Hetarylalkyl(aryl)cyanoguanidines as histamine H_4 receptor ligands: Synthesis, chiral separation, pharmacological characterization, structure-activity and -selectivity relationships

Dissertation

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„Was wir Realität nennen, ist oft nur die Wirklichkeit“

Kuno Klaboschke

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Igel, P.; Geyer, R.; Strasser, A.; Dove,S.; Seifert, R.; Buschauer, A.: *Synthesis and structure-activity relationships of cyanoguanidin-type histamine H_4 receptor agonists.* Jahrestagung der Deutschen Pharmazeutischen Gesellschaft e.V.; Jena, 28.09. – 01.10.2009

Elz, S.; Igel, P.; Geyer, R.; Kraus, A.; Kunze, M.; Birnkammer, T.; Buschauer, A.: *Cimetidine: A veterane histamine H_2R blocker for the characterisation of novel potent H_2R agonists.* Jahrestagung der Deutschen Pharmazeutischen Gesellschaft e.V.; Jena, 28.09. – 01.10.2009

Elz, S.; Igel, P.; Geyer, R.; Kraus, A.; Kunze, M.; Birnkammer, T.; Buschauer, A.: *Cimetidine: A veteran H_2-receptor antagonist for the characterisation of novel potent acylguanidine-type H_2-receptor agonists.* The European Histamine Research Society 38th Annual Meeting; Fulda, 13.05. – 16.05.2009

Igel, P.; Geyer, R.; Schneider, E.; Schnell, D.; Seifert, R.; Buschauer, A.: *2-Cyano-1-[4-(1H-imidazole-4-yl)butyl]-3-[(2-phenylthio)ethyl]-guanidine (UR-PI376): A potent and selective histamine H_4 receptor agonist.* The European Histamine Research Society 38th Annual Meeting; Fulda, 13.05. – 16.05.2009

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Contents

Chapter 1

Introduction

1.1 The biogenic amine histamine – a short overview

More than a century ago (1910), Dale and Laidlaw were the first to observe the biological effects of histamine (Figure 1.1).[1] Histamine was isolated from *Secale cornutum*,[2] and synthetic histamine (2-(1*H*-imidazol-4-yl)ethanamine) was available as well, as the chemical synthesis of histamine had been reported by Windaus and Vogt in 1907.[3] Histamine contains two basic functionalities, a primary aliphatic amine and imidazole, resulting in the monocation with different tautomers being the predominating form at physiological pH.[4]

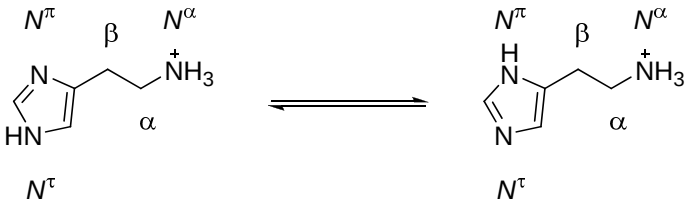

Figure 1.1 Tautomeric forms of the histamine monocation.

Histamine is formed in the body by decarboxylation of the amino acid L-histidine catalyzed by the enzymes L-histidine decarboxylase (HDC) or L-aromatic amino acid decarboxylase.[5, 6] Regarding the metabolic pathway, two major inactivation mechanisms can be distinguished. In one route, mainly in the periphery, the primary amine is oxidized in two steps catalyzed by the enzymes diamine oxidase and xanthine oxidase to give imidazole-4-acetic acid, which is further metabolized by ribosylation of the imidazole ring. The main, ubiquitously found metabolic pathway starts with the histamine *N*-methyltransferase (HNMT) catalyzed methylation of the imidazole nitrogen followed by subsequent oxidation of the primary amine to the corresponding carboxylic acid.[7] High concentrations of histamine are found in the lungs, the skin, connective tissues and gastrointestinal tract.[5] It is stored in mast cells,[8] basophils,[9] platelets,[9] enterochromaffin-like (ECL) cells of the stomach,[10] endothelial cells[11] and is also found in neurons.[12] Histamine is released during allergic reactions resulting in smooth muscle contraction, vasodilatation and an increase in vascular permeability.[13] Furthermore, histamine, released from ECL cells, is involved in the regulation of gastric acid secretion from parietal cells.[14] As neurotransmitter histamine plays a crucial role in sleep/waking cycle, learning and memory, anxiety, locomotion and neuroendocrine regulation.[15] All these effects are mediated by four histamine receptor (HR) subtypes, the H_1R, H_2R, H_3R and H_4R, all belonging to class A or rhodopsin-like G-protein coupled receptors (GPCR).[16, 17]

1.2 GPCR activation, ligand classification and signal transduction

The four histamine receptors belong to the class of G-protein coupled receptors, involving the interaction of the respective GPCR (R), a G-protein (G) and an agonist (A) when the receptor is activated. Different models have been developed to describe this interaction, with the cubic ternary model being the thermodynamically most complete one.[18-20] This model distinguishes between an active (R*) and an inactive (R) receptor state. These two states are in equilibrium and are allowed to isomerize independently from agonist binding. Such a spontaneous activation of the receptor in the absence of agonists is referred to as constitutive activity.[21] Both receptor states are able to bind G-proteins, but only the active receptor – G-protein complex (R*G) induces GDP/GTP exchange resulting in signal transduction.

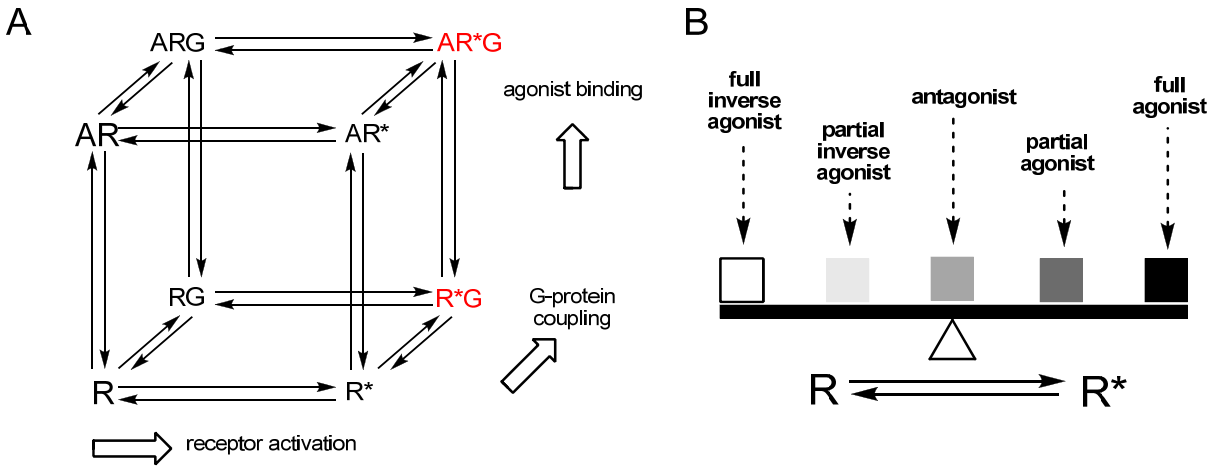

Figure 1.2 A) Two-state cubic ternary complex model of GPCR activation (R: inactive state of the receptor, R*: active state of the receptor, G: G-protein, A: agonist). Signaling complexes mediating GDP/GTP exchange are highlighted in red. **B)** Ligand classification according to their capability of shifting the equilibrium to either side of both states. According to Seifert et al.[21]

According to this model of GPCR activation, ligands are divided in five classes. Full agonists preferentially bind to the R^* state, stabilizing the active conformation and inducing a functional response. Inverse agonists particularly interact and stabilize the inactive conformation R of the receptor and reduce the basal G-protein activity. Partial agonists and partial inverse agonists are less effective in stabilizing the active or the inactive receptor conformation, respectively. Neutral antagonists show similar binding affinity for both conformations and do not change the equilibrium but inhibit the binding of other ligands.[22] However, the two state model of GPCR activation is only a rough simplification and cannot describe all experimental findings. The function of GPCRs is considered much more complex

in terms of ligand binding (allosteric, orthosteric), different conformational states, accessory protein interaction, phosphorylation, G-protein coupling, oligomerization and internalization.[23-26] Furthermore, there is growing evidence of several inactive and active receptor conformations,[27] suggesting that structurally different ligands stabilize distinct receptor conformations, resulting in different biological responses.[28] In conclusion, the presented model provides a working hypothesis for classical concepts of pharmacology but raises no claim to completeness.

After activation (agonist-dependent or agonist-free) the majority of GPCRs are able to transduce signals into cells through G-protein coupling. However, recent work has indicated that GPCRs also participate in various other protein-protein interactions which induce intracellular signals in conjunction with, or even independent of, G-protein activation. The G-protein dependent signal transduction is based on the ability of the activated receptors to interact with heterotrimeric G-proteins that transmit signals through the activation of intracellular effectors from the extracellular to the intracellular region.[29] These G-proteins consist of a Gα-subunit and a G$\beta\gamma$-complex (Figure 1.3).[30, 31] Upon binding of the G-protein to the active GPCR a conformational change of the G-protein is induced and GDP is released from its binding site on the Gα-subunit. Thereby, a ternary complex is formed that consists of the agonist, the receptor and the nucleotide-free G-protein. Binding of GTP to the Gα-subunit disrupts this complex and promotes the dissociation of the Gα-GTP-subunit and the G$\beta\gamma$-complex from the receptor and from each other. Both dissociated subunits interact with effector proteins like enzymes or ion channels, resulting in various cellular responses. Since the Gα-subunit is able to hydrolyze GTP to GDP and phosphate, the Gα–induced effector modulation is terminated after some time and the GDP-bound Gα-subunit can re-associate with G$\beta\gamma$ allowing the next G-protein cycle.[32] Moreover, the activity of G-proteins is receptor independently influenced by a family of proteins called the regulators of G-protein signaling (RGS).[33-35]

G-proteins are divided into four subfamilies termed G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$.[36] This classification is based on the structure and signaling pathway connected with the distinct Gα-subunit. All Gα and Gγ-subunits hold lipid anchors keeping the G-proteins in proximity to the membrane and the respective membrane proteins like GPCRs.[37] Members of the $G\alpha_s$ family activate adenylyl cyclases (AC 1–9) resulting in increased cellular turnover of the second messenger cAMP (3´-5´-cyclic adenosine monophosphate). In contrast, $G\alpha_i$ inhibits

the AC activity (AC 5 and AC 6). cAMP regulates various cellular effects such as activation of the protein kinase A (PKA) or the mitogen-activated protein kinase (MAPK) pathway, both modulating gene expression.[38] The $G\alpha_q$ family regulates phospholipase C activity (PLC_β) resulting in hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) into the second messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG).[39, 40] Finally, the $G\alpha_{12}$ proteins interact with Ras homology GEFs (Rho-GEFs) that regulate cytoskeletal assembly.[29, 41] Not only the Gα-subunit, but also the Gβγ-heterodimers are involved in signal transduction and regulate certain effectors like PLC_β and ion channels.[36]

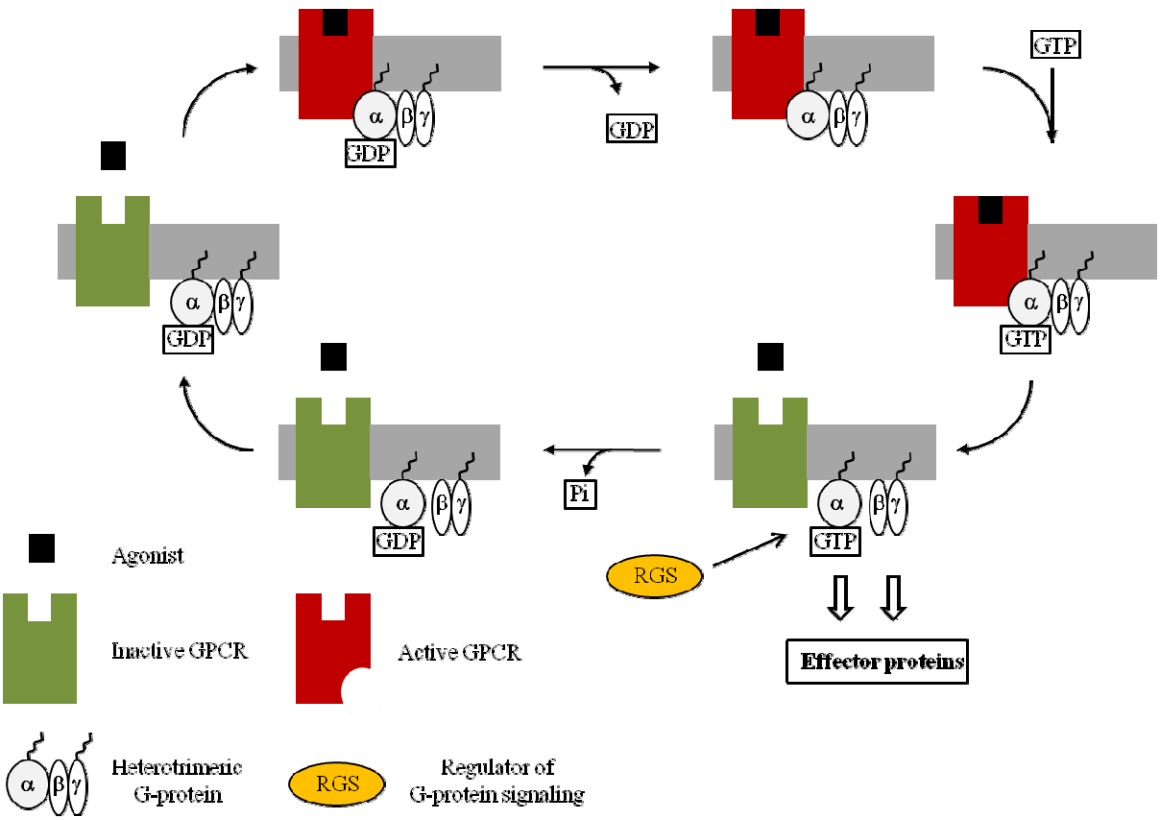

Figure 1.3 The G-protein cycle. Adapted from Igel.[42]

Furthermore, the insight into the signal transduction network rapidly increases and several G-protein independent signaling pathways have been reported.[38] For instance, GPCR dimerization, the interaction with receptor activity-modifying proteins (RAMPs) and the binding of various scaffolding proteins to GPCRs modulate GPCR signaling.[29] A hot topic is the recent discovery that β-arrestins function as alternative transducers of GPCR signals.[29, 43, 44] β-arrestins are mediators of GPCR desensitization (through internalization into clathrin-coated pits),[45, 46] and are therefore ubiquitously

expressed cellular regulatory proteins. Recently, β-arrestins were recognized as true adapter proteins that transduce signals to multiple effector pathways such as MAPKs, nuclear factor κB (Nf-κB) and phosphatidylinositol 3-kinase (PI_3K).[47] An updated model of signal transduction should comprise signaling by G-proteins and/or β-arrestins, as well as desensitization and internalization by β-arrestins. In a system with functional selectivity, signaling mainly proceeds through one of the possible pathways.[47] The term "functional selectivity" thereby describes the selective stimulation of some but not all possible signaling pathways[48] and was also described as "biased agonism"[49] or differential receptor-linked effector actions.[50, 51]

Apparently, depending on the ligand GPCR conformations change and different behavior and interactions are biased.[24] By now, several functionally selective ligands inducing G-protein- or arrestin-mediated processes are known for many GPCRs,[52] including μ-opioid,[53-55] serotonin 5-HT_{2A},[56-58] β-adrenergic,[59-62] angiotensin II AT_1,[63-65] dopamine D_2[66-69] and histamine H_1 receptors.[70] Such biased ligands might be useful pharmacological tools to investigate GPCR signaling and show potential for the fine-tuning of drug action.[71, 72]

1.3 Histamine receptors and their ligands

In the next paragraphs various molecular pharmacological aspects of the four histamine receptor subtypes, including the availability of selective agonists and antagonists, will be discussed.

In 1966 the term histamine H_1 receptor (H_1R) was introduced by Ash and Schild, who suggested the existence of a second HR subtype (non-H_1 receptor, H_2R) as not all effects provoked by histamine could be antagonized by classical antihistamines.[73] The H_2R was pharmacologically characterized in 1972.[74] Both receptors have been targets of blockbuster drugs for decades. H_1R antagonists ("antihistamines") are well established in the treatment of allergic disorders, H_2R antagonists have been used as antiulcer drugs ("H_2R blockers").[16] The identification of the presynaptic H_3R as a new receptor subtype by Arrang et al. in 1983[75, 76] gave rise to a new field of interest. The H_3R is now regarded as a regulatory system in the CNS and as potential target for new therapeutics.[77] The last member of the histamine receptor family was originally cloned as an orphan receptor, but based on its high sequence homology to the H_3R found to respond to histamine and confirmed to be a fourth histamine

receptor, the histamine H_4 receptor (H_4R).[78, 79] The average sequence homology between the HR subtypes is relatively low (20 %) except for H_3R and H_4R, which share overall sequence homology as high as 37 %.[17]

1.3.1 The histamine H_1 receptor

The histamine H_1 receptor (H_1R) is mainly expressed on smooth muscle cells, endothelial cells and in the CNS.[80] The H_1R predominantly couples to $G_{q/11}$-proteins leading to the activation of PLC and subsequent release of the second messengers IP_3 and DAG followed by the activation of PKC and the release of Ca^{2+}.[80, 81] Its (patho)physiological role includes vasodilatation (via release of nitric oxide from endothelial cells), bronchoconstriction, modulation of endothelial barrier function (responsible for hives), pain and itching due to insect stings.[82] Antagonists for the H_1R, the classical antihistamines, have been successfully used for the treatment of allergic diseases for decades.[5] The first generation H_1R antagonists like mepyramine (Pyrilamine®) or diphenhydramine (Dolestan®) are highly lipophilic compounds and are able to pass the blood brain barrier, resulting in sedative side effects. To challenge these drawbacks, more polar antagonists like cetirizine (Zyrtec®) or fexofenadine (Telfast®) were developed. These compounds belong to the non-sedating second generation of H_1R antagonists and are blockbuster drugs for the treatment of allergic disorders.[16] Mepyramine is still the most commonly used reference H_1R antagonist and radioligand ([^{3}H]mepyramine) for pharmacological studies.[83]

The only H_1R agonists used in therapy is betahistine (Aequamen®) which has some relevance in the treatment of Menière's disease.[84] Further highly potent and selective H_1R agonists have been developed as well, and are interesting pharmacological tools. Schunack and colleagues developed a series of H_1R selective histaprodifens.[85, 86] A further increase in H_1R agonist potency was obtained with suprahistaprodifen, a dimer of histaprodifen and histamine, which is currently one of the most potent H_1R agonists available.[87]

H_1R agonists

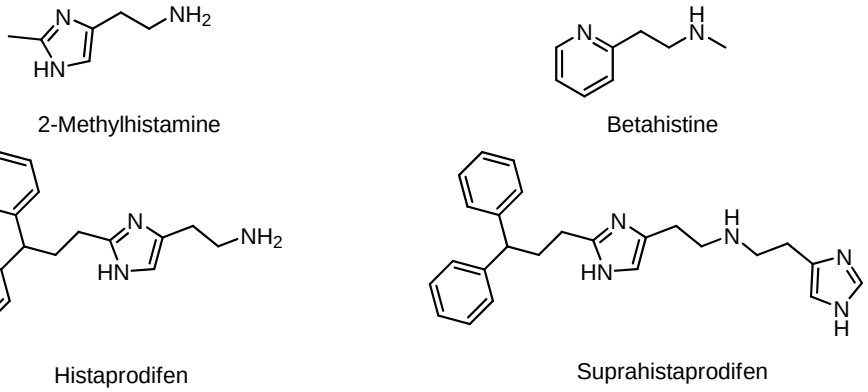

Figure 1.4 Structures of selected H_1R agonists.

H_1R antagonists

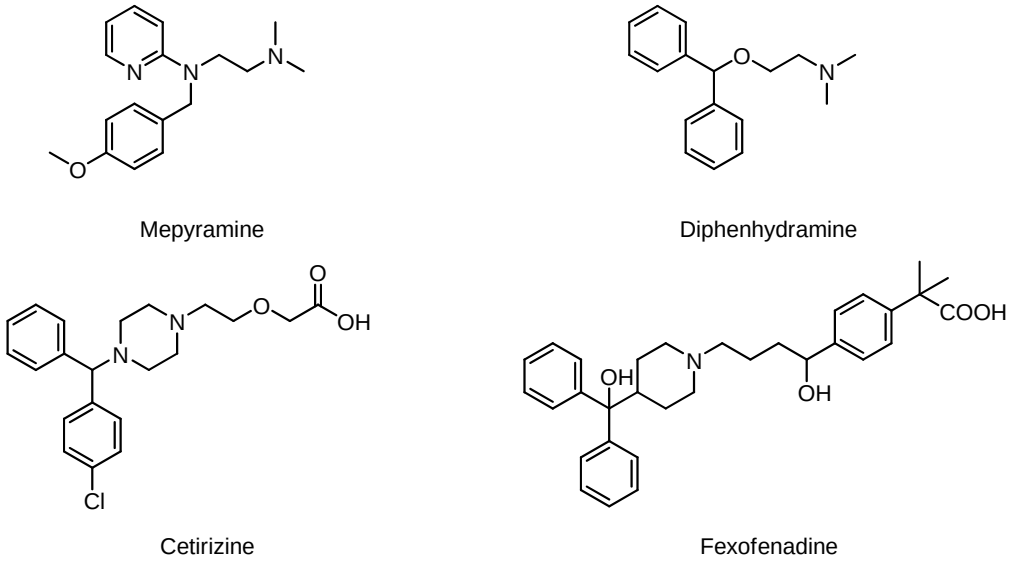

Figure 1.5 Structures of selected H_1R antagonists.

1.3.2 The histamine H_2 receptor

The histamine H_2 receptor (H_2R) is expressed in a variety of tissues including brain, gastric parietal cells and the heart.[16] It was pharmacologically characterized by Black et al. in 1972 using the first H_2R antagonist burimamide,[74] which was able to block the histamine mediated gastric acid secretion and positive chronotropic effect on the heart. In 1991, Gantz and co-workers were able to clone the canine and humane H_2Rs.[88, 89] The H_2R predominantly couples to $G\alpha_s$-proteins, resulting in an increase in intracellular cAMP levels and the activation of PKA.[88, 90, 91] Antagonists of the H_2R are used in the treatment of peptic ulcers.

The first H_2R antagonist burimamide, a moderately potent and selective ligand used to pharmacologically define the H_2R,[74] paved the way for several blockbuster drugs for the treatment of gastric and duodenal ulcer.[74] Cimetidine (Tagamet®), the first therapeutically used H_2R antagonist, and other H_2R blockers such as famotidine (Pepdul®) and ranitidine (Zantic®) were introduced on the market.[16, 92] Nowadays it has become apparent that gastric ulcers can be more effectively treated with proton-pump inhibitor such as omeprazole[93] and by eradication of *Helicobacter pylori*.[94] In addition to the therapeutically used drugs numerous structurally diverse highly active H_2R antagonists are known, for instance, tiotidine or aminopotentidine, which are used as pharmacological tools. A first step towards a selective H_2R agonist was the discovery of dimaprit, which was found to be almost as active as histamine at the H_2R, but hardly displays any H_1R agonism. Compared to the amine-type H_2R agonists, guanidine-type compounds such as impromidine[95, 96] or arpromidine[97-99] are much more potent but show insufficient oral bioavailability. This could be considerably improved according to a bioisosteric approach, by exchange of the guanidine by an acylguanidine moiety, resulting in N^G-acylated imidazolylpropylguanidines (e.g. UR-AK24, Figure 1.7).[100] Further improvement, concerning selectivity was achieved by the introduction of a 2-amino-4-methylthiazol-5-yl moiety as bioisostere of the imidazole ring.[101] Thus, N^G-acylated aminothiazolylpropylguanidines (e.g. UR-PG278, Figure 1.7) combine the high selectivity for the H_2R with improved pharmacokinetic properties, resulting in valuable pharmacological tools to evaluate the physiological role of H_2Rs.

H_2R antagonists

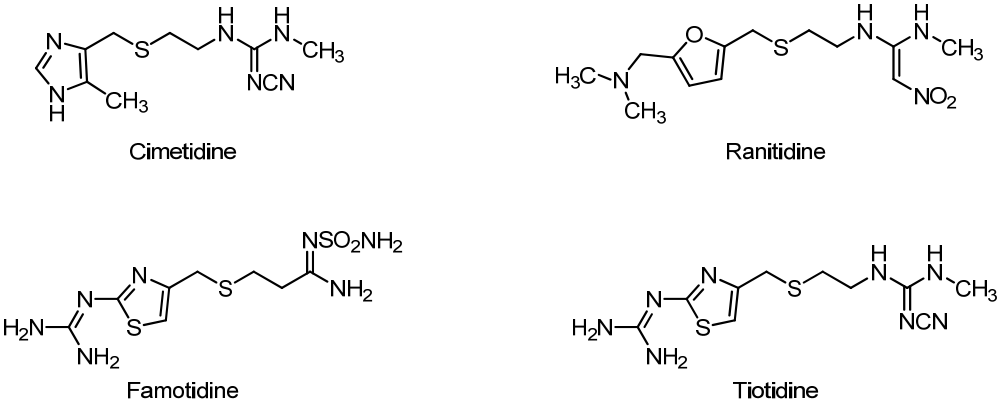

Figure 1.6 Structures of selected H_2R antagonists.

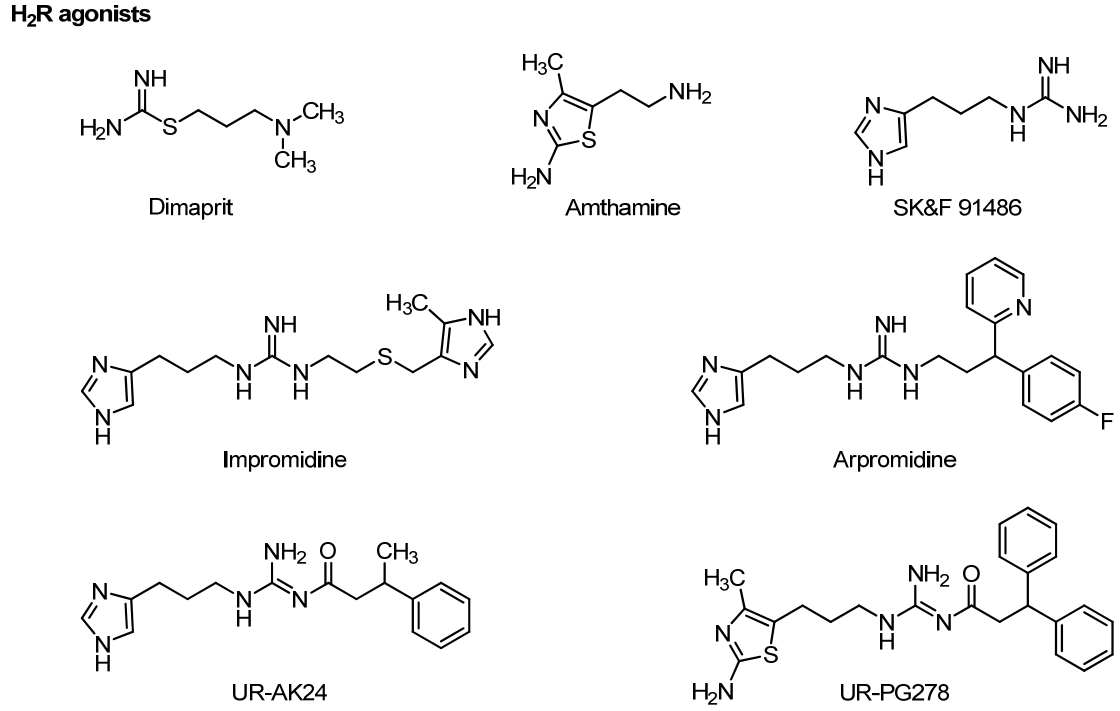

Figure 1.7 Structures of selected H_2R agonists.

1.3.3 The histamine H_3 receptor

The histamine H_3 receptor (H_3R) is mainly expressed in the CNS and to some extent in the peripheral nervous system.[16] The H_3R acts as a presynaptic autoreceptor inhibiting the synthesis and release of histamine from histaminergic neurons,[76] or as a heteroreceptor inhibiting the release of various important neurotransmitters like serotonin, noradrenalin, acetylcholine and dopamine.[16] The H_3R is suggested to be involved in various CNS functions, for instance, the regulation of locomotor activity, wakefulness and food intake, thermoregulation and memory.[102] In the periphery, H_3R activation has been shown to occur in the cardiovascular system, the gastrointestinal tract and the airways.[103-106] The H_3R signals via $G_{i/o}$ proteins, resulting in the inhibition of adenylyl cyclase after stimulation of the H_3R, a lower cellular cAMP level and modulation of CREB (cAMP responsive element-binding protein) dependent gene transcription. Moreover, the H_3R effectively activates MAPK, PI_3K, phospholipase A_2 (PLA_2), modulates intracellular calcium mobilization and inhibits the Na^+/H^+-exchanger.[107, 108] Research groups in both academia and pharmaceutical companies extensively investigated the H_3R as an attractive drug target.[108-110] Antagonists for the H_3R are promising agents in several therapeutic areas including dementia, Alzheimer`s disease, narcolepsy, insomnia, attention deficit hyperactivity disorder, schizophrenia as well as for

the treatment of myocardial ischemic arrhythmias, migraine and inflammatory and gastric acid related diseases.[111-115] The first potent H_3R antagonists, thioperamide and clobenpropit (Figure 1.8)[116, 117] were derived from the structure of histamine and have an imidazole ring in common. To improve the drug-like properties and to prevent potential drug-drug interactions, several pharmaceutical companies developed non-imidazole H_3R antagonists such as JNJ10181457.[109, 118]

H_3R antagonists

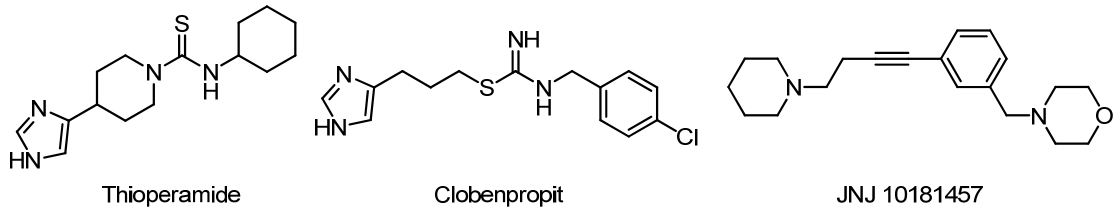

Figure 1.8 Structures of selected H_3R antagonists.

The natural ligand, histamine, is a highly potent H_3R agonist. Methylation at the α-carbon atom of histamine provided (*R*)-α-methylhistamine, a potent H_3R agonist with a strongly increased selectivity over the H_1R and the H_2R.[119] Immepip is another example of a potent H_3 agonist that is effective *in vitro* and *in vivo*. In Immepip the amine function of histamine was incorporated in a ring structure (Figure 1.9). After identification of the fourth histamine receptor, numerous H_3R ligands turned out to be also active at the H_4R. Taking that into consideration, new potent and selective H_3R agonists like methimepip (pEC_{50} = 9.5; 10,000-fold selectivity over the H_4R) have been developed.[120]

H_3R agonists

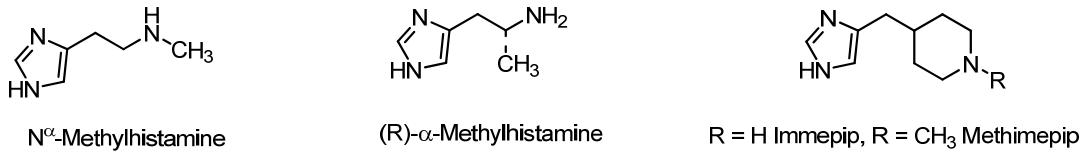

Figure 1.9 Structures of selected H_3R agonists.

1.3.4 The histamine H_4 receptor

In 1994 Raible et al. reported on contradictory observations concerning the histamine induced calcium mobilization in human eosinophils. H_1R and H_2R antagonists were not able to block the response, whereas the H_3R antagonist thioperamide turned out to be an

antagonist. However, the potent H_3R agonist (*R*)-α-methylhistamine was less potent than histamine in inducing calcium mobilization, which disagrees with an H_3R mediated effect. Therefore, Raible and co-workers suggested a novel HR subtype on human eosinophils.[121, 122] Finally, the human H_4R was identified and cloned in 2000 and 2001, independently by several research groups due to its rather high sequence homology with the hH_3R (about 40 % overall sequence identity and about 58 % sequence identity within the transmembrane domains).[78, 79, 123-127] The histamine H_4R is expressed in various cells of the immune system like mast cells, basophils, eosinophils, T-lymphocytes and dendritic cells, suggesting a role in inflammatory, autoimmune and allergic disorders.[128 129, 130] Recently, the hH_4R was reported to be also expressed in different areas of the CNS.[131] The H_4R couples to pertussis toxin sensitive $G_{i/o}$-proteins, resulting in inhibition of the adenylyl cyclase and activation of MAPKs.[79, 125] The biological role of the H_4R is still not fully understood, but the activation of H_4Rs has been shown to induce several responses closely associated to immune cells, like chemotaxis, chemokine production and Ca^{2+}-mobilization in mast cells, monocytes and eosinophils.[130] Results of pharmacological *in vitro* and *in vivo* studies and the potential therapeutic value of the H_4R as a drug target are discussed in section 1.4.

The successful cloning and expression of the H_4R stimulated the search for selective agonists and antagonists. A lot of interest is focused on H_4R antagonists and their potential use in the treatment of inflammatory conditions and itch.[132] Moreover, since the suggested role of the H_4R in immunological responses overlaps with the function of the H_1R, ligands combining H_1- and H_4-receptor antagonistic properties are considered beneficial for the treatment of inflammatory diseases.[133] In search for H_4R-selective compounds, at an early stage, agonists and antagonists of various GPCRs were pharmacologically studied, resulting in the identification of numerous H_4R ligands from different structural classes (Figure 1.10 and Figure 1.11).[130] The H_3R inverse agonist thioperamide was identified as potent H_4R inverse agonist and is frequently used as a reference compound. Meanwhile, highly selective H_4R antagonists such as the indole-2-carboxamide JNJ-7777120,[134] various quinazolines[135] and 2-aminopyrimidines[136-138] have been developed (Figure 1.11). In particular, JNJ-7777120 serves as valuable pharmacological tool and has already been employed in several animal models to study the biological function of the H_4R.[139-141] The first identified H_4R agonists were imidazole-containing H_2R and H_3R ligands such as clobenpropit, imetit or the isothiourea dimaprit, which proved to exert activity also at the H_4R.[142] Moreover, even the antipsychotic

drug clozapine was active as an agonist at the H_4R.[79] Since selective agonists represent valuable pharmacological tools for further investigations on the biological role of the H_4R, numerous agonists have been developed over the last decade (Figure 1.10).

The first selective H_4R agonist reported in literature was OUP-16, a chiral tetrahydrofuran analog which was derived from imifuramine.[143] Thereafter, 5-methylhistamine (also referred to as 4-methylhistamine), which was originally considered as a selective H_2R agonist,[144] turned out to be more potent at the H_4R.[142] In addition, N^G-acylated imidazolylpropylguanidines such as UR-AK24 – originally designed as H_2R agonists – were found to be highly potent and almost full H_4R agonists. The residual agonistic activities of these compounds at the H_2R and H_3R were reduced, when the acylguanidine moiety was replaced by a non-basic cyanoguanidine group. Further structural optimization provided highly potent and selective cyanoguanidine-type H_4R agonists such as UR-PI376.[42, 130, 145] Structural variations of JNJ-7777120 resulted in oxime analogues which are almost full agonists of comparable potency at both rodent and human H_4Rs.[146] Furthermore, Johnson & Johnson developed 2-arylbenzimidazoles as H_4R antagonists and thereby identified several derivatives with full agonistic activity at the hH_4R. Substitution of the 2-arylbenzimidazole with a histamine substructure yielded one of the most potent hH_4R agonists described so far, showing sub-nanomolar hH_4R affinity (Figure 1.10).[147] For radioligand binding studies [^{3}H]histamine and [^{3}H]JNJ-7777120 as well the iodinated H_3R ligand [^{125}I]iodophenpropit[142] and the recently developed high affinity $hH_{3/4}R$ radioligand [^{3}H]UR-PI294 have been used.[148]

Up to now, a common hH_4R-agonistic pharmacophore can be based only on a two-point model representing key interactions with $Asp^{3.32}$ and $Glu^{5.46}$.[145, 149, 150] Therefore, new lead structures are difficult to predict and further data on structure-activity and selectivity relationships are necessary. Another key issue in H_4R ligand development is the selectivity-profile of the known compounds. Even many 'selective' H_4R agonists also activate other histamine receptor subtypes, especially the H_3R. Moreover, the investigation of the (patho)physiological role of the histamine H_4 receptor in animal models is hampered by species-dependent discrepancies regarding potencies, receptor selectivities and even by opposite qualities of action of the available pharmacological tools.[128, 148] Due to these substantial pharmacological differences between various H_4R species orthologs the development of further H_4R agonists becomes even more abundant. In addition to the use in molecular pharmacological studies, it remains unclear whether H_4R agonists or antagonists

have beneficial effects for instance in bronchial asthma.[151] Despite extensive studies, discrepancies in the pharmacological profile of the H_4R already described in the first review-type article by Hough et al.[17] in 2001 have not been satisfactorily explained and a therapeutic value of H_4R agonists cannot be ruled out.[133] Thus, further specific agonists showing improved pharmacological properties are required to explore ligand receptor interactions in more detail, to obtain deeper insight into the molecular determinants of receptor subtype and species selectivity and as pharmacological tools for *in vitro* and *in vivo* studies.[130]

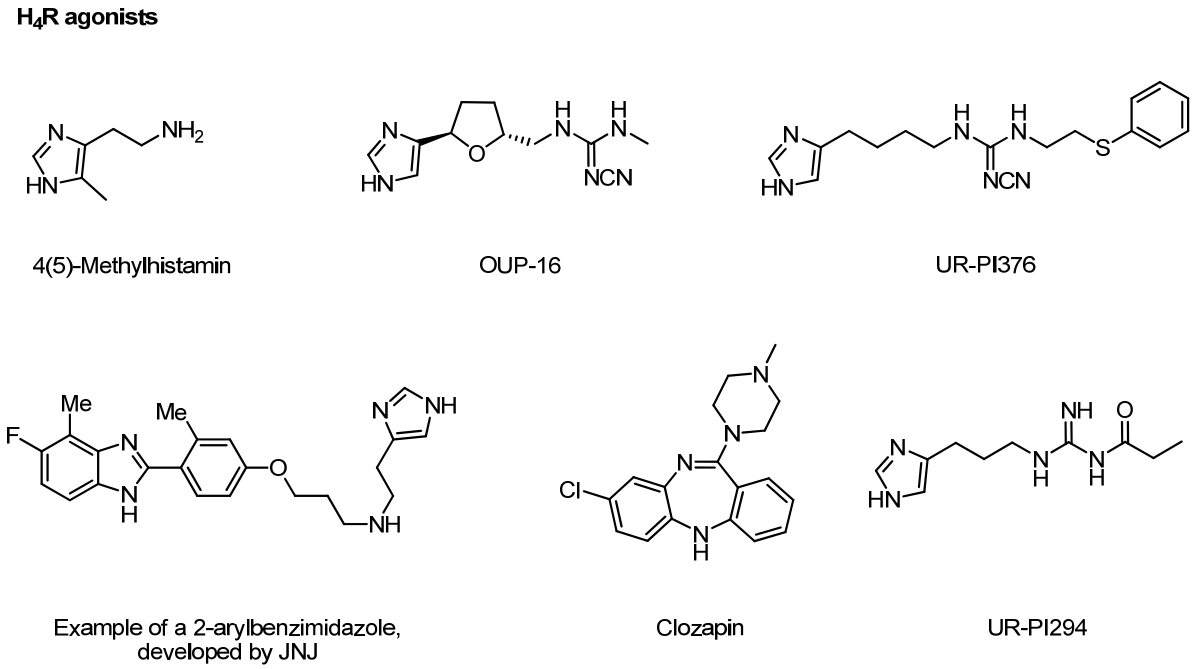

Figure 1.10 Structures of selected H_4R agonists.

