screen assay validation for plates ori.hya.scr.1-7



Run (measurement)

No.	Description	SW	Z‘
1	ori.hya.scr.1; plate 1	144.4	0.9590
2	ori.hya.scr.1; plate 2	68.4	0.8660
3	ori.hya.scr.2; plate 1	62.9	0.8980
4	ori.hya.scr.2; plate 2	63.5	0.8620
5	ori.hya.scr.3; plate 1	64.9	0.8580
6	ori.hya.scr.3; plate 2	71.3	0.9300
7	ori.hya.scr.4; plate 1	53.5	0.8110
8	ori.hya.scr.4; plate 2	61.4	0.5530
9	ori.hya.scr.5; plate 1	29.9	0.8220
10	ori.hya.scr.5; plate 2	98.1	0.9460
11	ori.hya.scr.6; plate 1	62.8	0.8630
12	ori.hya.scr.6; plate 2	46.1	0.8520
13	ori.hya.scr.7; plate 1	38.3	0.8330
14	ori.hya.scr.7; plate 2	67.4	0.9090

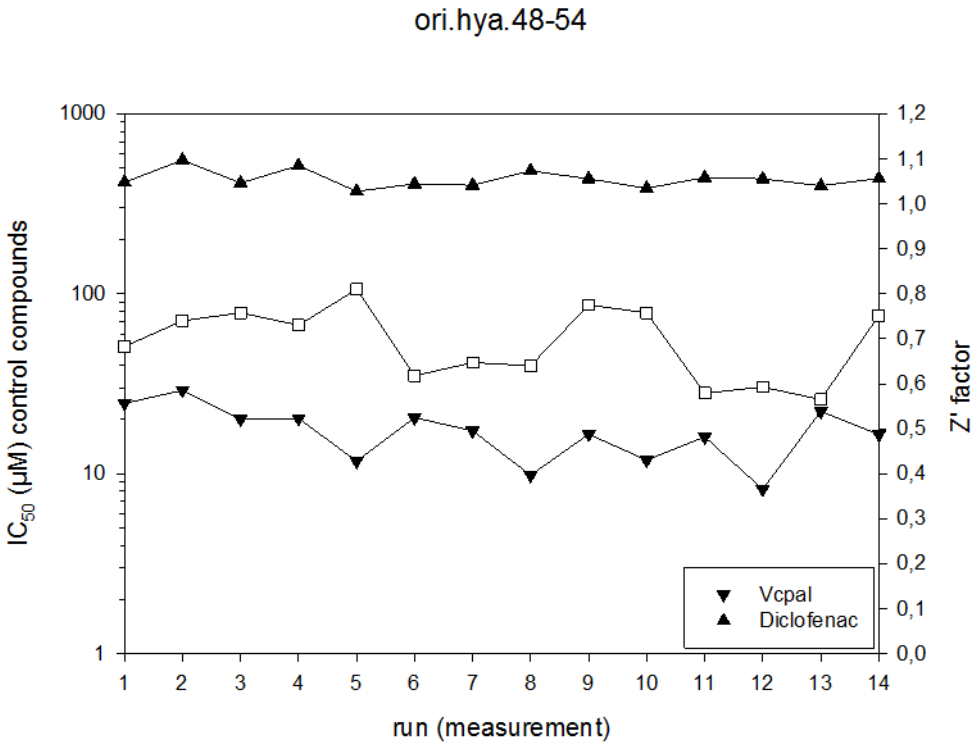
B.8.3 On-screen assay validation for plates ori.hya.44-47



Run (measurement)

No.	Description	SW	Z‘
1	ori.hya.44; plate 1	46.5	0.8880
2	ori.hya.44; plate 2	50.3	0.9640
3	ori.hya.45; plate 1	23.1	0.9220
4	ori.hya.45; plate 2	20.7	0.8680
5	ori.hya.46; plate 1	74.0	0.8010
6	ori.hya.46; plate 2	30.3	0.7700
7	ori.hya.47; plate 1	44.3	0.8010
8	ori.hya.47; plate 2	51.0	0.8630

B.8.4 On-screen assay validation for plates ori.hya.48-54



Run (measurement)

No.	Description	SW	Z‘
1	ori.hya.48; plate 1	11.7	0.6830
2	ori.hya.48; plate 2	28.7	0.7400
3	ori.hya.49; plate 1	24.5	0.7580
4	ori.hya.49; plate 2	26.4	0.7300
5	ori.hya.50; plate 1	13.9	0.8100
6	ori.hya.50; plate 2	14.4	0.6180
7	ori.hya.51; plate 1	8.2	0.6470
8	ori.hya.51; plate 2	13.7	0.6400
9	ori.hya.52; plate 1	22.4	0.7750
10	ori.hya.52; plate 2	14.8	0.7580
11	ori.hya.53; plate 1	9.3	0.5790
12	ori.hya.53; plate 2	11.3	0.5930
13	ori.hya.54; plate 1	14.1	0.5650
14	ori.hya.54; plate 2	56.6	0.7520

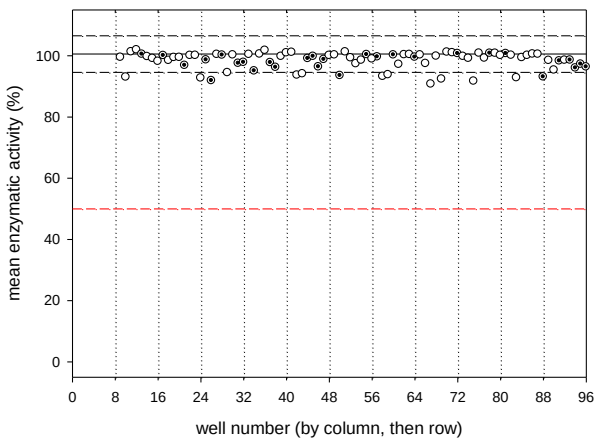
B.9 Pharmacological results

B.9.1 Tabulation of screening hits

Plate	Well	Compound	Comment
ori.hya.2	B2	**6.5**	missed hit criteria
ori.hya.scr.3	H3	**5.1**	hit
ori.hya.scr.6	E7	**5.2**	hit
ori.hya.scr.7	A6	**5.3**	hit
ori.hya.scr.1	G2	**5.4**	missed hit criteria
ori.hya.scr.1	A3	**5.5**	missed hit criteria
ori.hya.scr.1	C3	**5.6**	missed hit criteria
ori.hya.scr.1	A5	**5.7**	missed hit criteria
ori.hya.scr.1	B5	**5.8**	missed hit criteria
ori.hya.scr.1	F9	**5.9**	missed hit criteria
ori.hya.scr.3	C7	**5.10**	missed hit criteria
ori.hya.scr.6	C7	**5.11**	missed hit criteria
ori.hya.44	A2		hit
ori.hya.44	D2	**5.17**	hit
ori.hya.44	E2		hit
ori.hya.44	D3		hit
ori.hya.44	D5		hit
ori.hya.44	D6		hit
ori.hya.44	A7	**5.16**	hit
ori.hya.44	D7		hit
ori.hya.44	A9		hit
ori.hya.44	D9		hit
ori.hya.44	D12		hit
ori.hya.45	D2	**5.18**	hit
ori.hya.45	D5		hit
ori.hya.45	D6		hit
ori.hya.45	D7		hit
ori.hya.45	D9		hit
ori.hya.45	D10	**5.21**	hit
ori.hya.45	E10		hit
ori.hya.46	D2		hit
ori.hya.46	D3		hit
ori.hya.46	D7		hit
ori.hya.46	D9		hit
ori.hya.47	D5		hit
ori.hya.47	D7		hit
ori.hya.47	E7		hit
ori.hya.50	F4	**6.1**	hit
ori.hya.50	C5	**6.2**	hit
ori.hya.50	D5	**6.3**	hit
ori.hya.50	G11	**6.6**	missed hit criteria
ori.hya.50	H11	**6.4**	hit
ori.hya.52	A7	**6.7**	missed hit criteria
ori.hya.52	B7	**6.8**	missed hit criteria

B.9.2 Pharmacological results for plates ori.hya.1-9

plate ori.hya.1



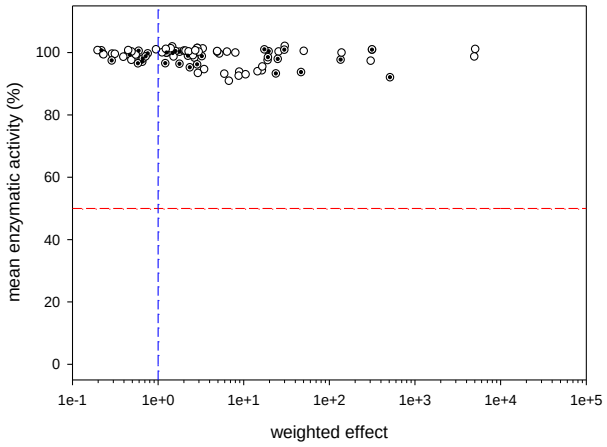
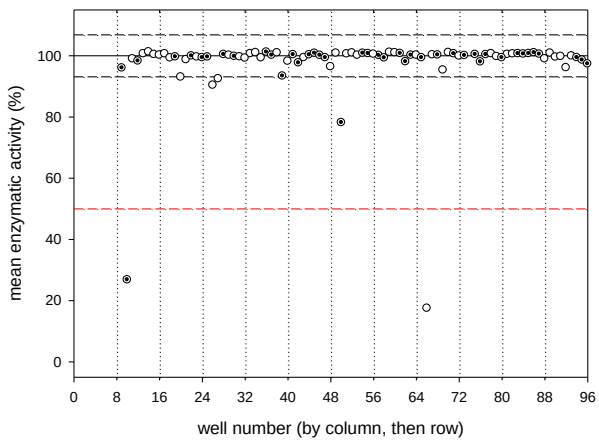


plate ori.hya.2



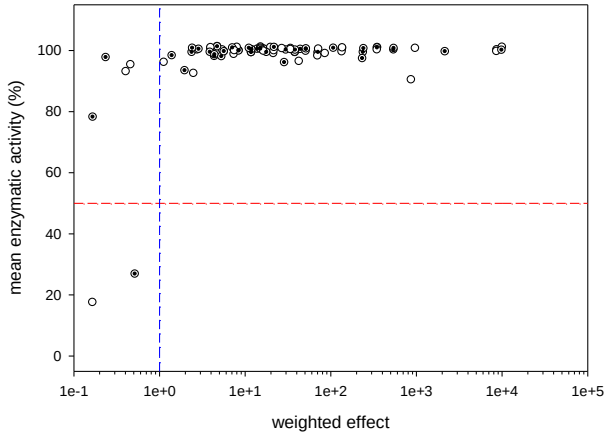
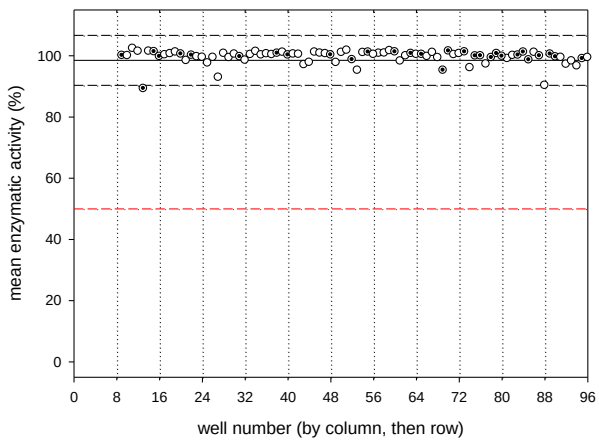


plate ori.hya.3



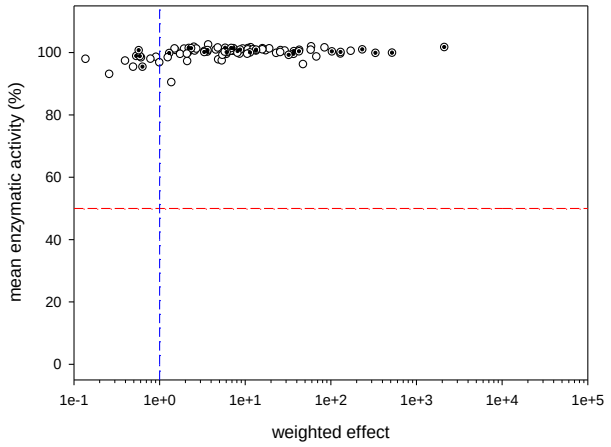


plate ori.hya.4

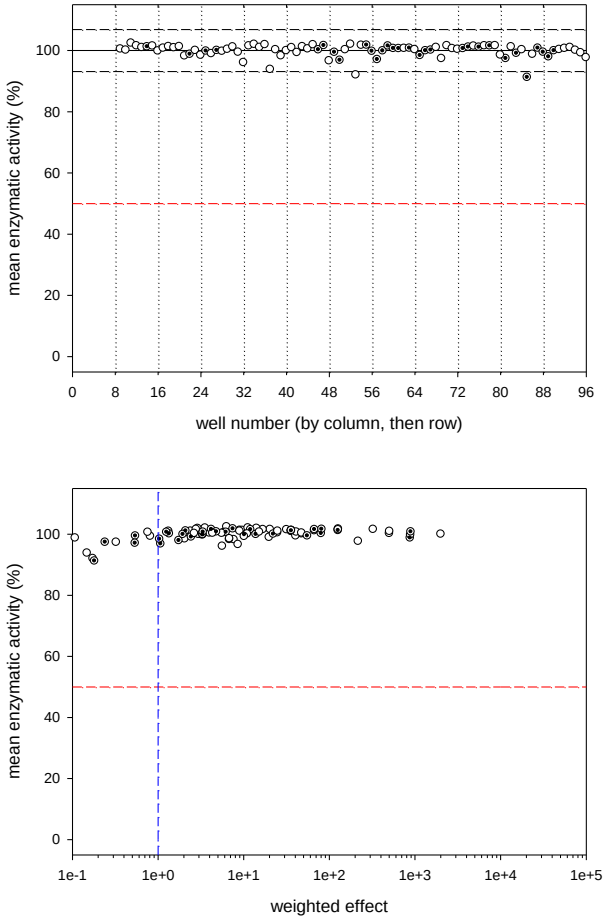


plate ori.hya.5

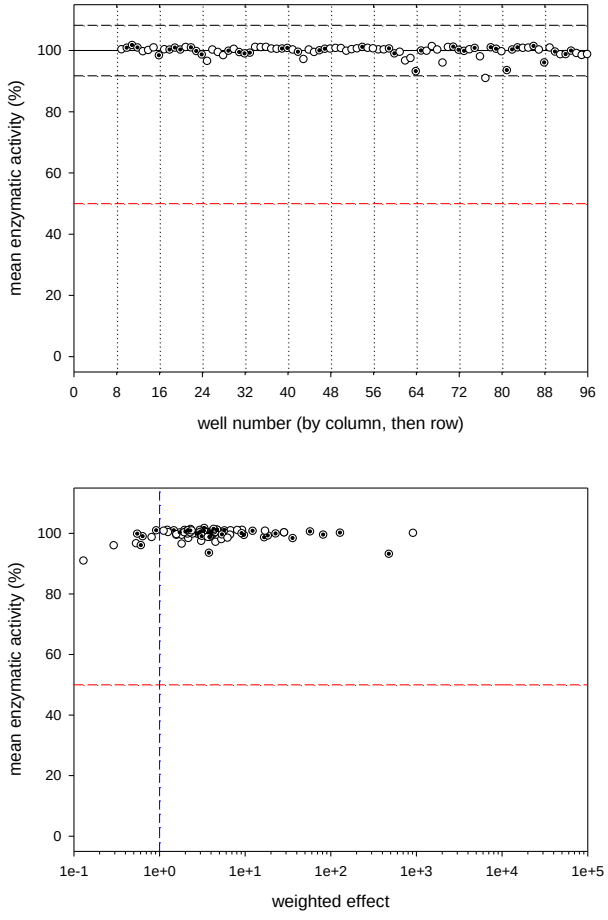


plate ori.hya.6

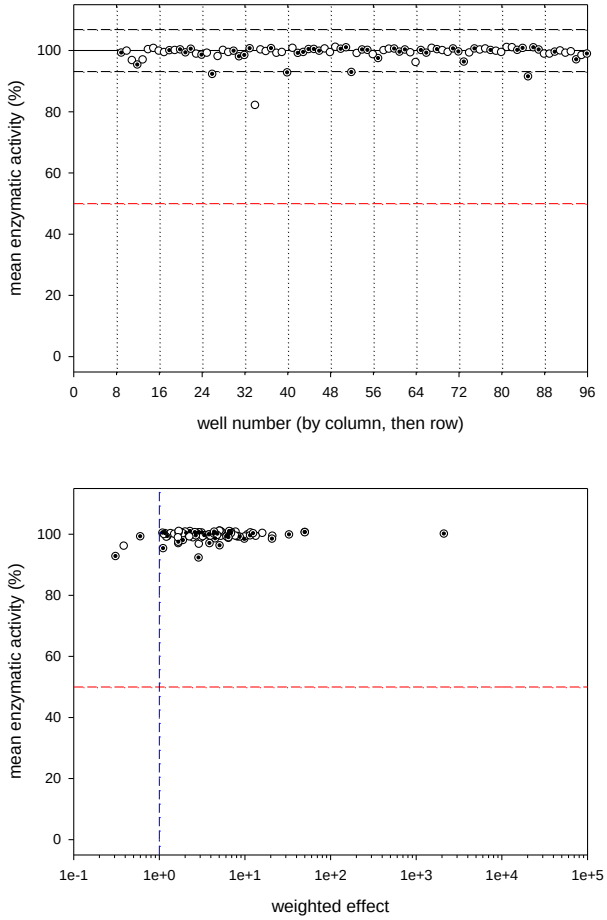
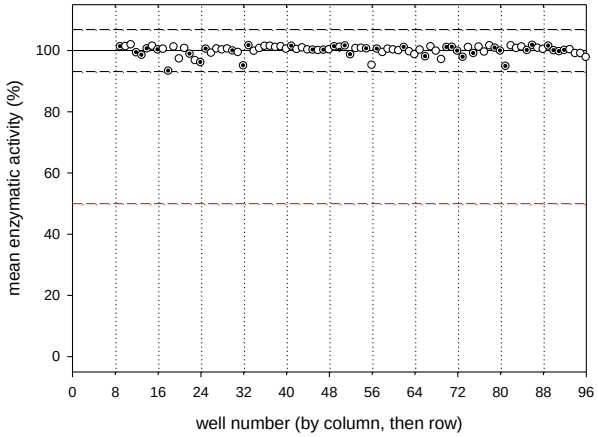


plate ori.hya.7



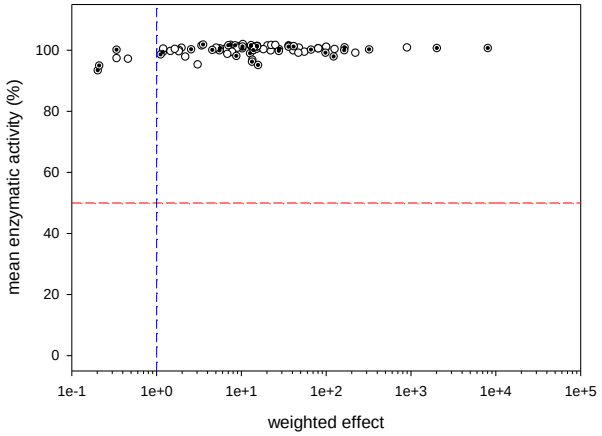
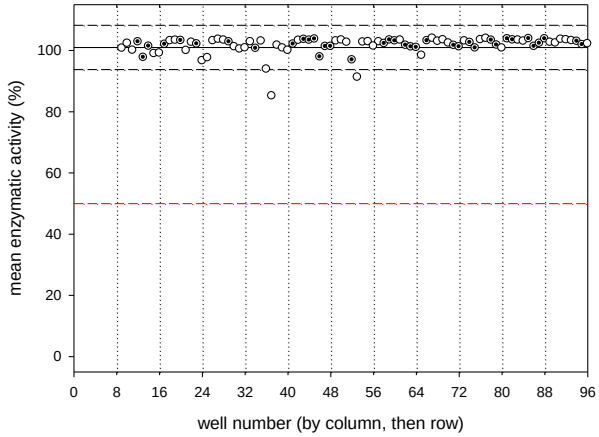


plate ori.hya.8



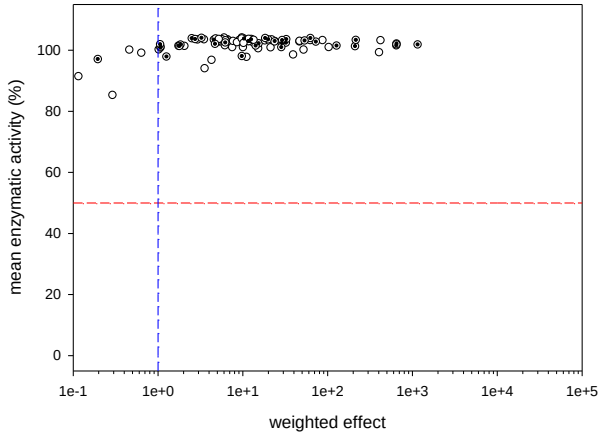
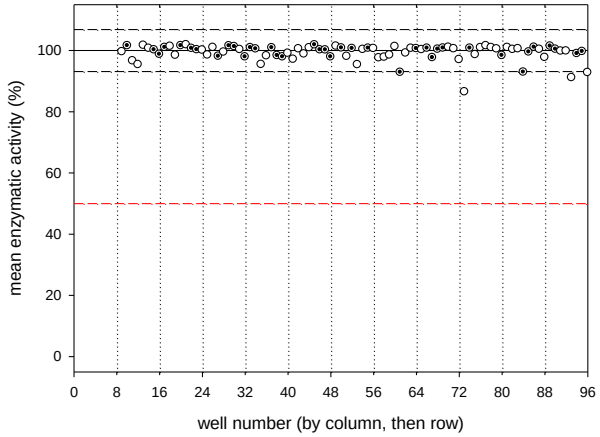
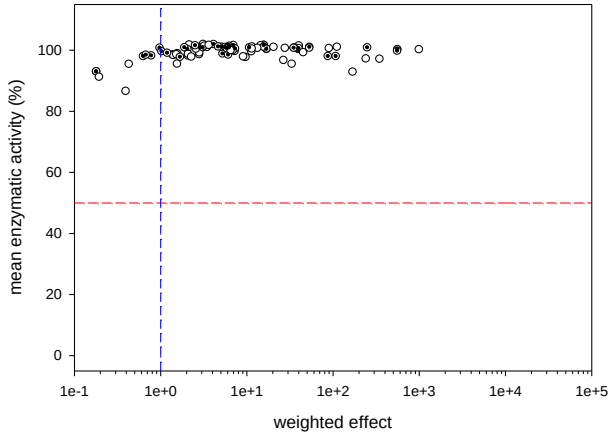