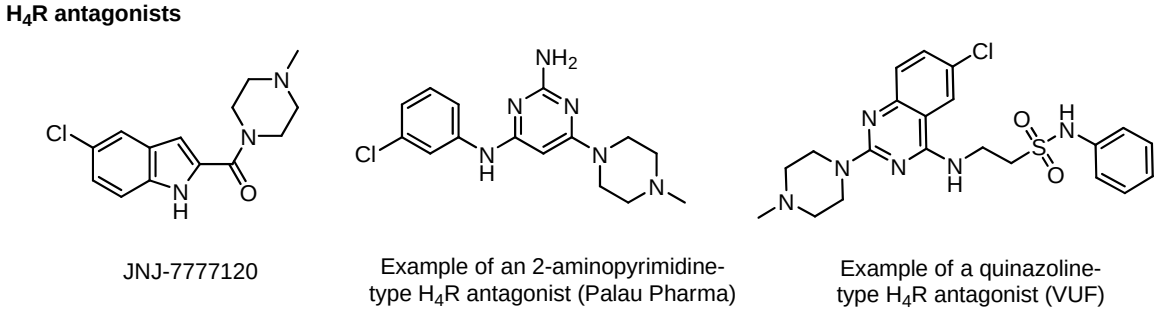

Figure 1.11 Structures of selected H_4R antagonists.

1.4 Future antihistamines – potential therapeutic indications for H_4 receptor ligands

The clinical development of potential therapeutic agents targeting the H_4R is not as advanced as for the long-known histamine receptors. However, a lot of preclinical work and ligand development was carried out. The results suggest an important role of the H_4R in immunological responses and inflammatory diseases. The following section gives a short overview about the potential use of compounds targeting the H_4R in the treatment of various pathophysiological conditions.

Asthma[152]

Histamine is a known airway constrictor and high histamine concentrations have been found in airways and plasma of asthma patients after antigen challenge[153] and many cell types that play a major role in asthma, like eosinophils, T cells, mast cells and smooth muscle cells express histamine receptors. Since, H_1R and H_2R antagonists have not been effective in treating asthma, the identification of the H_4R offered new perspectives of histamine and its receptors in asthma.[13, 154-156] Mast cells for instance are a main source of histamine in the lung and it has been shown that histamine enhances mast cell chemotaxis via the H_4R.[157] Eosinophil chemotaxis was also found to be mediated by the H_4R.[158] H_4R-deficient mice and mice treated with H_4R antagonists exhibited decreases in Th2 responses, including decreases in interleukin levels and accordingly decreased allergic bronchial inflammation.[139] In contrast to H_1R antagonists, H_4R antagonists were effective during both, the sensitization and the allergen challenge phase of a mouse asthma model.[139] Surprisingly, the H_4R agonist 4-methylhistamine reduced inflammation and airway hyperreactivity.[159] Furthermore, it is suggested that the H_1R and the H_4R may interact and synergistic effects might be used as the H_4R may account for effects of histamine that are not blocked by H_1R antagonists in asthmatic responses.[133] In addition, the H_4R might mediate various other effects that contribute to asthma.[160-163] However, further exploration of the role of the H_4R in asthmatic responses is needed and might provide useful additional therapeutic options.

Atopic dermatitis[164]

Histamine is a mediator of itch,[165] and an increase in histamine levels has been observed in the skin and plasma of patients with pruritic conditions such as atopic dermatitis or acute and chronic urticaria.[13, 166-168] However, pruritus in patients with atopic dermatitis is often not affected by H_1R or H_2R antagonists.[169] This observation led to the assumption that histamine is binding to other histamine receptors, presumably the H_4R. The H_4R was shown to be expressed on various cells that may play a role in atopic dermatitis, for instance Th2 cells,[160] human dermal fibroblasts and inflammatory dendritic epidermal cells.[170, 171] Moreover, the H_4R agonist clobenpropit enhanced the chemotaxis of dendritic cells through skin in an *in vitro* assay and this effect could be blocked by JNJ-7777120.[172] In addition, H_4R antagonists significantly inhibited inflammation in a Th2-cell-mediated mouse skin inflammation model that mimics several features of atopic dermatitis.[173] H_4R knockout mice or mice pretreated with the H_4R antagonist JNJ-7777120 showed significantly less scratching responses after challenge with histamine or H_4R agonists.[146, 174, 175] In summary, *in vitro* studies on human immune cells and *in vivo* data from murine models support the immunomodulatory role of the H_4R on various cell types relevant in atopic dermatitis. Hence, H_4R antagonists may have therapeutic utility for treating pruritus[176] in atopic dermatitis and other indications.

Allergic rhinitis[177]

A huge number of people suffer from seasonal allergic rhinitis. Traditionally, classical antihistamines, the H_1R antagonists, are used to treat allergic rhinitis. Investigations concerning the H_4R as potential drug target gave hints to a possible role of the H_4R in this disease. For instance, significantly increased levels of both the H_1R and H_4R in human nasal polyp tissue taken from patients with chronic rhinosinusitis were reported.[178] In a mouse allergic rhinitis model, JNJ-7777120 caused a dose-dependent inhibition of nasal symptoms.[179] Meanwhile, Palau Pharma started clinical studies, targeting asthma and allergic rhinitis with its most advanced H_4R antagonist, UR-63325 (K_i = 15 nM, undisclosed structure). Phase I studies demonstrated that the candidate was well tolerated, with no specific adverse events identified.[180]

Autoimmune diseases[181, 182]

Histamine is a mediator of inflammatory responses in autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis and histamine levels positively correlate with disease severity.[182-187] Although histamine is involved in autoimmune diseases, H_1R and H_2R antagonists have shown little effect.[188] Therefore, the H_4R provides a promising new target for an effective treatment of these diseases. H_4R expression was observed in synovial tissues from rheumatoid arthritis and osteoarthritis patients and has been suggested to reflect the severity and duration of rheumatoid arthritis.[189] Furthermore, two selective H_4R antagonists reduced the pathological symptoms in an acute colitis model in rats, another evidence for a role of the H_4R in colitis.[190]

Pain[188]

The H_4R is also suggested to play a role in pain modulation. H_4R expression in the CNS was detected, including the brain, spinal cord and dorsal root ganglia.[191] Moreover, H_4R antagonists have been shown to be effective in several models of pain.[136, 137, 141, 192] The H_4R antagonist JNJ-7777120 was highly efficacious in an acute carrageenan-induced inflammatory pain model, in a model of osteoarthritis and in a model of post-operative pain.[192] In conclusion, several H_4R antagonists have shown activity in different pain models supporting an H_4R specific effect and making the H_4R a promising target for the treatment of pain.

Cancer[193]

Notably, most malignant cell lines contain high concentrations of histamine and diverse biological responses related with cell proliferation might be regulated by histamine.[193-198] Recent studies indicate that the H_4R is expressed in cells exhibiting an important role in histamine-mediated biological processes such as cell proliferation, senescence or apoptosis.[199] In addition, the H_4R was detected in colorectal cancer and in human colon cancer cells in which histamine exerts a proproliferative and a proangiogenic effect via activation of the H_2R and the H_4R.[200, 201] The role of the H_4R in cell proliferation is far from being fully understood and seems to be cancer type dependent.[193] Nevertheless, the H_4R is considered a potential molecular target of new anticancer drugs.

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Chapter 2

Scope and objectives

N^G-acylated imidazolylpropylguanidines are among the most potent histamine H_2 receptor agonists reported in literature.[1] Interestingly, more detailed pharmacological investigations of the acylguanidine-type H_2R agonists revealed a lack of selectivity: these compounds were also highly active at the H_3R and the H_4R. This problem was solved by a bioisosteric approach, resulting in the development of highly potent and selective H_2R agonists bearing an aminothiazole ring instead of the bioisosteric imidazole.[2] Aiming at analogues with selectivity for the other HR subtypes, especially the H_4R, extensive studies led to cyanoguanidine-type H_4R ligands. Replacing the basic acylguanidine group with a non-basic cyanoguanidine moiety provided highly potent and selective H_4R agonists. Highest potency resided in imidazolylalkylcyanoguanidines such as UR-PI376, with a tetramethylene linker connecting imidazole and cyanoguanidine moiety.[3]

Hashimoto et al.[4] developed conformationally restricted H_4R ligands, using tetrahydrofurane to connect imidazole and amine or cyanoguanidine, respectively. OUP-16[4] was the most potent and selective agonist in this series with an EC_{50} of 78 nM and a 40-fold selectivity over the H_3R. Furthermore, Kitbunnadaj et al.[5] and Watanabe et al.[6] demonstrated by introducing phenylene, cyclohexylene or cyclopropylene spacers that stereochemical requirements of selective hH_3R and hH_4R ligands should be considered for the design of new H_4R agonists.

The aim of this thesis was the design, synthesis and pharmacological characterization of novel cyanoguanidine-type H_4R ligands and related compounds to elucidate structure-activity and structure-selectivity relationships at the different histamine receptor subtypes. Although the recently discovered H_4R is considered a promising drug target for the treatment of inflammatory and immunological diseases, its biological role is still far from being completely understood.[7-14] Therefore, a major goal was the development of potent and selective H_4R agonists as pharmacological tools.

In the first part of this doctoral project the flexible tetramethylene linker between imidazole and cyanoguanidine moiety in UR-PI376 should be replaced by conformationally restricted carbocycles. In a first approach, aiming at maximum conformational constraints, a phenylene linker should be introduced. The effect of this conformational restriction on potency and subtype-selectivity should be evaluated. Subsequently, with respect to stepwise decreased conformational constraints, a replacement of the phenyl ring with more flexible saturated

carbocycles should be considered. For instances, based on a combination of the structural feature of UR-PI376[3] and OUP-16,[4] a series of differently substituted racemates should be synthesized and characterized. The most promising racemates should be separated and the resulting stereoisomers should be investigated in more detail. This design strategy aimed at information about the optimal balance between rigidification and remaining flexibility and the stereochemical requirements of cyanoguanidine-type H_4R agonists.

The second part of the doctoral project should focus on the imidazole moiety in UR-PI376. By analogy with a strategy, which was successful in the H_2R agonist field,[2] a bioisosteric approach should be applied. Aiming at a higher subtype-selectivity as well as improved drug-like properties, a series of hetarylalkylamines and –cyanoguanidines should be synthesized and characterized at the HR subtypes. The imidazole ring should be replaced by various heterocycles with the goal to identify a potential bioisostere of imidazole in cyanoguanidine-type H_4R ligands.

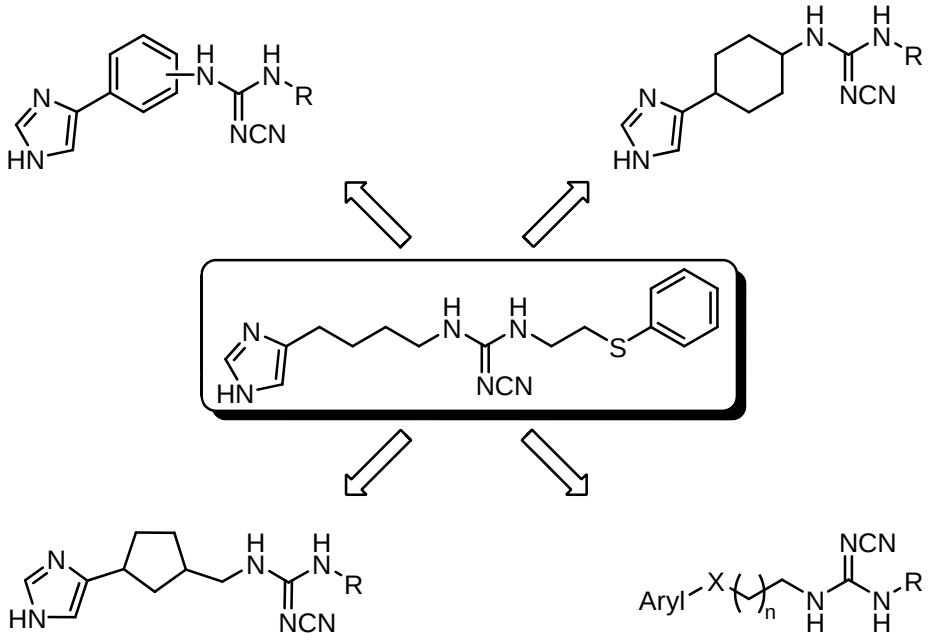

Figure 2.1 Overview about the intended modifications of the cyanoguanidine-type H_4R agonist UR-PI376.

To further explore the biological role of the histamine H_4 receptor there is a demand for fluorescence and radiolabeled ligands as pharmacological tools. Up to now, only a few radioligands and no fluorescent compound with affinity for the H_4R have been reported. The major drawback of the used labeled compounds, for instance, [^{3}H]-histamine and [^{3}H]-UR-PI294,[15] is the lack of receptor subtype selectivity. Therefore, the third part of the doctoral project was aiming at fluorescent ligands, starting from high affinity 2-arylbenzimidazoles, which were recently reported by Johnson and Johnson.[16, 17] To evaluate the effect of the

introduction of fluorescent dyes, with respect to maintained potency and selectivity, structural diverse dyes should be coupled to a benzimidazole building block. The resulting compounds should be investigated at the HR subtypes in radioligand binding studies.
In the final part, various bioanalytical investigations such as determination of plasma protein binding and hemolytic activity should be performed to characterize selected H_4R ligands in more detail with respect to further usage *in vitro* and *in vivo*.

In summary, the aim of this thesis was to design, synthesize and pharmacologically characterize new cyanoguanidine-type H_4R agonists to obtain more information about structure-activity and structure-selectivity relationships and to develop H_4R subtype selective compounds as pharmacological tools.

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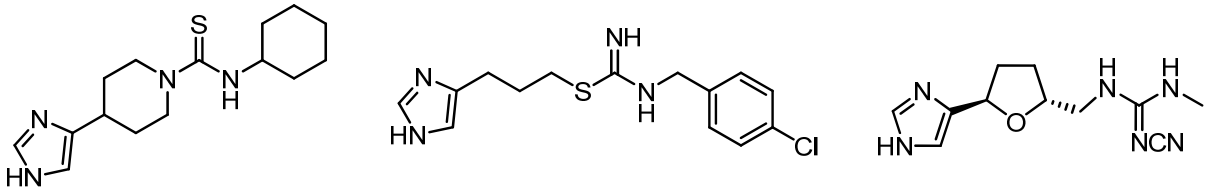
Chapter 3

Imidazolylphenylcyanoguanidines – synthesis and pharmacological characterization at the histamine H_3 and H_4 receptor

3.1 Introduction

The H_4R shares highest sequence similarity with the histamine H_3 receptor. Therefore, it is not surprising that the H_4R is targeted by various H_3R ligands.[1-4] This especially holds for compounds incorporating an imidazole moiety, for instance, the H_3R and H_4R inverse agonist thioperamide (**3.1**) or the H_3R antagonist and H_4R agonist clobenpropit (**3.2**) (Figure 3.1).[5] Even many of the "selective" H_4R ligands including[6] OUP-16 (**3.3**),[7] VUF-8430[8] or JNJ-7777120[9] still exhibit noteworthy affinity for the H_3R.



Thioperamide (**3.1**)
hH_4R K_i = 125 nM
hH_3R K_i = 50 nM

Clobenpropit (**3.2**)
hH_4R K_i = 13 nM
hH_3R K_i = 7.9 nM

OUP-16 (**3.3**)
hH_4R K_i = 125 nM
hH_3R K_i = 2,200 nM

Figure 3.1 Imidazole containing H_4R ligands described in literature[5] and their affinities for the H_4R and the H_3R.

Aiming at H_4R agonists with increased selectivity for the H_4R over the other HR subtypes the presented study was focused on the linker between imidazole ring and cyanoguanidine moiety in H_4R agonists such as UR-PI376 (**3.4**) (Figure 3.2).[10] In a previous study, hH_4R agonists were identified among N^G-acylated imidazolylpropylguanidines which were initially designed as H_2 receptor agonists.[11, 12] Structural optimization resulted in highly potent agonists with selectivity for the hH_4R over the hH_1R and the hH_2R but still lacking selectivity compared to the hH_3R.[10, 13, 14] Replacement of the basic acylguanidine group with a cyanoguanidine moiety, which is uncharged at physiological pH, turned out to be key in terms of H_4R agonism and selectivity. Highest potency resided in imidazolylalkylcyano-guanidines with a tetramethylene linker, connecting the imidazole and the cyanoguanidine moiety. UR-PI376 (**3.4**) was the most potent and selective agonist with an EC_{50} of 34 nM (intrinsic activity (α) = 0.93) displaying a more than 25-fold selectivity over the hH_3R and negligible activities at the hH_1R and hH_2R.[10] As demonstrated by Hashimoto et al.[7] and Kitbunnadaj et al.,[15] the stereochemical requirements of selective hH_3R and hH_4R ligands should be considered for the design of new H_4R ligands. Therefore, aiming at elucidating the structure-activity and structure-selectivity relationships of cyanoguanidines derived from UR-PI376, the replacement of the flexible tetramethylene chain by conformationally constrained spacers was explored (Figure 3.3). Based on a previously suggested model of UR-

PI376[10] binding to the hH_4R three small series of cyanoguanidines were constructed by introducing a phenyl ring as in the imidazolylphenylamine VUF-5801 (**3.5**) (Figure 3.2) which was reported to have some hH_4R affinity (pK_i = 5.8).[15]

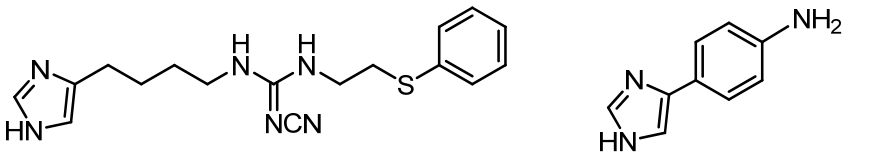


Figure 3.2 Structures of the cyanoguanidine UR-PI376 and the imidazolylphenylamine VUF-5801.

As a first approach, a 1,3- and a 1,4-substituted phenylene spacer was introduced to obtain information concerning the effect of rigidization of the linker and the influence of the substitution pattern at the phenyl ring. Furthermore, to retain the distance of four carbon atoms between imidazole and cyanoguanidine moiety, a series of 1,3-substituted derivatives with an additional exocyclic methylene group was synthesized. In each series five compounds with different N^G-substituents were investigated. The residues at the cyanoguanidine ("eastern part" of the molecule) were selected with respect to comparison of the structure-activity relationships with those of the flexible compounds.

This chapter describes the synthesis of imidazolylphenylcyanoguanidines and the functional pharmacological evaluation of these compounds in [^{35}S]GTPγS assays at the H_3R and the H_4R. The effect of the introduction of a conformationally restricted phenylene spacer and the different substitution pattern will be discussed.

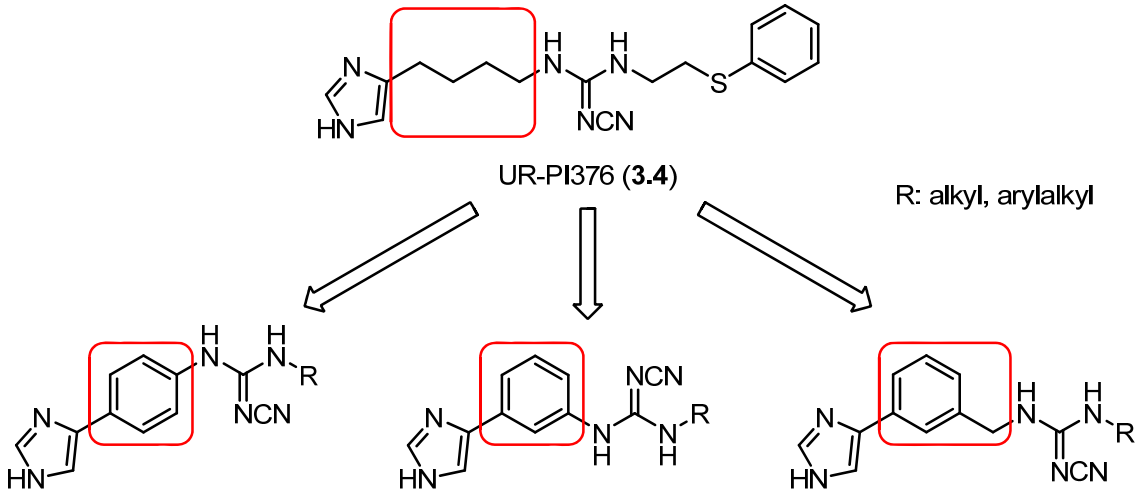
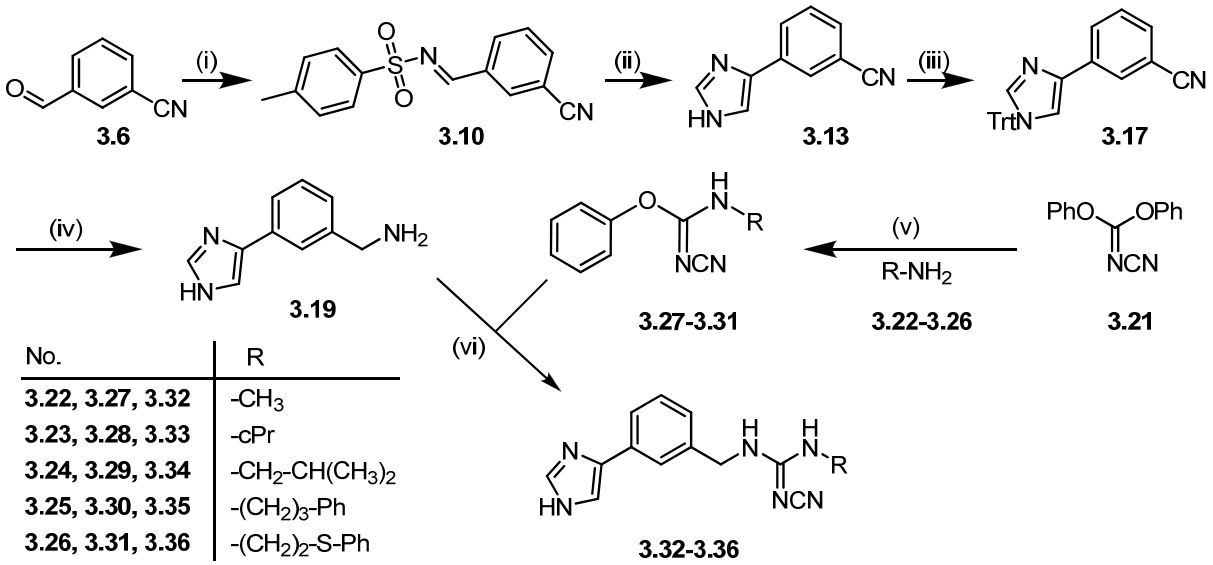


Figure 3.3 Replacement of the flexible tetramethylene linker in UR-PI376 with a conformationally restricted phenylene spacer.

3.2 Chemistry

The imidazolylphenylcyanoguanidines **3.32-3.46** were synthesized in five steps (Scheme 3.1 and Scheme 3.2), starting from commercially available benzaldehydes (**3.6-3.8**). Condensation of **3.6-3.8** with *N*-tosylamide (**3.9**) using a *Dean-Stark* trap gave the aldimines **3.10-3.12**.[16] According to ten Have et al.[17] the imidazole ring in **3.13-3.15** was introduced by cycloaddition of tosylmethyl isocyanide (TosMIC, **3.16**)[18] to the corresponding aldimines **3.10-3.12**. For the purpose of easier work-up, the imidazole ring in **3.13** was trityl protected yielding **3.18**. Subsequent reduction of the nitrile with $LiAlH_4$, detritylation under acidic conditions and liberation of the free base using an ion exchanger provided the amine **3.19**. Hydrogenation of the nitro group in **3.14** and **3.15** over palladium catalyst gave the amines **3.5** and **3.20**. Compound **3.19** was then transformed to the cyanoguanidines **3.32-3.36** by analogy with a previously described synthetic route.[19] Aminolysis of diphenyl cyano-carbonimidate (**3.21**)[20, 21] with primary amines **3.22-3.26** at ambient temperature gave the isourea intermediates **3.27-3.31** which crystallized from diethyl ether.[10] Conversion of the isoureas to the cyanoguanidines **3.32-3.36** was performed by heating in a microwave oven with **3.19** in acetonitrile (Scheme 3.1).

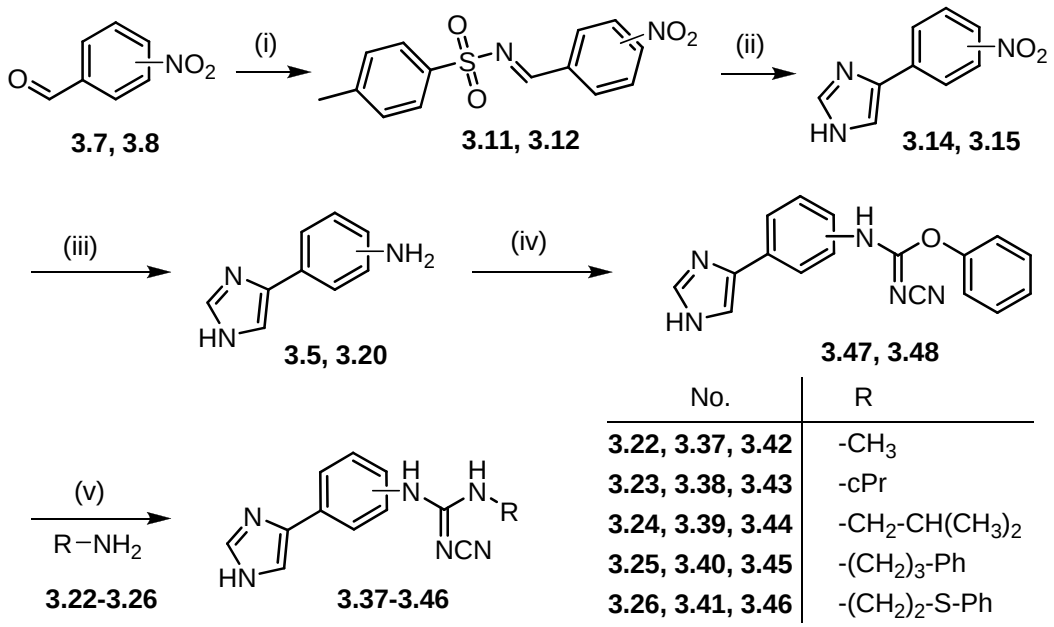


No.	R
3.22, 3.27, 3.32	$-CH_3$
3.23, 3.28, 3.33	-cPr
3.24, 3.29, 3.34	$-CH_2-CH(CH_3)_2$
3.25, 3.30, 3.35	$-(CH_2)_3-Ph$
3.26, 3.31, 3.36	$-(CH_2)_2-S-Ph$

Scheme 3.1 Synthesis of the cyanoguanidines **3.32-3.36**. Reagents and conditions: (i) 4-methylbenzene-sulfonamide (**3.9**) (1 eq), toluene, 24 h, reflux, 80 %; (ii) TosMIC (**3.16**) (1.1 eq), K_2CO_3 (3 eq), EtOH/DME 2:1, 2 h, reflux, 45 %; (iii) TrtCl (1.2 eq), NEt_3 (2 eq), DMF/DCM, 24 h, rt, 96 %; (iv) a) $LiAlH_4$ (2 eq), THF/diethyl ether, 0 °C - reflux, 3 h, 89 %; b) HCl, ion exchanger, 81 %; (v) 2-propanol, 1 h, rt, 66-84 %; (vi) MeCN, microwave 140 °C, 15 min, 70-87 %.

In case of the synthesis of **3.37-3.46** the coupling of the phenylamines (**3.5**, **3.20**) to the pertinent isourea intermediates (**3.27**-**3.31**) failed due to the low nucleophilicity of aniline

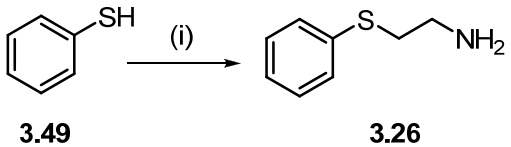
derivatives. To circumvent this problem the order of the coupling steps was reverted: **3.21** was treated first with building block **3.5** or **3.20** to yield **3.47** or **3.48**, respectively, and afterwards with an aliphatic amine (**3.22**-**3.26**) to yield the cyanoguanidines **3.37**-**3.46** (Scheme 3.2).



No.	R
3.22, 3.37, 3.42	-CH_3
3.23, 3.38, 3.43	-cPr
3.24, 3.39, 3.44	-CH_2-$CH(CH_3)_2$
3.25, 3.40, 3.45	-$(CH_2)_3$-Ph
3.26, 3.41, 3.46	-$(CH_2)_2$-S-Ph

Scheme 3.2 Synthesis of the cyanoguanidines **3.37-3.46.** Reagents and conditions: (i) 4-methylbenzene-sulfonamide (**3.9**) (1 eq), toluene, 24 h, reflux, 90 %; (ii) TosMIC (**3.16**) (1.1 eq), K_2CO_3 (3 eq), EtOH/DME 2:1, 2 h, reflux, 56 %; (iii) H_2 5 bar, Pd/C 10 %, MeOH, 24 h, rt, 93 %; (iv) diphenyl cyanocarbonimidate (**3.21**) (1 eq), 2-propanol, 36 h, rt, 76 %; (v) MeCN, microwave 140 °C, 15 min, 55-94 %.

The primary amine **3.26** (2-(phenylthio)ethanamine hydrochloride) was obtained by nucleophilic substitution from bromoethylamine and thiophenol (**3.49**).[22] The amines **3.22-3.25** were commercially available.



Scheme 3.3 Synthesis of the amine precursor **3.26**. Reagents and conditions: (i) bromoethylamine · HBr (1 eq), K^{t}BuO (2 eq), MeOH, 48 h, rt, 82 %.

3.3 Pharmacological results and discussion

3.3.1 Potencies and efficacies of the synthesized compounds at the hH_4R and the hH_3R in the [^{35}S]GTPγS binding assay

The synthesized cyanoguanidines were investigated for agonism and antagonism at hH_4R and hH_3R subtypes in [^{35}S]GTPγS binding assays using membrane preparations of Sf9 insect cells co-expressing the hH_4R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or co-expressing the hH_3R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$. Additionally, for reasons of comparison the amine precursors (**3.5, 3.19, 3.20**) and UR-PI376 (**3.4**) were characterized.

In the following agonistic potencies are expressed as EC_{50} values. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine. Compounds identified to be inactive as agonists (α <0.1 or negative values, respectively, determined in the agonist mode; cf. Table 3.1) were investigated in the antagonist mode. The corresponding K_B values of neutral antagonists and inverse agonists (Table 3.1) were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding.

Free rotation about single bonds in a flexible linker might result in various conformations, with the single conformers having different affinities for the common target. The synthesis of conformationally restricted analogues of a lead compound often results in increased specific binding to the biological target and is a useful approach to explore the bioactive conformation of flexible molecules and to refine models of ligand-receptor interactions.[23-25] Therefore, we evaluated if the replacement of the flexible tetramethylene chain in **3.5** with a phenyl ring is tolerated by the hH_4R. The substituents at the cyanoguanidine ("eastern part" of the molecule) were selected with respect to comparison of the structure-activity relationships with those of recently published flexible compounds.[10]

The amines **3.5**, **3.19** and **3.20** were devoid of agonistic activity in the [^{35}S]GTPγS assay on hH_3R and hH_4R. VUF-5801 (**3.5**) showed inverse agonism at both, the hH_4R (K_B = 2,500 nM, α = -0.64) and the hH_3R (K_B = 1,260 nM, α = -0.57); the K_B values correspond to published binding data of **3.5**.[15] The *meta*-isomer **3.20** is a weak antagonist at the hH_4R and the hH_3R (K_B > 10,000 nM), whereas the homologue **3.19** turned out to prefer the hH_3R: Compound **3.19** is a weak antagonist at the hH_4R (K_B > 10,000 nM) and an inverse agonist at the hH_3R (K_B = 430 nM, α = -1.3).

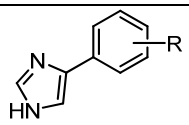
Similar to the results for the building blocks **3.5, 3.19** and **3.20**, none of the synthesized cyanoguanidines showed agonistic activity, neither at the hH_4R nor at the hH_3R. The determined K_B values for antagonistic/inverse agonistic activity were above 10 μM except for several compounds bearing bulkier alkyl or phenyl(thio)alkyl substituents at the cyanoguanidine moiety (K_B values in the range of 1-5 μM). A tendency was observed concerning *para* or *meta* substitution at the phenyl ring and its correlation to H_4R or H_3R preference. Derivatives with *para* substitution and bulkier residues in the eastern part (**3.39**-**3.41**) show a preference for the H_4R, with **3.41** being the only compound with a K_B value below 1 μM at the H_4R (K_B = 935 nM, α = -0.21). In contrast, the *meta* substituted derivative **3.46** was by a factor >10 more potent at the H_3R compared to the H_4R. Nevertheless, a significant effect of the substitution pattern on histamine receptor subtype selectivity cannot be concluded from these findings.

3.3.2 Summary and conclusion

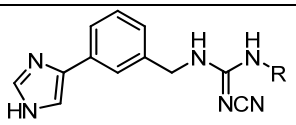
Aiming at compounds with higher histamine receptor subtype selectivity, the flexible tetramethylene linker between imidazole and cyanoguanidine moiety in **3.5** was replaced by conformationally constrained phenylene linkers. The amine precursors **3.5**, **3.19** and **3.20** were devoid of agonistic activity in the [^{35}S]GTPγS assay on hH_3R and hH_4R. All three compounds were weak antagonists or inverse agonists at both receptor subtypes. For the *meta*-isomer **3.19** a K_B value of 430 nM was found at the hH_3R (α = -1.3) and a clear preference for the hH_3R was observed. All other potencies were in the micromolar range. Unfortunately, the same effect was observed for the synthesized cyanoguanidines. None of the 15 compounds showed agonistic activity, neither at the hH_4R nor at the hH_3R. The determined K_B values for antagonistic/inverse agonistic activity were above 1 μM.

Obviously, the phenylene linker is too rigid to enable optimal orientation of the ligand in the binding pocket. Additionally, the change in electronic properties due to the additional π-system might contribute to the dramatic decrease in affinity for hH_3R and hH_4R compared to compound **3.4**. The planar geometry of a phenylene spacer proved to be inappropriate, suggesting further studies, using more flexible carbocycles connecting imidazole and cyanoguanidine.

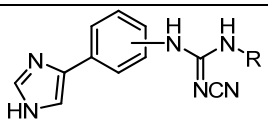
Table 3.1 Potencies and efficacies of the synthesized cyanoguanidines **3.32-3.46** and the amines **3.5**, **3.19** and **3.20** at the hH_3R and hH_4R in the [^{35}S]GTPγS assay.[a]



3.5, 3.19, 3.20



3.32-3.36



3.37-3.46

Compound no.	Phenyl substitution	R	hH_3R			hH_4R		
			(EC_{50}) or K_B (nM)	α	N	(EC_{50}) or K_B (nM)	α	N
Histamine			(13 ± 2)	1	3	(11± 3)	1	5
UR-PI376 (**3.4**)			720 ± 38	-0.52	2	(37± 3)	0.88	3
VUF5801[b] (**3.5**)	*para*	$-NH_2$	1,260 ± 50	-0.57	2	2,500 ± 126	-0.64	2
3.19	*meta*	$-CH_2NH_2$	430 ± 10	-1.3	2	> 10,000	0.09	2
3.20	*meta*	$-NH_2$	> 10,000	-0.09	2	> 10,000	-0.06	2
3.32		$-CH_3$	> 10,000	-0.06	4	> 10,000	-0.16	4
3.33		-cPr	> 10,000	-0.01	4	> 10,000	-0.18	4
3.34		$-CH_2CH(CH_3)_2$	> 10,000	-0.05	4	> 10,000	-0.21	4
3.35		$-(CH_2)_3$-Ph	> 10,000	-0.23	4	> 10,000	-0.28	4
3.36		$-(CH_2)_2$-S-Ph	> 10,000	-0.15	3	5,200 ± 150	-0.19	2
3.37	*para*	$-CH_3$	> 10,000	0.02	3	> 10,000	-0.03	2
3.38	*para*	-cPr	> 10,000	-0.11	3	> 10,000	-0.11	2
3.39	*para*	$-CH_2CH(CH_3)_2$	> 10,000	-0.05	3	1,970 ± 470	-0.1	3
3.40	*para*	$-(CH_2)_3$-Ph	4,100 ± 200	-0.06	3	2,400 ± 100	-0.19	2
3.41	*para*	$-(CH_2)_2$-S-Ph	4,585 ± 850	-0.06	3	935 ± 13	-0.21	2
3.42	*meta*	$-CH_3$	> 10,000	0.05	3	> 10,000	0.08	2
3.43	*meta*	-cPr	> 10,000	-0.01	3	> 10,000	-0.1	2
3.44	*meta*	$-CH_2CH(CH_3)_2$	6,700 ± 200	-0.09	3	> 10,000	-0.13	2
3.45	*meta*	$-(CH_2)_3$-Ph	> 10,000	0.01	2	> 10,000	-0.23	2
3.46	*meta*	$-(CH_2)_2$-S-Ph	1,100 ± 200	-0.13	3	> 10,000	-0.31	2

[a] Functional [^{35}S]GTPγS binding assay with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ was performed as described in section *Pharmacological methods*. N gives the number of independent experiments performed in duplicate each. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. The α values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM. The K_B values of neutral antagonists and inverse agonists were determined in the antagonist mode versus histamine (100 nM) as the agonist [b] Published pK_i values measured by [^{3}H]-histamine (H_4R) or [^{3}H]-N^{α}-methylhistamine (H_3R) binding to membranes of SK-N-MC cells expressing the human H_4 or H_3 receptor in presence of the ligand: pK_i (hH_4R) = 5.8, pK_i (hH_3R) = 6.0.[15]

3.4 Experimental Section

3.4.1 Chemistry

3.4.1.1 General Conditions

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany), TCI Europe (Zwijndrecht, Belgium) and used without further purification. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). Diphenyl cyanocarbonimidate (**3.21**) was a gift from Prof. Dr. Armin Buschauer, Department of Pharmaceutical/medicinal Chemistry II, University of Regensburg. All solvents were of analytical grade or distilled prior to use. Millipore water was used throughout for the preparation of buffers and HPLC eluents. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen). Anhydrous DMF was purchased from Sigma-Aldrich Chemie GmbH and stored over 3 Å molecular sieves. Flash chromatography was performed in glass columns on silica gel (Merck silica gel 60, 40 – 63 μM). Automated flash chromatography was performed on a Varian IntelliFlash 310 using pre-packed Varian Superflash columns (Varian, Darmstadt, Germany). Reactions were monitored by TLC on aluminum plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm). The compounds were detected by UV light (254 nm), a 0.3 % solution of ninhydrine in *n*-butanol (amines), a 1.0 % solution of Fast Blue B salt (imidazole containing compounds) in EtOH/H_2O = 30/70 (v/v) or iodine staining. All melting points are uncorrected and were measured on a Büchi 530 (Büchi GmbH, Essen, Germany) apparatus. Lyophilisation was done with a Christ alpha 2-4 LD equipped with a vacuubrand RZ 6 rotary vane vacuum pump. Microwave assisted reactions were performed on an Initiator 2.0 synthesizer (Biotage, Uppsala, Sweden).