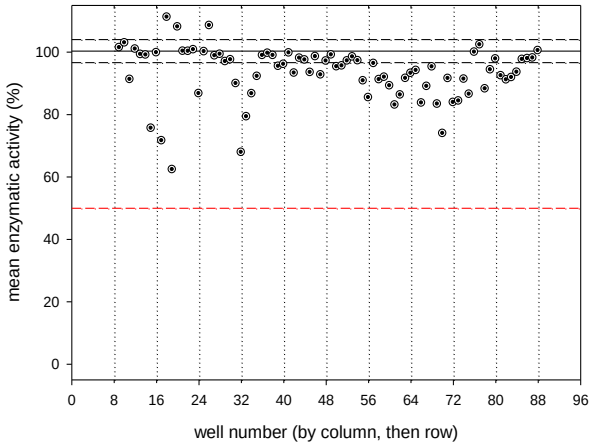
plate ori.hya.9





B.9.3 Pharmacological results for plates ori.hya.scr.1-7

plate ori.hya.scr.1



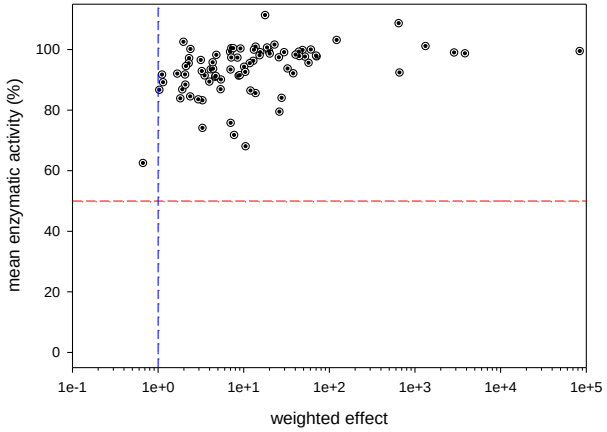
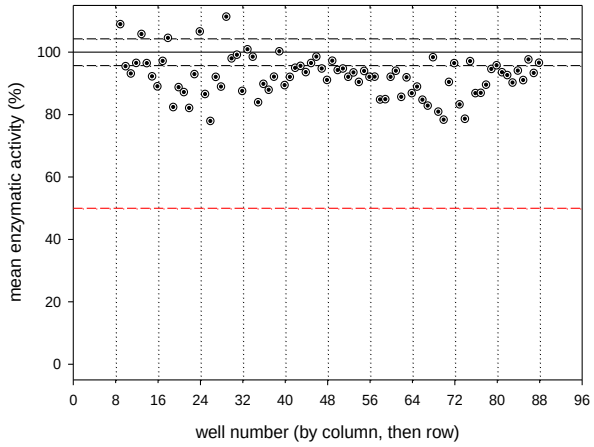


plate ori.hya.scr.2



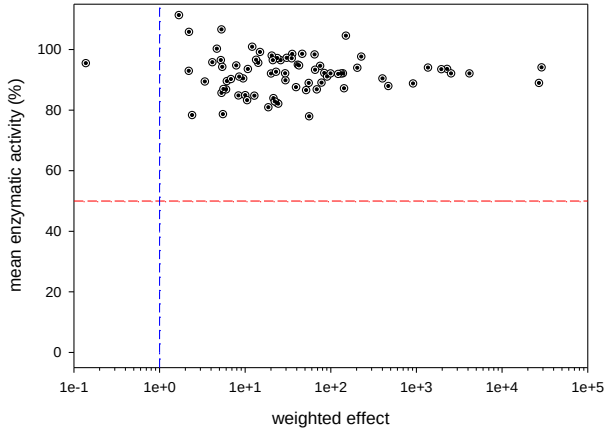
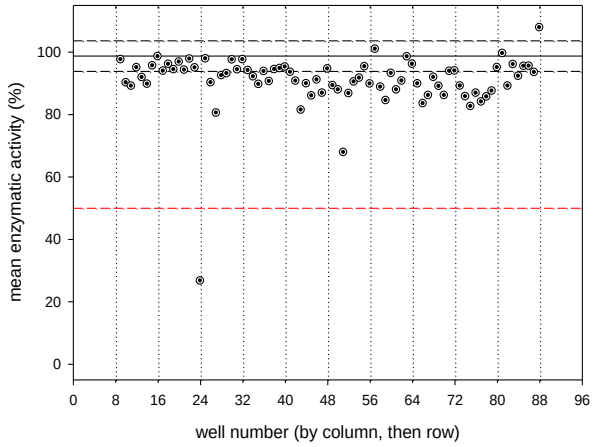


plate ori.hya.scr.3



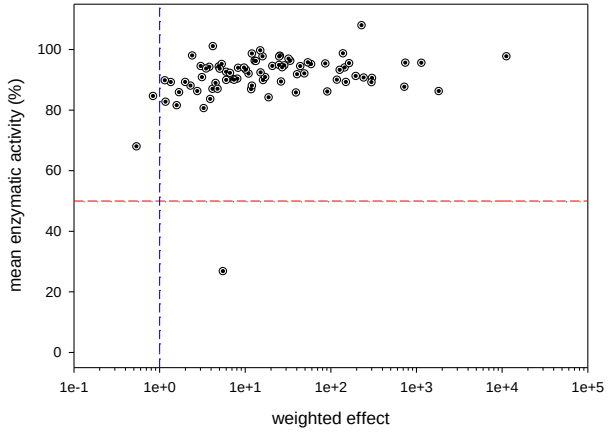


plate ori.hya.scr.4

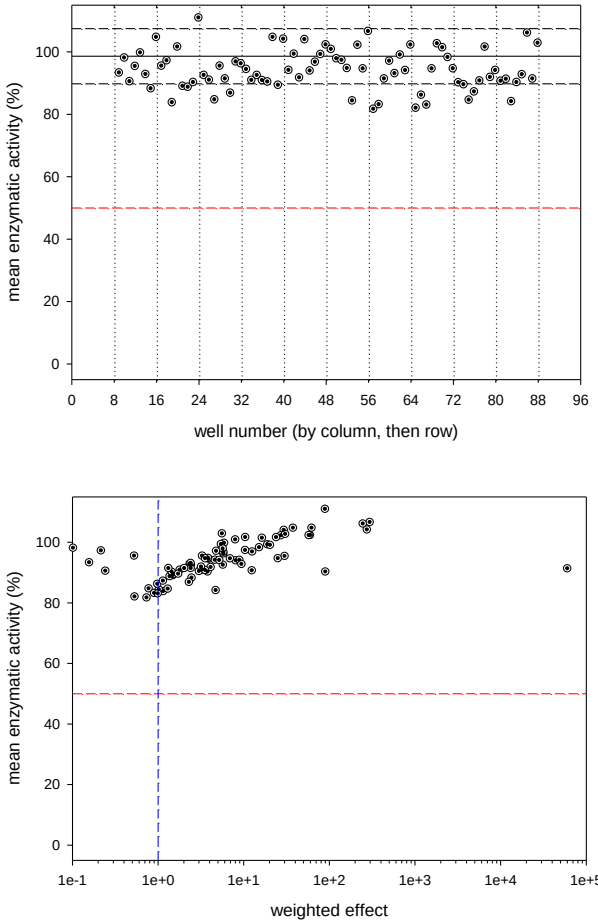


plate ori.hya.scr.5

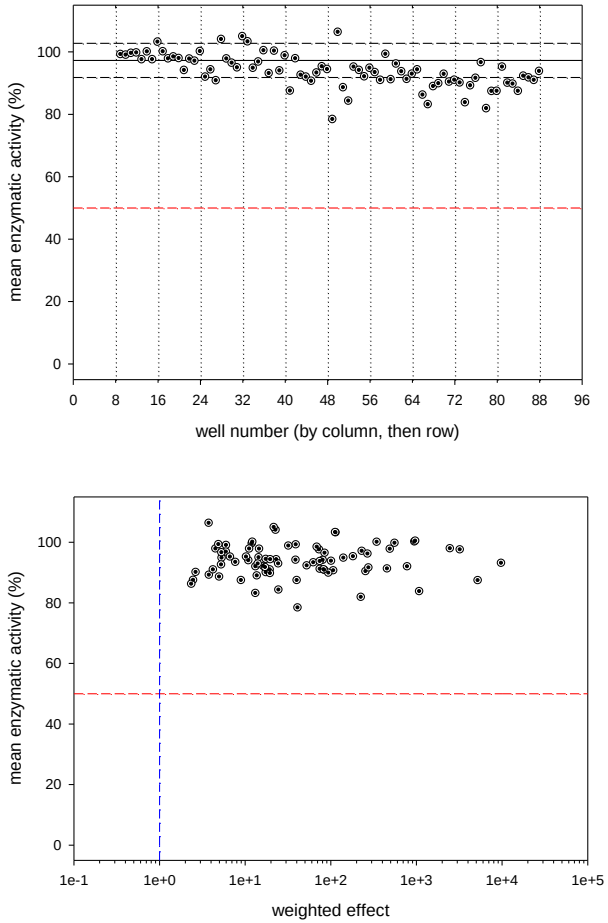
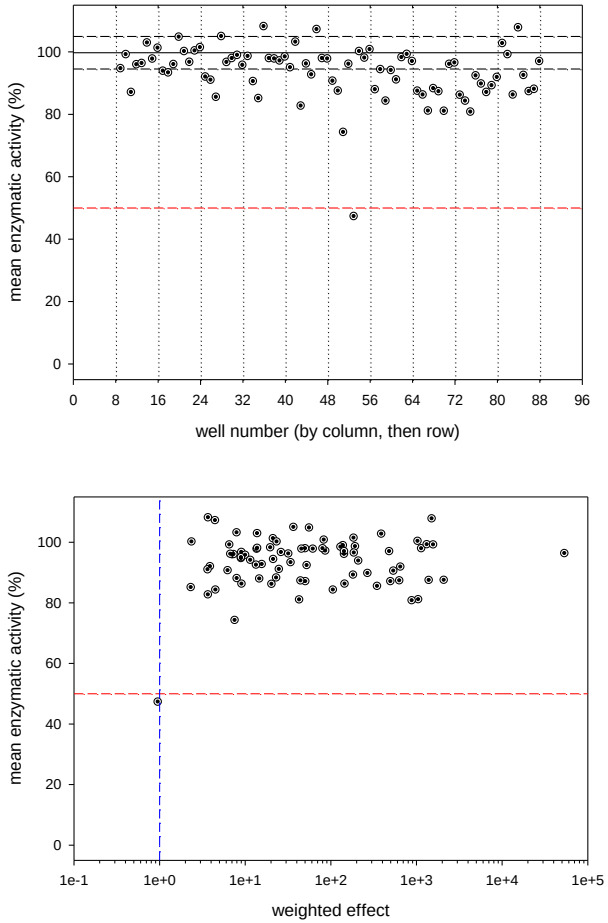


plate ori.hya.scr.6



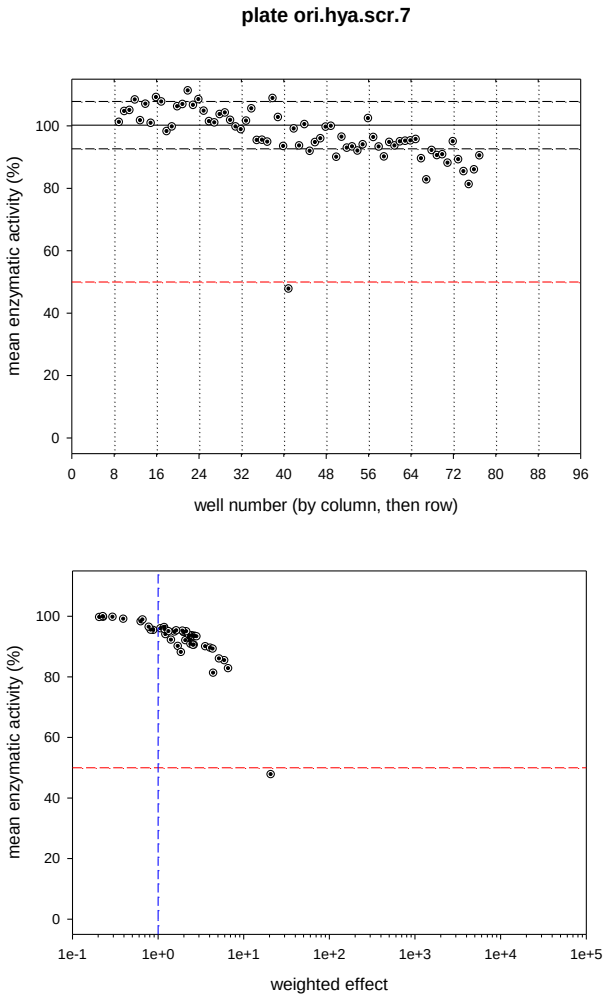
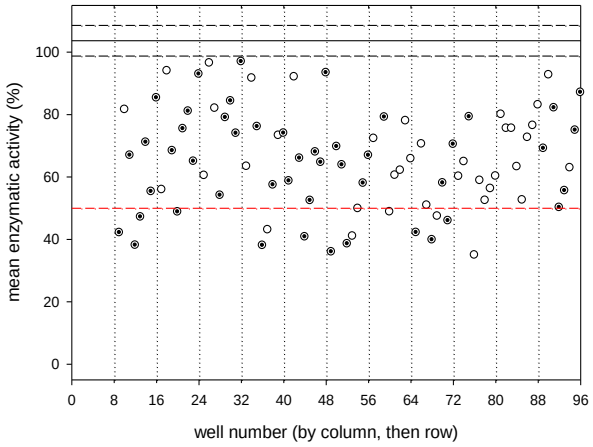


plate ori.hya.scr.7
mean enzymatic activity (%)
0
20
40
60
80
100
0 8 16 24 32 40 48 56 64 72 80 88 96
well number (by column, then row)
mean enzymatic activity (%)
0
20
40
60
80
100
1e-1 1e+0 1e+1 1e+2 1e+3 1e+4 1e+5
weighted effect

B.9.4 Pharmacological results for plates ori.hya.44-47

plate ori.hya.44



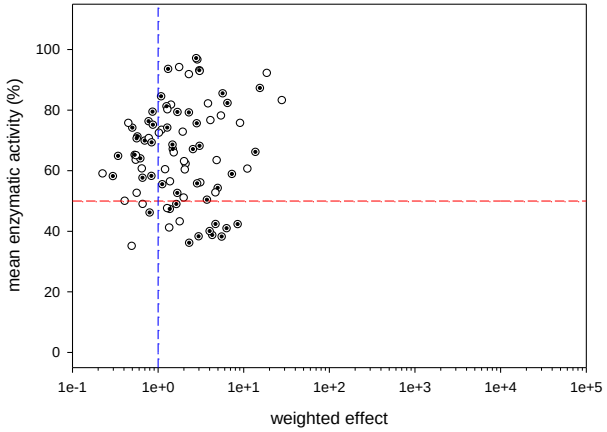
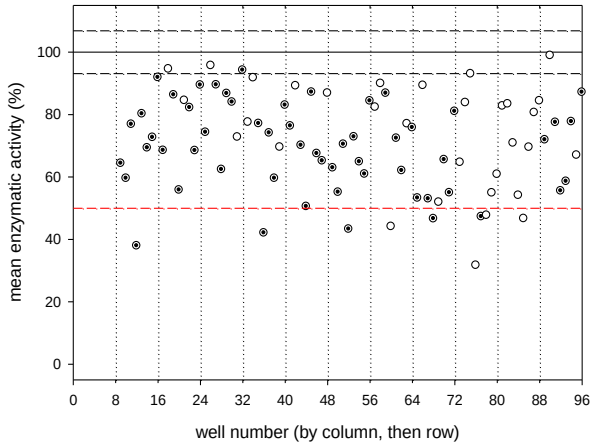


plate ori.hya.45



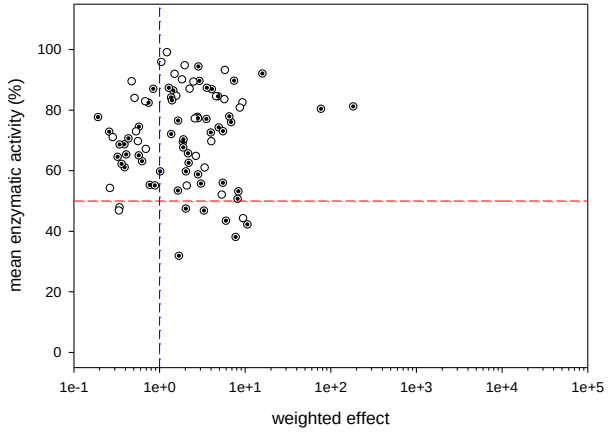
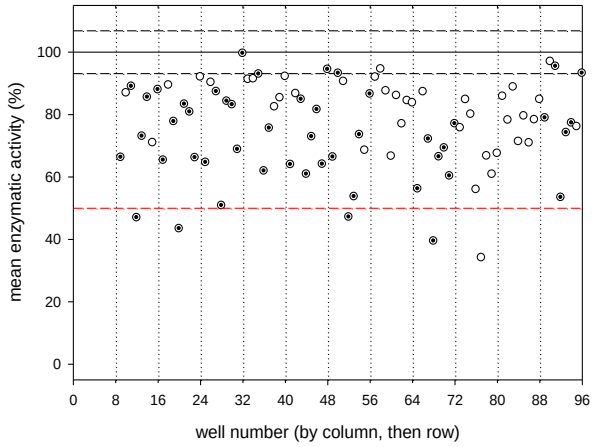


plate ori.hya.46



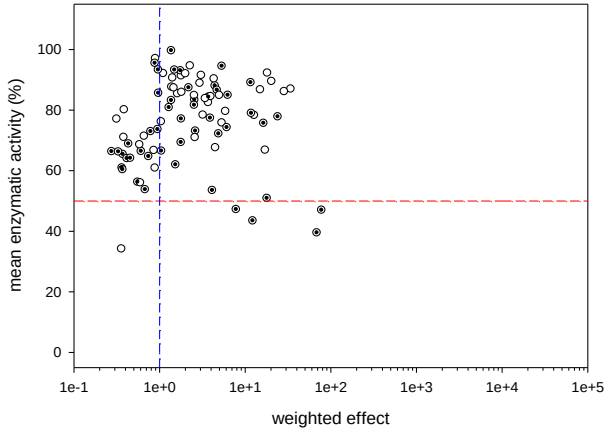
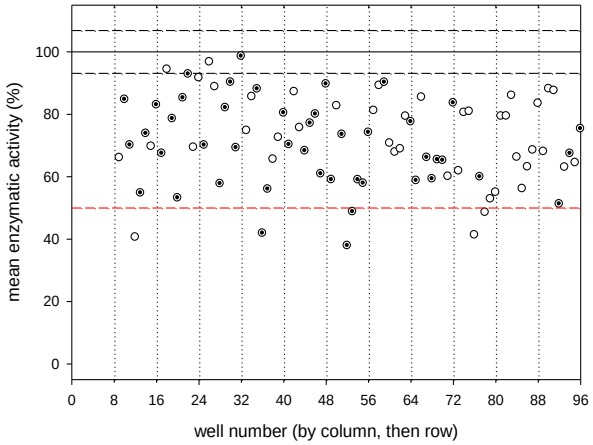
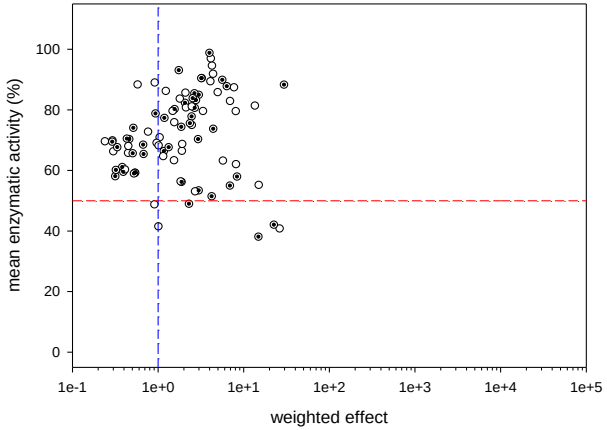


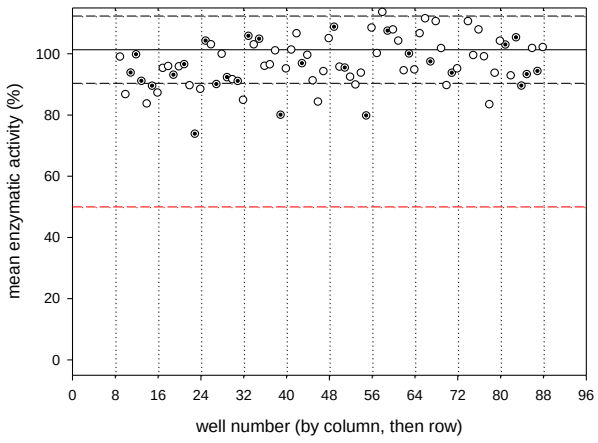
plate ori.hya.47





B.9.5 Pharmacological results for plates ori.hya.48-54

plate ori.hya.48



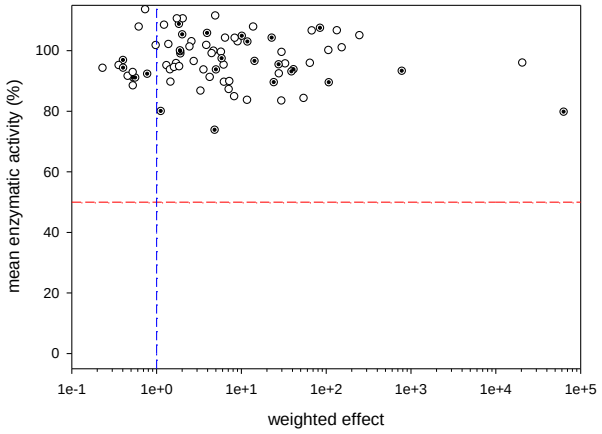
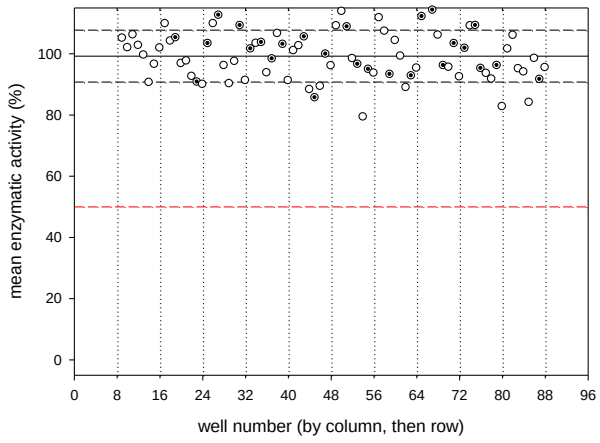