Nuclear Magnetic Resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz), Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz) or Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.9 MHz) NMR spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts are given in δ (ppm) relative to external standards. Abbreviations for the multiplicities of the signals: s (singlet), d (doublet), t (triplet), m (multiplet), brs (broad singlet) and combinations thereof. The multiplicity of

carbon atoms (^{13}C-NMR) was determined by DEPT 135 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "C_{quat}" quaternary carbon atom. In certain cases 2D-NMR techniques (COSY, HMQC, HSQC, HMBC, NOESY) were used to assign ^{1}H and ^{13}C chemical shifts. Infrared spectra (IR) were measured on a Bruker Tensor 27 spectrometer equipped with an ATR (attenuated total reflection) unit from Harrick Scientific Products Inc. (Ossinning/NY, US). Mass spectra (MS) were 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. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Elemental analysis (C, H, N, Heraeus Elementar Vario EL III) were performed by the Analytical Department of the University Regensburg and are within ± 0.4 % unless otherwise noted.

Analytical HPLC analysis was performed on a system from Thermo Separation Products (TSP, Egelsbach, Germany) composed of a SN400 controller, a P4000 pump, an AS3000 autosampler, a degasser (Degassex DG-4400, Phenomenex), a Spectra Focus UV-VIS detector and a RP-column thermostated at 30 °C ((a) Eurosphere-100 C18, 250 × 4.0 mm, 5 µm; Knauer, Berlin, Germany; t_0 = 3.32 min; (b) MN Nucleodur 100-5 C18 ec, 250 x 4.0 mm, 5 µm; Macherey Nagel, Düren, Germany; t_0 = 2.68 min; (c) Gemini NX C18, 250 x 4.6 mm, 5 µm; Phenomenex, Aschaffenburg, Germany; t_0 = 3.83 min; (d) Luna C18-2, 150 x 4.6 mm, 4 µm; Phenomenex, Aschaffenburg, Germany; t_0 = 2.88 min) at a flow rate of 0.8 mL/min. UV-detection was done at 220 nm. Mixtures of acetonitrile and 0.05 % aq. TFA were used as mobile phase. Helium degassing was used throughout. Compound purities were calculated as percentage peak area of the analyzed compound by UV detection at 220 nm. HPLC conditions, retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and purities of the synthesized compounds are listed in the appendix (Chapter **10**). Purity of tested compounds was > 95 % as determined by high-performance liquid chromatography.

3.4.1.2 Preparation of the amine precursor 3.26

2-(Phenylthio)ethanamine hydrochloride (3.26)[10, 26]

A solution of thiophenol (**3.49**) (4.1 mL, 40 mmol), bromoethylamine · HBr (8.2 g, 40 mmol) and K^{t}BuO (9.0 g, 80 mmol) in anhydrous MeOH (150 mL) was stirred for 48 h at room temperature under a nitrogen atmosphere. The solvent was removed in vacuo, 1 M NaOH

(60 mL) was added to the residue and extracted with Et_2O (3 x 80 mL). After evaporation of the combined organic layers, 1 M hydrochloric acid (80 mL) was added, the solvent was removed under reduced pressure and H_2O (20 mL) was added. The solvent was removed by lyophilization giving a white solid (6.2 g, 82 %); mp 105 °C (ref.[26]: 100 – 102 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.85 – 3.00 (m, 2H, C***H***$_2$-NH_3^+), 3.28 – 3.29 (m, 2H, S-C***H***$_2$), 7.21 – 7.29 (m, 1H, Ph-***H***-4), 7.31 – 7.48 (m, 4H, Ph-***H***), 8.32 (brs, 3H, N***H***$_3^+$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 29.00 (-, S-***C***H_2), 37.97 (-, ***C***H_2-NH_3^+), 126.27 (+, Ph-***C***-4), 128.50 (+, 2 Ph-***C***), 129.21 (+, 2 Ph-***C***), 134.21 (C_{quat}, Ph-***C***-1). CI-MS (NH_3) *m/z* (%): 154 (100) $[M + H]^+$. Anal. ($C_8H_{11}NS \cdot HCl$) C, H, N. $C_8H_{11}NS \cdot HCl$ (189.71).

3.4.1.3 Preparation of the isoureas 3.27-3.31

General procedure[20, 21]

A solution of the pertinent amine (1 eq) and diphenyl cyanocarbonimidate (**3.21**, 1 eq) in 2-propanol was stirred for 1 h. After evaporation of the solvent, the product was crystallized from Et_2O.

1-Cyano-3-methyl-2-phenylisourea (3.27)[27]

The title compound was prepared from a 33 % solution of methylamine (**3.22**) in EtOH (0.73 mL, 5.9 mmol) and **3.21** (1.0 g, 4.2 mmol) in 2-propanol (50 mL) according to the general procedure yielding a white solid (0.55 g, 75 %); mp 119 °C (ref.[10]: 125 – 126 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.1 (d, 3 H, ^3J = 4.8 Hz C***H***$_3$), 7.10 (d, 2H, ^3J = 7.8 Hz, Ph-***H***), 7.28 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.41 (t, 2H, ^3J = 7.5 Hz, Ph-***H***). CI-MS (NH_3) *m/z* (%): 193 (100) $[M + NH_4]^+$, 176 (80) $[M + H]^+$. $C_9H_9N_3O$ (175.19).

1-Cyano-3-cyclopropyl-2-phenylisourea (3.28)

The title compound was prepared from cyclopropylamine (**3.23**) (0.34 g, 5.9 mmol) and **3.21** (1.0 g, 4.2 mmol) in 2-propanol (50 mL) according to the general procedure yielding a white solid (0.64 g, 76 %); mp 137 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.77 (m, 2H, C***H***$_2$), 0.90 (m, 2H, C***H***$_2$), 2.88 (m, 1H, C***H***), 7.11 (d, 2H, ^3J = 7.4 Hz, Ph-***H***), 7.30 (t, 1H, ^3J = 7.4 Hz, Ph-***H***-4), 7.42 (t, 2H, ^3J = 7.3 Hz, Ph-***H***). CI-MS (NH_3) *m/z* (%): 202 (100) $[M + H]^+$. $C_{11}H_{11}N_3O$ (201.22).

1-Cyano-3-isobutyl-2-phenylisourea (3.29)[10]

The title compound was prepared from isobutylamine (**3.24**) (0.43 g, 5.9 mmol) and **3.21** (1.0 g, 4.2 mmol) in 2-propanol (50 mL) according to the general procedure yielding a white solid (0.6 g, 66 %); mp 101 °C (ref.[10]: 103 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.01 (d, 6H, ^3J = 6.7 Hz, 2 C***H***$_3$), 1.86 – 2.03 (m, 1H, C***H***), 3.28 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$), 7.08 (d, 2H, ^3J = 7.8 Hz, Ph-***H***), 7.28 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.41 (t, 2H, ^3J = 7.5 Hz, Ph-***H***). CI-MS (NH_3) *m/z* (%): 218 (100) $[M + H]^+$. $C_{12}H_{15}N_3O$ (217.27).

1-Cyano-2-phenyl-3-(3-phenylpropyl)isourea (3.30)[10]

The title compound was prepared from 3-phenylpropan-1-amine (**3.25**) (0.79 g, 5.9 mmol) and **3.21** (1.0 g, 4.2 mmol) in 2-propanol (50 mL) according to the general procedure yielding a white solid (1.0 g, 84 %); mp 85 °C (ref.[10]: 92 – 95 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.91 – 2.10 (m, 2H, Ph-CH_2-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.6 Hz, Ph-C***H***$_2$), 3.39 – 3.54 (m, 2H, Ph-$(CH_2)_2$-C***H***$_2$), 7.08 (m, 2H, Ph-***H***), 7.22 (m, 3H, Ph-***H***), 7.28 (m, 3H, Ph-***H***), 7.40 (m, 2H, Ph-***H***). CI-MS (NH_3) *m/z* (%): 280 (100) $[M + H]^+$. $C_{17}H_{17}N_3O$ (279.34).

1-Cyano-2-phenyl-3-[2-(phenylthio)ethyl]isourea (3.31)[10]

The hydrochloride of **3.26** was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). The title compound was then prepared from **3.26** (free base, 0.77 g, 5.0 mmol) and **3.21** (1.0 g, 4.2 mmol) in 2-propanol (50 mL) according to the general procedure yielding a white solid (0.93 g, 74 %); mp 95 – 96 °C (ref.[10]: 110 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.18 (t, 2H, ^3J = 6.7 Hz, Ph-S-C***H***$_2$), 3.55 – 3.69 (m, 2H, Ph-S-CH_2-C***H***$_2$), 7.10 (m, 3H, Ph-***H***), 7.30 (m, 3H, Ph-***H***), 7.40 (m, 4H, Ph-***H***). CI-MS (NH_3) *m/z* (%): 298 (100) $[M + H]^+$. $C_{16}H_{15}N_3OS$ (297.37).

3.4.1.4 Preparation of the sulfonamides 3.10, 3.11 and 3.12

General procedure[16, 17, 28]

A solution of the pertinent benzaldehyde (1 eq) and 4-methylbenzenesulfonamide (**3.9**, 1 eq) in toluene was refluxed in a *Dean-Stark* apparatus for 24 h. Upon cooling to room temperature the product crystallized, was filtered, washed with toluene/ethylacetate, dried *in vacuo* and used without further purification.

_N_-(3-Cyanobenzylidene)-4-methylbenzenesulfonamide (3.10)

The title compound was prepared from 3-formylbenzonitrile **3.6** (3.0 g, 23 mmol) and **3.9** (4.1 g, 24.0 mmol) in 100 mL toluene according to the general procedure yielding a white solid (5.24 g, 80 %); mp 153 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.45 (s, 3 H, C***H***$_3$), 7.37 (d, 2H, ^3J = 8.0 Hz, Tos-***H***-3,5), 7.64 (t, 1H, ^3J = 7.8 Hz, Ph-***H***-5), 7.88 (m, 3H, Tos-***H***-2,6 + Ph-***H***), 8.10 (d, 1H, ^3J = 7.9 Hz, Ph-***H***), 8.22 (t, 1H, J = 1.4 Hz, Ph-***H***-2), 9.03 (s, 1H, Imin-***H***). CI-MS (NH_3) *m/z* (%): 302 (100) $[M + NH_4]^+$. $C_{15}H_{12}N_2O_2S$ (284.33).

4-Methyl-_N_-(4-nitrobenzylidene)benzenesulfonamide (3.11)[17]

The title compound was prepared from 4-nitrobenzaldehyde **3.7** (1.5 g, 10 mmol) and **3.9** (1.8 g, 10.5 mmol) in 100 mL toluene according to the general procedure yielding a yellow solid (1.88 g, 62 %); mp 200 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.42 (s, 3 H, C***H***$_3$), 7.49 (d, 2H, ^3J = 8.1 Hz, Tos-***H***-3,5), 7.88 (d, 2H, ^3J = 8.3 Hz, Tos-***H***-2,6), 8.27 (d, 2H, ^3J = 8.9 Hz, Ph-***H***), 8.36 (d, 2H, ^3J = 8.9 Hz, Ph-***H***), 9.28 (s, 1H, Imin-***H***). CI-MS (NH_3) m/z (%): 322 (100) $[M + NH_4]^+$. $C_{14}H_{12}N_2O_4S$ (304.32).

4-Methyl-_N_-(3-nitrobenzylidene)benzenesulfonamide (3.12)

The title compound was prepared from 3-nitrobenzaldehyde **3.8** (3.0 g, 20 mmol) and **3.9** (3.6 g, 21.0 mmol) in 100 mL toluene according to the general procedure yielding a white solid (5.46 g, 90 %); mp 126 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.46 (s, 3 H, C***H***$_3$), 7.38 (d, 2H, ^3J = 8.1 Hz, Tos-***H***-3,5), 7.72 (t, 1H, ^3J = 8.0 Hz, Ph-***H***-5), 7.91 (d, 2H, ^3J = 8.3 Hz, Tos-***H***-2,6), 8.25 (d, 1H, ^3J = 7.7 Hz, Ph-***H***), 8.45 (d, 1H, ^3J = 8.3 Hz, Ph-***H***), 8.76 (t, 1H, J = 1.8 Hz, Ph-***H***-2), 9.10 (s, 1H, Imin-***H***). CI-MS (NH_3) *m/z* (%): 322 (100) $[M + NH_4]^+$. $C_{14}H_{12}N_2O_4S$ (304.32).

3.4.1.5 Preparation of the 4-phenyl-imidazole precursors 3.5, 3.18 and 3.20

General procedure for the synthesis of 3.13–3.15[17]

A solution of the pertinent benzenesulfonamide (**3.10**-**3.12**, 1 eq), TosMIC (**3.16**, 1.1 eq) and potassium carbonate (3 eq) in 90 mL EtOH/DME 2:1 was refluxed for 2 h. After cooling to room temperature the reaction mixture was quenched with 20 mL water and stirred for 15 min. Further 50 mL of water were added, the solution was extracted with ether and DCM and the combined organic layers were concentrated. The crude product was subsequently

taken up in 80 mL ether and extracted with 80 mL 3 M HCl. The acidic layer was alkalized with 50 % NaOH and the resulting mixture extracted with 5 x 80 mL DCM. The combined organic layers were dried over $MgSO_4$ and evaporated. The crude product was purified by flash-chromatography.

3-(1*H*-Imidazol-4-yl)benzonitrile (3.13)[29]

The title compound was prepared from **3.10** (2.1 g, 7.4 mmol), TosMIC (1.6 g, 8.3 mmol) and K_2CO_3 (3.1 g, 22.2 mmol) according to the general procedure and purified by flash chromatography ($CHCl_3$/DCM 100/0 – 90/10 v/v) yielding a brown solid (0.56 g, 45 %); mp 175 °C (ref.[29]: 191 – 193 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.56 (t, 1H, ^3J = 7.7 Hz, Ph-***H***-3,5), 7.63 (d, 1H, ^3J = 7.7 Hz, Ph-***H***), 7.78 (s, 1H, Im-***H***), 7.80 (s, 1H, Im-***H***), 8.11 (d, 1H, ^3J = 7.7 Hz, Ph-***H***), 8.18 (t, 1H, *J* = 1.5 Hz, Ph-***H***-2), 12.37 (br, 1H, Im-N***H***). EI-MS (70 ev) *m/z* (%): 169 (100) [$M^{+\bullet}$]. $C_{10}H_7N_3$ (169.18).

4-(4-Nitrophenyl)-1*H*-imidazole (3.14)[17]

The title compound was prepared from **3.11** (1.88 g, 6.1 mmol), TosMIC (1.3 g, 6.8 mmol) and K_2CO_3 (2.5 g, 18.3 mmol) according to the general procedure and purified by flash chromatography ($CHCl_3$/DCM 100/0 – 90/10 v/v) yielding a yellow solid (0.44 g, 38 %); mp 215 °C (ref.[17]: 226 – 227 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.84 (s, 1H, Im-***H***), 7.94 (s, 1H, Im-***H***), 8.03 (d, 2H, ^3J = 9.0 Hz, Ph-***H***), 8.22 (d, 2H, ^3J = 9.0 Hz, Ph-***H***), 12.50 (br, 1H, Im-N***H***). EI-MS (70 ev) *m/z* (%): 189 (100) [$M^{+\bullet}$]. $C_9H_7N_3O_2$ (189.17).

4-(3-Nitrophenyl)-1*H*-imidazole (3.15)[30]

The title compound was prepared from **3.12** (3.0 g, 10 mmol), TosMIC (2.1 g, 11 mmol) and K_2CO_3 (4.1 g, 30 mmol) according to the general procedure and purified by flash chromatography ($CHCl_3$/DCM 90/10 v/v) yielding a yellow solid (1.05 g, 56 %); mp 216 °C (ref.[30]: 223 – 224 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.64 (t, 1H, ^3J = 8.0 Hz, Ph-***H***-3,5), 7.80 (s, 1H, Im-***H***), 7.89 (s, 1H, Im-***H***), 8.03 (m, 1H, Ph-***H***), 8.21 (d, 1H, ^3J = 7.9 Hz, Ph-***H***), 8.59 (t, 1H, *J* = 1.9 Hz, Ph-***H***-2), 12.42 (br, 1H, Im-N***H***). CI-MS (NH_3) *m/z* (%): 190 (100) $[M + H]^+$, 207 (20) $[M + NH_4]^+$. $C_9H_7N_3O_2$ (189.17).

3-(1-Trityl-1*H*-imidazol-4-yl)benzonitrile (3.17)[31]

To a solution of **3.13** (1.1 g, 6.5 mmol) and NEt_3 (1.8 mL, 13 mmol) in DCM/DMF 1:1 (50 mL), trityl chloride (2.2 g, 8.0 mmol) was slowly added. The mixture was stirred for 24 h at room temperature. The reaction was stopped by adding 10 mL water. After evaporation of the solvents the residue was taken up in EtOAc and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (PE/EtOAc 80/20 v/v) yielded a yellow foam-like solid (2.57 g, 96 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 7.17 – 7.20 (m, 7H, Trt-***H*** + Im-***H***-5), 7.36 – -7.38 (m, 9H, Trt-***H***), 7.43 – 7.49 (m, 2H, Ph-***H***), 7.54 (s, 1H, Im-***H***-2), 7.96 – 7.99 (m, 2H, Ph-***H***). ES-MS (MeCN/H_2O) *m/z* (%): 412 (100) $[M + H]^+$. $C_{29}H_{21}N_3$ (411.50).

General procedure for the reduction of the nitrophenyls 3.14 and 3.15[15]

Pd/C (10 %) (cat.) was slowly added to a solution of the pertinent nitrophenyl-1*H*-imidazole in 50 mL MeOH. The mixture was stirred under hydrogen atmosphere (5 bar) at room temperature for 24 h, filtered through a Celite-packed column and evaporated. For analytical purposes a small amount was converted into the hydrochloride by adding 6 M HCl in 2-propanol.

4-(1*H*-Imidazol-4-yl)aniline (3.5)[15]

The title compound was prepared from **3.14** (0.44 g, 2.3 mmol) according to the general procedure yielding a purple oil (0.37 g, 100 %); mp (hydrochloride) >250 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 6.73 (d, 2H, ^3J = 8.6 Hz, Ph-***H***-2,6), 7.18 (s, 1H, Im-***H***-5), 7.42 (d, 2H, ^3J = 8.5 Hz, Ph-***H***-3,5), 7.63 (s, 1H, Im-***H***-2). CI-MS (NH_3) *m/z* (%): 160 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_9H_9N_3$ $[M^{+\bullet}]$ 159.0796; found 159.0801. IR (cm^{-1}) = 3101 (N-H), 2970, 2833, 2759, 2597 (C-H), 1739, 1612, 1588, 1506, 1464, 1107. Anal. ($C_9H_9N_3 \cdot 1.9$ HCl) C, H, N. $C_9H_9N_3$ (159.19).

[3-(1-Trityl-1*H*-imidazol-4-yl)phenyl]methanamine (3.18)

$LiAlH_4$ (0.48 g, 12.4 mmol) was added in portions to a solution of **3.17** (2.57 g, 6.2 mmol) in a mixture of THF_{abs} and anhydrous Et_2O. After stirring at 0 °C for 15 min the mixture was allowed to warm to room temperature and subsequently refluxed for 3 h. After cooling externally with ice 0.5 mL H_2O, 0.5 mL 15 % NaOH and 2 mL H_2O were added consecutively.

Insoluble material was removed by filtration and washed with THF. The filtrate was washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over $MgSO_4$ and evaporated to yield a white foam-like solid (2.29 g, 89 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.43 (brs, 2H, N***H***$_2$), 3.88 (s, 2H, C***H***$_2$), 7.14 (s, 1H, Im-***H***-5), 7.17 – 7.21 (m, 7H, Ph-***H***), 7.28 (m, 1H, Ph-***H***), 7.30 – 7.36 (m, 9H, Ph-***H***), 7.47 (s, 1H, Im-***H***-2), 7.57 (d, 1H, ^3J = 7.7 Hz, Ph-***H***), 7.71 (m, 1H, Ph-***H***). ES-MS (MeCN/H_2O) *m/z* (%): 416 (100) $[M + H]^+$, 243 (55) $[Trt]^+$. $C_{29}H_{25}N_3$ (415.53).

[3-(1*H*-Imidazol-4-yl)phenyl]methanamine hydrochloride (3.19)[32]

A solution of **3.18** (2.29 g, 5.5 mmol) in 20 mL MeOH and 2 mL 37 % HCl was refluxed for 2 h. The solvent was removed *in vacuo* and the residue washed with Et_2O. Ethanol was added and evaporation yielded a white solid (1.1 g, 81 %); mp >250 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 4.24 (s, 2H, C***H***$_2$), 7.61 (m, 1H, Ph-***H***), 7.63 (m, 1H, Ph-***H***), 7.82 (d, 1H, ^3J = 6.7 Hz, Ph-***H***), 7.94 (s, 1H, Ph-***H***), 8.02 (s, 1H, Im-***H***-5), 9.07 (s, 1H, Im-***H***-2). CI-MS (NH_3) *m/z* (%): 174 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{10}H_{11}N_3$ $[M^{+\bullet}]$ 173.0953; found 173.0950. IR (cm^{-1}) = 3082 (N-H), 2970, 2841, 2623 (C-H), 1737, 1696, 1588, 1496, 1373, 1110. Anal. ($C_{10}H_{11}N_3 \cdot 2$ HCl) C, H, N. $C_{10}H_{11}N_3 \cdot 2$ HCl (246.21).

3-(1*H*-Imidazol-4-yl)aniline (3.20)[33]

The title compound was prepared from **3.15** (1.05 g, 5.6 mmol) according to the general procedure yielding a white foam-like solid (0.82 g, 93 %); mp (hydrochloride) >250 °C (ref.[33]: 270 °C, dec.); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 6.66 (m, 1H, Ph-***H***-6), 6.99-7.05 (m, 2H, Ph-***H***), 7.13 (t, 1H, ^3J = 7.7 Hz, Ph-***H***-5), 7.42 (s, 1H, Im-***H***-5), 7.97 (s, 1H, Im-***H***-2). CI-MS (NH_3) *m/z* (%): 160 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_9H_9N_3$ $[M^{+\bullet}]$ 159.0796; found 159.0795. IR (cm^{-1}) = 3101 (N-H), 2970, 2833, 2597 (C-H), 1739, 1612, 1588, 1506, 1464, 1374, 1107. Anal. ($C_9H_9N_3 \cdot 2$ HCl) C, H, N. $C_9H_9N_3$ (159.19).

3.4.1.6 Preparation of the isoureas 3.47-3.48

1-Cyano-3-[4-(1*H*-imidazol-4-yl)phenyl]-2-phenylisourea (3.47)

The title compound was prepared from **3.5** (0.37 g, 2.3 mmol) and **3.21** (0.55 g, 2.3 mmol) in 2-propanol (20 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 95/5 v/v) yielding a white solid (0.53 g, 76 %); mp 221 –

225 °C (dec.). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.30 (m, 3H, Ph-***H***), 7.46 (m, 4H, Ph-***H***), 7.59 (s, 1H, Im-***H***-5), 7.72 (s, 1H, Im-***H***-2), 7.78 (d, 2H, ^{3}J = 8.6 Hz, Ph-***H***). EI-MS (70 eV) *m/z* (%): 303 (10) [$M^{+\bullet}$], 94 (100) [$C_6H_6O^{+\bullet}$]. $C_{17}H_{13}N_5O$ (303.32).

1-Cyano-3-[3-(1*H*-imidazol-4-yl)phenyl]-2-phenyl-isourea (3.48)

The title compound was prepared from **3.20** (0.5 g, 3.1 mmol) and **3.21** (0.75 g, 3.1 mmol) in 2-propanol (20 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/32 % $NH_{3(aq)}$ 90/8/2 v/v/v) yielding a white solid (0.81 g, 86 %); mp 215 – 220 °C (dec.). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 7.24 (m, 2H, Ph-***H***), 7.29 – 7.48 (m, 6H, Im-***H***-5 + Ph-***H***), 7.60 – 7.63 (m, 1H, Ph-***H***), 7.76 (m, 2H, Im-***H***-2 + Ph-***H***). ES-MS *m/z* (%): 304 (100) $[M + H]^+$. $C_{17}H_{13}N_5O$ (303.32).

3.4.1.7 Preparation of the cyanoguanidines 3.32-3.46

General Procedure for the synthesis of compounds 3.32-3.35[19, 34]

The hydrochloride of **3.19** was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). The respective isourea (1 eq) and **3.18** (1 eq) in MeCN were heated under microwave irradiation at 150 °C for 15 min. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (DCM/MeOH 98/2 – 80/20 v/v).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)benzyl]-3-methylguanidine (3.32)

The title compound was prepared from **3.19** (0.05 g, 0.3 mmol) and **3.27** (0.051 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.06 g, 82 %); mp 77 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.82 (s, 3H, C***H***$_3$), 4.44 (s, 2H, C***H***$_2$), 7.18 (d, 1H, ^{3}J = 7.6 Hz, Ph-***H***), 7.34 (t, 1H, ^{3}J = 7.7 Hz, Ph-***H***), 7.43 (s, 1H, Im-***H***-5), 7.60 (d, 1H, ^{3}J = 7.8 Hz, Ph-***H***), 7.64 (s, 1H, Ph-***H***), 7.75 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.82 (+, ***C***H$_3$), 46.04 (-, ***C***H$_2$), 116.45 (+, Im-***C***-5), 121.02 (C_{quat}, ***C***≡N), 124.76 (+, Ph-***C***), 124.94 (+, Ph-***C***), 126.79 (+, Ph-***C***), 127.33 (C_{quat}, Ph-***C***), 130.03 (+, Ph-***C***), 133.42 (C_{quat}, Im-***C***-4), 134.75 (+, Im-***C***-2), 140.29 (C_{quat}, Ph-***C***), 162.13 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{13}H_{14}N_6$ [$M^{+\bullet}$] 254.1280; found 254.1281. IR (cm^{-1}) = 3266 (N-H), 2922 (C-H), 2159 (C≡N), 1574 (C=N), 1405, 1367, 1066. Anal. ($C_{13}H_{14}N_6 \cdot 0.75\ CH_3OH$) C, H, N. $C_{13}H_{14}N_6$ (254.29).

2-Cyano-3-cyclopropyl-1-[3-(1*H*-imidazol-4-yl)benzyl]guanidine (3.33)

The title compound was prepared from **3.19** (0.05 g, 0.3 mmol) and **3.28** (0.058 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.07 g, 87 %); mp 62 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.63 (m, 2H, C***H***$_2$), 0.82 (m, 2H, C***H***$_2$), 2.52 (m, 1H, C***H***), 4.49 (s, 2H, C***H***$_2$), 7.19 (d, 1H, ^{3}J = 7.6 Hz, Ph-***H***), 7.34 (t, 1H, ^{3}J = 7.6 Hz, Ph-***H***), 7.44 (s, 1H, Im-***H***-5), 7.59 (d, 1H, ^{3}J = 7.7 Hz, Ph-***H***), 7.64 (s, 1H, Ph-***H***), 7.76 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 8.16 (-, 2 ***C***H$_2$), 23.87 (+, ***C***H), 46.01 (-, ***C***H$_2$), 116.77 (+, Im-***C***-5), 120.61 (C_{quat}, ***C***≡N), 124.84 (+, 2 Ph-***C***), 126.88 (+, Ph-***C***), 126.99 (C_{quat}, Ph-***C***), 129.99 (+, Ph-***C***), 134.56 (C_{quat}, Im-***C***-4), 134.62 (+, Im-***C***-2), 140.67 (C_{quat}, Ph-***C***), 162.64 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{15}H_{16}N_6$ [$M^{+\bullet}$] 280.1436; found 280.1435. IR (cm^{-1}) = 3214 (N-H), 2985 (C-H), 2163 (C≡N), 1571 (C=N), 1436, 1412, 1343. Anal. ($C_{15}H_{16}N_6 \cdot 1.5\ CH_3OH$) C, H, N. $C_{15}H_{16}N_6$ (280.33).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)benzyl]-3-isobutylguanidine (3.34)

The title compound was prepared from **3.19** (0.05 g, 0.3 mmol) and **3.29** (0.063 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.07 g, 82 %); mp 75 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.84 (d, 6H, ^{3}J = 6.7 Hz, C***H***$_3$), 1.80 (m, 1H, C***H***), 3.02 (d, 2H, ^{3}J = 7.1 Hz, C***H***$_2$-CH), 4.46 (s, 2H, Ph-C***H***$_2$), 7.19 (d, 1H, ^{3}J = 7.6 Hz, Ph-***H***), 7.35 (t, 1H, ^{3}J = 7.6 Hz, Ph-***H***), 7.42 (s, 1H, Im-***H***-5), 7.61 (d, 1H, ^{3}J = 7.9 Hz, Ph-***H***), 7.64 (s, 1H, Ph-***H***), 7.74 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 20.22 (+, 2 ***C***H$_3$), 29.56 (+, ***C***H), 46.04 (-, ***C***H$_2$), 50.11 (-, ***C***H$_2$), 115.39 (+, Im-***C***-5), 120.70 (C_{quat}, ***C***≡N), 124.74 (+, Ph-***C***), 124.98 (+, Ph-***C***), 126.67 (+, Ph-***C***), 127.99 (C_{quat}, Ph-***C***), 130.09 (+, Ph-***C***), 134.86 (+, Im-***C***-2), 135.24 (C_{quat}, Im-***C***-4), 140.08 (C_{quat}, Ph-***C***), 161.42 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{16}H_{20}N_6$ [$M^{+\bullet}$] 296.1749; found 296.1749. IR (cm^{-1}) = 3261 (N-H), 3141, 2962, 2925, 2970 (C-H), 2163 (C≡N), 1575 (C=N), 1449, 1379, 1339, 1070. Anal. ($C_{16}H_{20}N_6$) C, H, N. $C_{16}H_{20}N_6$ (296.37).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)benzyl]-3-(3-phenylpropyl)guanidine (3.35)

The title compound was prepared from **3.19** (0.04 g, 0.23 mmol) and **3.30** (0.065 g, 0.23 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.065 g, 79 %); mp 59 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.80 (m, 2H, C***H***$_2$-CH$_2$-Ph), 2.52 (t, 2H, ^{3}J = 7.5 Hz, C***H***$_2$-Ph), 3.22 (t, 2H, ^{3}J = 7.0 Hz, N-C***H***$_2$), 4.44 (s, 2H, Ph-

C***H***$_2$-N), 7.08 (m, 3H, Ph-***H***), 7.19 (m, 3H, Ph-***H***), 7.35 (t, 1H, ^3J = 7.7 Hz, Ph-***H***), 7.42 (s, 1H, Im-***H***-5), 7.61 (d, 1H, ^3J = 7.7 Hz, Ph-***H***), 7.66 (s, 1H, Ph-***H***), 7.73 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 32.23 (-, ***C***H_2), 33.81 (-, ***C***H_2), 42.35 (-, ***C***H_2), 46.03 (-, ***C***H_2), 115.44 (+, Im-***C***-5), 120.10 (C_{quat}, ***C***≡N), 124.71 (+, Ph-***C***), 125.02 (+, Ph-***C***), 126.70 (+, Ph-***C***), 126.94 (+, Ph-***C***), 127.44 (C_{quat}, Ph-***C***), 129.43 (+, 4 Ph-***C***), 130.13 (+, Ph-***C***), 134.90 (+, Im-***C***-2), 136.12 (C_{quat}, Im-***C***-4), 140.06 (C_{quat}, Ph-***C***), 142.78 (C_{quat}, Ph-***C***), 161.35 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{21}H_{22}N_6$ [$M^{+\bullet}$] 358.1906; found 358.1901. IR (cm^{-1}) = 3262 (N-H), 2925, 2856 (C-H), 2158 (C≡N), 1574 (C=N), 1451, 1426, 1342. Anal. ($C_{21}H_{22}N_6 \cdot 0.3\ CH_3OH$) C, H, N. $C_{21}H_{22}N_6$ (358.44).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)benzyl]-3-[2-(phenylthio)ethyl]guanidine (3.36)

The title compound was prepared from **3.19** (0.04 g, 0.23 mmol) and **3.31** (0.069 g, 0.23 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.06 g, 70 %); mp 64 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.05 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$), 3.40 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 4.40 (s, 2H, Ph-C***H***$_2$-N), 7.12 – 7.18 (m, 2H, Ph-***H***), 7.24 (m, 2H, Ph-***H***), 7.31 – 7.37 (m, 3H, Ph-***H***), 7.43 (s, 1H, Im-***H***-5), 7.62 (m, 2H, Ph-***H***), 7.73 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 33.63 (-, S-***C***H_2), 42.16 (-, ***C***H_2), 46.17 (-, ***C***H_2), 118.36 (+, Im-***C***-5), 121.36 (C_{quat}, ***C***≡N), 124.73 (+, Ph-***C***), 125.08 (+, Ph-***C***), 126.68 (+, Ph-***C***), 127.39 (+, Ph-***C***), 127.39 (C_{quat}, Ph-***C***), 130.14 (+, 3 Ph-***C***), 130.60 (+, 2 Ph-***C***), 136.74 (C_{quat}, Ph-***C***), 137.21 (+, Im-***C***-2), 137.26 (C_{quat}, Im-***C***-4), 139.65 (C_{quat}, Ph-***C***), 161.29 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{20}H_{20}N_6S$ [$M^{+\bullet}$] 376.1470; found 376.1467. IR (cm^{-1}) = 3132 (N-H), 2970, 2925, 2864 (C-H), 2172 (C≡N), 1720, 1655, 1579 (C=N), 1455, 1086. Anal. ($C_{20}H_{20}N_6S \cdot 0.5\ CH_3OH \cdot 0.8\ H_2O$) C, H, N. $C_{20}H_{20}N_6S$ (376.48).

General Procedure for the synthesis of compounds 3.37-3.46[19, 34]

The respective isourea **3.47** or **3.48** (1 eq) and the pertinent primary amine **3.22-3.26** (1 eq) in MeCN were heated under microwave irradiation at 150 °C for 15 min. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (DCM/MeOH 98/2 – 80/20 v/v).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)phenyl]- 3-methylguanidine (3.37)

The title compound was prepared from **3.47** (0.09 g, 0.3 mmol) and a 33 % solution of methylamine in ethanol (0.037 mL, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.06 g, 83 %); mp 138 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.84 (s, 3H, C***H***$_3$), 7.28 (d, 2H, ^{3}J = 8.5 Hz, Ph-***H***), 7.44 (s, 1H, Im-***H***-5), 7.72 (d, 2H, ^{3}J = 8.6 Hz, Ph-***H***), 7.76 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 29.00 (+, ***C***H$_3$), 116.42 (+, Im-***C***-5), 119.36 (C_{quat}, ***C***≡N), 126.45 (+, 2 Ph-***C***), 126.78 (+, 2 Ph-***C***), 132.61 (C_{quat}, Ph-***C***), 136.77 (C_{quat}, Im-***C***-4), 137.27 (+, Im-***C***-2), 139.30 (C_{quat}, Ph-***C***), 161.01 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{12}N_6$ [$M^{+\bullet}$] 240.1123; found 240.1120. IR (cm^{-1}) = 3492 (N-H), 3314, 3162 (C-H), 2163 (C≡N), 1578 (C=N), 1413, 1362, 1075. Anal. ($C_{12}H_{12}N_6 \cdot 0.4\ CH_3OH \cdot 0.6\ H_2O$) C, H, N. $C_{12}H_{12}N_6$ (240.26).