plate ori.hya.49



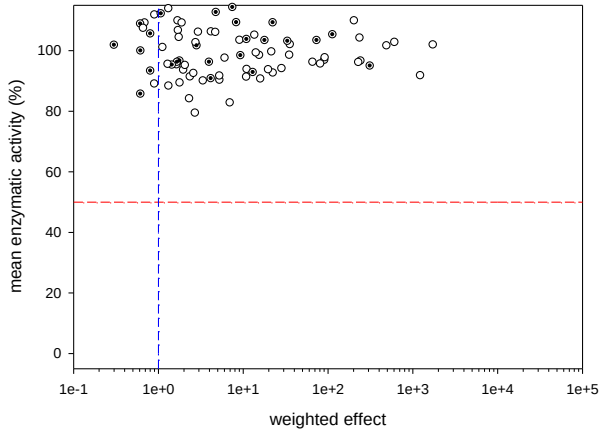
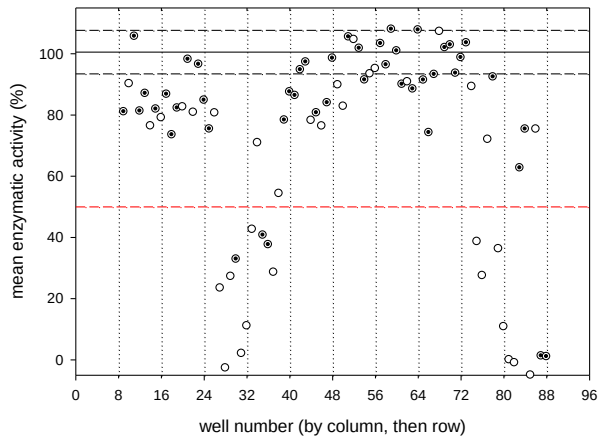


plate ori.hya.50



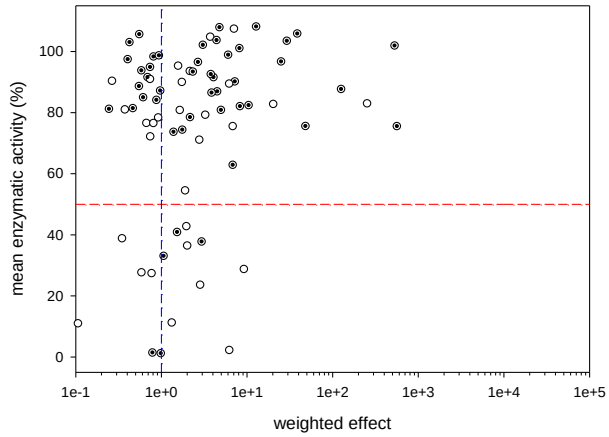
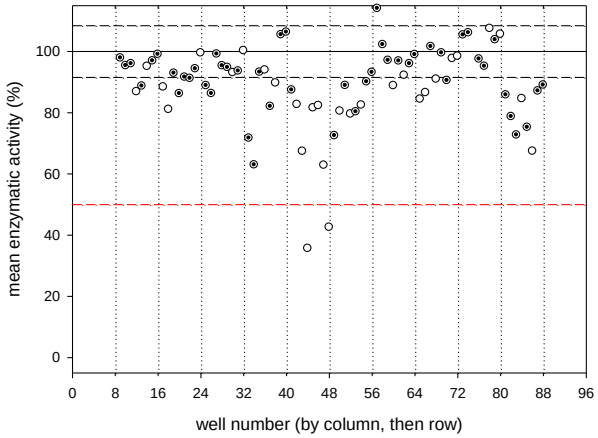


plate ori.hya.51



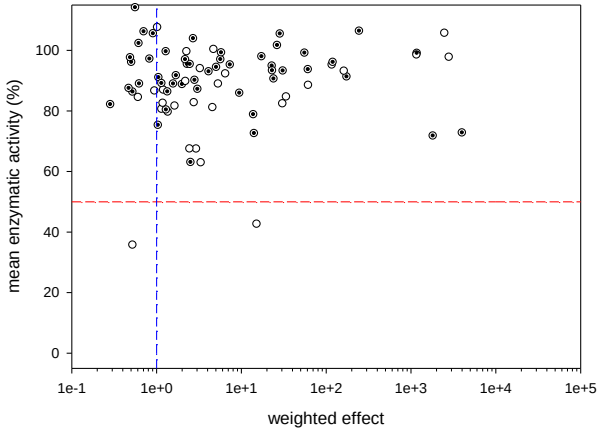
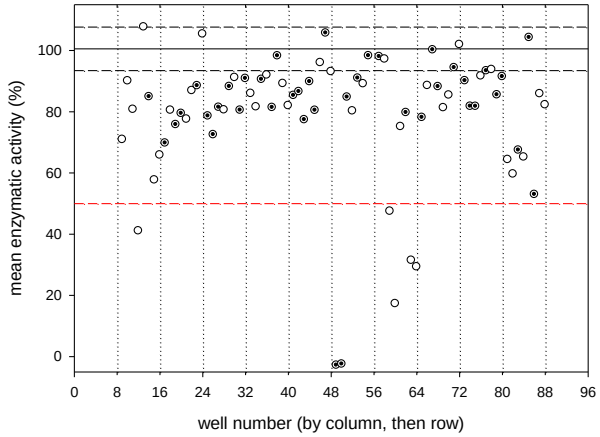


plate ori.hya.52



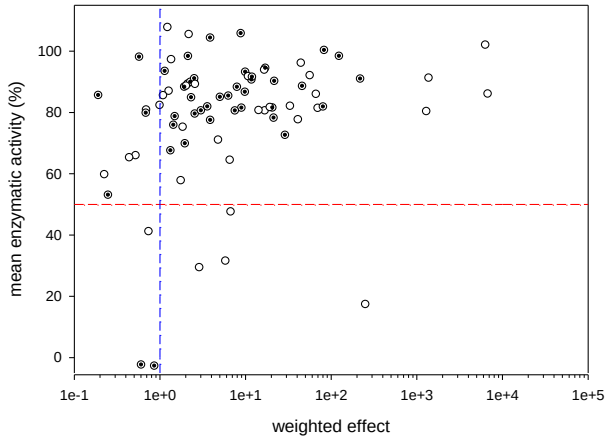
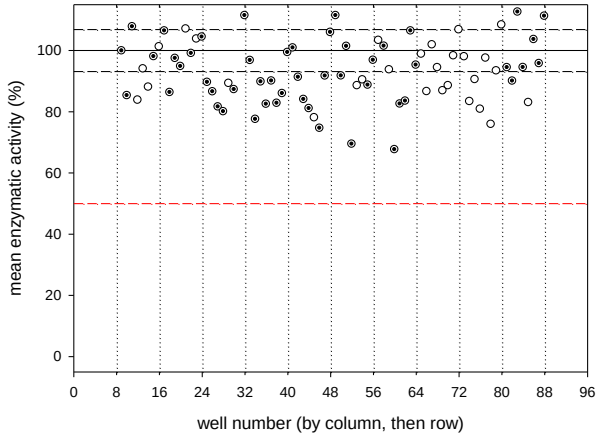
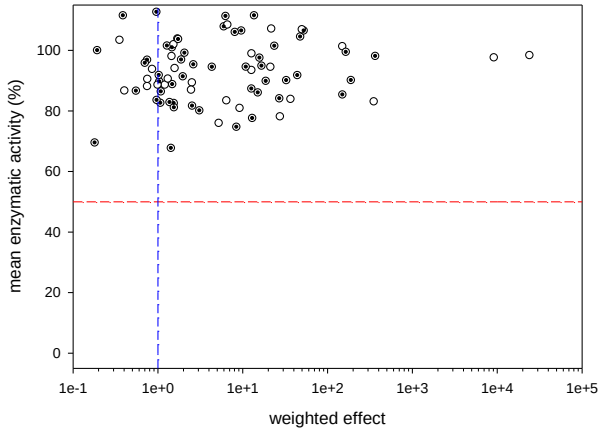


plate ori.hya.53





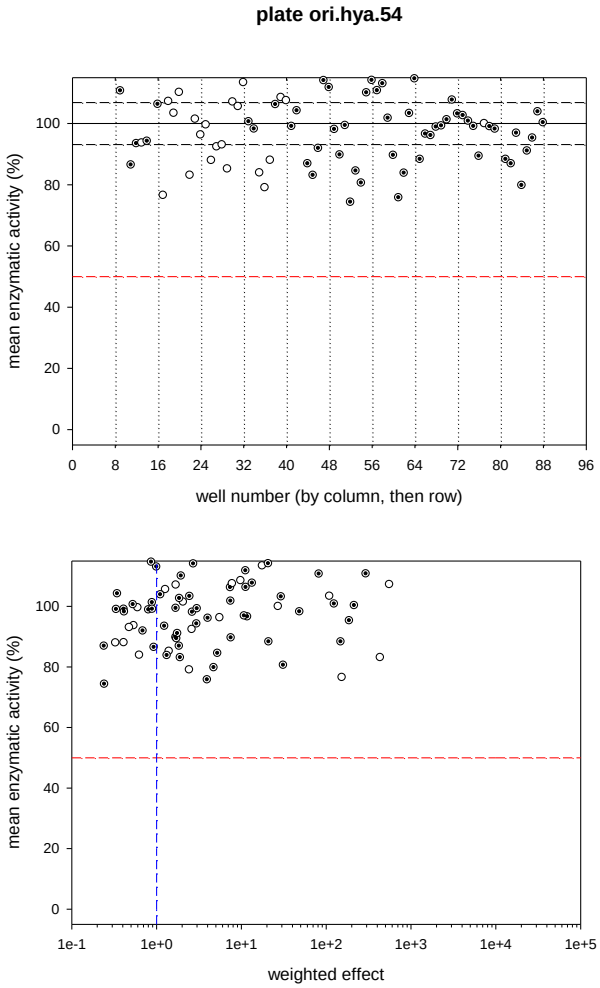


plate ori.hya.54
mean enzymatic activity (%)
0
20
40
60
80
100
0 8 16 24 32 40 48 56 64 72 80 88 96
well number (by column, then row)
mean enzymatic activity (%)
0
20
40
60
80
100
1e-1 1e+0 1e+1 1e+2 1e+3 1e+4 1e+5
weighted effect

B.10 Preparation of the compounds 5.71-5.74

B.10.1 General procedure

A solution of the pertinent aldehyde (1 eq) and amine (1 eq) in dry MeOH (1 mL per mmol) was stirred for 1 h at room temperature and was subsequently cooled to 0 °C. After the addition of KOCN (4 eq), pyridinium chloride (4 eq) and the corresponding isocyanide (1 eq), the mixture was stirred overnight below 20 °C. Insoluble material was removed by filtration and the solvent (MeOH) was removed under reduced pressure. After addition of ethyl acetate as solvent, precipitated material was removed by filtration and the crude product was obtained as residue after evaporation. The crude substance was submitted to RP-HPLC (method indicated) yielding the corresponding {$[M + H]^+ + 16$} product.

B.10.2 1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-4-[4-(trifluoromethyl)benzylamino]-5-(2,4,5-trihydroxyphenyl)-1*H*-imidazol-2(5*H*)-one (5.71)

The title compound was prepared from 2,4,5-trihydroxybenzaldehyde (0.3 mmol, 46 mg), 4-amino-2,6-dichlorophenol (0.3 mmol, 53 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave orange oil, which was stored at +7 °C for 7 days. A partial conversion to the corresponding {$[M + H]^+ + 16$} derivative was observed under the specified conditions. MS (CI-MS)[b] *m/z* (rel. int. in %) = 628.0 (10), 585.0 (90), 542.0 ($[M + H]^+$, 100), 385.1 (95), 361.1 (15), 218.1 (5). MS (CI-MS)[d] *m/z* (rel. int. in %) = 628.0 (10), 585.0 (90), 558.0 ($[M + H]^+ + 16$, 10), 542.0 ($[M + H]^+$, 100), 385.1 (95), 361.1 (15), 218.1 (5). $C_{23}H_{16}Cl_2F_3N_3O_6$ (M_r* = 558.29 g/mol).

Note: mass spectral data refer to $[M + H]^+$ (M_r = 542.29 g/mol) and {$[M + H]^+ + 16$} (M_r* = 558.29 g/mol). [b] 17 h after completion of the experiment; relative abundance (TIC): M_r (542.29 g/mol) = 15 %; relative abundance (TIC): M_r* (558.29 g/mol) = 0 %; [d] 168 h after completion of the experiment; relative abundance (TIC): M_r (542.29 g/mol) = 15 %; relative abundance (TIC): M_r* (558.29 g/mol) = 5 %.