2-Cyano-3-cyclopropyl-1-[4-(1*H*-imidazol-4-yl)phenyl]guanidine (3.38)

The title compound was prepared from **3.47** (0.09 g, 0.3 mmol) and cyclopropylamine (0.021 mL, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.05 g, 62 %); mp 215 °C (dec.); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.70 (m, 2H, C***H***$_2$), 0.87 (m, 2H, C***H***$_2$), 2.67 (m, 1H, C***H***), 7.36 (d, 2H, ^{3}J = 8.6 Hz, Ph-***H***), 7.42 (s, 1H, Im-***H***-5), 7.69 (d, 2H, ^{3}J = 8.6 Hz, Ph-***H***), 7.73 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 8.33 (-, 2 ***C***H$_2$), 24.47 (+, ***C***H), 116.45 (+, Im-***C***-5), 118.94 (C_{quat}, ***C***≡N), 125.56 (+, 2 Ph-***C***), 126.30 (+, 2 Ph-***C***), 131.84 (C_{quat}, Ph-***C***), 137.17 (C_{quat}, Im-***C***-4), 137.27 (+, Im-***C***-2), 139.25 (C_{quat}, Ph-***C***), 161.43 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{14}H_{14}N_6$ [$M^{+\bullet}$] 266.1280; found 266.1280. IR (cm^{-1}) = 3196 (N-H), 3127, 3005 (C-H), 2166 (C≡N), 1579 (C=N), 1547, 1439, 1343. Anal. ($C_{14}H_{14}N_6 \cdot 0.6\ CH_3OH$) C, H, N. $C_{14}H_{14}N_6$ (266.30).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)phenyl]-3-isobutylguanidine (3.39)

The title compound was prepared from **3.47** (0.09 g, 0.3 mmol) and isobutylamine (0.03 mL, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.08 g, 94 %); mp 103 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.91 (d, 6H, ^{3}J = 6.7 Hz, C***H***$_3$), 1.88 (m, 1H, C***H***), 3.08 (d, 2H, ^{3}J = 7.1 Hz, C***H***$_2$-CH), 7.27 (d, 2H, ^{3}J = 8.6 Hz, Ph-***H***), 7.45 (s, 1H, Im-***H***-5), 7.74 (d, 2H, ^{3}J = 8.6 Hz, Ph-***H***), 7.74 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 20.38 (+, 2 ***C***H$_3$), 29.66 (+, ***C***H), 50.23 (-, ***C***H$_2$), 116.37 (+, Im-***C***-5), 119.37 (C_{quat}, ***C***≡N), 126.48 (+, 2 Ph-***C***), 126.89 (+, 2 Ph-***C***), 132.79 (C_{quat}, Ph-***C***),

136.74 (C_{quat}, Im-***C***-4), 137.29 (+, Im-***C***-2), 139.37 (C_{quat}, Ph-***C***), 160.46 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{15}H_{18}N_6$ [$M^{+\bullet}$] 282.1593; found 282.1592. IR (cm^{-1}) = 3267 (N-H), 2925, 2866 (C-H), 2160 (C≡N), 1575 (C=N), 1506, 1428, 1256. Anal. ($C_{15}H_{18}N_6 \cdot 0.4\ CH_3OH$) C, H, N. $C_{15}H_{18}N_6$ (282.34).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)phenyl]-3-(3-phenylpropyl)guanidine (3.40)

The title compound was prepared from **3.47** (0.076 g, 0.25 mmol) and 3-phenylpropan-1-amine (0.036 mL, 0.25 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.07 g, 81 %); mp 88 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.86 (m, 2H, C***H***$_2$-CH_2-Ph), 2.63 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$-Ph), 3.28 (t, 2H, ^3J = 7.2 Hz, N-C***H***$_2$), 7.11 – 7.29 (m, 7H, Ph-***H***), 7.44 (s, 1H, Im-***H***-5), 7.73 (d, 2H, ^3J = 8.6 Hz, Ph-***H***), 7.73 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 32.29 (-, ***C***H_2), 34.12 (-, ***C***H_2), 42.67 (-, ***C***H_2), 116.39 (+, Im-***C***-5), 120.18 (C_{quat}, ***C***≡N), 126.43 (+, 2 Ph-***C***), 126.89 (+, 2 Ph-***C***), 127.01 (+, Ph-***C***), 129.45 (+, 2 Ph-***C***), 129.50 (+, 2 Ph-***C***), 132.80 (C_{quat}, Ph-***C***), 136.69 (C_{quat}, Im-***C***-4), 137.29 (+, Im-***C***-2), 140.41 (C_{quat}, Ph-***C***), 142.90 (C_{quat}, Ph-***C***), 160.32 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{20}H_{20}N_6$ [$M^{+\bullet}$] 344.1749; found 344.1745. IR (cm^{-1}) = 3235 (N-H), 2987, 2901 (C-H), 2156 (C≡N), 1570 (C=N), 1451, 1431, 1371, 1065. Anal. ($C_{20}H_{20}N_6 \cdot 0.5\ H_2O$) C, H, N. $C_{20}H_{20}N_6$ (344.41).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)phenyl]-3-[2-(phenylthio)ethyl]guanidine (3.41)

The title compound was prepared from **3.47** (0.076 g, 0.25 mmol) and 2-(phenylthio)ethanamine **3.26** (0.038 g, 0.25 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.08 g, 88 %); mp 140 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.10 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 3.44 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 7.20 (m, 3H, Ph-***H***), 7.29 (m, 2H, Ph-***H***), 7.38 (m, 2H, Ph-***H***), 7.45 (s, 1H, Im-***H***-5), 7.74 (d, 2H, ^3J =8.5 Hz, Ph-***H***), 7.74 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 33.32 (-, S***C***H_2), 42.29 (-, ***C***H_2), 116.45 (+, Im-***C***-5), 120.31 (C_{quat}, ***C***≡N), 126.77 (+, 2 Ph-***C***), 126.98 (+, 2 Ph-***C***), 127.29 (+, Ph-***C***), 130.16 (+, 2 Ph-***C***), 130.31 (+, 2 Ph-***C***), 133.13 (C_{quat}, Ph-***C***), 136.30 (C_{quat}, Im-***C***-4), 136.95 (C_{quat}, Ph-***C***), 137.33 (+, Im-***C***-2), 139.89 (C_{quat}, Ph-***C***), 160.32 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{18}N_6S$ [$M^{+\bullet}$] 362.1314; found 362.1306. IR (cm^{-1}) = 3241 (N-H), 3052, 2846 (C-H), 2157 (C≡N), 1582 (C=N), 1548, 1436, 1244. Anal. ($C_{19}H_{18}N_6S \cdot 0.1\ CH_3OH$) C, H, N. $C_{19}H_{18}N_6S$ (362.45).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)phenyl]-3-methylguanidine (3.42)

The title compound was prepared from **3.48** (0.09 g, 0.3 mmol) and a 33 % solution of methylamine in ethanol (0.037 mL, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.015 g, 21 %); mp 97 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.84 (s, 3H, C***H***$_3$), 7.15 (d, 1H, ^{3}J = 8.5 Hz, Ph-***H***), 7.40 (t, 1H, ^{3}J = 8.1 Hz, Ph-***H***), 7.48 (s, 1H, Im-***H***-5), 7.58 – 7.61 (m, 2H, Ph-***H***), 7.74 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.97 (+, ***C***H$_3$), 116.46 (+, Im-***C***-5), 119.32 (C_{quat}, ***C***≡N), 122.53 (+, Ph-***C***), 123.89 (+, Ph-***C***), 124.56 (+, Ph-***C***), 124.11 (C_{quat}, Ph-***C***), 130.85 (+, Ph-***C***), 135.81 (C_{quat}, Im-***C***-4), 137.35 (+, Im-***C***-2), 138.64 (C_{quat}, Ph-***C***), 161.03 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{12}N_6$ [$M^{+\bullet}$] 240.1123; found 240.1120. IR (cm^{-1}) = 3199 (N-H), 2971, 2901 (C-H), 2168 (C≡N), 1595 (C=N), 1566, 1406, 1065. Anal. ($C_{12}H_{12}N_6 \cdot 0.5\ CH_3OH \cdot 0.3\ H_2O$) C, H, N. $C_{12}H_{12}N_6$ (240.26).

2-Cyano-3-cyclopropyl-1-[3-(1*H*-imidazol-4-yl)phenyl]guanidine (3.43)

The title compound was prepared from **3.48** (0.09 g, 0.3 mmol) and cyclopropylamine (0.021 mL, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.05 g, 63 %); mp 137 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.71 (m, 2H, C***H***$_2$), 0.87 (m, 2H, C***H***$_2$), 2.68 (m, 1H, C***H***), 7.23 (d, 1H, ^{3}J = 8.0 Hz, Ph-***H***), 7.35 (t, 1H, ^{3}J = 8.0 Hz, Ph-***H***), 7.44 (s, 1H, Im-***H***-5), 7.54 (d, 1H, ^{3}J = 7.8 Hz, Ph-***H***), 7.66 (s, 1H, Ph-***H***), 7.73 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 8.31 (-, 2 ***C***H$_2$), 24.50 (+, ***C***H), 116.41 (+, Im-***C***-5), 118.94 (C_{quat}, ***C***≡N), 121.83 (+, Ph-***C***), 123.24 (+, Ph-***C***), 123.94 (+, Ph-***C***), 130.33 (+, Ph-***C***), 133.80 (C_{quat}, Ph-***C***), 137.07 (C_{quat}, Im-***C***-4), 137.29 (+, Im-***C***-2), 139.03 (C_{quat}, Ph-***C***), 161.58 (C_{quat}, ***C***=N). HRMS (LSI-MS) calcd. for $C_{14}H_{15}N_6$ [M + H]$^{+\bullet}$ 267.1358; found 267.1357. IR (cm^{-1}) = 3115 (N-H), 2956, 2845 (C-H), 2165 (C≡N), 1578 (C=N), 1547, 1437, 1341. Anal. ($C_{14}H_{14}N_6 \cdot 0.9\ CH_3OH$) C, H, N. $C_{14}H_{14}N_6$ (266.30).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)phenyl]-3-isobutylguanidine (3.44)

The title compound was prepared from **3.48** (0.09 g, 0.3 mmol) and isobutylamine (0.03 mL, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.06 g, 71 %); mp 95 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.91 (d, 6H, ^{3}J = 6.7 Hz, C***H***$_3$), 1.88 (m, 1H, C***H***), 3.08 (d, 2H, ^{3}J = 7.1 Hz, C***H***$_2$-CH), 7.14 (d, 1H, ^{3}J = 7.8 Hz, Ph-***H***), 7.40 (t, 1H, ^{3}J = 7.7 Hz, Ph-***H***), 7.47 (s, 1H, Im-***H***-5), 7.60 (m, 2H, Ph-***H***), 7.74

(s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 20.39 (+, 2 ***C***H_3), 29.64 (+, ***C***H), 50.24 (-, ***C***H_2), 116.58 (+, Im-***C***-5), 119.38 (C_{quat}, ***C***≡N), 122.53 (+, Ph-***C***), 123.91 (+, Ph-***C***), 124.50 (+, Ph-***C***), 130.94 (+, Ph-***C***), 130.28 (C_{quat}, Ph-***C***), 136.22 (C_{quat}, Im-***C***-4), 137.37 (+, Im-***C***-2), 138.68 (C_{quat}, Ph-***C***), 160.47 (C_{quat}, ***C***=N). HRMS (LSI-MS) calcd. for $C_{15}H_{19}N_6$ $[M + H]^{+\bullet}$ 283.1671; found 283.1674. IR (cm^{-1}) = 3117 (N-H), 2956, 2869 (C-H), 2166 (C≡N), 1565 (C=N), 1446, 1369. Anal. ($C_{15}H_{18}N_6 \cdot 0.75\ CH_3OH$) C, H, N. $C_{15}H_{18}N_6$ (282.34).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)phenyl]-3-(3-phenylpropyl)guanidine (3.45)

The title compound was prepared from **3.48** (0.076 g, 0.25 mmol) and 3-phenylpropan-1-amine (0.036 mL, 0.25 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.07 g, 81 %); mp 78 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.86 (m, 2H, C***H***$_2$-CH_2-Ph), 2.63 (t, 2H, ^3J = 7.9 Hz, C***H***$_2$-Ph), 3.29 (t, 2H, ^3J = 7.2 Hz, N-C***H***$_2$), 7.09 – 7.26 (m, 6H, Ph-***H***), 7.40 (t, 1H, ^3J =8.2 Hz, Ph-***H***), 7.46 (s, 1H, Im-***H***-5), 7.59 (m, 2H, Ph-***H***), 7.74 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 32.27 (-, ***C***H_2), 34.11 (-, ***C***H_2), 42.68 (-, ***C***H_2), 116.43 (+, Im-***C***-5), 119.39 (C_{quat}, ***C***≡N), 122.51 (+, Ph-***C***), 123.92 (+, Ph-***C***), 124.49 (+, Ph-***C***), 126.99 (+, Ph-***C***), 129.44 (+, 2 Ph-***C***), 129.47 (+, 2 Ph-***C***), 130.96 (+, Ph-***C***), 134.78 (C_{quat}, Im-***C***-4), 136.20 (C_{quat}, Ph-***C***), 137.37 (+, Im-***C***-2), 138.64 (C_{quat}, Ph-***C***), 142.90 (C_{quat}, Ph-***C***), 160.25 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{20}H_{20}N_6$ $[M^{+\bullet}]$ 344.1749; found 344.1740. IR (cm^{-1}) = 3093 (N-H), 2971, 2900 (C-H), 2165 (C≡N), 1557 (C=N), 1452, 1376, 1066. Anal. ($C_{20}H_{20}N_6 \cdot 0.6\ CH_3OH$) C, H, N. $C_{20}H_{20}N_6$ (344.41).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)phenyl]-3-[2-(phenylthio)ethyl]guanidine (3.46)

The title compound was prepared from **3.48** (0.076 g, 0.25 mmol) and 2-(phenylthio)ethanamine **3.26** (0.038 g, 0.25 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.05 g, 55 %); mp 51 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.11 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 3.44 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 7.08 – 7.19 (m, 2H, Ph-***H***), 7.27 (m, 2H, Ph-***H***), 7.39 (m, 3H, Ph-***H***), 7.48 (s, 1H, Im-***H***-5), 7.61 (m, 2H, Ph-***H***), 7.75 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 33.26 (-, S-***C***H_2), 42.21 (-, ***C***H_2), 116.75 (+, Im-***C***-5), 120.21 (C_{quat}, ***C***≡N), 122.76 (+, Ph-***C***), 124.18 (+, Ph-***C***), 124.71 (+, Ph-***C***), 127.26 (+, Ph-***C***), 130.14 (+, 2 Ph-***C***), 130.28 (+, 2 Ph-***C***), 131.08 (+, Ph-***C***), 133.43 (C_{quat}, Ph-***C***), 136.91 (C_{quat}, Ph-***C***), 137.04 (C_{quat}, Im-***C***-4), 137.40 (+, Im-***C***-2), 138.31 (C_{quat}, Ph-***C***), 160.30 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{18}N_6S$ $[M^{+\bullet}]$ 362.1314; found

362.1309. IR (cm^{-1}) = 3037 (N-H), 2987, 2901 (C-H), 2172 (C≡N), 1668, 1583 (C=N), 1561, 1199, 1131. $C_{19}H_{18}N_6S$ (362.45).

3.4.2 Pharmacological methods

3.4.2.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide hydrochloride was synthesized according to a previously described method.[35] Guanosine diphosphate (GDP) was from Sigma-Aldrich Chemie GmbH (Munich, Germany), unlabeled GTPγS was from Roche (Mannheim, Germany). [^{35}S]GTPγS was from PerkinElmer Life Sciences (Boston, MA) or Hartmann Analytic GmbH (Braunschweig, Germany). GF/C filters were from Whatman (Maidstone, UK).

3.4.2.2 [^{35}S]GTPγS binding assay[36, 37]

[^{35}S]GTPγS binding assays were performed as previously described for the H_2R,[38, 39] H_3R[40, 41] and H_4R.[42] H_2R assays: Sf9 insect cell membranes expressing the hH_2R-$G\alpha_s$ fusion protein were employed, H_3R assays: Sf9 insect cell membranes coexpressing the hH_3R, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were employed, H_4R assays: Sf9 insect cell membranes coexpressing the hH_4R, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were employed.

The respective membranes were thawed, sedimented by a 10 min centrifugation at 4 °C and 13,000 *g*. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each assay tube contained Sf9 membranes expressing the respective HR subtype (15 – 30 µg protein/tube), 1 µM GDP, 0.05 % (w/v) bovine serum albumin, 0.2 nM [^{35}S]GTPγS and the investigated ligands (dissolved in millipore water or in a mixture (v/v) of 80 % millipore water and 20 % DMSO) at various concentrations in binding buffer (total volume 250 µL). All H_4R assays additionally contained 100 mM NaCl.

For the determination of K_B values (antagonist mode of the [^{35}S]GTPγS Binding Assay) histamine was added to the reaction mixtures (final concentrations: H_2R: 1 µM; H_3/$_4R$: 100 nM). Incubations were conducted for 90 min at 25 °C and shaking at 250 rpm. Bound [^{35}S]GTPγS was separated from free [^{35}S]GTPγS by filtration through GF/C filters, followed by three washes with 2 ml of binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid

scintillation counting. The experimental conditions chosen ensured that no more than 10 % of the total amount of [^{35}S]GTPγS added was bound to filters. Non-specific binding was determined in the presence of 10 µM unlabeled GTPγS.

3.4.2.3 Data analysis and pharmacological parameters

All data are presented as mean of N independent experiments ± SEM. Agonist potencies were given as EC_{50} values (molar concentration of the agonist causing 50 % of the maximal response). Maximal responses (intrinsic activities) were expressed as α values. The α value of histamine was set to 1.00, α values of other compounds were referred to this value.
IC_{50} values were converted to K_i and K_B values using the Cheng-Prussoff equation.[43] pK_i values were analyzed by nonlinear regression and best fit to one-site (monophasic) competition isotherms. pEC_{50} and pK_B values from the functional [^{35}S]GTPγS and GTPase assays were analyzed by nonlinear regression and best fit to sigmoidal dose-response curves (GraphPad Prism 5.0 software, San Diego, CA).

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Chapter 4

Imidazolylcyclohexylcyanoguanidines – towards conformationally restricted H_4R ligands

4.1 Introduction

Recently, we identified highly potent agonists of the human histamine H_4 receptor among a series of imidazolylbutylcyanoguanidines.[1] The cyanoguanidine moiety was found to be a useful bioisosteric replacement of the strongly basic guanidine group and the corresponding acylguanidine group to obtain potent H_4R agonists with improved selectivity over the other histamine receptor subtypes. However, even the most potent and selective compound in this series, UR-PI376 (**4.1**), exhibits noteworthy inverse agonistic activity at the H_3R. If a compound has low selectivity for a distinct target molecule it may simply be because of different conformers enable binding to several targets.[2] Therefore, restricting the conformation of a compound can be effective in the search for improved selectivity. Introduction of a *para*- or a *meta*-phenylene spacer yielded only very weakly active compounds at both, hH_3R and hH_4R (see chapter 3). Aiming at an optimized balance between rigidification and remaining flexibility, the tetramethylene linker connecting imidazole ring and cyanoguanidine group was replaced by a cyclohexyl ring, as in the imidazolylcyclohexylamines VUF-5803 (**4.2**) and VUF-5804 (**4.3**), which were described as non-selective H_3R and H_4R agonists ((**4.2**) H_4R: pK_i = 7.7, α = 1.3; H_3R: pK_i = 7.0; (**4.3**) H_4R: pK_i = 6.5, α = 1.1; H_3R: pK_i = 7.4).[3]

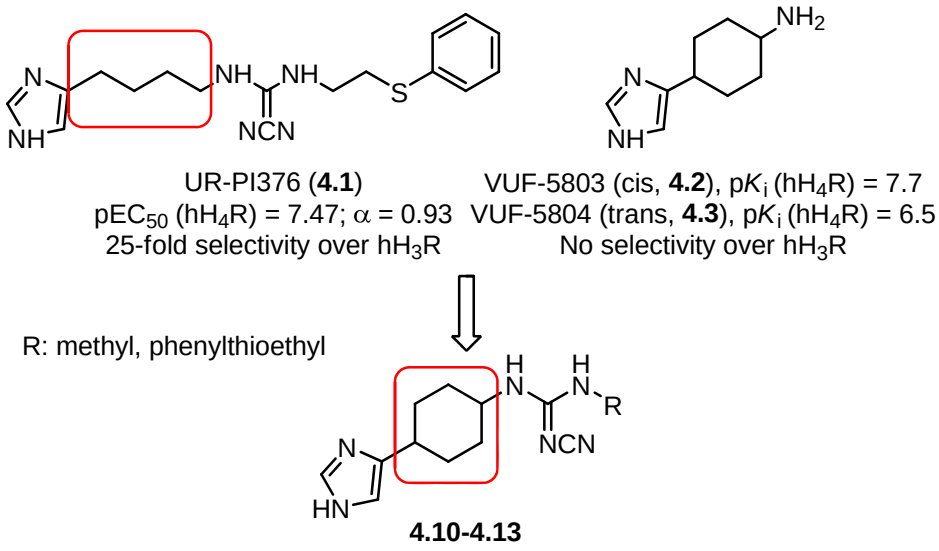
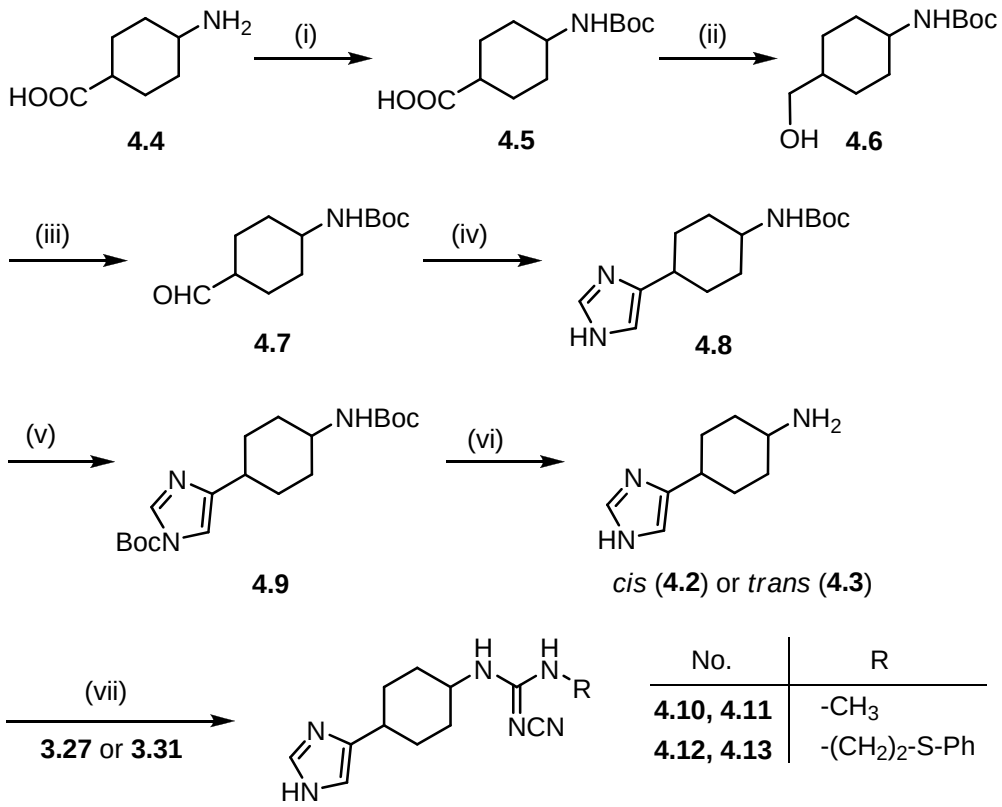


Figure 4.1 Structures of the cyanoguanidine UR-PI376, the imidazolylcyclohexylamines VUF-5803 and VUF-5804 and the synthesized cyanoguanidines **4.10-4.13.**

4.2 Chemistry

The 1,4-disubstituted cyclohexanes **4.10-4.13** were synthesized as outlined in Scheme 4.1. A diastereomeric mixture of 4-aminocyclohexanecarboxylic acid (**4.4**) was Boc-protected,[4] and the carboxylic acid **4.5** was reduced with borane to yield the alcohol **4.6**.[5] Swern oxidation[6] gave the corresponding aldehyde **4.7**, which was successively treated with TosMIC (**3.16**) and 7 M ammonia in methanol to introduce the imidazole ring.[7] After Boc-protection of the imidazole nitrogen in **4.8**, the mixture of *cis*- and *trans*-**4.9** was separated by flash chromatography.[3] Deprotection under acidic conditions and liberation of the amines as free bases with the help of a basic ion exchanger gave the *cis*- and *trans*-imidazolyl-cyclohexylamines **4.2** and **4.3**.[1, 3] The cyanoguanidines **4.10-4.13** were synthesized by analogy with the procedure described in Chapter 3.



Scheme 4.1 Synthesis of the cyanoguanidines **4.10-4.13**. Reagents and conditions: (i) Boc_2O (1.1 eq), NaOH, overnight, rt, 88 %; (ii) BH_3·THF (2.25 eq), THF, 12 h, 0 °C → rt, 99 %; (iii) DMSO (4 eq), NEt_3 (8 eq), $(COCl)_2$ (2 eq), DCM, 3 h, -78 °C → 12 h, rt, 69 %; (vi) a) TosMIC (**3.16**, 1 eq), NaCN (0.15 eq), EtOH, 30 min, 0 °C; b) 7 M NH_3 in MeOH, 18 h, 100 °C, 70 %; (v) Boc_2O (1.1 eq), NaOH, overnight, rt, 92 %; (vi) a) separation of diastereomers, b) HCl, ion exchanger, 93 %; (vii) MeCN, microwave 140 °C, 15 min, 68-93 %.

4.3 Pharmacological results and discussion

4.3.1 Potencies and efficacies of the synthesized compounds at the hH_4R and the hH_3R in the [^{35}S]GTPγS binding assay

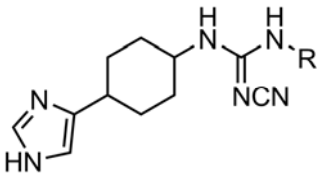
The synthesized cyanoguanidines were investigated for agonism and antagonism at hH_4R and hH_3R subtypes in [^{35}S]GTPγS binding assays using membrane preparations of Sf9 insect cells co-expressing the hH_4R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or co-expressing the hH_3R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$. Additionally, for reasons of comparison the amine precursors (**4.2** and **4.3**) were characterized.

In the following agonistic potencies are expressed as EC_{50} values. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine. Compounds identified to be inactive as agonists (α <0.1 or negative values, respectively, determined in the agonist mode; cf. Table 4.1) were investigated in the antagonist mode. The corresponding K_B values of neutral antagonists and inverse agonists (Table 4.1) were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding.

The compounds with a cyclohexylene instead of a phenylene spacer retain some conformational flexibility. The *cis*- and *trans*-configured amines VUF-5803 (**4.2**) and VUF-5804 (**4.3**), which were used as building blocks, proved to be partial agonists (α: 0.7 - 0.9) in the [^{35}S]GTPγS assay at both histamine receptor subtypes. The *cis* isomer prefers the hH_4R (EC_{50} values: hH_4R: 15 nM, hH_3R: 115 nM) while higher potency at the hH_3R resides in the *trans* isomer (EC_{50} values: hH_4R 300 nM, hH_3R 46 nM). The potencies in the [^{35}S]GTPγS assay correspond to published binding data for **4.2** and **4.3**.[3] The compounds **4.10**-**4.13** were synthesized as prototypical cyanoguanidines reminiscent of characteristic structural features of OUP-16[8] and UR-PI376 (**4.1**), respectively. The investigation in the [^{35}S]GTPγS assay revealed that the phenylthioethyl substituted cyanoguanidines **4.12** and **4.13** were superior to the methyl substituted analogues. This is in agreement with structure-activity relationships of flexible cyanoguanidines,[1] corroborating that arylalkyl residues are suitable to increase the affinity for both receptors compared to the methyl substitution. Similar to **4.2** and **4.3** the preference for hH_3R and hH_4R depends on the stereochemistry with higher activities at the hH_4R residing in the *cis* isomers. However the cyanoguanidines are less potent partial agonists than the corresponding amines and show some efficacy-selectivity

depending on the configuration. At the hH_4R both *cis*-configured compounds (**4.10**, **4.12**) are hH_4R partial agonists (α: 0.55 and 0.32) while the *trans* isomers (**4.11**, **4.13**) are neutral antagonists. At the hH_3R only the methyl substituted *cis* configured compound **4.10** is a weak partial agonist, whereas the other three cyanoguanidines act as inverse agonists with intrinsic activities from -0.38 to -0.86. The *trans* isomer **4.13** is by a factor of 10 more potent than the corresponding *cis* diastereomer **4.12** at the hH_3R.

Table 4.1 Potencies and efficacies of the synthesized cyanoguanidines **4.10**-**4.13** and the amines **4.2**, **4.3** at the hH_3R and hH_4R in the [^{35}S]GTPγS assay.[a]



Compound	Config.	R	hH_3R			hH_4R		
			(EC_{50}) *or* K_B (nM)	α	N	(EC_{50}) *or* K_B (nM)	α	N
Histamine			(13 ± 2)	1	3	(11± 3)	1	5
VUF-5803[b] (**4.2**)	*cis*		(115 ± 11)	0.87	2	(15± 1)	0.88	2
VUF-5804[b] (**4.3**)	*trans*		(46 ± 7)	0.71	2	(300 ± 20)	0.92	2
4.10	*cis*	$-CH_3$	(> 10,000)	0.41	3	(1,840 ± 40)	0.55	2
4.11	*trans*	$-CH_3$	> 10,000	-0.38	2	> 10,000	-0.01	2
4.12	*cis*	$-(CH_2)_2$-S-Ph	1,900 ± 300	-0.86	2	(110 ± 7)	0.32	3
4.13	*trans*	$-(CH_2)_2$-S-Ph	180 ± 16	-0.62	2	188 ± 5	-0.02	2

[a] Functional [^{35}S]GTPγS binding assay with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ was performed as described in section *Pharmacological methods*. N gives the number of independent experiments performed in duplicate each. The intrinsic activity (*α*) of histamine was set to 1.00 and *α* values of other compounds were referred to this value. *α* values of neutral antagonists and inverse agonists were determined at 10 µM. The K_B values of neutral antagonists and inverse agonists were determined in the antagonist mode versus histamine (100 nM) as the agonist. [b] Published pK_i values measured by [^{3}H]-histamine (H_4) or [^{3}H]-N^{α}-methylhistamine (H_3) binding to membranes of SK-N-MC cells expressing the human H_4 or H_3 receptor in presence of the ligand: VUF-5803: pK_i (hH_4R) = 7.7, pK_i (hH_3R) = 7.0; VUF-5804: pK_i (hH_4R) = 6.5, pK_i (hH_3R) = 7.4.[3]

4.3.2 Summary and conclusion

Conformational constraints are, in principle, tolerated in cyanoguanidine-type hH_3R and hH_4R ligands. Conformationally restricted analogues of cyanoguanidine-type hH_4R agonists such as UR-PI376 (**4.1**), in which the flexible tetramethylene chain was replaced by a *cis*-configured 1,4-cyclohexylene spacer, turned out to be moderately potent and selective hH_4R agonists. The same holds for the building block, *cis*-4-(1*H*-imidazol-4-yl)cyclohexylamine. The

situation is less clear in case of the *trans*-configured analogues, but there is a tendency toward preference for the hH_3R.

In conclusion, the results suggest the optimization of imidazolylcycloalkylcyanoguanidines with regard to ring size, balance between rigidification and flexibility, regioisomers and stereochemical properties to explore the three-dimensional requirements of high hH_4R affinity and selectivity.

4.4 Experimental Section

4.4.1 Chemistry

4.4.1.1 General Conditions

See section 3.4.1.1.

4.4.1.2 Preparation of the amine precursors 4.2 and 4.3

4-(*tert*-Butoxycarbonylamino)cyclohexanecarboxylic acid (4.5)[9]

To a solution of 4-aminocyclohexanecarboxylic acid (cis/trans 1:1, **4.4**) (10 g, 69.8 mmol, 1 eq) in 210 mL dioxane/water 2:1 (v/v) was added 70 mL 1 M aqueous NaOH. After cooling to 0 °C Boc_2O (16.8 g, 76.8 mmol, 1.1 eq) was added and the mixture was stirred at room temperature overnight. After removing the solvent *in vacuo* the residue was taken up in EtOAc, washed with citric acid 5 % and brine and dried over $MgSO_4$. Evaporation of the solvent yielded a white solid that was used without further purification. (14.9 g, 88 %); mp 157°C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.43 (s, 9H, Boc), 1.46 – 1.70 (m, 6H, 3 C***H***$_2$), 1.95 (m, 2H, C***H***$_2$), 2.46 (m, 1H, C***H***), 3.45 (m, 1H, C***H***-NHBoc). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 26.26 (-, 2 ***C***H_2), 28.84 (+, C(***C***$H_3)_3$), 30.70 (-, 2 ***C***H_2), 41.13 (+, ***C***H-COOH), 48.94 (+, ***C***H-NHBoc), 79.91 (C_{quat}, ***C***$(CH_3)_3$), 157.87 (C_{quat}, ***C***=O), 178.91 (C_{quat}, ***C***OOH). CI-MS (NH_3) *m/z* (%): 244 (10) $[M + H]^+$, 261 (10) $[M + NH_4]^+$, 205 (80) $[M – C_4H_8 + NH_4]^+$, 143 (40) [M – Boc + H]$^+$, 126 (100) [M – Boc – H_2O + H]$^+$. $C_{12}H_{21}NO_4$ (243.30).

***tert*-Butyl 4-(hydroxymethyl)cyclohexylcarbamate (4.6)[10]**

Borane · THF (134 mL, 134 mmol) was slowly added to a solution of **4.5** (14.5 g, 59.6 mmol, 1 eq) in 100 mL THF at 0 °C. After stirring at room temperature overnight water was slowly

added under ice-cooling and most of the THF was evaporated. The aqueous phase was extracted with 3 x 150 mL EtOAc. The organic layer was washed with 10 mL brine and the combined aqueous layers again extracted with 200 mL EtOAc. The combined organic layers are dried over $MgSO_4$ and evaporated yielding a white semisolid. (13.5 g, 99 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.19 (m, 1H, C***H***), 1.40 (s, 9H, Boc), 1.56 (m, 8H, 4 C***H***$_2$), 3.45 (d, 2H, ^3J = 6.0 Hz, C***H***$_2$-OH), 3.58 (m, 1H, C***H***-NHBoc), 3.70 (br, 1H, O***H***), 4.70 (m, 1H, N***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.23, 24.32 (-, 2 ***C***H$_2$), 28.42 (+, C(***C***H$_3$)$_3$), 29.43, 29.50 (-, 2 ***C***H$_2$), 38.59 (+, ***C***H-COOH), 46.36, 46.54 (+, ***C***H-NHBoc), 66.88, 67.03 (-, ***C***H$_2$-OH), 79.06 (C$_{quat}$, ***C***(CH$_3$)$_3$), 155.27 (C$_{quat}$, ***C***=O). CI-MS (NH_3) *m/z* (%): 230 (60) $[M + H]^+$, 243 (60) $[M + NH_4]^+$, 191 (100) $[M – C_4H_8 + NH_4]^+$. $C_{12}H_{23}NO_3$ (229.32).

tert-Butyl 4-formylcyclohexylcarbamate (4.7)[11]

A mixture of DMSO (16.1 mL, 226.8 mmol, 4 eq) and DCM (40 mL) was added slowly to a solution of oxalyl chloride (9.73 mL, 113.4 mmol, 2 eq) in DCM (50 mL) at -78 °C over 30 min. Then a solution of **4.6** (13.0 g, 56.7 mmol, 1 eq) in DCM (50 mL) was added. The resulting mixture was stirred at the same temperature for 2 h, and then NEt_3 (63 mL, 453.5 mmol, 8 eq) was added. After the resulting mixture was stirred at the same temperature for a further 30 min it was allowed to warm to room temperature and stirred overnight. 120 mL water were added and the resulting layers were separated. The aqueous layer was extracted twice with 60 mL DCM and the combined organic layers washed with brine dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (PE/EtOAc 70/30 v/v) to give **4.6** as yellow oil (8.8 g, 69 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.33 (m, 2H, C***H***$_2$), 1.40, 1.41 (s, 9H, Boc), 1.72 (m, 4H, 2 C***H***$_2$), 1.96 (m, 2H, C***H***$_2$), 2.10 (m, 1H, C***H***$_2$), 2.15 (m, 1H, C***H***-CHO), 3.53 (br, 1H, C***H***-NHBoc), 4.47 (br, 1H, N***H***), 9.59, 9.64 (s, 1H, C***H***O). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 22.51, 24.78 (-, 2 ***C***H$_2$), 28.39 (+, C(***C***H$_3$)$_3$), 29.77, 32.08 (-, 2 ***C***H$_2$), 46.88, 49.25 (+, ***C***H-CHO), 47.69 (+, ***C***H-NHBoc), 79.20 (C$_{quat}$, ***C***(CH$_3$)$_3$), 155.14 (C$_{quat}$, ***C***=O), 203.84, 204.51 (+, ***C***HO). CI-MS (NH_3) *m/z* (%): 245 (100) $[M + NH_4]^+$, 228 (10) $[M + H]^+$, 189 (50) $[M – C_4H_8 + NH_4]^+$. $C_{12}H_{21}NO_3$ (227.30).

tert-Butyl 4-(1*H*-imidazol-4-yl)cyclohexylcarbamate (4.8)

Finely powdered sodium cyanide (0.04 g, 0.7 mmol) was added in one portion to a stirred suspension of tosylmethyl isocyanide **3.16** (0.86 g, 4.4 mmol) and **4.7** (1.0 g, 4.4 mmol) in

30 mL of absolute ethanol at 0 °C. The reaction mixture became clear, and the solution was stirred for another 30 min. The solvent was evaporated under reduced pressure. The resulting slurry is dissolved in 15 mL 7 M NH_3 in methanol and stirred under microwave irradiation at 100 °C and 12 bar for 18 h. The solvent was evaporated and the residue was purified by flash chromatography (DCM/MeOH 90/10 v/v) to yield **4.7** as brown oil (0.8 g, 70 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.21 (m, 1H, C***H***$_2$), 1.42, 1.43 (s, 9H, Boc), 1.47 (m, 1H, C***H***$_2$), 1.64 – 1.91 (m, 4H, C***H***$_2$), 2.07 (m, 2H, C***H***$_2$), 2.54, 2.72 (m, 1H, C***H***-Im), 3.41, 3.75 (m, 1H, C***H***-NHBoc), 4.54, 4.88 (2d, 1H, ^3J = 7.2 Hz, N***H***), 6.59 (br, 1H, Im-***H***-1), 6.72, 6.76 (s, 1H, Im-***H***-5), 7.53, 7.55 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.53 (-, ***C***H$_2$), 28.45 (+, C(***C***H$_3$)$_3$), 29.80 (-, ***C***H$_2$), 31.58 (-, ***C***H$_2$), 33.27 (-, ***C***H$_2$), 34.10, 35.50 (+, ***C***H-Im), 46.40, 49.55 (+, ***C***H-NHBoc), 79.24 (C$_{quat}$, ***C***(CH$_3$)$_3$), 115.04 (+, Im-***C***-5), 134.37, 134.43 (+, Im-***C***-2), 142.02, 142.24 (C$_{quat}$, Im-***C***-4), 155.42 (C$_{quat}$, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 266 (100) $[M + H]^+$. $C_{14}H_{23}N_3O_2$ (265.35).

***tert*-Butyl 4-[4-(*tert*-butoxycarbonylamino)cyclohexyl]-1*H*-imidazole-1-carboxylate (4.9)[3]**

4.8 (3.0 g, 11.3 mmol) and NEt_3 (3.5 mL, 25 mmol) are dissolved in 100 mL $CHCl_3$ and the solution is cooled to 0 °C. A solution of Boc_2O (2.8 g, 12.5 mmol) in 50 mL $CHCl_3$ was added dropwise and the mixture was stirred at room temperature overnight. After washing the solution with 2x 80 mL aqueous $NaHCO_3$ the organic layer was dried over $MgSO_4$ and evaporated. The products ***cis*-4.9** and ***trans*-4.9** were separated by flash-chromatography (n-hexane/DCM/EtOH 7.5/2/0.5 v/v/v) as light yellow solids (total yield 3.81 g, 92 %);

Compound ***cis*-4.9**: mp 145 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.21 – 1.39 (m, 2H, C***H***$_2$), 1.42 (s, 9H, Boc), 1.47 – 1.56 (m, 2H, C***H***$_2$), 1.59 (s, 9H, Boc), 1.63 – 1.86 (m, 4H, C***H***$_2$), 2.64 (m, 1H, C***H***-Im), 3.77 (m, 1H, C***H***-NHBoc), 4.75 (br, 1H, N***H***), 7.05 (s, 1H, Im-***H***-5), 7.99 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.00 (-, 2 ***C***H$_2$), 27.91 (+, C(***C***H$_3$)$_3$), 28.44 (+, C(***C***H$_3$)$_3$), 29.78 (-, 2 ***C***H$_2$), 35.17 (+, **C**H-Im), 46.09 (+, ***C***H-NHBoc), 79.04 (C$_{quat}$, ***C***(CH$_3$)$_3$), 85.33 (C$_{quat}$, ***C***(CH$_3$)$_3$), 111.87 (+, Im-***C***-5), 136.55 (+, Im-***C***-2), 147.15 (C$_{quat}$, Im-***C***-4), 147.79 (C$_{quat}$, ***C***=O), 155.28 (C$_{quat}$, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 366 (100) $[M + H]^+$. $C_{19}H_{31}N_3O_4$ (365.47).

Compound ***trans*-4.9**: mp 151 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.15 – 1.49 (m, 4H, 2 C***H***$_2$), 1.44 (s, 9H, Boc), 1.60 (s, 9H, Boc), 2.09 (m, 4H, 2 C***H***$_2$), 2.48 (m, 1H, C***H***-Im), 3.46 (m, 1H, C***H***-NHBoc), 4.43 (br, 1H, N***H***), 7.02 (s, 1H, Im-***H***-5), 7.97 (s, 1H, Im-***H***-2).

^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.92 (+, C(***C***H_3)$_3$), 28.44 (+, C(***C***H_3)$_3$), 31.00 (-, 2 C***H***$_2$), 33.26 (-, 2 C***H***$_2$), 36.48 (+, ***C***H-Im), 49.49 (+, ***C***H-NHBoc), 79.11 (C_{quat}, ***C***$(CH_3)_3$), 85.27 (C_{quat}, ***C***$(CH_3)_3$), 111.21 (+, Im-***C***-5), 136.48 (+, Im-***C***-2), 147.22 (C_{quat}, Im-***C***-4), 148.30 (C_{quat}, ***C***=O), 155.27 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 366 (100) $[M + H]^+$. $C_{19}H_{31}N_3O_4$ (365.47).

cis-4-(1*H*-Imidazole-4-yl)cyclohexylamine dihydrochloride (4.2)[3]

cis*-4.9** (1.7 g, 4.7 mmol) was dissolved in 50 mL MeOH, 5 mL HCl 37 % were added and the solution was stirred overnight at room temperature. The solvent was evaporated to yield **4.2** as light yellow solid (0.91 g, 81 %); mp 230 – 232 °C. ^{1}H-NMR (300 MHz, D_2O): δ [ppm] = 1.58 (m, 2H, CH***$_2$), 1.87 (m, 6H, C***H***$_2$), 2.99 (m, 1H, C***H***), 3.34 (m, 1H, C***H***), 7.22 (s, 1H, Im-***H***-5), 8.51 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, D_2O): δ [ppm] = 25.53 (-, 2 ***C***H_2), 26.12 (-, 2 ***C***H_2), 29.95 (+, ***C***H-Im), 48.43 (+, ***C***H-NH_2), 115.20 (+, Im-***C***-5), 133.07 (+, Im-***C***-2), 136.16 (C_{quat}, Im-***C***-4). HRMS (EI-MS) calcd. for $C_9H_{15}N_3$ $[M^{+\bullet}]$ 165.1266; found 165.1269. IR (cm^{-1}) = 2983, 2933, 2829, 2780 (C-H), 1615, 1485, 1393, 1096.Anal. ($C_9H_{15}N_3 \cdot 2\ HCl \cdot H_2O$) C, H, N. $C_9H_{15}N_3 \cdot 2$ HCl (238.16).

trans-4-(1*H*-Imidazole-4-yl)cyclohexylamine dihydrochloride (4.3)[3]

trans*-4.9** (1.2 g, 3.3 mmol) was dissolved in 50 mL MeOH, 5 mL HCl 37 % were added and the solution was stirred overnight at room temperature. The solvent was evaporated to yield **4.3** as light yellow solid (0.73 g, 93 %); mp >250 °C. ^{1}H-NMR (300 MHz, D_2O): δ [ppm] = 1.47 (m, 4H, CH***$_2$), 2.05 (m, 4H, C***H***$_2$), 2.72 (m, 1H, C***H***), 3.17 (m, 1H, C***H***), 7.12 (s, 1H, Im-***H***-5), 8.47 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, D_2O): δ [ppm] = 29.26 (-, 2 ***C***H_2), 29.52 (-, 2 ***C***H_2), 32.30 (+, ***C***H-Im), 49.47 (+, ***C***H-NH_2), 114.02 (+, Im-***C***-5), 132.83 (+, Im-***C***-2), 137.28 (C_{quat}, Im-***C***-4). HRMS (EI-MS) calcd. for $C_9H_{15}N_3$ $[M^{+\bullet}]$ 165.1266; found 165.1266. IR (cm^{-1}) = 2992, 2822, 2781 (C-H), 1620, 1517, 1450, 1390, 1150. Anal. ($C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.1\ H_2O$) C, H, N. $C_9H_{15}N_3 \cdot 2$ HCl (238.16).

4.4.1.3 Preparation of the cyanoguanidines 4.10-4.13

General Procedure[12, 13]

Hydrochlorides of **4.2** and **4.3** were converted into the bases by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). The isourea (1 eq) and the

pertinent amine (1 eq) in MeCN were heated under microwave irradiation at 150 °C for 15 min. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (DCM/MeOH 98/2 – 80/20 v/v).

2-Cyano-1-[*cis*-4-(1*H*-imidazol-4-yl)cyclohexyl]-3-methylguanidine (4.10)

The title compound was prepared from **4.2** (0.08 g, 0.48 mmol) and **3.27** (0.085 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.09 g, 76 %); mp 125 – 127 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.71 (m, 4H, C***H***$_2$), 1.85 (m, 2H, C***H***$_2$), 1.95 (m, 2H, C***H***$_2$), 2.79 (s, 3H, C***H***$_3$-N), 2.84 (s, 1H, C***H***-Im), 3.76 (m, 1H, C***H***-N), 6.86 (s, 1H, Im-***H***-5), 7.59 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.79 (+, ***C***H$_3$), 28.84 (-, 2 ***C***H$_2$), 29.96 (-, 2 ***C***H$_2$), 34.05 (+, ***C***H-Im), 50.31 (+, ***C***H-N), 117.56 (+, Im-***C***-5), 120.31 (C$_{quat}$, ***C***≡N), 135.75 (+, Im-***C***-2), 141.54 (C$_{quat}$, Im-***C***-4), 161.20 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{18}N_6$ [$M^{+\bullet}$] 246.1593; found 246.1593. IR (cm^{-1}) = 3301 (N-H), 2932, 2861 (C-H), 2159 (C≡N), 1567 (C=N), 1423, 1356, 1232, 1165. Anal. ($C_{12}H_{18}N_6 \cdot 0.55\ H_2O$) C, H, N. $C_{12}H_{18}N_6$ (246.31).

2-Cyano-1-[*trans*-4-(1*H*-imidazol-4-yl)cyclohexyl]-3-methylguanidine (4.11)

The title compound was prepared from **4.3** (0.08 g, 0.48 mmol) and **3.27** (0.085 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.11 g, 93 %); mp 120 – 121 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.47 (m, 4H, C***H***$_2$), 2.05 (m, 4H, C***H***$_2$), 2.55 (m, 1H, C***H***-Im), 2.80 (s, 3H, C***H***$_3$-N), 3.59 (m, 1H, C***H***-N), 6.76 (s, 1H, Im-***H***-5), 7.55 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.78 (+, ***C***H$_3$), 32.84 (-, 2 ***C***H$_2$), 33.63 (-, 2 ***C***H$_2$), 36.68 (+, ***C***H-Im), 52.08 (+, ***C***H-N), 115.93 (+, Im-***C***-5), 120.31 (C$_{quat}$, ***C***≡N), 135.68 (+, Im-***C***-2), 143.15 (C$_{quat}$, Im-***C***-4), 161.23 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{18}N_6$ [$M^{+\bullet}$] 246.1593; found 246.1592. IR (cm^{-1}) = 3260 (N-H), 2931, 2858 (C-H), 2159 (C≡N), 1574 (C=N), 1423, 1361, 1217, 1089. Anal. ($C_{12}H_{18}N_6 \cdot 0.65\ H_2O$) C, H, N. $C_{12}H_{18}N_6$ (246.31).

2-Cyano-1-[*cis*-4-(1*H*-imidazol-4-yl)cyclohexyl]-3-[2-(phenylthio)ethyl]guanidine (4.12)

The title compound was prepared from **4.2** (0.08 g, 0.48 mmol) and **3.31** (0.144 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.13 g, 73 %); mp 88 – 90 °C. ^{1}H-NMR (300 MHz, CD_3OD):

δ [ppm] = 1.70 (m, 4H, C***H***$_2$), 1.85 (m, 4H, C***H***$_2$), 2.80 (m, 1H, C***H***-N), 3.10 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-N), 3.43 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-S), 3.69 (s, 1H, C***H***-Im), 6.84 (s, 1H, Im-***H***-5), 7.17 (m, 1H, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.39 (m, 2H, Ph-***H***), 7.58 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.64 (-, 2 ***C***H$_2$), 30.03 (-, 2 ***C***H$_2$), 33.70 (-, ***C***H$_2$-S), 34.37 (+, ***C***H-Im), 42.33 (-, ***C***H$_2$-N), 49.91 (+, ***C***H-N), 117.19 (+, Im-***C***-5), 120.04 (C_{quat}, ***C***≡N), 127.35 (+, Ph-***C***-4), 130.17 (+, 2 Ph-***C***), 130.42 (+, 2 Ph-***C***), 135.77 (+, Im-***C***-2), 136.97 (C_{quat}, Ph-***C***-1), 141.92 (C_{quat}, Im-***C***-4), 160.32 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6S$ [$M^{+\bullet}$] 368.1783; found 368.1777. IR (cm^{-1}) = 3254 (N-H), 3131, 2930, 2858 (C-H), 2159 (C≡N), 1571 (C=N), 1438, 1355, 1300, 1089. Anal. ($C_{14}H_{24}N_6S$. 0.25 H_2O) C, H, N. $C_{14}H_{24}N_6S$ (368.50)

2-Cyano-1-[*trans*-4-(1*H*-imidazol-4-yl)cyclohexyl]-3-[2-(phenylthio)ethyl]guanidine (4.13)

The title compound was prepared from **4.3** (0.08 g, 0.48 mmol) and **3.31** (0.144 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.12 g, 68 %); mp 95 – 96 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.45 (m, 4H, C***H***$_2$), 2.03 (m, 4H, C***H***$_2$), 2.54 (m, 1H, C***H***-N), 3.11 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-N), 3.42 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-S), 3.45 (m, 1H, C***H***-Im), 6.75 (s, 1H, Im-***H***-5), 7.19 (m, 1H, Ph-***H***-4), 7.30 (m, 2H, Ph-***H***), 7.40 (m, 2H, Ph-***H***), 7.55 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 32.68 (-, 2 ***C***H$_2$), 33.58 (-, 2 ***C***H$_2$ + ***C***H$_2$-S), 36.59 (+, ***C***H-Im), 42.31 (-, ***C***H$_2$-N), 52.12 (+, ***C***H-N), 115.13 (+, Im-***C***-5), 119.97 (C_{quat}, ***C***≡N), 127.34 (+, Ph-***C***-4), 130.18 (+, 2 Ph-***C***), 130.44 (+, 2 Ph-***C***), 135.35 (C_{quat}, Im-***C***-4), 135.70 (+, Im-***C***-2), 137.01 (C_{quat}, Ph-***C***-1), 160.28 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6S$ [$M^{+\bullet}$] 368.1783; found 368.1781. IR (cm^{-1}) = 3253 (N-H), 3138, 2932, 2857 (C-H), 2159 (C≡N), 1568 (C=N), 1437, 1361, 1231. Anal. ($C_{14}H_{24}N_6S$. 0.25 H_2O) C, H, N. $C_{14}H_{24}N_6S$ (368.50).

4.4.2 Pharmacological methods

4.4.2.1 Materials

See section 3.4.2.1.

4.4.2.2 [^{35}S]GTPγS binding assay[14, 15]

See section 3.4.2.2.

4.4.2.3 Data analysis and pharmacological parameters

See section 3.4.2.3.

4.5 References

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Chapter 5

Synthesis, chiral separation and pharmacological characterization of imidazolylcyclopentylmethyl-cyanoguanidines

5.1 Introduction

Cyanoguanidines such as UR-PI376 and OUP-16 are potent histamine H_4R ligands.[1] The major drawback of these and many other H_4R ligands is their residual activity at the H_3R subtype. Aiming at higher selectivity for the H_4R over the other HR subtypes, in particular the H_3R, conformationally constrained carbocycles were introduced in compounds related to UR-PI376 (**5.1**). A phenylene spacer proved to be inappropriate (see chapter 3), whereas a more flexible cyclohexylene linker (see chapter 4) was tolerated, suggesting further exploration of conformational constraints. Watanabe et al.[2] synthesized a series of conformationally restricted histamine analogues, using a chiral cyclopropane structure (e.g. **5.2**), and demonstrated that the pharmacological profiles of these compounds depends on the stereochemistry. The importance of preorientation, regioisomers and the three dimensional structure was also demonstrated by Kitbunnadaj et al.[3] in a series of histamine analogues lacking a basic side chain. Moreover, imifuramine (**5.3**), a chiral 2,5-disubstituted tetrahydrofuran derivative, was found to be a potent full H_3R agonist with 50-fold selectivity for the hH_3R versus the hH_4R (Figure 5.1).[4, 5] Hashimoto et al.[6] synthesized imifuramine analogues and investigated these compounds at the hH_3R and hH_4R. All four imifuramine-type stereoisomers were partial to full agonists at both histamine receptor subtypes with selectivity for the hH_3R. The activity of the compounds at the hH_3R was reduced by introduction of a non-basic cyanoguanidine moiety instead of the primary amino group. Additionally, this approach resulted in most cases in an increase in potency and intrinsic activity at the hH_4R. The respective cyanoguanidine analogue of imifuramine, the (2*R*,5*R*)-configured isomer OUP-16 (**5.4**),[6] proved to be a rather potent full hH_4R agonist with 40-fold selectivity over the hH_3R, where **5.4** behaves as a moderate partial agonist. Interestingly, a distinct stereoselectivity in favor of **5.4** compared to its optical antipode, the (2*S*,5*S*)-configured enantiomer, was observed.

These results prompted us to further elucidate the optimal ring size and balance between rigidification and remaining flexibility of imidazolylcycloalkylcyanoguanidines related to UR-PI376. Aiming at H_4R agonists with improved selectivity and considering that substances with reduced flexibility might help to refine H_4R-ligand interaction models, we synthesized a series of compounds with a cyclopentan-1,3-diyl linker, thereby combining the structural features of UR-PI376 and OUP-16 (Figure 5.1).

The first approach was focused on the racemic compounds in order to get information if the constraints are tolerated at all. Taking into consideration the published data on tetrahydrofuranes such as OUP-16, differences between the pharmacological properties of the enantiomers could be expected. Therefore, a method for the separation of the most interesting racemates had to be developed to allow for the characterization of the enantiomerically pure stereoisomers.

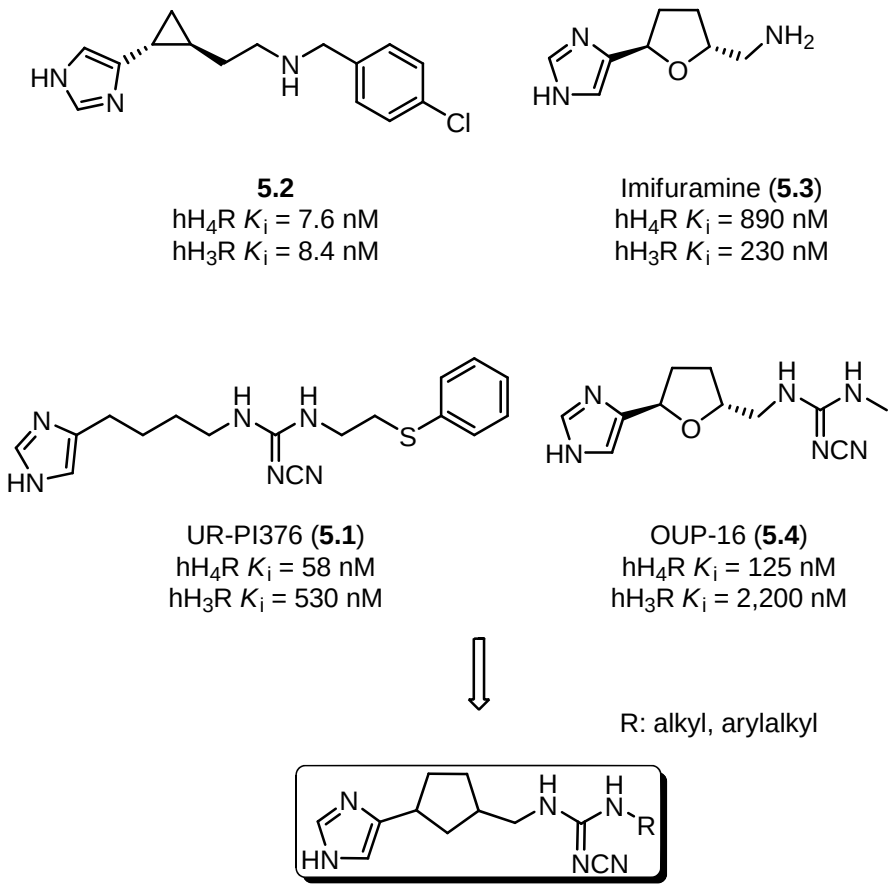


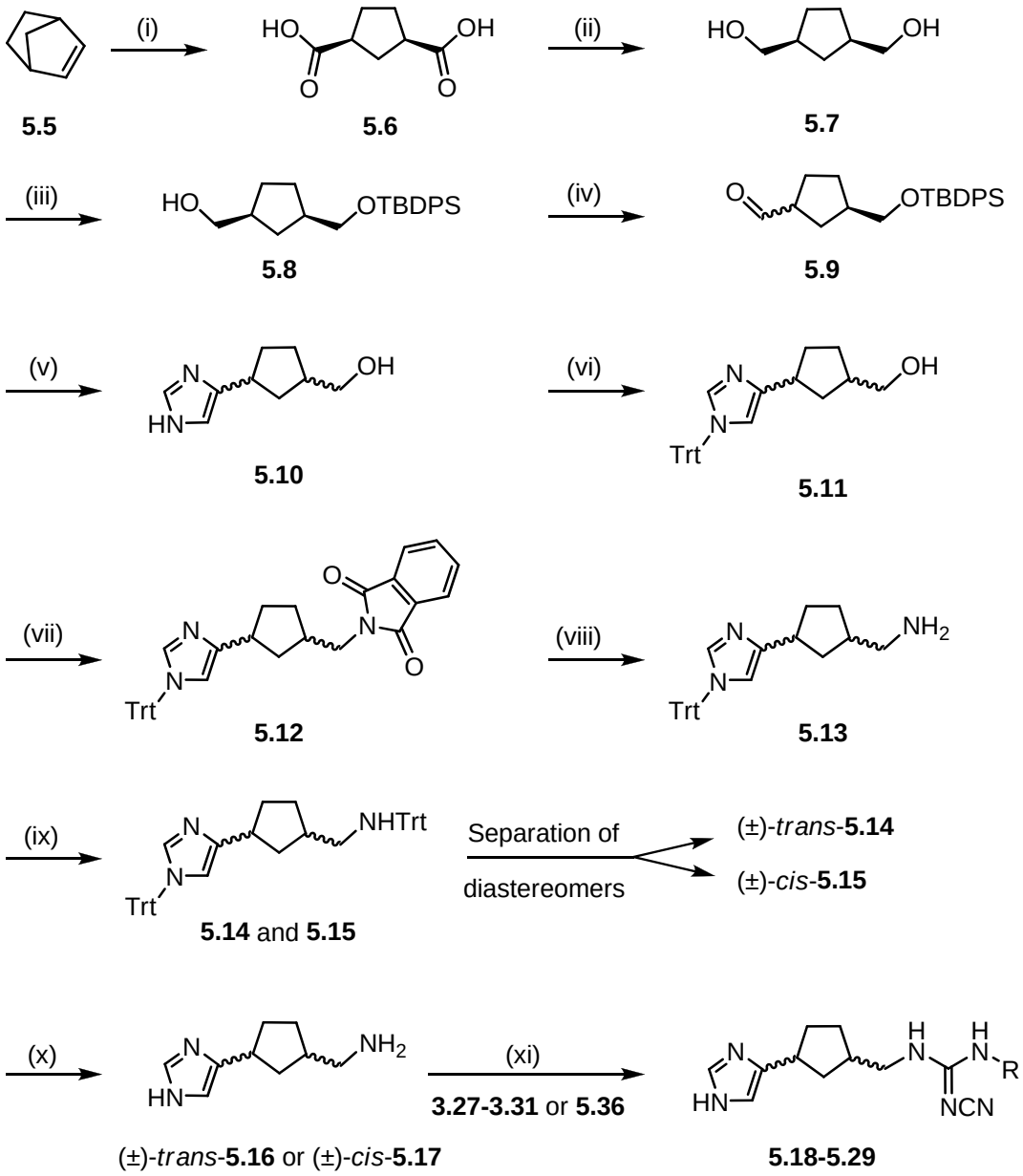
Figure 5.1 Structure of selected histamine H_4R ligands and the intended combination thereof.

This chapter describes the synthesis of racemic imidazolylcyclopentylmethylcyanoguanidines Moreover, the chiral separation of the stereoisomers and the assignment of the absolute configuration, based on a stereoselective synthesis. All synthesized compounds were pharmacologically characterized in functional assays ([^{35}S]GTPγS assay, GTPase assay) at the four human HR subtypes. Additionally, the separated stereoisomers were investigated in radioligand binding studies on human histamine receptors and in luciferase reporter gene assays at the human and the murine H_4R.

5.2 Chemistry

5.2.1 Synthesis of racemic imidazolylcyclopentylmethylcyanoguanidines

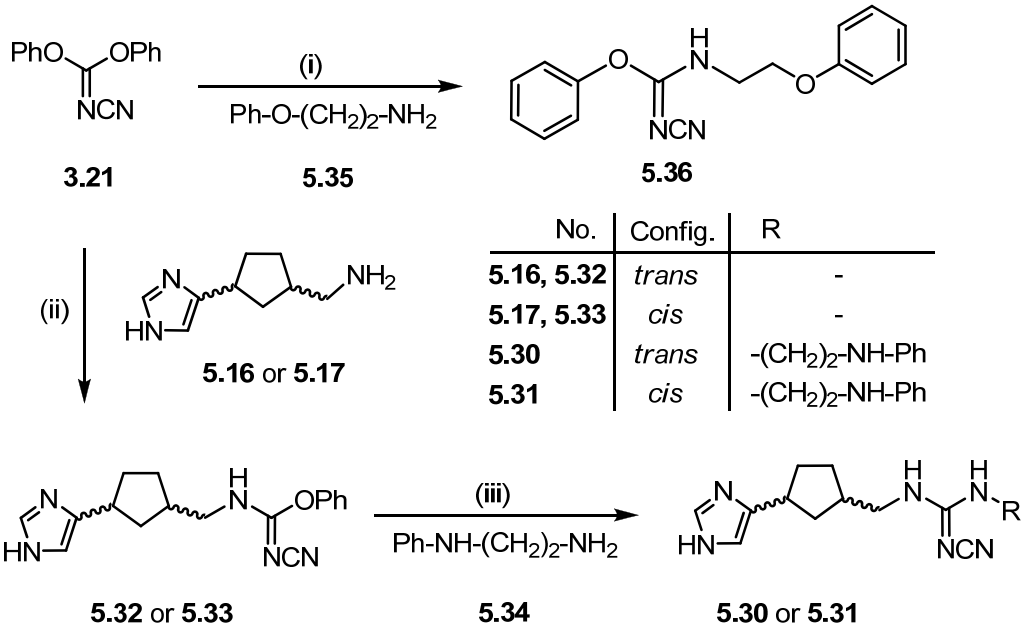
The synthesis of the *cis*- and *trans*-configured imidazolylcyclopentylmethylcyanoguanidines **5.18**-**5.31** is outlined in Scheme 5.1 and Scheme 5.2. The compounds were synthesized as racemic mixtures in 11 steps starting from norbornene (**5.5**). *Cis*-selective oxidation of **5.5** yielded norcamphoric acid (**5.6**),[7] subsequent reduction with borane[9] gave the diol **5.7,**[8, 9] monoprotection with a *tert*-butyldiphenylsilyl group and Swern oxidation[10] of the unprotected alcohol in **5.8** resulted in the corresponding aldehyde **5.9**. The construction of the imidazole ring turned out to be the crucial step, which was achieved by treating the aldehyde **5.9** with tosylmethyl isocyanide (**3.16**) followed by ammonia in methanol.[11] Due to the basic conditions in this reaction step isomerization occurred, and all four stereoisomers were present in the reaction mixture from now on. After protection of the imidazole nitrogen with a trityl group, the alcohol **5.11** was converted to the phthalimide **5.12** under *Mitsunobu* conditions,[12] and hydrazinolysis gave the primary amine **5.13**. The separation of the diastereomers was only possible when an additional trityl group was coupled to the primary amine in **5.13** and allowed the separation of the diastereomers **5.14** and **5.15** by flash chromatography. Deprotection under acidic conditions and liberation of the free amines with the help of a basic ion exchanger gave the *cis*- and *trans*-configured imidazolylcyclopentylmethylamines **5.16** and **5.17**. The cyanoguanidines **5.18**-**5.29** (Scheme 5.1 and Scheme 5.2) were synthesized by analogy with a previously described synthetic route (see Chapter 3).[13-16] The synthesis of the isourea derivative **5.36** is depicted in Scheme 5.2.



No.	Config.	R	No.	Config.	R
5.18	*trans*	$-CH_3$	**5.24**	*trans*	$-(CH_2)_3$-Ph
5.19	*cis*	$-CH_3$	**5.25**	*cis*	$-(CH_2)_3$-Ph
5.20	*trans*	-cPr	**5.26**	*trans*	$-(CH_2)_2$-S-Ph
5.21	*cis*	-cPr	**5.27**	*cis*	$-(CH_2)_2$-S-Ph
5.22	*trans*	$-CH_2-CH(CH_3)_2$	**5.28**	*trans*	$-(CH_2)_2$-O-Ph
5.23	*cis*	$-CH_2-CH(CH_3)_2$	**5.29**	*cis*	$-(CH_2)_2$-O-Ph

Scheme 5.1 Synthesis of the cyanoguanidines **5.18-5.29** as racemic mixtures (only one isomer is depicted). Reagents and conditions: (i) $RuCl_3$ (2.2 mol %), $NaIO_4$ (4.1 eq), EtOAc/MeCN/water 2:2:3, 2 d, rt, 99 %; (ii) BH_3·THF (4.5 eq), THF, 12 h, 0 °C → rt, 80 %; (iii) TBDPSCl (0.98 eq), DIPEA, DCM, 15 h, 0 °C → rt, 57 %; (iv) DMSO (4 eq), NEt_3 (8 eq), $(COCl)_2$ (2 eq), DCM, 3 h, -78 °C → 12 h, rt, 92 %; (v) a) TosMIC (**3.16**, 1 eq), NaCN (0.15 eq), EtOH, 30 min, 0 °C; b) 7 M NH_3 in MeOH, 18 h, 100 °C, 78 %; c) TrtCl (1 eq), NEt_3 (2 eq), DMF, 24 h, rt, 83 %; (vi) phthalimide (1.1 eq), PPh_3 (1.1 eq), DIAD (1.1 eq), THF, overnight, 0 °C → rt, 75 %; (vii) N_2H_4 · H_2O (5 eq), EtOH, 1.5 h, reflux → 1 h, rt, 92 %; (viii) TrtCl (1.5 eq), NEt_3 (2 eq), DCM, 24 h, rt, 95 %; (ix) 37 % HCl, MeOH, 3 h, reflux, ion exchanger, 88 %; (x) MeCN, microwave 150 °C, 15 min, 59-95 %.

Diphenyl cyanimidocarbonate (**3.21**) is prone to undergo two-fold aminolysis when treated with one equivalent of an ethylenediamine derivative such as N-phenylethylenediamine, resulting in the corresponding cyaniminoimidazoles. Therefore, in case of the synthesis of **5.30-5.31** the coupling steps were carried out in reversed order: the amines **5.16** and **5.17** were first allowed to react with **3.21** to give **5.32** and **5.33**, which were treated with the aliphatic amine **5.34** to yield the cyanoguanidines **5.30**-**5.31** (Scheme 5.2).



No.	Config.	R
5.16, 5.32	*trans*	-
5.17, 5.33	*cis*	-
5.30	*trans*	-$(CH_2)_2$-NH-Ph
5.31	*cis*	-$(CH_2)_2$-NH-Ph

Scheme 5.2 Synthesis of the isoureas **5.32, 5.33** and **5.36** and the cyanoguanidines **5.30** and **5.31**. Reagents and conditions: (i) 2-Propanol, 1 h, rt, 94 %; (ii) 2-propanol, 1 h, rt, 86-97 %; (iii) MeCN, microwave 150 ^{o}C, 15 min, 55-69 %.

5.2.2 Chiral separation of selected racemates

The synthesized compounds are chiral and comprise two stereogenic centers in the cyclopentane ring. Since the monoprotection of meso compound **5.7** with TBDPSCl does not proceed in a stereoselective manner, the derivative **5.8** was obtained as racemate. In the next step, under basic reaction conditions, epimerization did take place. Thenceforward, four stereoisomers have been present in the reaction mixture. The *cis*- and *trans*-diastereomers were successfully separated by flash chromatography. The obtained pairs of racemic cyanoguanidines were pharmacologically characterized prior to opting for enantioseparation.

Considering the obtained pharmacological data and structure-activity relationships (see 5.3.1) and taking into account the reported stereoselectivity of the tetrahydrofurane analogues,[6] the separation of the most promising racemates was attempted.

To obtain more detailed structure-activity and selectivity data, the complete set of four stereoisomers was necessary. A stereoselective synthesis yields only one isomer, the consequence being that four variations of the whole procedure are needed and have to be performed to obtain the four desired target compounds. Moreover, the development of a asymmetric synthesis pathway seems not easily possible for this substance class and the intended high enantiomeric excess is hardly accessible. Hence, a stereoselective synthesis was discarded due little prospect of success. Another option is the separation of enantiomers by fractionized crystallization of diastereomeric salts. Unfortunately all attempts to crystallize the cyanoguanidines failed. The amine precursors **5.16** or **5.17** can be crystallized as hydrochlorides, but again crystallization failed when using various chiral acids. The low amount of available substance obtained from the ten step synthesis prevented further extensive studies on fractionized crystallization. Instead chiral chromatography was tested.

Separations of enantiomers by achiral chromatographical methods can be achieved via derivatization of the compounds with chiral auxiliaries and subsequent separation of the diastereomeric products, for example by HPLC. In principle, a separation without pre-column derivatization can be attained when adding chiral additives to the eluent, using achiral stationary phases. The most elegant and versatile way is the separation of enantiomers with the help of HPLC on a chiral stationary phase. The challenge is to find a suitable chiral selector for the respective separation problem, providing sufficient resolution. As only small amounts of the enantiomers were required for pharmacological investigations, the prospects of chiral separation by HPLC on a semi-preparative scale were evaluated. The most promising racemates **5.26** and **5.27** were selected for a small screening using various chiral HPLC columns and conditions. These preliminary experiments were performed by Sirius Fine Chemicals GmbH (Bremen, Germany), and the results are shown in Table 5.1.

Table 5.1 Overview about the evaluated HPLC methods and the observed results.

Compound No.	Column	Eluent	t_r [min]	Separation
5.27	Chiralpak IA 20µm	70:30 MeOH/H_2O	3-7	-
5.27	Chiralpak IA 20µm	70:30 MeOH/H_2O/0,1 % $EtNH_2$	4.8	-
5.27	Chiralpak IA 20µm	60:40 MeCN/DCM	5	-
5.27	Chiralpak IA 20µm	90:10 DCM/EtOH	3	-
5.27	Chiralpak IA 20µm	95:5 DCM/MeOH	2.8	-
5.27	Chiralpak IA 20µm	93:7 DCM/2-PrOH	6	- (1)
5.27	Chiralpak IA 20µm	97:3 DCM/EtOH/0,1 % $EtNH_2$	4	-
5.26	Chiralpak IA 20µm	70:30 MeOH/H_2O/0,1 % $EtNH_2$	4.8	- (2)
5.26	Chiralcel OJ 20µm	80:20 Hexane/2-PrOH	3	- (3)
5.27	Chiralcel OJ 20µm	80:20 Hexane/2-PrOH	4.0; 4.7	+, low resolution (4)
5.27	Chiralcel OJ 20µm	80:20 Cyclohexane/2-PrOH	3.5	-
5.26	Chiralcel OJ 20µm	80:20 Heptane/2-PrOH	7.5	-
5.27	Chiralcel OJ 20µm	80:20 Heptane/2-PrOH	7.0; 9.0	+, low resolution
5.27	Chiralcel OD-H 5 µm	40:60-70:30 MeOH/H_2O/0.05 % TFA	14.2	-
5.26	Chiralcel OD-H 5 µm	70:30 Heptane/2-PrOH	5.5	-
5.27	Phenomenex Lux 2 Cellulose 20 µm	100 MeCN	10; 12	+, low resolution (5)
5.27	Phenomenex Lux 2 Cellulose 20 µm	50:50 PE/1-PrOH	3	-
5.27	Phenomenex Lux 2 Cellulose 20 µm	65:35 PE/2-PrOH	7	- (6)
5.26	Phenomenex Lux 2 Cellulose 20 µm	50:50 PE/1-PrOH	3	- (7)
5.27	Phenomenex Lux 2 Cellulose 20 µm	65:35 PE/EtOH	3.5	- (8)

5.26	Phenomenex Lux 2 Cellulose 20 µm	98:2 MeCN/2-PrOH	8; 9	+, low resolution
5.26	Phenomenex Lux 2 Cellulose 20 µm	98:2 MeCN/EtOH/0,1 % $EtNH_2$	7; 8	+, low resolution

This screening provided a small selection of chiral selectors with promising properties. The amylose based Chiralpak IA and the Chiralcel OD-H column did not separate the investigated racemates. For the cellulose based Phenomenex Lux 2 and the Chiralcel OJ column a separation was observed under specific conditions. For selected HPLC traces see Figure 5.2.

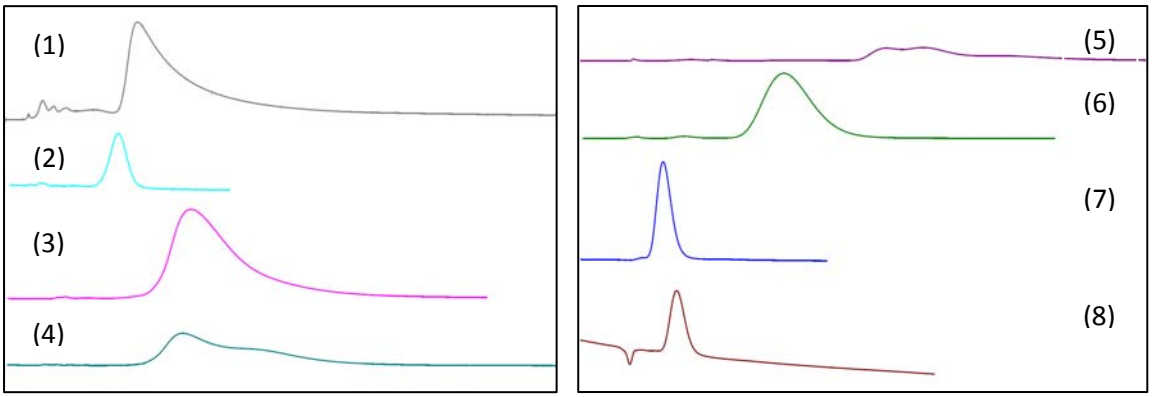


Figure 5.2 Selected HPLC traces of the racemates. For details see Table 5.1**.**

The best results were found for the Chiralcel OJ column, using heptane/2-propanol as eluent. According to the manufacturer's information, the chiral stationary phase consists of a silica based matrix coated with cellulose-tris(4-methylbenzoate) as the chiral selector. To optimize the resolution, all following separations were performed in our laboratory on Chiralcel OJ-H columns with a particle size of 5 instead of 20 µm. HPLC was performed in the normal phase mode, using mixtures of heptane/2-propanol and isocratic elution.

Using this system the racemates **5.18**, **5.19**, **5.26** and **5.27** could be successfully separated in less than 30 minutes run time. The best result was obtained for **5.27**, showing complete baseline separation. Changing the composition of the eluent only broadened the peaks but did not improve the resolution of the separation. However, the performance of this method was sufficient for the transfer to semi-preparative scale (10-20 mg/injection). Chromatograms of the racemates are depicted in Figure 5.3.

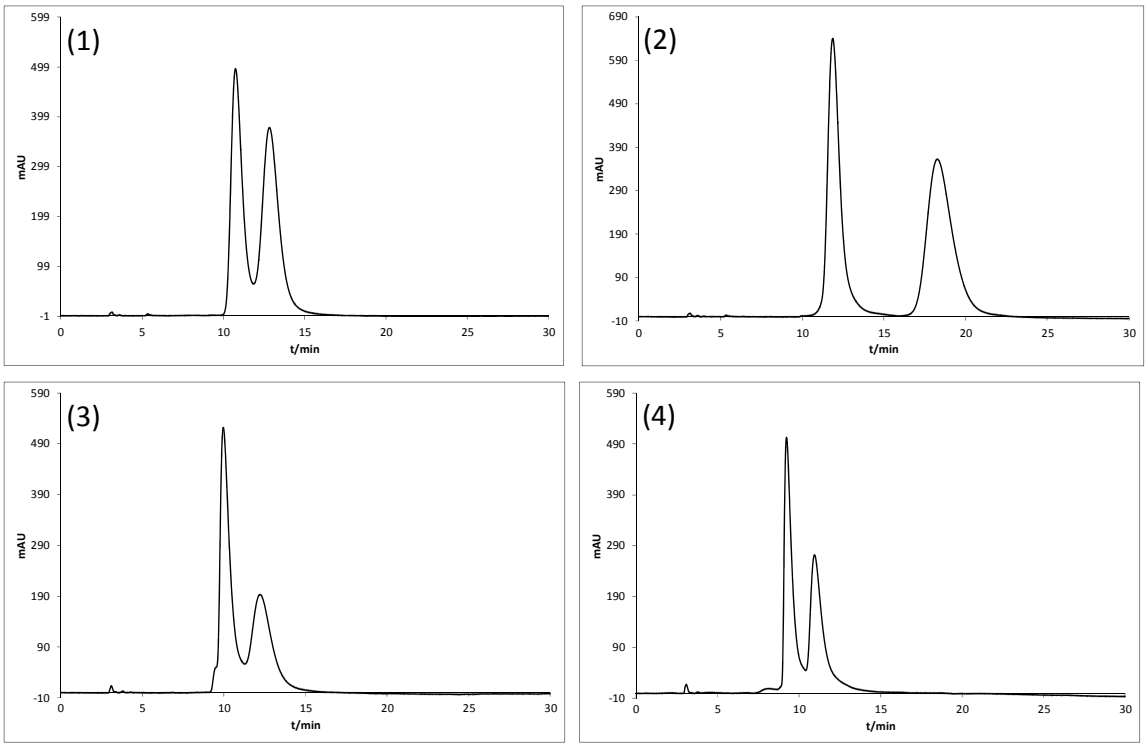


Figure 5.3 Successful analytical separation of the racemates on a Chiralcel OJ-H column (5 µm). (1) **5.26**, (2) **5.27**, heptane:2-propanol 80/20; (3) **5.18**, (4) **5.19**, heptane:2-propanol 85/15; flow: 1 mL/min, temperature: 30 °C, UV-detection at 215 nm.