B.10.3 1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-phenyl-4-[4-(trifluoromethyl) benzylamino]-1H-imidazol-2(5*H*)-one (5.72)

The title compound was prepared from benzaldehyde (0.3 mmol, 32 mg), 4-amino-2,6-dichlorophenol (0.3 mmol, 53 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave dark yellow oil which was stored at +7 °C for 20 days. A partial conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. MS (CI-MS)[b] *m/z* (rel. int. in %) = 539.0 (50), 494.0 ([M + H]$^+$, 100). MS (CI-MS)[d] *m/z* (rel. int. in %) = 528.1 (5), 524.1 (90), 510.0 ([M + H]$^+$ + 16, 80), 494.0 ([M + H]$^+$, 100), 492.0 (10). $C_{23}H_{16}Cl_2F_3N_3O_3$ (M_r* = 510.29 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 494.29 g/mol) and {[M + H]$^+$ + 16} (M_r* = 510.29 g/mol). [b] 20 h after completion of the experiment; relative abundance (TIC): M_r (494.29 g/mol) = 50 %; relative abundance (TIC): M_r* (510.29 g/mol) = 0 %; [d] 20 d after completion of the experiment; relative abundance (TIC): M_r (494.29 g/mol) = 40 %; relative abundance (TIC): M_r* (510.29 g/mol) = 4 0%.

B.10.4 1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-4-[4-(trifluoromethyl)benzylamino]-5-(2,4,5-trimethoxyphenyl)-1*H*-imidazol-2(5*H*)-one (5.73)

The title compound was prepared from 2,4,5-trimethoxybenzaldehyde (0.3 mmol, 59 mg), 4-amino-2,6-dichlorophenol (0.3 mmol, 53 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil which was stored at +7 °C for 20 days. A partial conversion to the corresponding {[M + H]$^+$ + 16} derivative was observed under the specified conditions. MS (CI-MS)[b] *m/z* (rel. int. in %) = 584.0 ([M + H]$^+$, 100), 405.2 (5), 345.1 (5), 275.1 (5). MS (CI-MS)[d] *m/z* (rel. int. in %) = 600.1 ([M + H]$^+$ + 16, 100), 584.0 ([M + H]$^+$, 5), 531.0 (5), 354.1 (5). $C_{26}H_{22}Cl_2F_3N_3O_6$ (M_r* = 600.37 g/mol).

Note: mass spectral data refer to [M + H]$^+$ (M_r = 584.37 g/mol) and {[M + H]$^+$ + 16} (M_r* = 600.37 g/mol).[b] 20 h after completion of the experiment; relative abundance (TIC): M_r (584.37 g/mol) = 20 %; relative abundance (TIC): M_r* (600.37 g/mol) = 5 %; [d] 20 d after completion of the experiment; relative abundance (TIC): M_r (584.37 g/mol) = 3 %; relative abundance (TIC): M_r* (600.37 g/mol) = 20 %.

B.10.5 1-(3,5-Dichloro-4-hydroxyphenyl)-5-hydroxy-5-(1H-indazol-5-yl)-4-[4-(trifluoromethyl) benzylamino]-1*H*-imidazol-2(5*H*)-one (5.74)

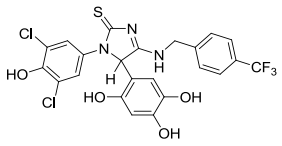
The title compound was prepared from 2,4,5-trimethoxybenzaldehyde (0.3 mmol, 59 mg), 4-amino-2,6-dichlorophenol (0.3 mmol, 53 mg) and 1-(isocyanomethyl)-4-(trifluoromethyl)benzene (0.3 mmol, 56 mg) according to the general procedure. The crude product was obtained as residue after evaporation from ethyl acetate, and gave yellow oil which was stored at +7 °C for 20 days. A partial conversion to the corresponding {$[M + H]^+ + 16$} derivative was observed under the specified conditions. MS (CI-MS)[b] *m/z* (rel. int. in %) = 558.1 (10), 534.1 ($[M + H]^+$, 100), 434.2 (5), 376.0 (5), 304.4 (5), 218.1 (15). MS (CI-MS)[d] *m/z* (rel. int. in %) = 550.1 ($[M + H]^+ + 16$, 5), 385.1 (70), 218.1 (100). $C_{24}H_{16}Cl_2F_3N_5O_6$ (M_r* = 550.32 g/mol).

Note: mass spectral data refer to $[M + H]^+$ (M_r = 534.32 g/mol) and {$[M + H]^+ + 16$} (M_r* = 550.32 g/mol). [b] 20 h after completion of the experiment; relative abundance (TIC): M_r (534.32 g/mol) = 5 %; relative abundance (TIC): M_r* (550.32 g/mol) = 0 %; [d] 20 d after completion of the experiment; relative abundance (TIC): M_r (534.32 g/mol) = 0 %; relative abundance (TIC): M_r* (550.32 g/mol) = 5 %.

B.11 Mass spectral analysis of the oxidation of 5.22

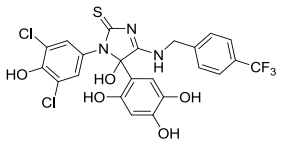
B.11.1 Molecular properties of 5.22 and 5.62

"compound 1"
5.22 ($[M + H]^+$)
t_R = 2.78 min



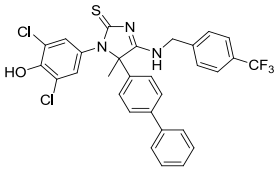
Chemical Formula: $C_{23}H_{16}Cl_2F_3N_3O_4S$
Exact Mass: 557,0191
Molecular Weight: 558,3570

"compound 2"
5.22 ($\{[M + H]^+ + 16\}$)
t_R = 2.67 min



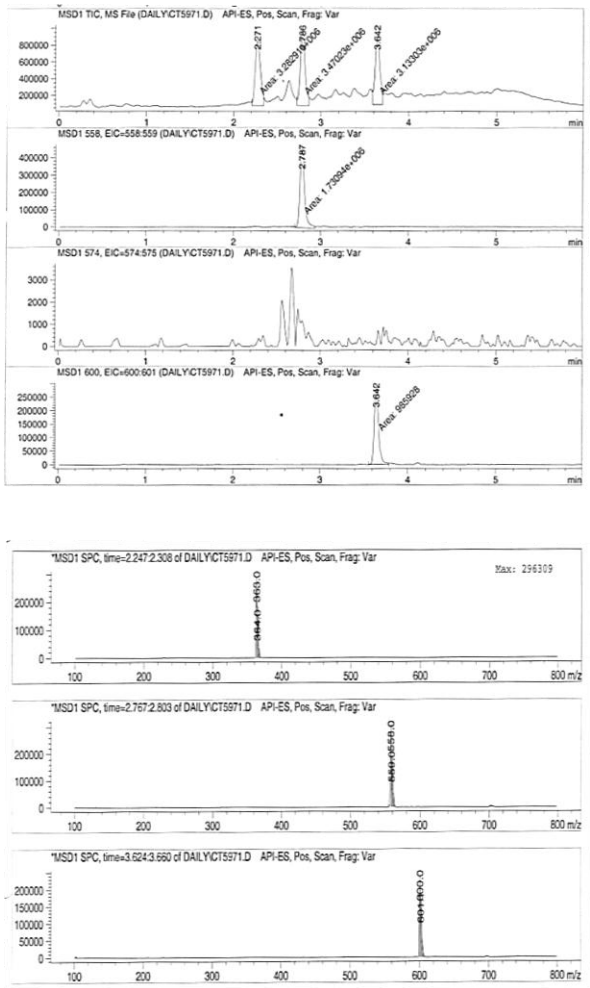
Chemical Formula: $C_{23}H_{16}Cl_2F_3N_3O_5S$
Exact Mass: 573,0140
Molecular Weight: 574,3564

"internal standard" (compound 3)
5.62
t_R = 3.64 min

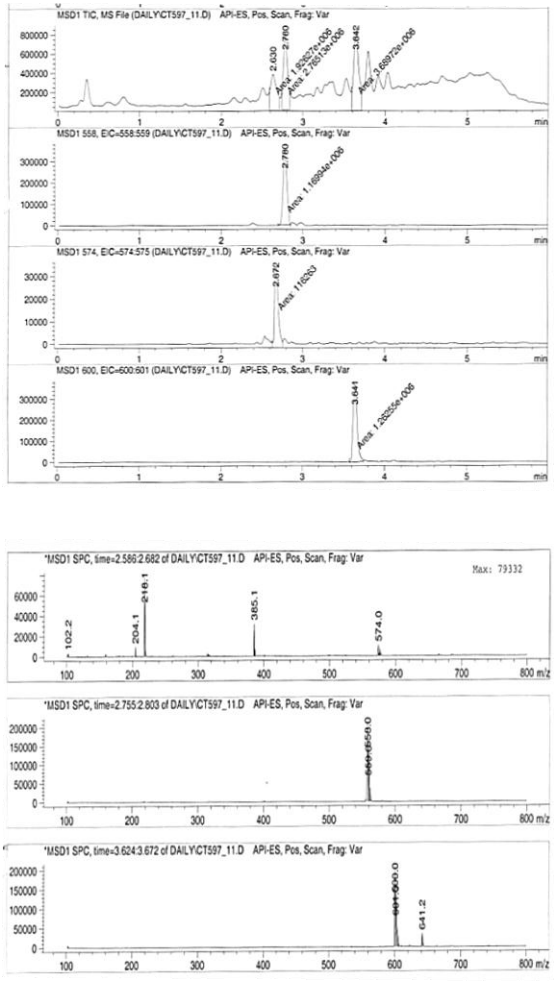


Chemical Formula: $C_{30}H_{22}Cl_2F_3N_3OS$
Exact Mass: 599,0813
Molecular Weight: 600,4814