After optimization of the method for analytical HPLC (for details see Figure **5.3** and experimental section) the mixtures were separated by preparative HPLC on a semi-preparative Chiralcel OJ-H column (5 µm, 20x250 mm). The conditions could be directly transferred from analytical to preparative HPLC (see Figure 5.4). If the enantiomers were not totally resolved by the chiral selector after the first separation, the whole procedure was repeated.

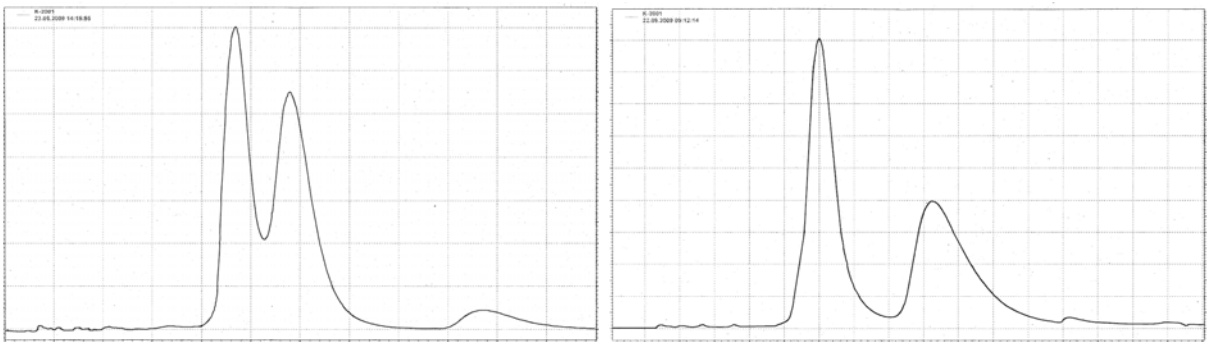


Figure 5.4 Separation of racemic **5.26** (left) and **5.27** (right) on a semi-preparative Chiralcel OJ-H column (5 µM, 20x250 mm). Heptane:2-propanol 85/15; flow: 18 mL/min, temperature: 22 °C, UV-detection at 220 nm.

The enantiomeric purity of the compounds after the separation was determined by peak integration for each enantiomer. The circular dichroism (CD) of the individual enantiomers was determined at 240 nM using a CD detector directly coupled to the HPLC system. The observed CD signal confirmed the high enantiomeric purity.

Representative chromatograms (UV and CD absorption) are depicted in Figure 5.5 and Figure 5.6. The enantiomeric purities, the circular dichroism at 240 nm and the relative optical rotation (+ or -) of all isolated stereoisomers are summarized in Table 5.2. The separated isomers are numbered **a** and **b** in order of their elution.

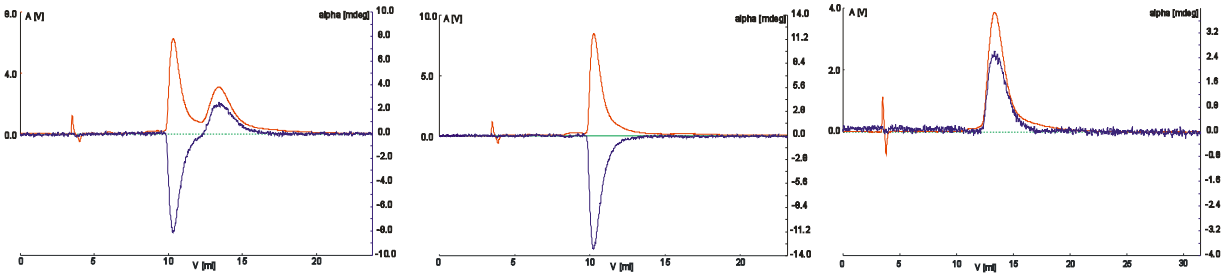


Figure 5.5 Chromatograms of racemic *cis*-**5.19** (left) and the pure enantiomers **5.19a** (middle) and **5.19b** (right) after the separation. Red: UV absorption at 240 nm; blue: circular dichroism at 240 nm. Concentration 1 mg/mL, heptane:2-propanol 85/15; flow: 0.5 mL/min, temperature: 25 °C.

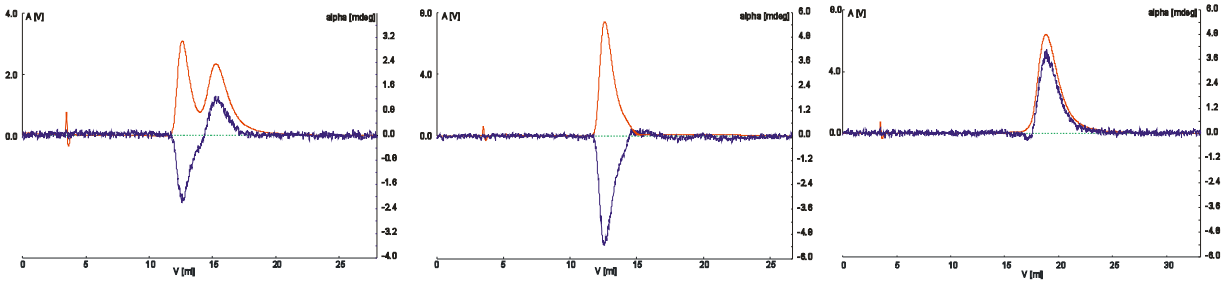


Figure 5.6 Chromatograms of racemic *trans*-**5.26** (left) and the enantiomerically pure isomers **5.26a** (*trans*-(+)-(1*S*,3*S*)-UR-RG98) (middle) and **5.26b** (right) after the separation. Red: UV absorption at 240 nm; blue: circular dichroism at 240 nm. Concentration 1 mg/mL, heptane:2-propanol 80/20; flow: 0.5 mL/min, temperature: 25 °C.

All isomers were obtained in high enantiomeric purity (ee > 95 %). For both, the methyl and the phenylthioethyl substituted derivatives, the first eluted *cis*-enantiomer (**5.19a**, **5.27a**) gave the negative CD signal, the second eluted *cis*-enantiomer (**5.19b**, **5.27b**) the positive CD signal. The same was observed for the optical rotation at 589 nm. A different behaviour was found for the *trans*-isomers. The first eluted methylcyanoguanidine **5.18a** gave the positive

CD signal and a negative optical rotation, the first eluted phenylthioethyl-substituted cyanoguanidine **5.26a** gave the negative CD signal and a positive optical rotation (Table 5.2).

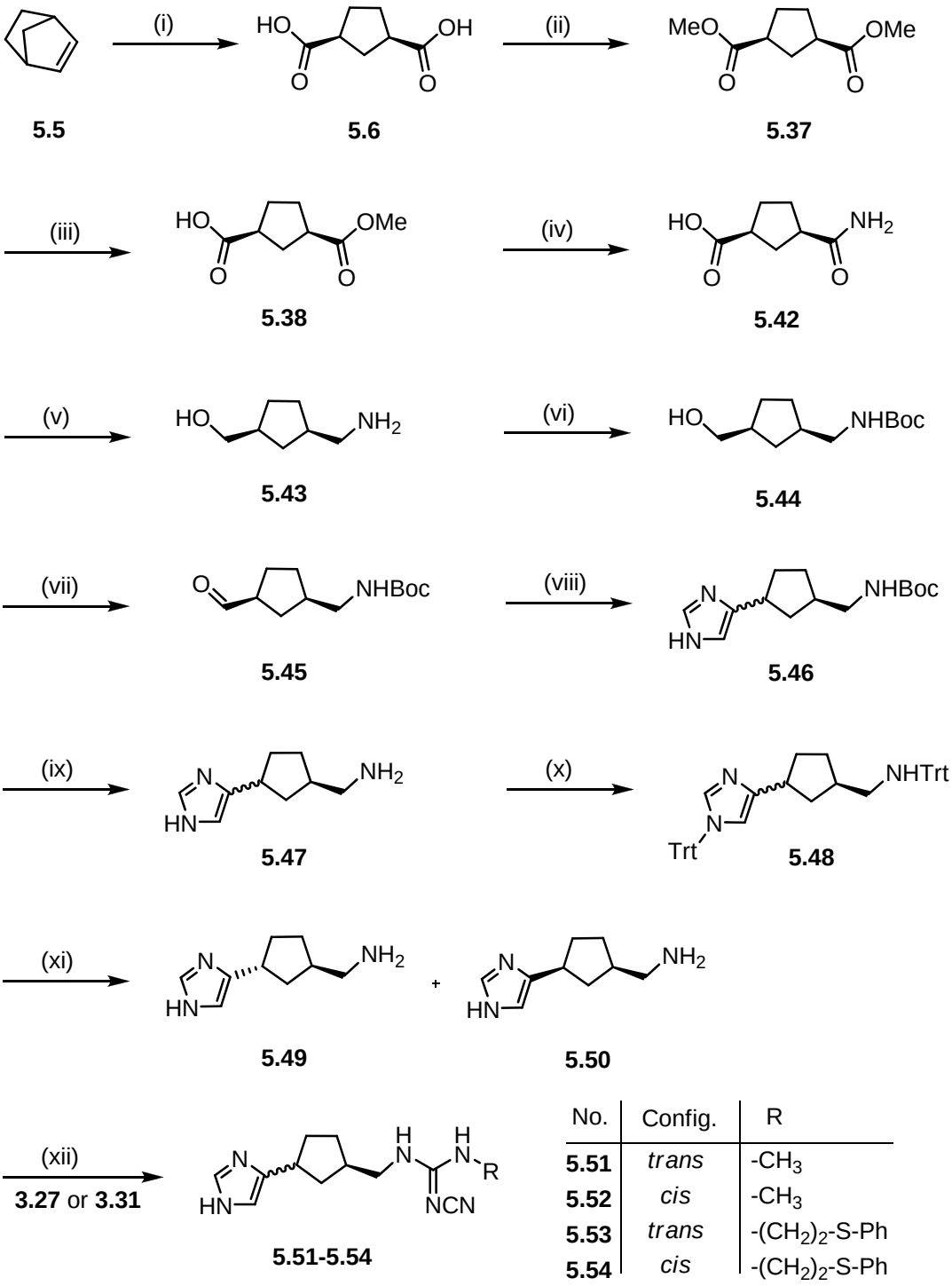
Table 5.2 Resolution of HPLC peaks, selectivity factors, enantiomeric purities, circular dichroism and optical rotation of all isolated stereoisomers.

Compound	R_s[a]	α[b]	CD[c]	OR[d]	ee[e] [%]
5.18a	1.23	1.33	+	-	98
5.18b			-	+	96
5.19a	1.23	1.27	-	-	> 99
5.19b			+	+	97
5.26a	1.23	1.27	-	+	> 99
5.26b			+	-	96
5.27a	2.67	1.72	-	-	> 99
5.27b			+	+	97

[a] R_s = resolution of HPLC peaks; injection: 10 µL of a 1 mg/mL solution; [b] α = selectivity factor (k'_b/k'_a); [c] circular dichroism at 240 nm; [d] relative optical rotation at 589 nm; [e] enantiomeric excess.

5.2.3 Determination of the absolute configuration by an enzyme assisted stereoselective synthesis

As mentioned before, all attempts to crystallize the cyanoguanidines for x-ray analysis failed. Therefore, aiming at elucidation of the absolute configuration of the title compounds, a stereoselective synthesis was developed, which selectively provided one *cis* and one *trans* isomer with known absolute configuration. Comparison of the optical rotation and HPLC data of these products with the data collected for the investigated isomers allowed the unequivocal assignment of the absolute configuration of all stereoisomers.



5.5 **5.6** **5.37**

5.38 **5.42**

5.43 **5.44**

5.45 **5.46**

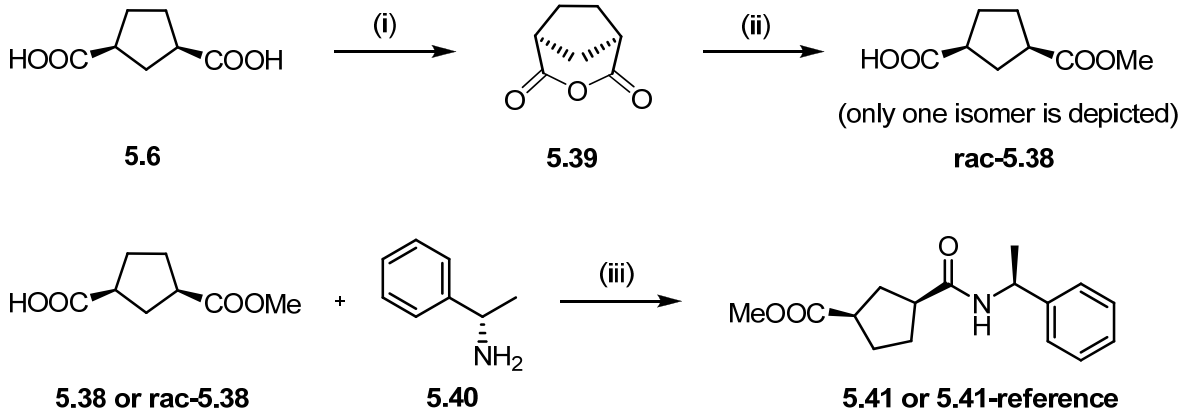
5.47 **5.48**

5.49 **5.50**

No.	Config.	R
5.51	*trans*	-CH_3
5.52	*cis*	-CH_3
5.53	*trans*	-$(CH_2)_2$-S-Ph
5.54	*cis*	-$(CH_2)_2$-S-Ph

Scheme 5.3 Synthesis of the cyanoguanidines **5.51-5.54**. Reagents and conditions: (i) $RuCl_3$ (2.2 mol %), $NaIO_4$ (4.1 eq), EtOAc/MeCN/water 2:2:3, 2 d, rt, 99 %; (ii) Dowex® 50x8 H^+-resin, MeOH, 12 h, reflux, 88 %; (iii) cholesterol esterase (4000 U), 1 M NaOH, 0.05 M phosphate buffer pH 7.0, 1 % MeCN, 37 °C, 89 %, ee 72 %; (iv) 32 % $NH_{3(aq)}$, overnight, rt, 100 %; (v) BH_3·THF (4.5 eq), THF, 1 h, 0 °C → rt, overnight, reflux, 70 %; (vi) Boc_2O (1.1 eq), 1 M NaOH, dioxane/water 2/1 (v/v), overnight, 0 °C → rt, 74 %; (vii) DMSO (4 eq), NEt_3 (8 eq), $(COCl)_2$ (2 eq), DCM, 3 h, -78 °C → 12 h, rt, 61 %; (viii) a) TosMIC (**3.16**, 1 eq), NaCN (0.15 eq), EtOH, 30 min, 0 °C; b) 7 M NH_3 in MeOH, 18 h, 100 °C, 87 %; (ix) 37 % HCl, MeOH, overnight, rt, ion exchanger, 88 %; (x) TrtCl (6 eq), NEt_3 (10 eq), DCM, 24 h, rt, 80 %; (xi) a) separation of diastereomers by flash chromatography; b) 37 % HCl, MeOH, 3 h, reflux, ion exchanger, 95-98 %; (xii) MeCN, microwave 150 °C, 15 min, 59-76 %.

The key step of this approach was an enzymatic cleavage enabling the discrimination between the enantiotopic ester groups in meso *cis*-1,3-cyclopentanedicarboxylic acid dimethyl ester (**5.37**) according to the method published by Chenevert et al.[17] Oxidation of norbornene (**5.5**) gave the dicarboxylic acid **5.6** (Scheme 5.3). Esterification of **5.6** with methanol in the presence of an acidic resin as a catalyst gave diester **5.37**. Cholesterol esterase (CE) catalysed hydrolysis of diester **5.37** has been reported with high enantiomeric purity of the product (mono-ester **5.38**) (ee = 91 %).[18] According to this procedure **5.38** was obtained in 89 % yield and 72 % ee. Racemic **rac-5.38**, obtained by dehydration of **5.6** with acetic anhydride followed by reaction of anhydride **5.39** with methanol, was used as reference compound to assign the NMR data (Scheme 5.4). The absolute configuration of **5.38** was determined by comparison of the specific optical rotation with reported values.[19] The enantiomeric purity of **5.38** was measured by derivatization with (*S*)-1-phenylethanamine (**5.40**) followed by ^{1}H-NMR (600 MHz) analysis of the resulting diastereomeric amides **5.41** and **5.41-reference** (Scheme 5.4).



Scheme 5.4 Determination of the enantiomeric purity of **5.38** and synthesis of racemic **rac-5.38** as reference compound. Reagents and conditions: (i) Acetic anhydride, microwave, 30 min, 150 °C, 73 %; (ii) MeOH, 4 h, reflux, 99 %; (iii) EDC (1.15 eq), DMAP (1.6 eq), DCM, 30 min, 0 °C, overnight, rt, 92-94 %.

The ester (1*S*,3*R*)-**5.38** was converted to the amide (1*S*,3*R*)-**5.42** by ammonolysis with aqueous ammonia at room temperature.[17] To confirm the absolute configuration of **5.42**, the ammonium carboxylate was crystallized and investigated by x-ray diffraction analysis. The obtained x-ray structure of (1*S*,3*R*)-**5.42** is depicted in Figure 5.7.

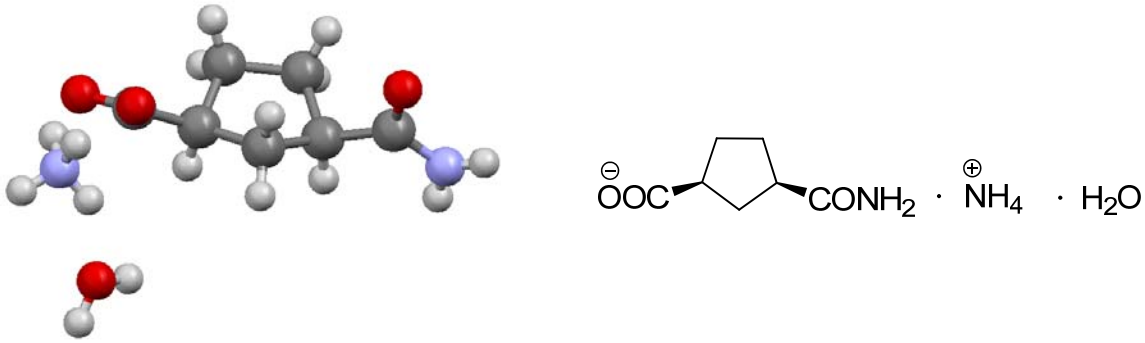


Figure 5.7 X-ray crystal structure of (1*S*,3*R*)-**5.42.**

Carboxylic acid **5.42** was reduced to the aminoalcohol **5.43** with borane.[8, 9] Boc-protection of the amine and subsequent Swern oxidation[10] of the unprotected alcohol in **5.44** gave the corresponding aldehyde **5.45**. The imidazole ring was introduced, as described under 5.2.1, by treating the aldehyde **5.45** with tosylmethyl isocyanide (**3.16**) followed by ammonia in methanol.[11] Due to the basic conditions in this step isomerization occurred at one of the two asymmetric carbon atoms (C-1) and two diastereomers were present in the reaction mixture from now on. After removing the Boc group in **5.46**, the imidazole nitrogen and the amino group in **5.47** were protected with a trityl group, and the resulting diastereomers separated by flash chromatography. Deprotection under acidic conditions and liberation of the free amines with the help of a basic ion exchanger gave the *cis*-(1*R*,3*S*)- and *trans*-(1*R*,3*R*)-configured imidazolylcyclopentylmethylamines **5.49** and **5.50**, respectively. To exclude undesired isomerization during the synthetic procedure, **5.49** was crystallized as hydrochloride and the configuration was confirmed by x-ray analysis. The cyanoguanidines **5.51-5.54** were synthesized as described in section 5.2.1.[13-16]

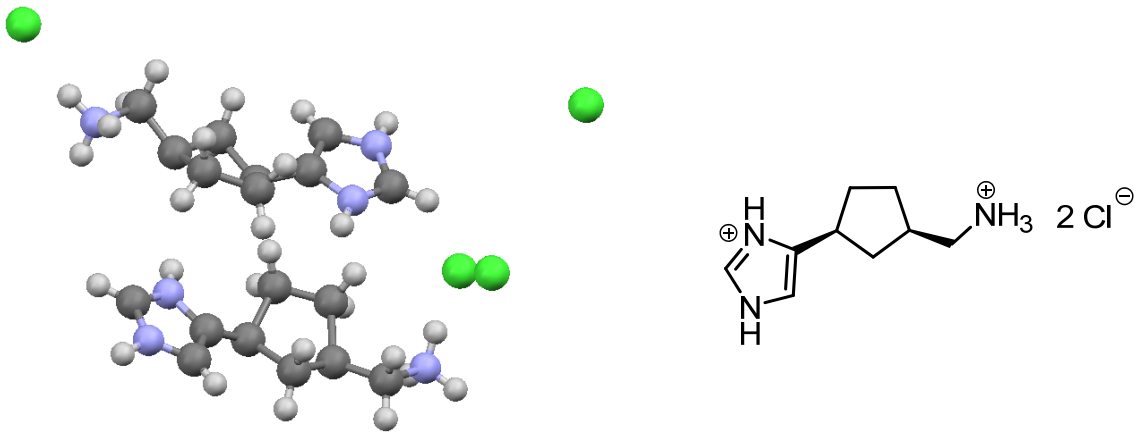


Figure 5.8 X-ray crystal structure of (1*R*,3*S*)-**5.49** · 2 HCl.

Finally, the assignment of the absolute configurations of cyanoguanidines **5.18a-5.19b** and **5.26a-5.27b** was possible by comparing the respective data of optical rotation and chromatographic separations (HPLC) with the results for **5.51-5.54**. The matched chromatograms are depicted in Figure 5.9.

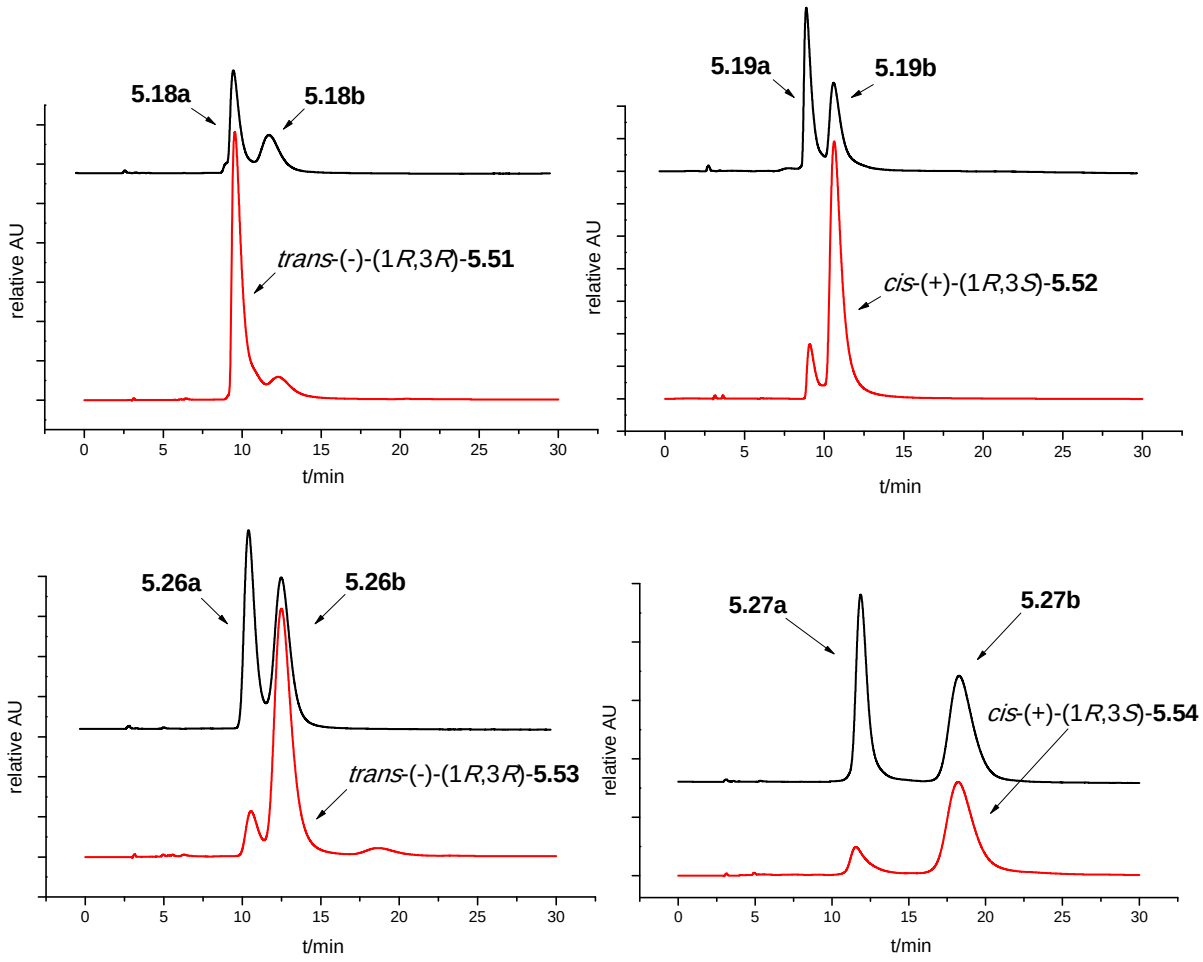
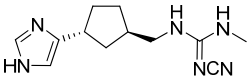
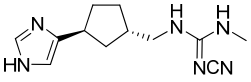
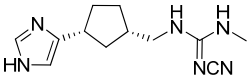
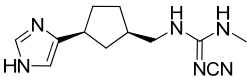
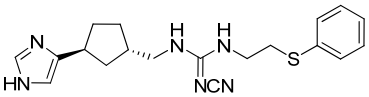
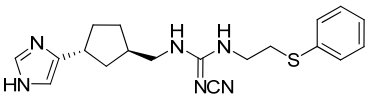
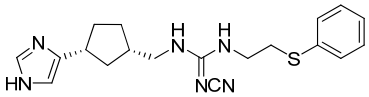
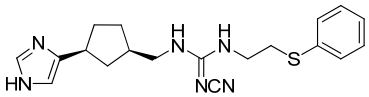


Figure 5.9 UV traces of the racemates **5.18**, **5.19**, **5.26** and **5.27** (black) and the products of the stereoselective synthesis of **5.51-5.54** (red).

This strategy allowed the assignment of the absolute configuration of all pharmacologically characterized pure stereoisomers. With the help of an enzyme assisted stereoselective synthesis reference compounds with ascertained absolute configuration were obtained. Mismatches could be ruled out, as x-ray structure analysis, performed for two precursors, confirmed the expected configuration. The obtained data provide important information for future pharmacological studies, ligand design and molecular modeling projects using these compounds. The stereochemical description of the enantiomers **5.18a-5.19b** and **5.26a-5.27b** is summarized in Table 5.3.

Table 5.3 Compound numbers, stereochemical descriptors, lab codes and structures of the stereoisomers **5.18a-5.19b** and **5.26a-5.27b**.

Compound	Stereochemical descriptors - Lab code[a]	Structure	CD[b]
5.18a	*trans*-(-)-(1*R*,3*R*)-UR-RG94		+
5.18b	*trans*-(+)-(1*S*,3*S*)-UR-RG94		-
5.19a	*cis*-(-)-(1*S*,3*R*)-UR-RG94		-
5.19b	*cis*-(+)-(1*R*,3*S*)-UR-RG94		+
5.26a	*trans*-(+)-(1*S*,3*S*)-UR-RG98		-
5.26b	*trans*-(-)-(1*R*,3*R*)-UR-RG98		+
5.27a	*cis*-(-)-(1*S*,3*R*)-UR-RG98		-
5.27b	*cis*-(+)-(1*R*,3*S*)-UR-RG98		+

[a] Laboratory code including relative and absolute configuration, relative optical rotation and identification number

[b] circular dichroism at 240 nm.

5.3 Pharmacological results and discussion

The synthesized cyanoguanidines were investigated for agonism and antagonism at the hH_2R, hH_3R and hH_4R subtypes in [^{35}S]GTPγS binding assays using membrane preparations of Sf9 insect cells coexpressing the hH_4R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or coexpressing the hH_3R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or expressing the hH_2R-$Gs\alpha_s$ fusion protein. At the hH_1R a steady-state GTPase assay and radioligand binding experiments with membrane preparations of Sf9 insect cells coexpressing the hH_1R plus RGS4 were used for characterization. Additionally, for reasons of comparison the amine precursors **5.16** and **5.17** were pharmacologically characterized (Table 5.4).

In the following agonistic potencies are expressed as EC_{50} values. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine. Compounds identified

to be inactive as agonists (α <0.1 or negative values, respectively, determined in the agonist mode; cf. Table 5.4) were investigated in the antagonist mode. The corresponding K_B values of neutral antagonists and inverse agonists (Table 5.4) were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding. Previously, steady-state GTPase assays, measuring the receptor-stimulated enzymatic cleavage of [^{32}P] or [^{33}P] labelled GTP, were used as the preferred pharmacological test system for the investigation of histamine receptor ligands synthesized in our laboratory. Compared to the GTPase assay the [^{35}S]GTPγS binding assay has several advantages such as lower amount of radioactivity, lower costs and easier handling. Therefore, we established this system as a standard for the characterization of agonists and antagonists at H_2R, H_3R and H_4R. Initial investigations with standard histamine receptor ligands confirmed that both test systems, the GTPase and the [^{35}S]GTPγS binding assay, provide comparable results. Unfortunately, at the H_1R the sensitivity of the binding assay is too low, preventing a reliable read out. The H_1R couples to Gq proteins which show sluggish intrinsic guanine nucleotide exchange rates and a low affinity for GTPγS.[20] The data of selected reference compounds are shown in Table 5.4.

5.3.1 Potencies, efficacies and affinities of the synthesized racemic compounds at the histamine receptor subtypes

The carba analogues of imifuramine (**5.3**), the racemic amine precursors **5.16** and **5.17** were devoid of significant activity at the H_1R and the H_2R, but moderately potent partial agonists at the H_4R and antagonists at the H_3R. The racemic *cis*-configured **5.17** was preferred at both receptor subtypes, at the H_4R by a factor of more than 10. Compared to the flexible analogue imbutamine,[21] efficacy and potency were drastically reduced by the introduction of a conformationally restricted linker. In comparison to the tetrahydrofurane analogues the potency at the H_4R increased, but the intrinsic activity decreased. The quality of action at the H_3R even changed from full agonism to antagonism. At the H_3R the results for the racemic *trans*-configured **5.16** were in the same range as for imifuramine, whereas the *cis*-diastereomer displayed about 10-fold higher potency. However, the free amines are less potent histamine receptor ligands compared to imbutamine or histamine and do not show an improved selectivity for the H_4R compared to the H_3R.

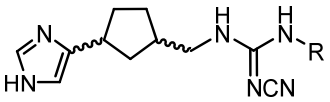
In several previous studies the introduction of a cyanoguanidine group as a non-basic central structural motif of the molecule proved to be beneficial in terms of H_4R activity.[1, 6] Obviously, a second basic moiety is not required for H_4R agonism. Furthermore, substitution of the cyanoguanidine with larger residues in the "eastern part" of the molecule decreased the affinity for the H_3R and yielded highly potent H_4R agonists with pronounced selectivity over the other HR subtypes. Moreover, a chain length of four carbon atoms between imidazole and cyanoguanidine moiety proved to be optimal in terms of H_4R agonism.

All these findings could be confirmed in the series of conformationally constrained cyanoguanidines (Table 5.4). Most of the investigated racemates showed partial to full agonistic activity at the H_4R with intrinsic activities (α) covering the range from 0.40 to 0.94. Only three compounds, the *cis*-phenoxyethyl (**5.29**) and the two phenylaminoethyl (**5.30**, **5.31**) substituted cyanoguanidines, showed antagonistic (α = -0.03 for **5.29**, α = 0.09 for **5.30**) or inverse agonistic (α = -0.40 for **5.31**) behavior. These results were not surprising, since Igel et al.[1] also observed a high sensitivity of the imidazolylbutylcyanoguanidines towards even minor variations in the eastern part of the molecule. The K_B values for **5.29** and **5.30** were in the two digit nanomolar range, **5.31** was very weakly active (K_B >10,000 nM). At the H_3R a weak antagonistic activity was observed with K_B values around 400 nM. All other racemates activated the H_4R with EC_{50} values ranging from 14 to 500 nM. The most potent compound was, similar to the imidazolylbutyl series, the phenylthioethyl-substituted *trans*-configured derivative **5.26** (EC_{50} = 14 nM, α = 0.9). In general, bulkier substituents provided higher potency at the H_4R, e.g. the isobutyl derivative **5.22** had an EC_{50} of 15 nM. Interestingly, also the configuration drastically influenced the pharmacological behavior. In case of the racemic methyl-substituted cyanoguanidines, higher potency resided in the *cis*–configured diastereomers (**5.19** EC_{50} = 111 nM, **5.18** EC_{50} = 206 nM). By contrast, all racemic analogues bearing substituents other than methyl at the cyanoguanidine moiety showed higher activity when *trans*-configured. The phenylthioethyl derivatives, for example, differed by almost a factor of 10 (**5.26** EC_{50} = 14 nM, **5.27** EC_{50} = 129 nM). At the H_3R, compounds bearing alkyl residues at the cyanoguanidine moiety were weak partial agonists, confirming previous data reported by Igel et al.[1] With EC_{50} values from 370 to >10,000 nM these compounds showed some selectivity for the H_4R over the H_3R but no improvement compared to flexible imidazolylbutylcyanoguanidines such as UR-PI376 or tetrahydrofuranes such as OUP-16.[6] Similar to UR-PI376, compounds bearing an aromatic substituent (**5.24**-

5.31) did not show agonistic activity at the H_3R. Compound **5.25** was the most potent H_3R antagonist with a K_B of 70 nM. Both phenylthioethyl-substituted cyanoguanidines, **5.26** and **5.27**, were moderately potent H_3R inverse agonists (α = -0.4 and -0.34). In contrast to the H_4R, the *cis*-configured isomers of most compounds were preferred at the H_3R. No agonistic activity was observed at the H_1R and the H_2R. All investigated racemates were very weak H_2R antagonists with K_B values above 10 µM. At the H_1R, weak antagonistic (**5.18-5.21** and **5.26-5.30**) or inverse agonistic (**5.22-5.25**, **5.31**) behavior was found. Except for **5.26** (K_B = 6 µM) and **5.27** (K_B = 3.8 µM) the K_B values were in the high micromolar range.

In summary, the racemates of the phenylthioethyl substituted cyanoguanidines **5.26** and **5.27**, by analogy with UR-PI376, turned out to be the most promising candidates for more detailed investigations. The racemic mixture **5.26** revealed highest H_4R agonistic potency and displayed an 18-fold selectivity for the H_4R over the H_3R as well as negligible activities at the other HR subtypes. Furthermore, in contrast to other selective H_4R agonists like OUP-16, **5.26** was devoid of agonistic activity at the H_3R. Aiming at more detailed structure-activity and selectivity data and due to the prospect of further improved pharmacological properties, the chiral separation of the racemates was performed as outlined above. In addition, for reason of comparison with the tetrahydrofurane series and OUP-16, the methyl-substituted cyanoguanidines **5.18** and **5.19** were included in this study.

Table 5.4 Potencies, efficacies and affinities of the synthesized racemic cyanoguanidines **5.18-5.31**, the amines **5.16** and **5.17** and selected reference compounds at the hHR subtypes in the [^{35}S]GTPγS assay[a], the GTPase assay[b] or in radioligand binding experiments.[c]



5.18-5.31

No.	Config.	R	No.	Config.	R	No.	Config.	R	No.	Config.	R
5.18	*trans*	-CH_3	**5.22**	*trans*	-CH_2-$CH(CH_3)_2$	**5.26**	*trans*	-$(CH_2)_2$-S-Ph	**5.30**	*trans*	-$(CH_2)_2$-NH-Ph
5.19	*cis*	-CH_3	**5.23**	*cis*	-CH_2-$CH(CH_3)_2$	**5.27**	*cis*	-$(CH_2)_2$-S-Ph	**5.31**	*cis*	-$(CH_2)_2$-NH-Ph
5.20	*trans*	-cPr	**5.24**	*trans*	-$(CH_2)_3$-Ph	**5.28**	*trans*	-$(CH_2)_2$-O-Ph			
5.21	*cis*	-cPr	**5.25**	*cis*	-$(CH_2)_3$-Ph	**5.29**	*cis*	-$(CH_2)_2$-O-Ph			

Compound	**hH_1R**			**hH_2R**			**hH_3R**			**hH_4R**		
	K_i (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
Histamine	190 ± 8[d]	1.00		1,200 ± 300[d]	1.00		13 ± 2	1	3	11± 3	1	5
UR-PI376 (**5.1**)	>10,000[e]	0.07		(>10,000)[e]	0.08		(720 ± 38)	-0.52 ± 0.05	2	37± 3	0.88 ± 0.08	3
Thioperamid	n.d.	n.d.		n.d.	n.d.		(78 ± 9)	-0.59 ± 0.7	3	(73 ± 6)	-0.88 ± 0.06	4
VUF-8430	n.d.	n.d.		n.d.	n.d.		170 ± 11	0.64 ± 0.07	3	44 ± 8	0.81 ± 0.03	3
JNJ-7777120	n.d.	n.d.		n.d.	n.d.		(2,000 ± 400)	-0.56 ± 0.02	3	(19.1 ± 0.3)	-0.04 ± 0.16	2
5-Methylhistamine	16,000 ± 540[e]	0.90		2900 ± 1200[e]	1.00		>1 mM[e]	0.12		70 ± 18[e]	0.9	
OUP-16	n.d.	n.d.		n.d.	n.d.		3160[f]	0.79		78[f]	0.99	
5.16 (*trans*-amine)	>10,000	0.04 ± 0.05	2	>10,000	0.14 ± 0.05	2	(103 ± 0.5)	-0.09 ± 0.02	2	1,710 ± 14	0.38 ± 0.01	2
5.17 (*cis*-amine)	>10,000	0.11 ± 0.06	2	>10,000	0.19 ± 0.1	2	(62 ± 10)	-0.08 ± 0.02	2	150 ± 23	0.36 ± 0.01	2
5.18 (*trans*-UR-RG94)	>10,000	-0.04 ± 0. 2	2	(>10,000)	-0.01 ± 0.01	2	370 ± 6	0.46 ± 0.06	2	206 ± 12	0.74 ± 0.05	3
5.19 (*cis*-UR-RG94)	>10,000	0.00 ± 0.02	2	(>10,000)	-0.01 ± 0.0	2	547 ± 55	0.51 ± 0.02	2	111 ± 17	0.94 ± 0.01	2
5.20	>10,000	-0.02 ± 0.00	2	(>10,000)	-0.01 ± 0.01	2	> 10,000	0.36 ± 0.03	2	230 ± 62	0.74 ± 0.07	2
5.21	>10,000	0.01 ± 0.03	2	(>10,000)	-0.01 ± 0.01	2	1,530 ± 14	0.28 ± 0.09	2	245 ± 78	0.64 ± 0.02	2

Table 5.4 (continued)

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	K_i (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
5.22	8,750 ± 290	-0.20 ± 0.16	2	(>10,000)	-0.01 ± 0.0	2	578 ± 13	0.54 ± 0.04	2	15 ± 3	0.84 ± 0.07	2
5.23	8,350 ± 900	-0.15 ± 0.1	2	(>10,000)	-0.01 ± 0.01	2	858 ± 68	0.50 ± 0.00	2	202 ± 66	0.81 ± 0.07	2
5.24	>10,000	-0.19 ± 0.17	2	(>10,000)	0.01 ± 0.01	2	(325 ± 135)	-0.19 ± 0.02	2	125 ± 39	0.70 ± 0.05	2
5.25	9,350 ± 210	-0.15 ± 0.14	2	(> 10,000)	0.0 ± 0.01	2	(70 ± 7)	-0.08 ± 0.03	2	372 ± 63	0.40 ± 0.11	2
5.26 (*trans*-UR-RG98)	6,080 ± 270	-0.05 ± 0.04	2	(>10,000)	-0.01 ± 0.01	2	(246 ± 49)	-0.40 ± 0.05	2	14 ± 3	0.90 ± 0.06	3
5.27 (*cis*-UR-RG98)	3,800 ± 700	0.04 ± 0.07	2	(>10,000)	-0.03 ± 0.03	2	(162 ± 3)	-0.34 ± 0.04	2	129 ± 10	0.72 ± 0.09	4
5.28	>10,000	0.04 ± 0.03	2	(>10,000)	-0.01 ± 0.0	2	(540 ± 50)	-0.01 ± 0.02	2	524 ± 8	0.63 ± 0.1	3
5.29	>10,000	0.08 ± 0.05	2	(>10,000)	-0.02 ± 0.01	2	(328 ± 38)	0.10 ± 0.08	2	(97 ± 7)	-0.03 ± 0.2	3
5.30	>10,000	-0.05 ± 0.03	2	(>10,000)	-0.02 ± 0.0	2	(408 ± 21)	-0.10 ± 0.12	2	(53 ± 6)	0.09 ± 0.01	3
5.31	>10,000	-0.13 ± 0.1	2	(>10,000)	-0.02 ± 0.01	2	(402 ± 38)	-0.03 ± 0.2	2	(>10,000)	-0.40 ± 0.2	3

[a] [^{35}S]GTPγS functional binding assays with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_2R-$Gs\alpha_s$ fusion protein were performed as described in section *Pharmacological methods.* [b] Steady-state GTPase activity in Sf9 cell membranes expressing the hH_1R + RGS4 was determined as described in section *Pharmacological methods.* [c] Displacement of [^{3}H]mepyramine (5 nM) from Sf9 cell membranes expressing the hH_1R + RGS4 was determined as described in section *Pharmacological methods.* [a,b,c] Reaction mixtures contained ligands at a concentration from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. N gives the number of independent experiments performed in duplicate each. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. The α values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM. The K_B values of neutral antagonists and inverse agonists were determined in the antagonist mode versus histamine (100 nM) as the agonist. [d] Data taken from Xie et al.[22] [e] Data taken from Igel.[23] [f] Data taken from Hashimoto et al.[6]

5.3.2 Pharmacological characterization of the separated stereoisomers

5.3.2.1 Functional activities at recombinant human histamine receptor subtypes

After the successful separation of the stereoisomers, the enantiomerically pure compounds were again tested in the [^{35}S]GTPγS binding assay (cf. Table 5.5). At first the carba analogues of the reported tetrahydrofurane series were investigated. The results were roughly in agreement with the data for OUP-16 and its analogues.[6] The stereoisomers *trans*-(-)-**5.18** (**5.18a**) and *cis*-(-)-**5.19** (**5.19a**) were both weakly potent H_4R partial agonists with EC_{50} values above 1 µM. The eutomers, *trans*-(+)-**5.18** (**5.18b**) and *cis*-(+)-**5.19**,(**5.19b**) were potent H_4R agonists with EC_{50} around 100 nM. Compared to the H_4R, the potencies and efficacies at the H_3R were lower: **5.19a** was an inverse agonist, whereas the other three isomers were weak partial agonists with α values from 0.29 to 0.61. Similar to the stereoselectivity at the H_4R, **5.18b** (EC_{50} = 994 nM) and **5.19b** (EC_{50} = 212 nM) were by a factor of 10 more potent than their optical antipodes at the H_3R. At the H_2R and the H_1R no relevant activity was detected. The most potent and selective H_4R agonist in this series was the carba analogues of OUP-16 (EC_{50} = 78 nM, α = 0.99), *trans*-(+)-(1*S*,3*S*)-UR-RG94 (**5.18b**) (EC_{50} = 100 nM, α = 0.71) displaying a 10-fold selectivity over the H_3R. Obviously, the exchange of the oxygen atom by a carbon atom in the five membered ring, does not significantly affect the pharmacological profile of this class of compounds. This information may be useful with respect to future ligand design and development of more convenient synthetic pathways. The small differences in potency and efficacy compared to the data on OUP-16 and analogues from the literature are probably due to the different test systems used for characterization (OUP-16 was characterized measuring the inhibition of forskolin-stimulated cAMP production in SK-N-MC cells expressing the respective HR subtype).[6] The correlation between the absolute configuration of the four isomers and their pharmacological properties at the H_3R and the H_4R is in complete agreement with the data for the structurally related tetrahydrofuranes reported by Hashimoto et al.[6]

The evaluation of the separated isomers of the most promising racemates **5.26** and **5.27** yielded a highly potent and selective H_4R agonist: *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) activated the H_4R with an EC_{50} value of 11 nM and an intrinsic activity of 0.75 in the [^{35}S]GTPγS binding assay. By contrast, at the H_3R **5.26a** was a weak antagonist (K_B = 1,150 nM), resulting in a more than 100-fold selectivity for the H_4R. The activities at the other two histamine receptor subtypes were negligible. Surprisingly, the investigation of the optical

antipode of **5.26a**, *trans*-(-)-(1*R*,3*R*)-UR-RG98 (**5.26b**) revealed just the opposite profile: **5.26b** was a weak antagonist at the H_4R (K_B = 7,700 nM), but by a factor of almost 10 more potent at the H_3R (K_B = 143 nM, α = -0.21) compared to **5.26a**. Both *cis* enantiomers **5.27a** and **5.27b** were moderately potent H_4R partial agonists with EC_{50} values in the three digit nanomolar range and H_3R inverse agonists with K_B values of 528 and 718 nM, respectively. At the H_2R and H_1R, these compounds were devoid of agonistic activity, but showed weak antagonistic activity in the micromolar range.

Comparing the whole set of eight enantioseparated imidazolylcyclopentylcyanoguanidines, highest H_4R agonistic potency always resided in the stereoisomers with *S*-configuration in position 3. Presumably, this configuration favors optimal orientation of the imidazole and the directly connected cyclopentyl ring in the binding pocket of the H_4R. The configuration at position 1 is obviously less important, although (1*S*)-configuration is the preferred one, in particular, in case of compounds bearing larger substituents in the eastern part of the molecule. Regarding the H_3R there was no clear correlation between configuration and potency. Nevertheless, as the most potent H_3R ligand in this series, **5.26b**, is *trans*-(1*R*,3*R*)-configured, the optimal stereochemical requirements for H_3R and H_4R affinity seem to be different. Hence, both highest H_4R agonistic potency and receptor subtype selectivity resides in *trans*-(1*S*,3*S*)-configured compounds. These results are in agreement with the spatial orientation of the substituents at the tetrahydrofurane ring in OUP-16. Moreover, in accordance with the imidazolylalkylcyanoguanidine series a phenylthioethyl substituent and a chain length of four carbon atoms between imidazole and cyanoguanidine moiety confer high H_4R potency and selectivity. Compared to the phenylene or cyclohexylene linked compounds (see chapters 3 and 4) a drastic increase in potency, efficacy and selectivity was observed. This might be due to the remaining flexibility of the linker, caused by the exocyclic methylene group.

For validation the racemates **5.26** and **5.27** as well as for the corresponding stereoisomers **5.26a,b** and **5.27a,b** were investigated in steady state GTPase assays on hH_4R and hH_3R. The determined potencies and efficacies were comparable to those from the [^{35}S]GTPγS binding assays.

Table 5.5 Potencies, efficacies and affinities of the separated cyanoguanidines **5.18a-5.19b** and **5.26a-5.27b**, the racemates **5.18**, **5.19** and **5.26**, **5.27** and selected reference compounds at the hHR subtypes in the [^{35}S]GTPγS assay[a],the GTPase assay[b] or in radioligand binding experiments.[c]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	K_i (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
Histamine	190 ± 8[d]	1.00		1,200 ± 300[d]	1.00		13 ± 2	1	3	11± 3	1	5
UR-PI376 (**5.1**)	n.d.	n.d.		n.d.	n.d.		720 ± 38[e]	-0.52 ± 0.05	2	37± 3[e]	0.88 ± 0.08	3
OUP-16	n.d.	n.d.		n.d.	n.d.		3,160[f]	0.79		78[f]	0.99	
5.18	>10,000	-0.04 ± 0. 2	2	(>10,000)	-0.01 ± 0.01	2	370 ± 6	0.46 ± 0.06	2	206 ± 12	0.74 ± 0.05	3
5.19	>10,000	0.00 ± 0.02	2	(>10,000)	-0.01 ± 0.0	2	547 ± 55	0.51 ± 0.02	2	111 ± 17	0.94 ± 0.01	2
5.18a (*trans*-(-)-(1*R*,3*R*)-UR-RG94)	>10,000	0.00 ± 0.01	2	(>10,000)	0.01 ± 0.02	2	9,200 ± 400	0.29 ± 0.07	2	1,100 ± 99	0.82 ± 0.05	3
5.18b (*trans*-(+)-(1*S*,3*S*)-UR-RG94)	>10,000	-0.02 ± 0.0	2	(>10,000)	-0.01 ± 0.01	2	994 ± 71	0.59 ± 0.04	2	100 ± 16	0.71 ± 0.01	3
5.19a (*cis*-(-)-(1*S*,3*R*)-UR-RG94)	>10,000	-0.01 ± 0.02	2	(>10,000)	-0.01 ± 0.02	2	(2,812 ± 9)	-0.14 ± 0.07	2	2,675 ± 272	0.32 ± 0.06	3
5.19b (*cis*-(+)-(1*R*,3*S*)-UR-RG94)	>10,000	0.03 ± 0.01	2	(>10,000)	0.01 ± 0.0	2	212 ± 33	0.61 ± 0.07	2	119 ± 20	0.86 ± 0.02	3
5.26	6,080 ± 270	-0.05 ± 0.04	2	(>10,000)	-0.01 ± 0.01	2	(246 ± 49)	-0.40 ± 0.05	2	14 ± 3	0.90 ± 0.06	3
5.27	3,800 ± 700	0.04 ± 0.07	2	(>10,000)	-0.03 ± 0.03	2	(162 ± 3)	-0.34 ± 0.04	2	129 ± 10	0.72 ± 0.09	4
5.26a (*trans*-(+)-(1*S*,3*S*)-UR-RG98)	6,520 ± 570	0.03 ± 0.01	2	(>10,000)	-0.06 ± 0.02	2	(1,150 ± 112)	-0.01 ± 0.02	3	11 ± 2	0.75 ± 0.04	3
5.26b (*trans*-(-)-(1*R*,3*R*)-UR-RG98)	7,910 ± 710	0.05 ± 0.02	2	(>10,000)	0.01 ± 0.08	2	(143 ± 12)	-0.21 ± 0.04	2	(7,700 ± 400)	0.02 ± 0.04	2
5.27a (*cis*-(-)-(1*S*,3*R*)-UR-RG98)	6,780 ± 300	0.04 ± 0.08	2	(>10,000)	-0.05 ± 0.02	2	(528 ± 50)	-0.34 ± 0.04	2	549 ± 8	0.54 ± 0.09	3
5.27b (*cis*-(+)-(1*R*,3*S*)-UR-RG98)	3,050 ± 260	0.14 ± 0.04	2	(>10,000)	-0.03 ± 0.06	2	(718 ± 12)	-0.21 ± 0.03	2	293 ± 32	0.39 ± 0.03	3

See Table 5.4 for details and explanations.

5.3.2.2 Histamine receptor subtype affinities of the separated stereoisomers

The racemic cyanoguanidines and the separated enantiomers were investigated in [^{35}S]GTPγS binding assays, and *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) was identified as the most potent and selective hH$_4$R agonist in this series (EC$_{50}$ = 11 nM, α = 0.75, 100-fold selectivity toward the hH$_3$R). Nevertheless, agonist potencies determined in functional assays depend on different factors, as for example the G-protein availability.[22] Especially for the H$_4$R, differences between pharmacological data from various studies are reported,[24, 25] and even the quality of action may differ,[25] depending on test system and read-out. In addition, this can result in discrepancies between potencies and affinities determined in functional experiments and binding studies, respectively. Therefore, dissociation constants (K_i values) of **5.26**, **5.27** and the respective isomers at the different hHR subtypes were determined. Competition binding studies on the hH$_4$R were performed with two different radioligands, which gave comparable results: the standard compound [^{3}H]histamine and [^{3}H]UR-PI294,[26] an acylguanidine developed in our laboratory.

All evaluated compounds displaced [^{3}H]mepyramine from the hH$_1$R, [^{3}H]tiotidine from the hH$_2$R, [^{3}H]*N*$^{\alpha}$-methylhistamine from the hH$_3$R and [^{3}H]UR-PI294 from the hH$_4$R, giving monophasic competition binding curves (Figure 5.10).

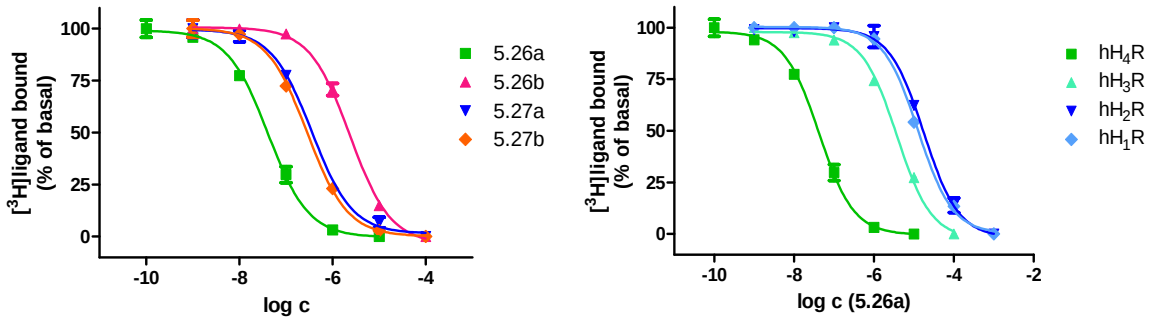
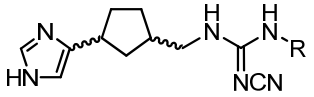


Figure 5.10 Representative radioligand binding experiments: (left) displacement of [^{3}H]UR-PI294 (5 nM) from Sf9 insect cell membranes expressing the hH$_4$R + Gα_{i2} + G$\beta_1\gamma_2$. (right) Displacement of [^{3}H]mepyramine (5 nM), [^{3}H]tiotidine (10 nM), [^{3}H]*N*$^{\alpha}$-methylhistamine (3 nM) and [^{3}H]UR-PI294 (5 nM) from Sf9 insect cell membranes expressing the hH$_1$R + RGS4, hH$_2$R-Gsα_s fusion protein, hH$_3$R + Gα_{i2} + G$\beta_1\gamma_2$ or the hH$_4$R + Gα_{i2} + G$\beta_1\gamma_2$. Radioligand binding was determined as described in section *Pharmacological methods*. Data were analyzed for best fit to one site (monophasic) competition curves.

Although for most compounds the K_i values from radioligand binding experiments were slightly higher than the EC$_{50}$ values determined in the [^{35}S]GTPγS assay, in general, the binding data and the rank order of the compounds were in agreement with the potencies evaluated in the functional assays. The only significant difference was found for **5.26b**. The K_i

value (1,050 nM) was much lower than the observed K_B value in the [^{35}S]GTPγS assay (K_B = 7,700 nM). However, this was not surprising, as no complete concentration-response curve for **5.26b** at the H_4R could be obtained in the antagonist mode of the [^{35}S]GTPγS assay. Though, the observed K_B value was just a rough estimation and it is recommended to use the K_i for further discussion. *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) bound with the highest affinity to the hH_4R (K_i = 22 nM) and, as expected, showed a remarkably lower affinity for the hH_3R (K_i = 2,130 nM). The affinities of **5.26a** for the hH_4R and the hH_3R were slightly reduced compared to the EC_{50}/K_B values determined in the [^{35}S]GTPγS assay. At the hH_2R and the hH_1R all compounds displayed low affinity. Overall, the determined K_i values confirm *trans*-(+)-(1*S*,3*S*)-UR-RG98 to be a highly affine and selective ligand for the hH_4R.

Table 5.6 Binding data of the racemates **5.18**, **5.19**, **5.26** and **5.27** and of the separated isomers **5.18a-5.19b** and **5.26a-5.27b** at the histamine receptor subtypes.[a]

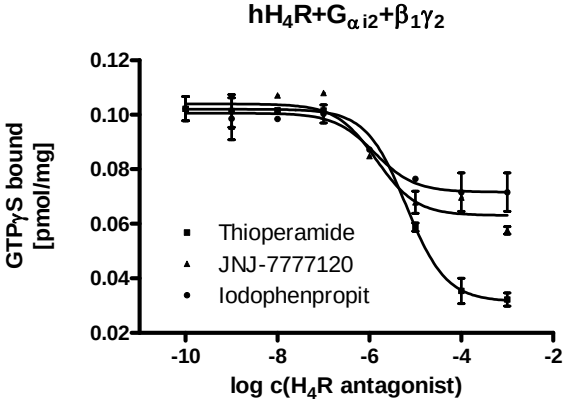


5.18(a/b)-5.19(a/b): R = -CH_3
5.26(a/b)-5.27(a/b): R = -$(CH_2)_2$-S-Ph

No.	hH_1R		hH_2R		hH_3R		hH_4R	
	K_i (nM)	N	K_i (nM)	N	K_i (nM)	N	K_i (nM)	N
5.18	> 10,000	2	n.d.		830 ± 34	2	240 ± 15	4
5.19	> 10,000	2	n.d.		860 ± 48	2	155 ± 25	4
5.18a (*trans*-(-)-(1*R*,3*R*)-UR-RG94)	> 10,000	2	> 10,000	2	5,680 ± 316	2	870 ± 57	4
5.18b (*trans*-(+)-(1*S*,3*S*)-UR-RG94)	> 10,000	2	> 10,000	2	958 ± 29	2	98 ± 11	4
5.19a (*cis*-(-)-(1*S*,3*R*)-UR-RG94)	> 10,000	2	> 10,000	2	9,500 ± 1300	2	3,500 ± 75	4
5.19b (*cis*-(+)-(1*R*,3*S*)-UR-RG94)	> 10,000	2	> 10,000	2	480 ± 100	2	120 ± 7	4
5.26	6,080 ± 270	2	n.d.		1,230 ± 134	2	99 ± 8	4
5.27	3,800 ± 700	2	n.d.		1,350 ± 165	2	250 ± 20	4
5.26a (*trans*-(+)-(1*S*,3*S*)-UR-RG98)	6,520 ± 570	2	10,300 ± 500	2	2,130 ± 17	3	22 ± 3	5
5.26b (*trans*-(-)-(1*R*,3*R*)-UR-RG98)	7,910 ± 710	2	9,820 ± 90	2	590 ± 36	3	1,050 ± 120	5
5.27a (*cis*-(-)-(1*S*,3*R*)-UR-RG98)	6,780 ± 300	2	7,360 ± 480	2	745 ± 8	3	290 ± 30	5
5.27b (*cis*-(+)-(1*R*,3*S*)-UR-RG98)	3,050 ± 260	2	7,250 ± 140	2	870 ± 22	3	180 ± 22	5

[a] Displacement of [^{3}H]mepyramine (5 nM), [^{3}H]tiotidine (10 nM), [^{3}H]N^{α}-methylhistamine (3 nM) and [^{3}H]UR-PI294 (5 nM) from Sf9 insect cell membranes expressing the hH_1R + RGS4, hH_2R-$G\alpha_s$ fusion protein, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$. Radioligand binding was determined as described in section *Pharmacological methods*. Data were analyzed for best fit to one site (monophasic) competition curves. N gives the number of independent experiments performed in duplicate.

5.3.2.3 Inhibition of the trans-(+)-(1S,3S)-UR-RG98 (5.26a) stimulated [^{35}S]GTPγS binding at the hH$_4$R by standard H$_4$R antagonists



Compound	K_B (nM)	K_i (nM)[a]
Thioperamide	100 ± 14	125
JNJ-7777120	29 ± 2	13
Iodophenpropit	19 ± 6	16

Figure 5.11 Inhibition of the *trans*-(+)-(1*S*,3*S*)-UR-RG98 stimulated [^{35}S]GTPγS binding at the hH$_4$R by the H$_4$R antagonists thioperamide, iodophenpropit and JNJ-7777120. Functional [^{35}S]GTPγS binding assays with membrane preparations of Sf9 cells expressing the hH$_4$R + Gα_{i2} + G$\beta_1\gamma_2$ was performed as described in section *Pharmacological methods*. Reaction mixtures contained 1 µM of *trans*-(+)-(1*S*,3*S*)-UR-RG98. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Data points shown are the means of two independent experiments performed in duplicate each. [a] K_i values of the reference H$_4$R antagonists were taken from Lim et al.[21]

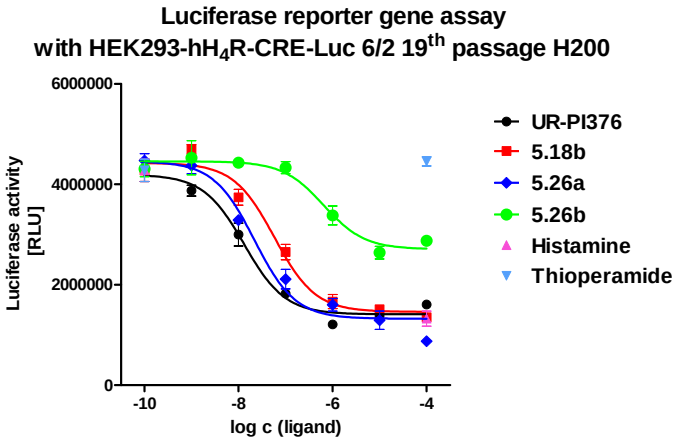
The majority of the synthesized compounds, including the most potent H$_4$R agonist *trans*-(+)-(1*S*,3*S*)-UR-RG98 contain the imidazole ring as a polar basic moiety combined with more lipophilic side chains. Due to this lipophilic moiety and the cationic-amphiphilic properties of the molecule it is conceivable that *trans*-(+)-(1*S*,3*S*)-UR-RG98 interacts directly with G-proteins. Such effects have been reported for many compounds, mainly peptides, but also for cationic-amphiphilic HR ligands.[27-29] Using membranes instead of intact cells makes G-proteins directly accessible to the evaluated compounds and facilitates receptor-independent G-protein activation. Therefore, [^{35}S]GTPγS binding was stimulated with *trans*-(+)-(1*S*,3*S*)-UR-RG98 (1 µM) and the effect of increasing concentrations of the H$_4$R antagonists thioperamide, iodophenpropit and JNJ-7777120 was evaluated. As shown in Figure 5.11, [^{35}S]GTPγS binding was successfully inhibited by all antagonists in a concentration-dependent manner. As expected, thioperamide was more effective than the other standard H$_4$R antagonists due to its inverse agonistic activity at the hH$_4$R. The K_B values determined for thioperamide, iodophenpropit and JNJ-7777120 against *trans*-(+)-(1*S*,3*S*)-UR-RG98 in the functional assay were in good agreement with K_i values from binding studies reported by Lim et al. (Figure 5.11).[21] These results confirm that *trans*-(+)-(1*S*,3*S*)-UR-RG98 competes with the H$_4$R antagonists for the same binding site. Consequently, stimulation of

[^{35}S]GTPγS binding to the G-protein in the functional assay seems to be receptor mediated. The same holds for the hH_3R activity of the investigated compounds, as both the hH_3R and hH_4R were coexpressed with Gα_{i2}. Furthermore, *trans*-(+)-(1*S*,3*S*)-UR-RG98 was found to displace [^{3}H]histamine from the hH_4R, being another evidence of binding to the same binding site. In summary, these data confirm *trans*-(+)-(1*S*,3*S*)-UR-RG98 to act as a hH_4R agonist in the [^{35}S]GTPγS binding assay.

5.3.2.4 Potencies and efficacies of selected isomers at the hH_4R in a luciferase reporter gene assay

For the characterization of the synthesized cyanoguanidines well-proven test systems, the [^{35}S]GTPγS and the GTPase assay were applied. A major advantage of this modus operandi is that an identical, very proximal read-out in G-protein-mediated signalling is used for any given HR subtype. This read-out avoids bias in data interpretation caused by limited availability of downstream effectors. However, both systems use membrane preparations of Sf9 insect cells expressing the respective HR subtype. Compared to whole cell systems, the handling is much easier and the system is very robust. Nevertheless, the obtained results from this artificial system should be validated in completely different pharmacological systems. This becomes even more important considering the reported discrepancies between data from different test systems for several H_4R ligands.[25]

Therefore, selected isomers, the carba analogue of OUP-16, **5.18b**, and the two *trans* enantiomers **5.26a** and **5.26b** were investigated in a luciferase reporter gene assay using HEK293-hH_4R-CRE-Luc cells expressing the hH_4R. The obtained concentration-response curves, potencies and intrinsic activities are summarized in Figure 5.12. This assay was developed by Uwe Nordemann in our research group as part of his dissertation project (to be reported in detail elsewhere).



Compound	hH_4R		
	EC_{50} (nM)	α	N
Histamine	13 ± 4	1	5
5.18b	63 ± 4	1	4
5.26a	25 ± 2	1	4
5.26b	500 ± 130	0.63	4

Figure 5.12 Inhibition of forskolin (400 nM) stimulated luciferase activity by H_4R agonists using HEK293-hH4R-CRE-Luc cells expressing the hH_4R. Luciferase reporter gene assays were performed as described in section *Pharmacological methods*. Reaction mixtures contained ligands at a concentration from 1 nM to 1 mM as appropriate to generate saturated concentration-response curves. N gives the number of independent experiments performed in duplicate. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value.

The data obtained for **5.18b** and **5.26a** were comparable to the results of the [^{35}S]GTPγS and radioligand binding assays. Both were full H_4R agonists with EC_{50} values in the low nanomolar range. Interestingly, **5.26b**, which was a weak neutral antagonist in the [^{35}S]GTPγS assay, showed partial agonistic activity with an EC_{50} of 500 nM. Presumably, this effect can be observed due to signal amplification downstream from G-protein activation. Regardless of this minor assay-dependent difference the high H_4R agonistic potency of the eutomer *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) and the stereodiscrimination by the receptor (cf. *trans*-(-)-(1*R*,2*R*)-UR-RG98, **5.26b**) was confirmed in the cell based luciferase reporter gene assay.

5.3.2.5 Potencies, efficacies and affinities at the mH_4R

Nowadays GPCR ligands are routinely optimized for activity at the human receptor of interest. The investigation of the (patho)physiological role of the H_4R and its validation as a possible drug target using antagonists and agonists in translational animal models are seriously hampered by tremendous species-dependent discrepancies regarding potencies and receptor selectivities of the pharmacological tools.[30] Therefore, with respect to future in vivo studies, the activities of H_4R ligands on species orthologues, in particular on the murine H_4R, should be taken into account.[31] Preliminary investigations revealed that UR-PI376, like many other hH_4R agonists, shows substantially reduced potency at the mH_4R.[30] Therefore,

UR-PI376 is in particular an interesting tool for pharmacological studies on human H_4Rs and less suitable for investigations on mouse H_4Rs. Most strikingly, the prototypical and widely used H_4R antagonist JNJ-7777120, a partial inverse agonist at hH_4R, exhibited partial agonistic activity at cH_4R, rH_4R, and mH_4R.[30]

To evaluate the potential use of the synthesized cyclopentyl derivatives in animal models, the separated enantiomers were investigated in radioligand binding studies and in a luciferase reporter gene assay using HEK293 cells stably expressing the mouse H_4R. The results are summarized in Table 5.7.

Similar to the results for UR-PI376, the investigation of the stereoisomers revealed differences between activities on the human and murine H_4R orthologues. The K_i values on the mH_4R were substantially increased compared to the data for the hH_4R. The same was observed for mH_4R agonistic activity in the functional reporter gene assay. All compounds were still partial to full agonists, but with lower potency at the murine receptor. The carba analogue of OUP-16, **5.18b**, was a full mH_4R agonist with an EC_{50} of 500 nM, corresponding to a 5-fold decrease in potency compared to the hH_4R. Similar differences between potencies were found for all methyl-substituted cyanoguanidines, however, the intrinsic activities at the mH_4R exceeded those at the hH_4R. The most potent enantiomer among the four analogues of OUP-16, **5.19b**, had an EC_{50} value of 160 nM and showed full mH_4R agonistic activity. Interestingly, the species-dependent differences were not the same for the stereoisomeric phenylthioethyl-substituted cyanoguanidines. For **5.26a,b** and **5.27a** lower efficacy at the mH_4R was observed, whereas **5.27b** was found to be prefer the mouse H_4R, showing an EC_{50} of 100 nM and an intrinsic activity of 0.81. **5.26b** and **5.27a** were equipotent at both species orthologs, with EC_{50} values of 630 and 400 nM, respectively. *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) was a moderately potent partial mH_4R agonist with an EC_{50} of 160 nM and an efficacy of 0.67. Compared to the hH_4R the potency was 10-fold lower (Table 5.7).

In summary, similar to the lead compound UR-PI376, the synthesized conformationally restricted cyanoguanidines are promising hH_4R agonists. With respect to investigations of these compounds in animal models species-dependent differences have to be taken into account, although, comparing the murine and the human H_4R orthologue, the decrease in potency was lower than for UR-PI376. Surprisingly, the enantiomer **5.27b** was more potent and efficacious at the murine H_4R and might be considered as pharmacological tool in mouse models.

Table 5.7 Potencies, efficacies[a] and affinities[b] of the stereoisomers **5.18a-5.19b**, **5.26a-5.27b** and reference compounds at the mouse H_4R.

Compound	mH_4R				
	K_i (nM)	N	EC_{50} (nM)	α	N
Histamine	126 ± 33	2	80 ± 20	1	4
UR-PI376 (**5.1**)	320 ± 65	2	500 ± 44	0.48	3
5.18a	<10,000	2	4,000 ± 1160	1	4
5.18b	<10,000	2	500 ± 33	0.96	3
5.19a	<10,000	1	10,000 ± 3000	0.87	4
5.19b	630 ± 10	2	160 ± 23	0.97	4
5.26a	500 ± 10	2	160 ± 20	0.67	3
5.26b	1,000 ± 550	2	630 ± 180	0.29	3
5.27a	1,300 ± 460	2	400 ± 160	0.31	4
5.27b	1,000 ± 500	2	100 ± 19	0.81	4

[a] Luciferase reporter gene assays using HEK293-hH4R-CRE-Luc cells expressing the hH_4R were performed as described in section *Pharmacological methods*. [b] Displacement of [^{3}H]histamine (5 nM) from HEK293-hH4R-CRE-Luc cells expressing the hH_4R was determined as described in section *Pharmacological methods.* [a,b] Reaction mixtures contained ligands at a concentration from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. N gives the number of independent experiments performed in duplicate. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. The α values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM.

5.3.3 Summary and conclusion

Starting from the imidazolylbutylcyanoguanidine UR-PI376, a potent hH_4R agonist with some selectivity over the H_3R, conformationally constrained analogues were synthesized. The aim was to obtain potent H_4R agonists with improved selectivity and to acquire information about structure-activity and structure-selectivity relationships of the compounds at the distinct HR subtypes. Therefore, the structural features of UR-PI376 were combined with those of OUP-16, a furane-type potent H_4R agonist. In a first approach a series of racemic imidazolylcyclopentylcyanoguanidines was prepared. In [^{35}S]GTPγS binding assays, the amine precursors **5.16**, **5.17** and most of the cyanoguanidines (**5.18-5.28**) displayed (partial) agonistic activity at the hH_4R. Only the phenoxyethyl and phenaminoethyl substituted cyanoguanidines were antagonists at the H_4R. The most potent hH_4R agonist was the *trans*-configured phenylthioethyl substituted derivative **5.26** with an EC_{50} of 14 nM. The variation of the substituent in the "eastern part" of the molecule revealed that larger residues are

required for selectivity over the H_3R and provide a higher potency at the H_4R. Activities at the H_2R and H_1R were negligible. At both, the H_4R and the H_3R, the pharmacological properties were strongly dependent on the stereochemistry of the cyclopentane-1,3-diyl moiety. At the H_4R a preference for *trans* configuration was observed except for the methyl-substituted cyanoguanidines. At the H_3R *cis* configuration was favored in most cases. The phenylthioethyl substituted derivative **5.26** turned out to be the most potent and selective racemate with an EC_{50} of 14 nM and an 18-fold selectivity over the H_3R. Therefore, in a second approach, aiming at more detailed information about the stereochemical requirements, *trans*-configured **5.26** and its *cis* diastereomer **5.27**, as well as the *trans*- and *cis*-configured carba analogues of OUP-16, **5.18** and **5.19**, were further investigated. The chiral separation of these racemates was possible by chiral semi-preparative HPLC using an OJ-H column and isocratic elution with heptane/2-propanol. All isomers were obtained in high enantiomeric purity (ee > 95 %). Finally, an enzyme assisted stereoselective synthesis was developed that allowed the precise assignment of the absolute configuration of all stereoisomers. The data for the separated enantiomerically pure compounds are in agreement with those for OUP-16 and its analogues. *trans*-(+)-(1*S*,3*S*)-UR-RG94 (**5.18b**) (EC_{50} = 100 nM, α = 0.71) was the most potent and selective H_4R agonist among the methyl-substituted cyanoguanidines, displaying a 10-fold selectivity over the H_3R. Obviously, the exchange of the oxygen atom with a carbon atom in the five membered ring, does not significantly affect the pharmacological profile of this compound class. The evaluation of the phenylthioethyl substituted isomers yielded a highly potent and selective H_4R agonist: *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) activated the H_4R with an EC_{50} value of 11 nM and an intrinsic activity of 0.75 in the [^{35}S]GTPγS binding assay and displayed more than 100-fold selectivity over the H_3R. Surprisingly, a high degree of stereoselectivity was observed. The optical antipode, *trans*-(-)-(1*R*,3*R*)-UR-RG98 (**5.26b**), had just the opposite effects: **5.26b** was a weak antagonist at the H_4R, but by a factor of almost 10 more potent at the H_3R (K_B = 143 nM, α = -0.21) compared to **5.26a**. The *cis*–configured enantiomers **5.27a** and **5.27b** were both moderately potent partial agonists at the H_4R and inverse agonists at the H_3R. All investigated compounds did not show agonistic activity at the H_2R and H_1R. At the H_4R highest potency resided in the stereoisomers with *S*-configuration in position 3. The configuration at position 1 seems less important, although the results suggest a preference for *S*-configuration. At the H_3R no evident correlation between configuration and potency

was observed. In summary, highest H_4R agonistic activity and selectivity was observed for *trans*-(1*S*,3*S*)-configured imidazolylcyclopentylmethylcyanoguanidines. Control experiments performed with selected racemic compounds in the GTPase assay revealed data comparable to those obtained in the [^{35}S]GTPγS assay. The measured ligand-stimulated increase in [^{35}S]GTPγS binding was H_4R-mediated as demonstrated by inhibition of the agonistic effect of *trans*-(+)-(1*S*,3*S*)-UR-RG98 by different hH_4R antagonists.

The potencies in the functional assays were in agreement with the affinities determined in radioligand binding studies. Moreover, a cell based luciferase reporter gene assay provided similar results as the [^{35}S]GTPγS assay and confirmed the high potency of *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) and the stereoselectivity compared to its optical antipode *trans*-(-)-(1*R*,2*R*)-UR-RG98 (**5.26b**). The evaluation of the separated isomers at the mouse H_4R revealed significantly lower potencies at the rodent compared to the human receptor orthologue for almost every compound. These species-dependent differences should be taken into account with respect to animal studies. Surprisingly, *cis*-(+)-(1*R*,3*S*)-UR-RG98 (**5.27b**) showed higher potency and efficacy at the murine H_4R and might be considered as pharmacological tool in mouse models.

In summary, a new highly potent and selective cyanoguanidine-type hH_4R agonists, *trans*-(+)-(1*S*,3*S*)-UR-RG98 was discovered and will be a valuable additional pharmacological tool to study the biological functions of the hH_4R. Moreover, the collected data on the stereoselectivity of the ligand - H_4R interactions provide valuable information for future ligand design and molecular modeling studies.

5.4 Experimental Section

5.4.1 Chemistry

5.4.1.1 General Conditions

See section 3.4.1.1.

Cholesterol esterase from porcine pancreas (EC 3.1.1.13) was from Sigma-Aldrich Chemie GmbH (Munich, Germany). Optical rotations were measured on a Perkin Elmer 141 polarimeter in the specified solvent. Concentrations are indicated in [g/100 mL].

5.4.1.2 Preparation of the isoureas 5.36 and 5.32-5.33

General procedure[14, 15]

A solution of the pertinent amine (1 eq) and diphenyl cyanocarbonimidate (**3.21**, 1 eq) in 2-propanol was stirred for 1 h. After evaporation of the solvent, the product was crystallized from Et_2O.

(±)-1-Cyano-3-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-2-phenylisourea (5.32)

The title compound was prepared from **3.21** (0.05 g, 0.3 mmol) and **5.16** (0.072 g, 0.3 mmol) in 2-propanol (10 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/32 % $NH_{3(aq)}$ 90/8/2 v/v/v) yielding a colorless oil (0.08 g, 86 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.38 (m, 1H, C***H***$_2$-2), 1.73 (m, 2H, C***H***$_2$), 1.92 (m, 2H, C***H***$_2$), 2.09 (m, 1H, C***H***$_2$-2), 2.44 (m, 1H, C***H***-CH_2-N), 3.19 (m, 1H, C***H***-Im), 3.27 (m, 1H, CH-C***H***$_2$-N), 3.40 (m, 1H, CH-C***H***$_2$-N), 6.79 (s, 1H, Im-***H***-5), 7.10 (d, 1H, ^3J = 8.0 Hz, Ph-***H***), 7.14 (d, 1H, ^3J = 8.0 Hz, Ph-***H***), 7.31 (m, 1H, Ph-***H***), 7.44 (m, 2H, Ph-***H***), 7.60 (s, 1H, Im-***H***-2). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 310 (100) $[M + H]^+$. $C_{17}H_{19}N_5O$ (309.37).