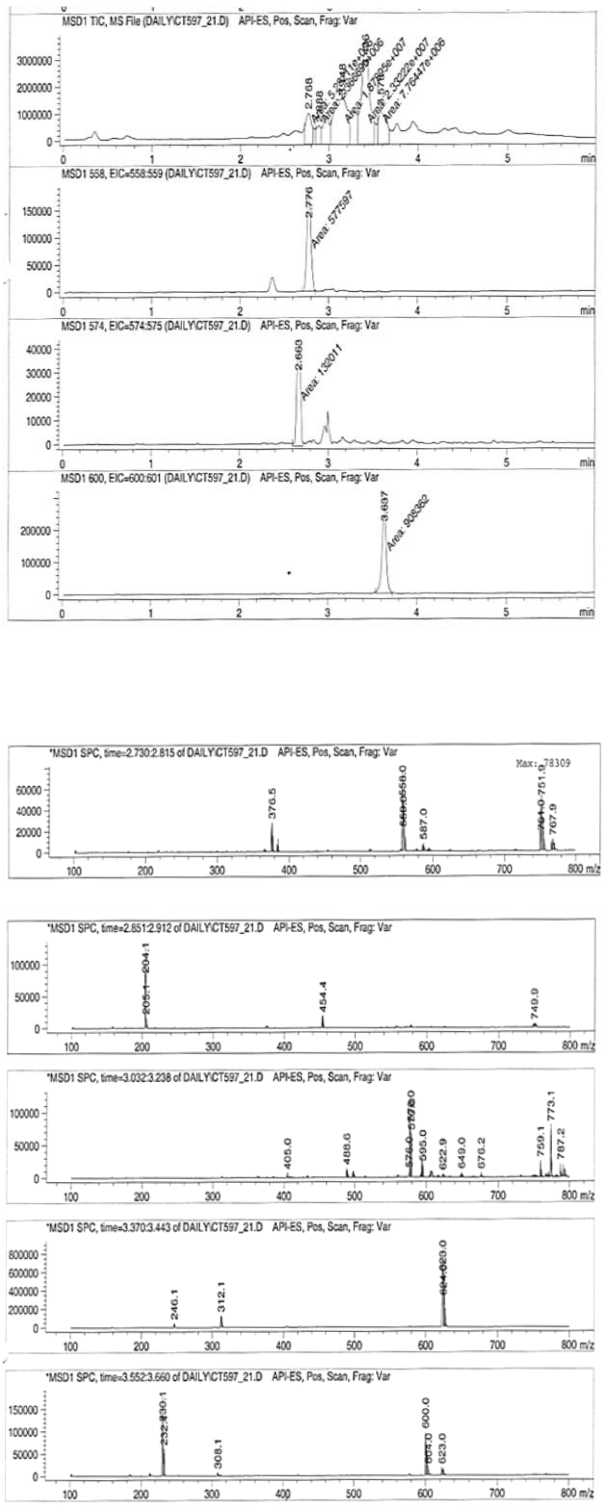
B.11.2 Documentation of LC-MS data



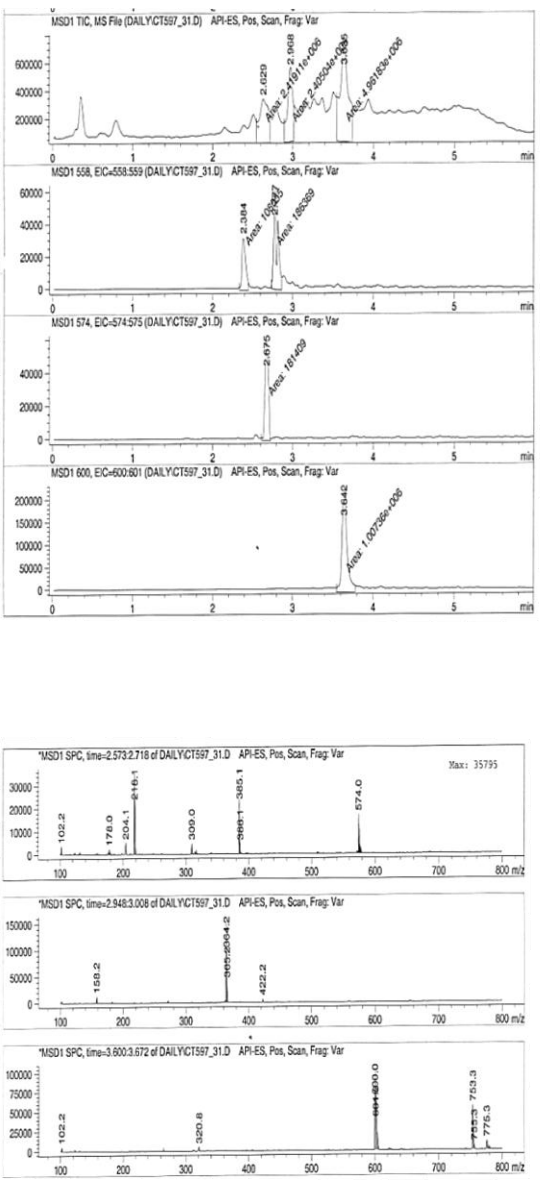
MS data after completion of the experiment; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3



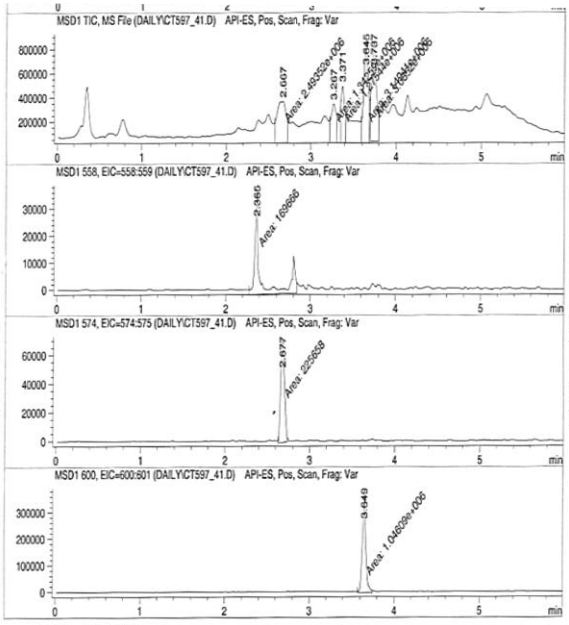
MS data after 24 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3

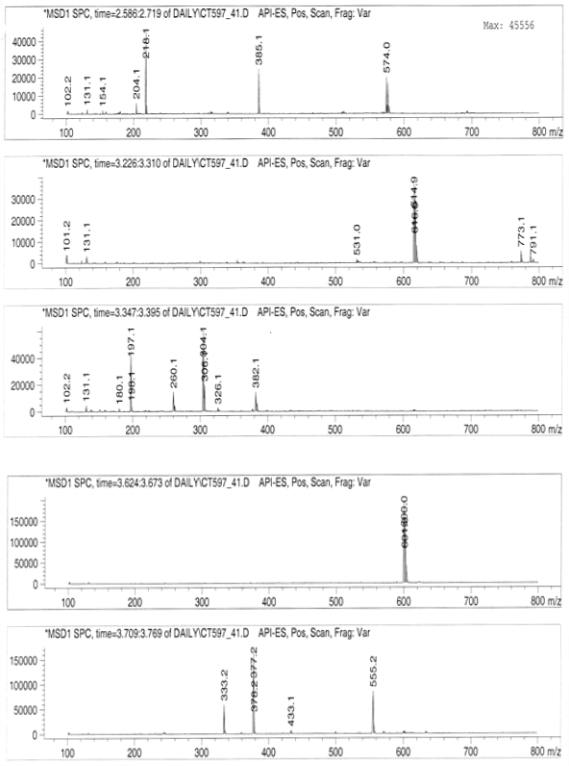


MS data after 48 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3

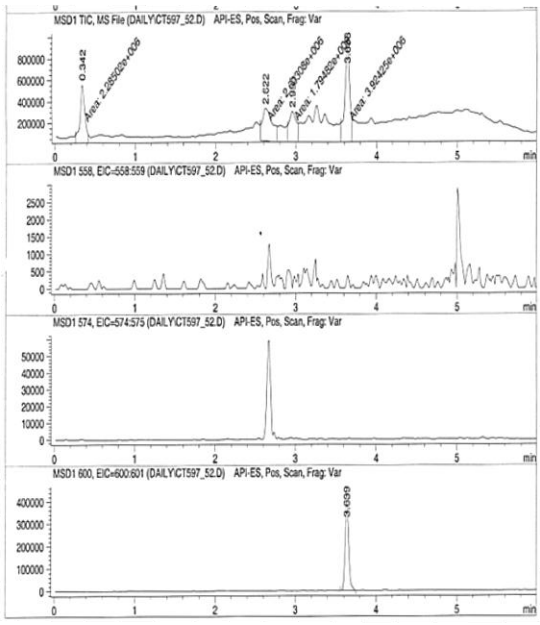


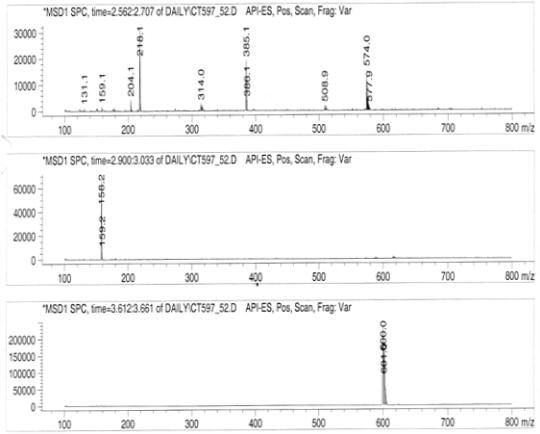
MS data after 72 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3



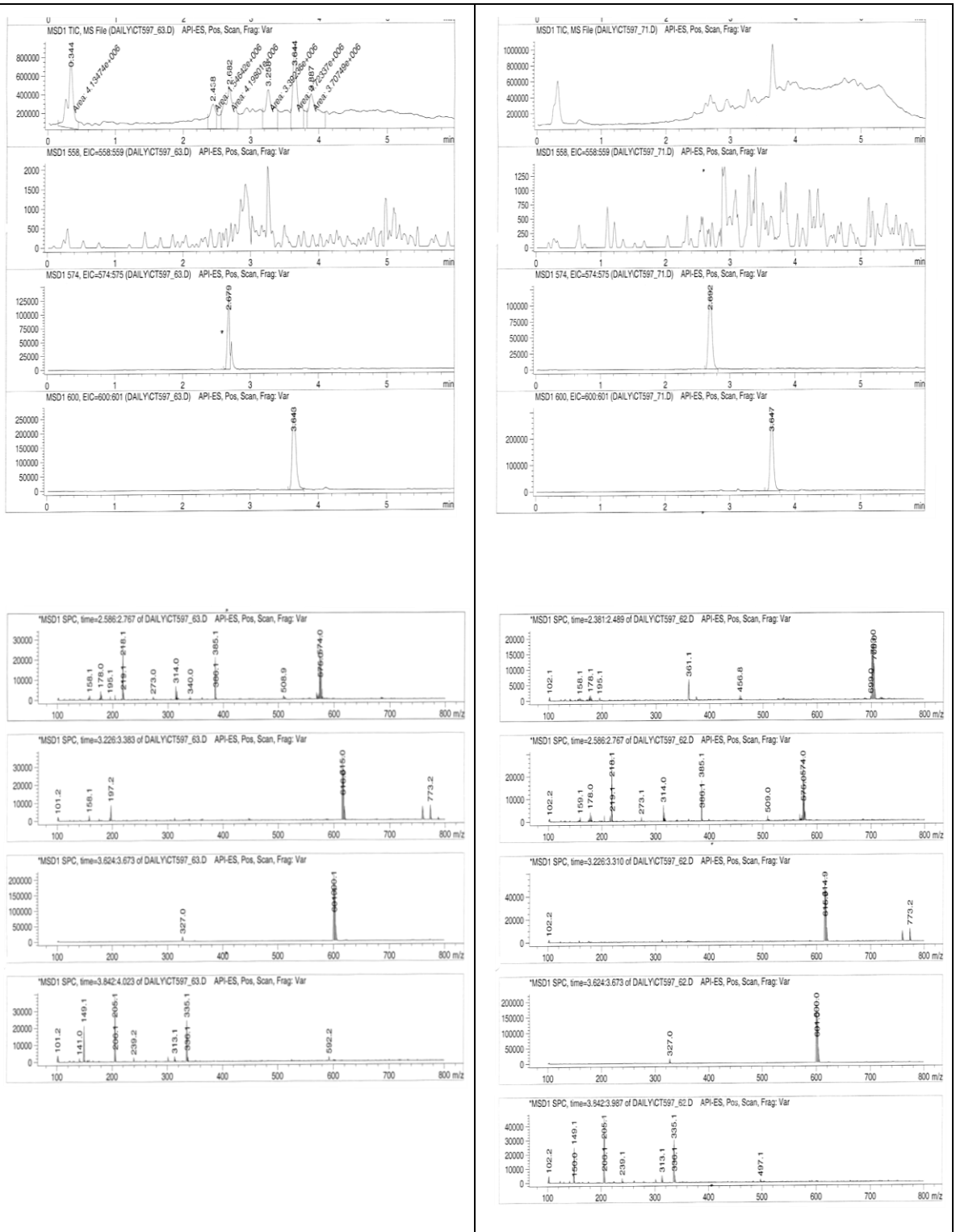


MS data after 96 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3





MS data after 184 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3



MS data after 226 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3

MS data after 302 h; above: TIC (total ion count) and ion counts of compounds 1-3, below: peak counts of compounds 1-3

C Appendix III

C.1 Small scale expression of *S. pneumoniae* hyaluronate lyase

In collaboration with Dr. Janina Hamberger from our workgroup, *Spn*Hyl-6-His was expressed in *E. coli* BL21(DE3) cells as described by Jedrzejas.[1, 2] A transformed *E. coli* cell colony was selected on LB medium with 100 µg/mL ampicillin. Multiple colonies were inoculated into 200 mL of LB medium with the same antibiotic and incubated at 37 °C on an incubator shaker overnight. Protein expression was induced by addition of isopropyl-β-*D*-1-thiogalactopyranoside (IPTG, Sigma-Aldrich, Munich, Germany) to the final concentration of 1 mM and growth was continued for another 3 hours. The cells were harvested by centrifugation at 6000 rpm for 10 minutes at 4 °C. All additional procedures were performed on ice. The resulting cell pellet was resuspended in McIlvaine's buffer (pH = 5.0), supplemented with 20 % of glycerol and frozen at -80 °C. After thawing on ice, the suspension was again centrifuged and the pellet resuspended in McIlvaine's buffer pH 6.0. The sample was sonicated for 5 x 20 s with 1 min intervals on ice and the resulting cell lysate was centrifuged at 15000 rpm at 4 °C for 30 min. the supernatant was used for activity measurements, SDS-PAGE and Western Blot analysis were performed as described in the doctoral thesis of Dr. Janina Hamberger.[2]

C.2 Large scale expression of *S. pneumoniae* hyaluronate lyase

Large scale expression was performed in a bioreactor (BIOSTAT C-Laborfermenter, B. Braun, Melsungen, Germany) in collaboration with the Institute of Biotechnology (Prof. Dr. R. Rudolph) at the Martin-Luther University Halle-Wittenberg Germany. 6 L of sterile complete medium (50 g/L LB medium, 2.5 g/L $(NH_4)_2SO_4$, 0.5 g/L NH_4Cl, 14.6 g/L K_2HPO_4, 4 g/L NaH_2PO_4 x H_2O, 4 g Na_2SO_4, 20 g/L glucose, 0.1 g/L thiamine, 2.5 g/L $MgSO_4$ x $7H_2O$) containing 100 µg/mL of ampicillin were initiated by addition of 100 mL of *E. coli* BL21(DE3) overnight culture grown for 17 h in LB-medium with 100 µg/mL ampicillin. Fermentation was monitored by measuring pH, temperature, pressure, and glucose concentration. The pH value was controlled by automated addition of 10% (v/v) H_3PO_4 and 25% (v/v) NH_3, respectively. For the fermentation process the pH was adjusted to 7.0. As illustrated in Figure 1, the culture was continuously fed by addition of 20 g/h sterile feeding solution (550 g/L glucose, 22 g/L Na_2SO_4, 24,92 g/L $(NH_4)_2SO_4$, 5 g/L NH_4Cl, 146 g/L, K_2HPO_4, 36 g/L NaH_2PO_4 x H_2O, 2,2 mL 1 M $MgSO_4$ solution) for 5 h. Subsequently, the addition of feeding solution was increased. After fermentation for 18 h,

1 mM IPTG (from a 1 M stock solution in water) was added to induce expression of *Spn*Hyl-6-His. The bacteria were cultured for additional 4 h before the suspension was centrifuged (20 min, 4 °C) and the pellets were stored at -80 °C.

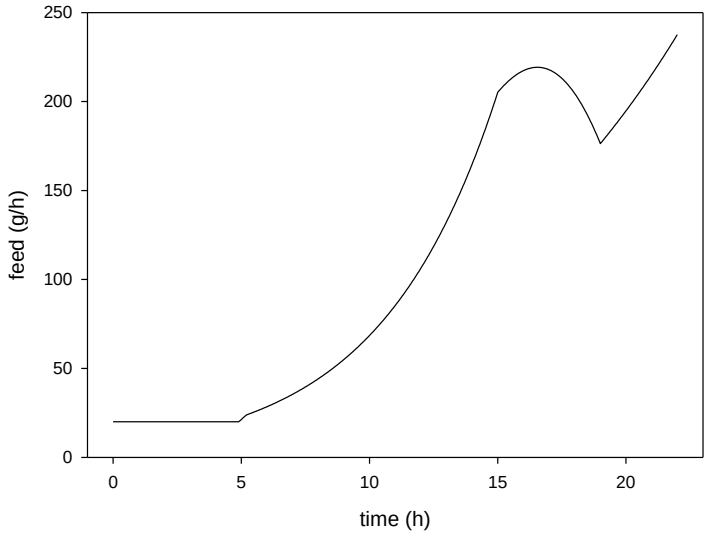


Figure 1 Illustration of glucose feed in g/h during culture fermentation (22 h); after 18 h, IPTG (1 M) was added to induce expression of *Spn*Hyl-6-His.

C.3 Purification of *S. pneumoniae* hyaluronate lyase

A bacterial pellet of 10 g (wet weight) was thawed on ice and resuspended in 50 mL of binding buffer (500 mM NaCl, 20 mM NaH_2PO_4, 20 mM imidazole, pH 7.4), supplemented with an EDTA-free protease inhibitor cocktail (Sigma-Aldrich, Munich, Germany) following sonication as described above. The cell lysate was centrifuged at 15000 rpm at 4 °C for 30 min and the supernatant was used for purification with ion metal affinity chromatography (IMAC).

Two Ni-IMAC columns (HisTrap FF^{TM}, CV = 5 mL, GE Healthcare, Munich, Germany) were equilibrated with wash buffer (500 mM NaCl, 20 mM NaH_2PO_4, 15 mM imidazole, pH = 7.4) at 4 °C. After loading sample (10 mL supernatant of *Spn*Hyl-6-His), the column was washed with 30 column volumes (CV) of wash buffer. Subsequently, *Spn*Hyl-6-His was eluted by washing with elution buffer (500 mM NaCl, 20 mM NaH_2PO_4, 500 mM imidazole, pH = 7.4). Elution fractions of 6.5 mL (cf. Figure 2) were collected and analyzed with respect to protein content, purity and enzymatic activity.

As shown in Figure 3, elution fractions 25 and 26 were of satisfying purity. After desalting by dialysis (MWCO 14000, 4 °C, buffer: citrate-phosphate buffer, pH = 6, 0.1 M NaCl), the protein content of the elution fractions was determined using the method of Bradford in the microtiter plate format.[3]

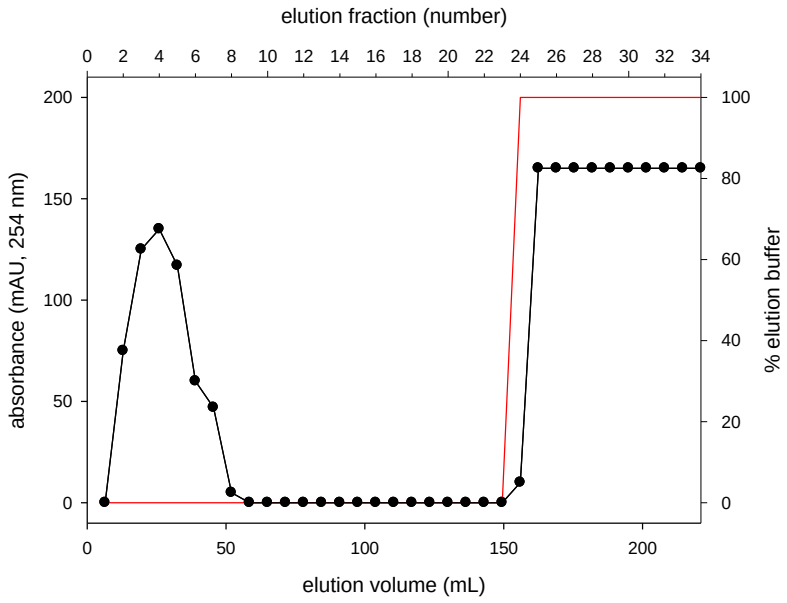


Figure 2: Purification of *Spn*Hyl-6-His by Ni-IMAC (CV 5 mL). Elution profile plotted as absorbance signal (254 nm) versus elution volume (mL). After 150 mL, the wash buffer (20 mM imidazole) was replaced by elution buffer (500 mM imidazole) as indicated (red line). A total of 34 fractions was collected (fraction volume 6.5 mL).

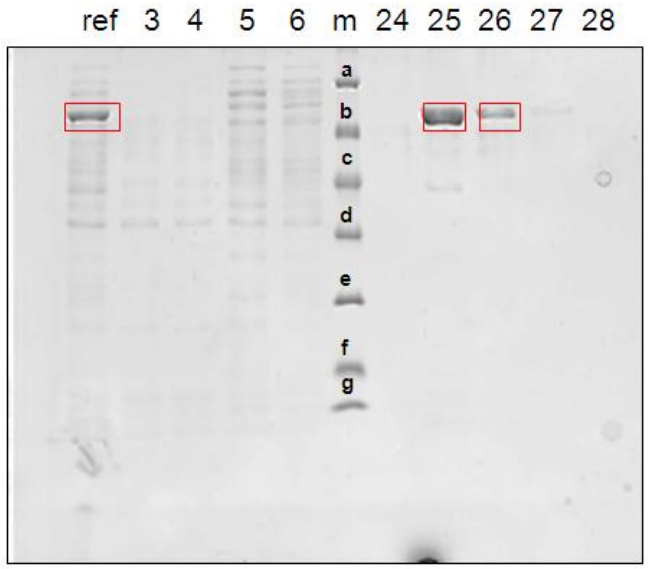


Figure 3: SDS-page of *Spn*Hyl-6His after large-scale purification .[a] (ref) total cell lysate prior to purification (target protein highlighted); (3)-(6) elution fractions 3-6; (m) peqlab marker, a: 116 kDa, b: 60.2 kDa, c: 45 kDa, d: 35 kDa, e: 25 kDa, f: 18.4 kDa, g: 14.4 kDa; (24)-(28) elution fractions 24-28 (purified target protein (84 kDa) highlighted in fractions 25, 26).

[a] 20 µL were applied to the SDS-gel and staining was accomplished with Coomassie brilliant blue; SDS-gel electrophoresis was performed by Dr. Janina Hamberger.

C.4 Colorimetric hyaluronidase activity assay and protein characterization

Enzymatic activity was measured using the colorimetric hyaluronidase assay. Specific activity in U/mg was calculated by dividing enzymatic activity by protein content (Table 1).

Table 1: Enzymatic activity and specific activity of *Spn*Hyl in the elution fractions as determined with the colorimetric assay.[a] Fractions 25 and 26 contained 13.5 and 5.5 µg/mL protein, respectively. Results are presented as mean ± SEM from two independent experiments.

elution fraction	enzymatic activity (mU/mL)	specific activity (U/mg)
25	63.5 ± 3.9	4.7 ± 0.3
26	21.3 ± 1.7	3.9 ± 0.3

[a] data were kindly provided by Dr. Janina Hamberger

C.5 References

1. Jedrzejas, M. J.; Mewbourne, R. B.; Chantalat, L.; McPherson, D. T. Expression and purification of Streptococcus pneumoniae hyaluronate lyase from Escherichia coli. *Protein Expres. Purif.* **1998,** 13, 83-89.
2. Hamberger, J. Characterization of Mammalian Hyaluronidase-2 Activity and Identification of Inhibitors of Streptococcal Hyaluronan Lyase. Doctoral thesis, University of Regensburg, Regensburg, 2012.
3. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976,** 72, 248-254.

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