(±)-1-Cyano-3-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-2-phenylisourea (5.33)

The title compound was prepared from **3.21** (0.05 g, 0.3 mmol) and **5.17** (0.072 g, 0.3 mmol) in 2-propanol (10 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/32 % $NH_{3(aq)}$ 90/8/2 v/v/v) yielding a colorless oil (0.09 g, 97 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.45 (m, 2H, C***H***$_2$), 1.83 (m, 2H, C***H***$_2$), 2.06 (m, 1H, C***H***$_2$-2), 2.23 (m, 1H, C***H***$_2$-2), 2.41 (m, 1H, C***H***-CH_2-N), 3.13 (m, 1H, C***H***-Im), 3.29 (m, 1H, CH-C***H***$_2$-N), 3.44 (m, 1H, CH-C***H***$_2$-N), 6.80 (s, 1H, Im-***H***-5), 7.10 (d, 1H, ^3J = 7.7 Hz, Ph-***H***), 7.17 (d, 1H, ^3J = 7.9 Hz, Ph-***H***), 7.31 (m, 1H, Ph-***H***), 7.44 (m, 2H, Ph-***H***), 7.59 (s, 1H, Im-***H***-2). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 310 (100) $[M + H]^+$. $C_{17}H_{19}N_5O$ (309.37).

1-Cyano-2-phenyl-3-[2-(phenoxy)ethyl]isourea (5.36)

The title compound was prepared from **3.21** (2.0 g, 8.4 mmol) and **5.35** (1.4 mL, 11.0 mmol) in 2-propanol (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 90/10 v/v) yielding a white solid (2.22 g, 94 %); mp 121 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.86 (m, 2H, Ph-O-CH_2-C***H***$_2$), 4.16 (t, 2H, ^3J = 5.1 Hz, Ph-O-C***H***$_2$), 6.93 (m, 2H, Ph-***H***), 7.00 (t, 1H, ^3J = 7.3 Hz, Ph-***H***), 7.10 (m, 2H, Ph-***H***), 7.30

(m, 3H, Ph-***H***), 7.42 (m, 2H, Ph-***H***). CI-MS (NH_3) *m/z* (%): 282 (100) $[M + H]^+$, 299 (80) $[M + NH_4]^+$. $C_{16}H_{15}N_3O_2$ (281.31).

5.4.1.3 Preparation of the amine precursors 5.16 and 5.17

(1*R*,3*S*)-Cyclopentane-1,3-dicarboxylic acid (5.6)[7]

To a solution of norbornene (18.8 g, 0.2 mol, 1 eq) and ruthenium chloride (1.0 g, 4.4 mmol, 2.2 mol %) in 800 mL ethyl acetate/acetonitrile 1:1 (v/v) was added a solution of $NaIO_4$ (175.2 g, 0.82 mol, 4.1 eq) in 600 mL water. The mixture was allowed to stir for 2 days with occasional shaking. The organic and the aqueous phases were separated and filtered to remove any ruthenium oxide. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (3 x 400 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness to yield a white crystalline solid. (31.3 g, 99 %); mp 112°C (ref.[32]: 114 °C). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.91 – 2.08 (m, 5H, C***H***$_2$), 2.18 – 2.28 (m, 1H, C***H***$_2$), 2.81 (m, 2H, 2 C***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.40 (-, 2 ***C***H_2), 34.52 (-, ***C***H_2), 45.08 (+, 2 ***C***H), 179.30 (C_{quat}, 2 ***C***OOH). ES-MS (MeOH + NH_4OAc) *m/z* (%): 157 (100) $[M – H]^-$, 113 (35) $[M – H – CO_2]^-$. $C_7H_{10}O_4$ (158.15).

(1*R*,3*S*)-Cyclopentane-1,3-diyldimethanol (5.7)[8, 9]

Borane · THF (285 mL, 285 mmol, 4.5 eq) was slowly added to a solution of **5.6** (10.0 g, 63.2 mmol, 1 eq) in 100 mL THF at 0 °C. After stirring at room temperature overnight water was slowly added under ice-cooling and most of the THF was evaporated. The aqueous phase was extracted with 3 x 200 mL EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated yielding a colorless oil. (6.61 g, 80 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.93 (m, 1H, C***H***$_2$), 1.34 (m, 2H, C***H***$_2$), 1.74 (m, 2H, C***H***$_2$), 1.95 (m, 1H, C***H***$_2$), 2.14 (m, 2H, 2 C***H***), 3.52 (m, 4H, 2 C***H***$_2$-OH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.27 (-, 2 ***C***H_2), 32.75 (-, ***C***H_2), 42.23 (+, 2 ***C***H), 67.24 (-, 2 ***C***H_2-OH). CI-MS (NH_3) *m/z* (%): 148 (100) $[M + NH_4]^+$, 131 (15) $[M + H]^+$. $C_7H_{14}O_2$ (130.18).

{3-[(*tert*-Butyldiphenylsilyloxy)methyl]cyclopentyl}methanol (5.8)[33]

A solution of **5.7** (7.2 g, 55.3 mmol) in 30 mL DCM_{abs} was cooled to 0 °C. Under nitrogen atmosphere DIPEA (10.2 mL, 60 mmol) and a solution of *tert*-butyldiphenylsilylchloride (14.9 g, 54 mmol) in 10 mL DCM_{abs} were slowly added. The mixture was stirred at room

temperature for 15 h. After evaporation of the solvent the residue was taken up in 50 mL water and extracted with 3 x 80 mL ethyl acetate. The combined organic layers were dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (PE/ EtOAc 80/20 v/v) to give **5.8** as colorless oil. Unprotected starting material was recovered and the procedure was repeated twice. Total yield (11.6 g, 57 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.93 (m, 1H, C***H***$_2$), 1.06 (s, 9H, C(C***H***$_3$)$_3$), 1.37 (m, 2H, C***H***$_2$), 1.74 (m, 2H, C***H***$_2$), 1.94 (m, 1H, C***H***$_2$), 2.09 – 2.25 (m, 2H, 2 C***H***), 3.51 (d, 2H, ^3J = 6.8 Hz, C***H***$_2$-O), 3.57 (d, 2H, ^3J = 6.3 Hz, C***H***$_2$-O), 7.41 (m, 6H, Ph-***H***), 7.66 (m, 4H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 19.33 (C_{quat}, ***C***$(CH_3)_3$), 26.90 (+, C(***C***H$_3$)$_3$), 28.23 (-, ***C***H$_2$), 28.37 (-, ***C***H$_2$), 32.77 (-, ***C***H$_2$), 42.24 (+, ***C***H), 42.43 (+, ***C***H), 67.57 (-, ***C***H$_2$-O), 67.75 (-, ***C***H$_2$-O), 127.61 (+, 4 Ph-***C***), 129.54 (+, 2 Ph-***C***-4), 134.07 ($C_{quat,}$ 2 Ph-***C***-1), 135.64 (+, 4 Ph-***C***). CI-MS (NH_3) *m/z* (%): 369 (100) $[M + H]^+$, 386 (35) $[M + NH_4]^+$. $C_{23}H_{32}O_2Si$ (368.58).

3-[(*tert*-Butyldiphenylsilyloxy)methyl]cyclopentanecarbaldehyde (5.9)

A mixture of DMSO (8.9 mL, 125.4 mmol, 4 eq) and DCM (20 mL) was added slowly to a solution of oxalyl chloride (5.4 mL, 62.9 mmol, 2 eq) in DCM (50 mL) at -78 °C over 30 min. Then a solution of **5.8** (11.6 g, 31.5 mmol, 1 eq) in DCM (30 mL) was added. The resulting mixture was stirred at the same temperature for 2 h, and then NEt_3 (35 mL, 251.8 mmol, 8 eq) was added and stirring was continued for additional 30 min. The mixture was allowed to warm to room temperature and stirred overnight. 80 mL of water were added and the resulting layers were separated. The aqueous layer was extracted with 3 x 50 mL DCM and the combined organic layers washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (PE/EtOAc 90/10 v/v) to give **5.9** as yellow oil (10.6 g, 92 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.06 (s, 9H, C(C***H***$_3$)$_3$), 1.41 (m, 1H, C***H***$_2$), 1.61 (m, 1H, C***H***$_2$), 1.84 (m, 3H, C***H***$_2$), 2.00 (m, 1H, C***H***$_2$), 2.26 (m, 1H, C***H***), 2.76 (m, 1H, C***H***), 3.54 – 3.60 (m, 2H, C***H***$_2$-O), 7.36 – 7.46 (m, 6H, Ph-***H***), 7.64 – 7.68 (m, 4H, Ph-***H***)), 9.60 (d, ^3J = 2.7 Hz, 1H, C***H***O). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 19.31 (C_{quat}, ***C***$(CH_3)_3$), 26.09 (-, ***C***H$_2$), 26.87 (+, C(***C***H$_3$)$_3$), 28.70 (-, ***C***H$_2$), 29.66 (-, ***C***H$_2$), 42.53 (+, ***C***H-CH$_2$O), 51.71 (+, ***C***H-CHO), 66.95 (-, ***C***H$_2$-O), 127.66 (+, 4 Ph-***C***), 129.62 (+, 2 Ph-***C***-4), 133.84 ($C_{quat,}$ 2 Ph-***C***-1), 135.64 (+, 4 Ph-***C***), 203.94 (+, ***C***HO). CI-MS (NH_3) *m/z* (%): 384 (100) $[M + NH_4]^+$, 367 (20) $[M + H]^+$. $C_{23}H_{30}O_2Si$ (366.57).

[3-(1*H*-Imidazol-4-yl)cyclopentyl]methanol (5.10)

Finely powdered sodium cyanide (0.26 g, 5.4 mmol) was added in one portion to a stirred suspension of tosylmethyl isocyanide **3.16** (6.85 g, 35.3 mmol) and **5.9** (12.9 g, 35.3 mmol) in 100 mL of absolute ethanol at 0 °C. The reaction mixture became clear, and the solution was stirred for another 30 min. The solvent was evaporated under reduced pressure. The resulting slurry is dissolved in 45 mL 7 M NH_3 in methanol and stirred in portions of 15 mL under microwave irradiation at 100 °C and 12 bar for 18 h. The solvent was evaporated and the residue taken up in 50 mL THF. After adding 30 mL of a 1.1 M solution of tetrabutylammonium fluoride in THF the mixture was stirred at room temperature overnight. After evaporation of the solvent the mixture was subjected to flash chromatography (DCM/MeOH 90/10 v/v) to yield **5.10** as brown oil (4.56 g, 78 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.34 (m, 1H, C***H***$_2$), 1.47 – 1.73 (m, 2H, C***H***$_2$ + C***H***), 1.83 (m, 1H, C***H***$_2$), 2.04 (m, 1H, C***H***$_2$), 2.22 (m, 2H, C***H***$_2$), 3.07 (m, 1H, C***H***-Im), 3.48 (m, 2H, C***H***$_2$-OH), 6.75 (s, 1H, Im-***H***-5), 7.54 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 29.21, 29.96 (-, ***C***H$_2$), 33.17, 34.14 (-, ***C***H$_2$), 36.60, 37.90 (-, ***C***H$_2$), 39.15 (+, ***C***H), 42.13, 43.16 (+, ***C***H), 67.45, 67.55 (-, ***C***H$_2$-OH), 121.44 (+, Im-***C***-5), 135.76 (+, Im-***C***-2), 135.80 (C_{quat}, Im-***C***-4). CI-MS (NH_3) *m/z* (%): 167 (100) $[M + H]^+$. $C_9H_{14}N_2O$ (166.22).

[3-(1-Trityl-1*H*-imidazol-4-yl)cyclopentyl]methanol (5.11)

To a solution of **5.10** (4.4 g, 26.3 mmol) and NEt_3 (7.3 mL, 52.6 mmol) in DMF (50 mL), trityl chloride (7.3 g, 26.3 mmol) was slowly added. The mixture was stirred for 24 h at room temperature. The reaction was stopped by adding 10 mL water. After evaporation of the solvents the residue was taken up in $CHCl_3$ and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (PE/EtOAc 80/20 v/v – $CHCl_3$/MeOH 90/10 v/v) yielded a brown oil (8.9 g, 83 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.20 – 2.32 (m, 7H, C***H***$_2$ + C***H***), 3.04 (m, 1H, C***H***-Im), 3.48 – 3.62 (m, 2H, C***H***$_2$-OH), 6.49 (s, 1H, Im-***H***-5), 7.10 (m, 6H, Ph-***H***), 7.29 (m, 10H, Ph-***H*** + Im-***H***-2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.15, 28.92 (-, ***C***H$_2$), 31.43, 33.01 (-, ***C***H$_2$), 35.47, 36.05 (-, ***C***H$_2$), 38.11, 38.71 (+, ***C***H), 41.00, 41.71 (+, ***C***H), 66.96, 67.14 (-, ***C***H$_2$-OH), 75.15 (C_{quat}, ***C***Ph$_3$), 116.67, 116.59 (+, Im-***C***-5), 127.97 (+, 9 Ph-***C***), 129.78 (+, 6 Ph-***C***), 138.12, 138.25 (+, Im-***C***-2), 142.48, 142.52 (C_{quat}, 3 Ph-***C***-1), 145.41, 145.55 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 409 (100) $[M + H]^+$. $C_{28}H_{28}N_2O$ (408.53).

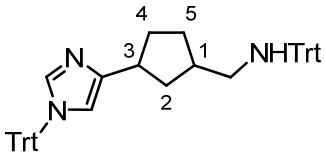
2-{[3-(1-Trityl-1*H*-imidazol-4-yl)cyclopentyl]methyl}isoindoline-1,3-dione (5.12)

To a stirred solution of **5.11** (10.6 g, 25.9 mmol) in THF_{abs} (150 mL), phthalimide (6.1 g, 41.6 mmol) and triphenylphosphine (10.9 g, 41.6 mmol) were added at 0 °C. Under ice cooling DIAD (10.9 mL, 55.5 mmol) in 50 mL THF_{abs} was added dropwise over a period of 1.5 h. The mixture was allowed to warm to ambient temperature, stirred overnight and concentrated *in vacuo*. Flash chromatography (PE/EtOAc 80/20 – 50/50 v/v) yielded a yellow foam-like solid (10.5 g, 75 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.27 – 1.57 (m, 2H, C***H***$_2$), 1.62 – 1.94 (m, 3H, C***H***$_2$), 2.06 – 2.21 (m, 1H, C***H***$_2$), 2.47 – 2.64 (m, 1H, C***H***), 3.00 – 3.25 (m, 1H, C***H***-Im), 3.63 – 3.69 (m, 2H, C***H***$_2$-Phthal), 6.50, 6.55 (2s, 1H, Im-***H***-5), 7.12 (m, 6H, Ph-***H***), 7.33 (m, 10H, Ph-***H*** + Im-***H***-2), 7.68 – 7.71 (m, 2H, Phthal-***H***), 7.81 – 7.84 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.20, 30.37 (-, ***C***H_2), 31.47, 32.62 (-, ***C***H_2), 36.39, 37.99 (-, ***C***H_2), 37.68, 38.11 (+, ***C***H), 39.04, 39.16 (+, ***C***H), 42.90 (-, ***C***H_2-Phthal), 75.15 (C_{quat}, ***C***Ph_3), 116.54, 116.70 (+, Im-***C***-5), 123.16 (+, Phthal-***C***-4,7), 127.96 (+, 3 Ph-***C***-4), 127.99 (+, 6 Ph-***C***), 129.80 (+, 6 Ph-***C***), 133.17 (C_{quat}, Phthal-***C***-3a,7a), 133.84 (+, Phthal-***C***-5,6), 138.33, 138.36 (+, Im-***C***-2), 142.53 (C_{quat}, 3 Ph-***C***-1), 145.06, 145.42 (C_{quat}, Im-***C***-4), 168.57, 168.63 (C_{quat}, 2 ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 538 (100) $[M + H]^+$. $C_{36}H_{31}N_3O_2$ (537.65).

[3-(1-Trityl-1*H*-imidazol-4-yl)cyclopentyl]methanamine (5.13)

To a solution of **5.12** (10.5 g, 19.5 mmol) in ethanol (100 mL) hydrazine monohydrate (4.7 mL, 100 mmol) was added and the mixture was refluxed for 1.5 h. Under stirring the mixture was allowed to cool to room temperature for 1 h. The precipitate was filtered off and washed with cold ethanol. The solvent was evaporated and the residue purified by flash chromatography ($CHCl_3$/MeOH 90/10 – 70/30 v/v) to yield a yellow foam-like solid (7.3 g, 92 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.16 – 1.45 (m, 1H, C***H***$_2$), 1.57 – 2.22 (m, 6H, C***H***$_2$ + C***H***), 2.65 (d, 1H, ^{3}J = 7.2 Hz, C***H***$_2$-NH_2), 2.70 (d, 1H, ^{3}J = 6.6 Hz, C***H***$_2$-NH_2), 3.02 (m, 1H, C***H***-Im), 6.49 (s, 1H, Im-***H***-5), 7.09 – 7.13 (m, 6H, Ph-***H***), 7.30 – 7.32 (m, 10H, Ph-***H*** + Im-***H***-2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.21, 30.36 (-, ***C***H_2), 32.13, 32.94 (-, ***C***H_2), 36.55, 37.74 (-, ***C***H_2), 38.00, 39.02 (+, ***C***H), 41.49, 42.15 (+, ***C***H), 47.18, 47.46 (-, ***C***H_2-NH_2), 75.09 (C_{quat}, ***C***Ph_3), 116.16 (+, Im-***C***-5), 127.97 (+, 9 Ph-***C***), 129.80 (+, 6 Ph-***C***), 138.35 (+, Im-***C***-2), 142.57 (C_{quat}, 3 Ph-***C***-1), 145.37, 145.65 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 408 (100) $[M + H]^+$, 243 (50) $[Trt]^+$. $C_{28}H_{29}N_3$ (407.55).

Cis- and _trans_-1,1,1-Triphenyl-_N_-{[3-(1-trityl-1_H_-imidazol-4-yl)cyclopentyl]methyl}-methanamine (5.14, 5.15)



To a cooled solution of **5.13** (7.3 g, 17.9 mmol) and NEt_3 (5.0 mL, 35.8 mmol) in DCM_{abs} (80 mL), trityl chloride (7.5 g, 26.3 mmol) was slowly added. The mixture was stirred for 24 h at room temperature. The reaction was stopped by adding 50 mL water. The layers were separated and the combined organic layers were washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The products **5.14** and **5.15** were separated by flash-chromatography (PE/EtOAc/32 % $NH_{3(aq)}$ 80/19/1 v/v/v) as white foam-like solids (total yield 11.0 g, 95 %);

Compound *trans*-**5.14**: mp 159 °C. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.22 (m, 1H, C***H***$_2$-5), 1.65 (m, 2H, C***H***$_2$-2 + C***H***$_2$-4), 1.88 (m, 1H, C***H***$_2$-2), 1.93 (m, 1H, C***H***$_2$-5), 1.99 (m, 1H, C***H***$_2$-4), 2.11 (d, 2H, ^3J = 7.2 Hz, C***H***$_2$-N), 2.22 (m, 1H, C***H***-1), 3.01 (m, 1H, C***H***-Im), 6.50 (s, 1H, Im-***H***-5), 7.14 – 7.16 (m, 6H, Ph-***H***), 7.17 (m, 1H, Ph-***H***), 7.18 (m, 1H, Ph-***H***), 7.27 (m, 6H, Ph-***H***), 7.30 (m, 1H, Ph-***H***), 7.34 (m, 9H, Ph-***H***), 7.37 (s, 1H, Im-***H***-2), 7.50 (m, 6H, Ph-***H***). ^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 30.94 (-, ***C***H_2-5), 32.84 (-, ***C***H_2-4), 37.04 (-, ***C***H_2-2), 37.75 (+, ***C***H-Im), 39.75 (+, ***C***H-1), 48.97 (-, ***C***H_2-N), 70.72 (C_{quat}, N-***C***Ph_3), 75.10 (C_{quat}, Im-***C***Ph_3), 116.54 (+, Im-***C***-5), 126.05, 127.66, 127.93, 128.65, 129.77 (+, 30 Ph-***C***), 138.35 (+, Im-***C***-2), 142.51 (C_{quat}, 3 Ph-***C***-1), 145.84 (C_{quat}, Im-***C***-4), 146.30 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 650 (80) $[M + H]^+$, 408 (100) $[M – Trt + H]^+$, 243 (45) $[Trt]^+$. $C_{47}H_{43}N_3$ (649.86).

Compound *cis*-**5.15**: mp 168 °C. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.20 (m, 1H, C***H***$_2$-2), 1.32 (m, 1H, C***H***$_2$-5), 1.61 (m, 1H, C***H***$_2$-4), 1.85 (m, 1H, C***H***$_2$-5), 2.00 (m, 1H, C***H***$_2$-4), 2.09 (m, 1H, C***H***$_2$-N), 2.17 (m, 2H, C***H***$_2$-N + C***H***-1), 2.24 (m, 1H, C***H***$_2$-2), 3.04 (m, 1H, C***H***-Im), 6.48 (s, 1H, Im-***H***-5), 7.12 – 7.14 (m, 6H, Ph-***H***), 7.15 (m, 1H, Ph-***H***), 7.16 (m, 1H, Ph-***H***), 7.17 (m, 1H, Ph-***H***), 7.25 (m, 6H, Ph-***H***), 7.32 (m, 9H, Ph-***H***), 7.34 (s, 1H, Im-***H***-2), 7.49 (m, 6H, Ph-***H***). ^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 29.65 (-, ***C***H_2-5), 31.64 (-, ***C***H_2-4), 38.71 (-, ***C***H_2-2), 39.12 (+, ***C***H-Im), 41.07 (+, ***C***H-1), 49.11 (-, ***C***H_2-N), 70.71 (C_{quat}, N-***C***Ph_3), 75.03 (C_{quat}, Im-***C***Ph_3), 116.34 (+, Im-***C***-5), 126.04, 127.65, 127.91, 128.65, 129.77 (+, 30 Ph-***C***), 138.31 (+, Im-***C***-2), 142.57 (C_{quat}, 3 Ph-***C***-1), 145.53 (C_{quat}, Im-***C***-4), 146.30 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH +

NH_4OAc) *m/z* (%): 650 (80) $[M + H]^+$, 408 (100) $[M – Trt + H]^+$, 243 (45) $[Trt]^+$. $C_{47}H_{43}N_3$ (649.86).

(±)-*trans*-[3-(1*H*-Imidazol-4-yl)cyclopentyl]methanamine (5.16)

A solution of **5.14** (3.0 g, 4.6 mmol) in 30 mL MeOH and 3 mL 37 % HCl was refluxed for 3 h. The solvent was removed *in vacuo* and the residue washed with Et_2O. Ethanol was added and evaporation yielded the hydrochloride of **5.16** as white semisolid. The hydrochloride was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). Evaporation of the solvent yielded **5.16** as yellow oil (0.67 g, 88 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.30 (m, 1H, C***H***$_2$), 1.71 (m, 2H, C***H***$_2$), 1.85 (m, 1H, C***H***$_2$), 1.96 (m, 1H, C***H***$_2$), 2.04 (m, 1H, C***H***$_2$) 2.17 (m, 1H, C***H***), 2.59 (d, 2H, ^3J = 7.0 Hz, C***H***$_2$-NH_2), 3.12 (m, 1H, C***H***-Im), 6.75 (s, 1H, Im-***H***-5), 7.54 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.38 (-, ***C***H_2), 34.07 (-, ***C***H_2), 37.63 (-, ***C***H_2), 37.86 (+, ***C***H), 42.70 (+, ***C***H), 48.26 (-, ***C***H_2-NH_2), 117.12 (+, Im-***C***-5), 135.83 (+, Im-***C***-2), 142.04 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 166 (60) $[M + H]^+$, 145 (100) $[M + 2\ H + 3\ MeCN]^{2+}$. HRMS (EI-MS) calcd. for $C_9H_{15}N_3$ $[M^{+\bullet}]$ 165.1266; found 165.1270. IR (cm^{-1}) = 2948, 2861 (C-H), 1738, 1555, 1448, 1377, 1320, 1107. Anal. ($C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.2\ H_2O$) C, H, N. $C_9H_{15}N_3$ (165.24).

(±)-*cis*-[3-(1*H*-Imidazol-4-yl)cyclopentyl]methanamine (5.17)

A solution of **5.15** (3.8 g, 5.8 mmol) in 30 mL MeOH and 3 mL 37 % HCl was refluxed for 3 h. The solvent was removed *in vacuo* and the residue washed with Et_2O. Ethanol was added and evaporation yielded the hydrochloride of **5.17** as white semisolid; The hydrochloride was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). Evaporation of the solvent yielded **5.17** as yellow oil (0.84 g, 88 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.29 (m, 1H, C***H***$_2$), 1.46 (m, 1H, C***H***$_2$), 1.71 (m, 1H, C***H***$_2$), 1.88 (m, 1H, C***H***$_2$), 2.06 (m, 2H, C***H***$_2$ + C***H***), 2.20 (m, 1H, C***H***$_2$), 2.62 (d, 2H, ^3J = 6.9 Hz, C***H***$_2$-NH_2), 3.08 (m, 1H, C***H***-Im), 6.75 (s, 1H, Im-***H***-5), 7.53 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.25 (-, ***C***H_2), 33.06 (-, ***C***H_2), 39.04 (+, ***C***H), 39.16 (-, ***C***H_2), 43.78 (+, ***C***H), 48.26 (-, ***C***H_2-NH_2), 117.10 (+, Im-***C***-5), 135.80 (+, Im-***C***-2), 141.78 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 166 (75) $[M + H]^+$, 145 (100) $[M + 2\ H + 3\ MeCN]^{2+}$. HRMS (EI-MS) calcd. for $C_9H_{15}N_3$ $[M^{+\bullet}]$ 165.1266; found 165.1265. IR (cm^{-1}) = 2947, 2861 (C-H),

1739, 1653, 1562, 1486, 1448, 1107. Anal. ($C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.2\ H_2O$) C, H, N. $C_9H_{15}N_3$ (165.24).

5.4.1.4 Preparation of the cyanoguanidines 5.18-5.31

General Procedure[13, 34]

The isourea (1 eq) and the pertinent amine (1 eq) in MeCN were heated under microwave irradiation at 150 °C for 15 min. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (DCM/MeOH/32 % $NH_{3(aq)}$ 95/4/1 v/v/v).

(±)-2-Cyano-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.18)

The title compound was prepared from **5.16** (0.05 g, 0.3 mmol) and **3.27** (0.053 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.07 g, 95 %); mp 41 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.35 (m, 1H, C***H***$_2$), 1.68 (m, 1H, C***H***$_2$), 1.83 (m, 2H, C***H***$_2$), 1.94 (m, 1H, C***H***$_2$), 2.08 (m, 1H, C***H***$_2$), 2.39 (m, 1H, C***H***), 2.79 (s, 3H, C***H***$_3$-N), 3.18 (m, 3H, C***H***-Im + C***H***$_2$-N), 6.80 (s, 1H, Im-***H***-5), 7.60 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.74 (+, ***C***H$_3$), 30.96 (-, ***C***H$_2$), 33.86 (-, ***C***H$_2$), 37.84 (-, ***C***H$_2$), 37.71 (+, ***C***H), 39.68 (+, ***C***H), 47.71 (-, ***C***H$_2$-N), 116.71 (+, Im-***C***-5), 120.28 (C_{quat}, ***C***≡N), 134.98 (+, Im-***C***-2), 141.94 (C_{quat}, Im-***C***-4), 162.05 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{18}N_6$ [$M^{+\bullet}$] 246.1593; found 246.1587. IR (cm^{-1}) = 3289 (N-H), 3142, 2940, 2864 (C-H), 2163 (C≡N), 1582 (C=N), 1368. Anal. ($C_{12}H_{18}N_6 \cdot 0.5\ H_2O \cdot CH_3OH$) C, H, N. $C_{12}H_{18}N_6$ (246.31).

(±)-2-Cyano-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.19)

The title compound was prepared from **5.17** (0.05 g, 0.3 mmol) and **3.27** (0.053 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.065 g, 82 %); mp 61 – 63 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.33 (m, 1H, C***H***$_2$), 1.50 (m, 1H, C***H***$_2$), 1.71 (m, 1H, C***H***$_2$), 1.85 (m, 1H, C***H***$_2$), 2.03 (m, 1H, C***H***$_2$), 2.19 (m, 1H, C***H***$_2$), 2.33 (m, 1H, C***H***), 2.79 (s, 3H, C***H***$_3$-N), 3.09 (m, 1H, C***H***-Im), 3.18 (m, 2H, C***H***$_2$-N), 6.79 (s, 1H, Im-***H***-5), 7.57 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.73 (+, ***C***H$_3$), 30.07 (-, ***C***H$_2$), 32.93 (-, ***C***H$_2$), 38.78 (-, ***C***H$_2$), 39.01 (+, ***C***H), 40.71 (+, ***C***H), 47.82 (-, ***C***H$_2$-N), 116.75 (+, Im-***C***-5), 120.29 (C_{quat}, ***C***≡N), 135.87 (+, Im-***C***-2), 141.94 (C_{quat}, Im-***C***-4), 162.02 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{18}N_6$ [$M^{+\bullet}$] 246.1593; found 246.1593. IR (cm^{-1}) =

3258 (N-H), 3149, 2930, 2869 (C-H), 2160 (C≡N), 1575 (C=N), 1448, 1364, 1026. Anal. ($C_{12}H_{18}N_6 \cdot 1.2\ H_2O \cdot 0.2\ CH_3OH$) C, H, N. $C_{12}H_{18}N_6$ (246.31).

(±)-2-Cyano-3-cyclopropyl-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}guanidine (5.20)

The title compound was prepared from **5.16** (0.05 g, 0.3 mmol) and **3.28** (0.061 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.06 g, 74 %); mp 43 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.60 (m, 2H, cPr-C***H***$_2$), 0.83 (m, 2H, cPr-C***H***$_2$), 1.36 (m, 1H, C***H***$_2$), 1.68 (m, 1H, C***H***$_2$), 1.81 (m, 2H, C***H***$_2$), 1.93 (m, 1H, C***H***$_2$), 2.07 (m, 1H, C***H***$_2$), 2.39 (m, 1H, C***H***), 2.47 (m, 1H, cPr-C***H***), 3.17 (m, 1H, C***H***-Im), 3.22 (d, 2H, ^3J = 7.5 Hz, C***H***$_2$-N), 6.78 (s, 1H, Im-***H***-5), 7.56 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 8.12 (-, 2 cPr-***C***H$_2$), 23.77 (+, cPr-***C***H), 30.91 (-, ***C***H$_2$), 33.91 (-, ***C***H$_2$), 37.30 (-, ***C***H$_2$), 37.76 (+, ***C***H), 39.89 (+, ***C***H), 47.51 (-, ***C***H$_2$-N), 116.94 (+, Im-***C***-5), 120.11 (C_{quat}, ***C***≡N), 135.70 (+, Im-***C***-2), 139.67 (C_{quat}, Im-***C***-4), 162.52 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{14}H_{20}N_6$ [$M^{+\bullet}$] 272.1749; found 272.1743. IR (cm^{-1}) = 3261 (N-H), 2945, 2867 (C-H), 2160 (C≡N), 1570 (C=N), 1428, 1344, 1106. Anal. ($C_{14}H_{20}N_6 \cdot 0.4\ CH_3OH$) C, H, N. $C_{14}H_{20}N_6$ (272.35).

(±)-2-Cyano-3-cyclopropyl-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}guanidine (5.21)

The title compound was prepared from **5.17** (0.05 g, 0.3 mmol) and **3.28** (0.061 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.065 g, 80 %); mp 46 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.60 (m, 2H, cPr-C***H***$_2$), 0.82 (m, 2H, cPr-C***H***$_2$), 1.32 (m, 1H, C***H***$_2$), 1.50 (m, 1H, C***H***$_2$), 1.71 (m, 1H, C***H***$_2$), 1.83 (m, 1H, C***H***$_2$), 2.03 (m, 1H, C***H***$_2$), 2.18 (m, 1H, C***H***$_2$), 2.35 (m, 1H, C***H***), 2.47 (m, 1H, cPr-C***H***), 3.09 (m, 1H, C***H***-Im), 3.24 (m, 2H, C***H***$_2$-N), 6.78 (s, 1H, Im-***H***-5), 7.56 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 8.11 (-, 2 cPr-***C***H$_2$), 23.77 (+, cPr-***C***H), 30.01 (-, ***C***H$_2$), 32.94 (-, ***C***H$_2$), 38.79 (-, ***C***H$_2$), 39.06 (+, ***C***H), 40.93 (+, ***C***H), 47.61 (-, ***C***H$_2$-N), 116.78 (+, Im-***C***-5), 120.33 (C_{quat}, ***C***≡N), 136.20 (+, Im-***C***-2), 141.88 (C_{quat}, Im-***C***-4), 162.49 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3253 (N-H), 2924, 2855 (C-H), 2161 (C≡N), 1575 (C=N), 1447, 1343, 1104. Anal. ($C_{14}H_{20}N_6 \cdot 0.35\ CH_3OH$) C, H, N. $C_{14}H_{20}N_6$ (272.35).

(±)-2-Cyano-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-isobutylguanidine (5.22)

The title compound was prepared from **5.16** (0.05 g, 0.3 mmol) and **3.29** (0.066 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.07 g, 81 %); mp 47 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.91 (d, 6H, ^{3}J = 6.7 Hz, 2 C***H***$_3$), 1.36 (m, 1H, C***H***$_2$), 1.63 – 1.87 (m, 4H, C***H***$(CH_3)_2$ + C***H***$_2$), 1.94 (m, 1H, C***H***$_2$), 2.07 (m, 1H, C***H***$_2$), 2.39 (m, 1H, C***H***), 3.01 (d, 2H, ^{3}J = 7.1 Hz, N-C***H***$_2$-CH$(CH_3)_2$), 3.17 (m, 1H, C***H***-Im), 3.18 (d, 2H, ^{3}J = 7.6 Hz, C***H***$_2$-N), 6.78 (s, 1H, Im-***H***-5), 7.57 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 20.31 (+, 2 ***C***H$_3$), 29.60 (+, ***C***H$(CH_3)_2$), 30.98 (-, ***C***H$_2$), 33.86 (-, ***C***H$_2$), 37.40 (-, ***C***H$_2$), 37.80 (+, ***C***H), 39.67 (+, ***C***H), 47.73 (-, ***C***H$_2$-N), 50.08 (-, N-***C***H$_2$-CH$(CH_3)_2$), 116.71 (+, Im-***C***-5), 120.29 (C_{quat}, ***C***≡N), 135.09 (+, Im-***C***-2), 141.06 (C_{quat}, Im-***C***-4), 161.35 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{15}H_{24}N_6$ [$M^{+\bullet}$] 288.2062; found 288.2057. IR (cm^{-1}) = 3266 (N-H), 2955, 2868 (C-H), 2156 (C≡N), 1576 (C=N), 1447, 1426, 1385, 1270, 1159, 1105. Anal. ($C_{15}H_{24}N_6 \cdot 0.5\ CH_3OH$) C, H, N. $C_{15}H_{24}N_6$ (288.39).

(±)-2-Cyano-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-isobutylguanidine (5.23)

The title compound was prepared from **5.17** (0.05 g, 0.3 mmol) and **3.29** (0.066 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.08 g, 92 %); mp 62 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.91 (d, 6H, ^{3}J = 6.7 Hz, 2 C***H***$_3$), 1.31 (m, 1H, C***H***$_2$), 1.50 (m, 1H, C***H***$_2$), 1.71 (m, 1H, C***H***$_2$), 1.85 (m, 2H, C***H*** + C***H***$_2$), 2.03 (m, 1H, C***H***$_2$), 2.19 (m, 1H, C***H***$_2$), 2.33 (m, 1H, C***H***), 3.01 (d, 2H, ^{3}J = 7.2 Hz, N-C***H***$_2$-CH$(CH_3)_2$), 3.09 (m, 1H, C***H***-Im), 3.21 (d, 2H, ^{3}J = 8.3 Hz, C***H***$_2$-N), 6.78 (s, 1H, Im-***H***-5), 7.56 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 20.33 (+, 2 ***C***H$_3$), 29.61 (+, ***C***H$(CH_3)_2$), 30.12 (-, ***C***H$_2$), 32.95 (-, ***C***H$_2$), 38.82 (-, ***C***H$_2$), 39.06 (+, ***C***H), 40.74 (+, ***C***H), 47.84 (-, ***C***H$_2$-N), 50.09 (-, N-***C***H$_2$-CH$(CH_3)_2$), 116.82 (+, Im-***C***-5), 120.49 (C_{quat}, ***C***≡N), 135.85 (+, Im-***C***-2), 140.41 (C_{quat}, Im-***C***-4), 161.33 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{15}H_{24}N_6$ [$M^{+\bullet}$] 288.2062; found 288.2057. IR (cm^{-1}) = 3275 (N-H), 2956, 2868 (C-H), 2156 (C≡N), 1575 (C=N), 1449, 1426, 1384, 1270, 1157, 1107. Anal. ($C_{15}H_{24}N_6 \cdot 0.5\ CH_3OH$) C, H, N. $C_{15}H_{24}N_6$ (288.39).

(±)-2-Cyano-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(3-phenylpropyl)guanidine (5.24)

The title compound was prepared from **5.16** (0.04 g, 0.24 mmol) and **3.30** (0.068 g, 0.24 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography

yielded a yellow solid (0.075 g, 89 %); mp 50 – 51 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.35 (m, 1H, C***H***$_2$), 1.69 (m, 1H, C***H***$_2$), 1.86 (m, 4H, C***H***$_2$ + C***H***$_2$-CH_2-Ph), 1.94 (m, 1H, C***H***$_2$), 2.08 (m, 1H, C***H***$_2$), 2.38 (m, 1H, C***H***), 2.64 (t, 2H, ^{3}J = 7.5 Hz, C***H***$_2$-Ph), 3.12 – 3.25 (m, 5H, C***H***-Im + N-C***H***$_2$ + C***H***$_2$-N), 6.85 (s, 1H, Im-***H***-5), 7.11 – 7.27 (m, 5H, Ph-***H***), 7.74 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.98 (-, ***C***H_2), 32.30 (-, ***C***H_2), 33.80 (-, ***C***H_2), 33.99 (-, ***C***H_2), 37.27 (-, ***C***H_2), 37.49 (+, ***C***H), 39.68 (+, ***C***H), 42.38 (-, N-***C***H_2), 47.63 (-, ***C***H_2-N), 116.57 (+, Im-***C***-5), 120.37 (C_{quat}, ***C***≡N), 127.03 (+, Ph-***C***-4), 129.45 (+, 2 Ph-***C***), 129.51 (+, 2 Ph-***C***), 135.60 (+, Im-***C***-2), 139.37 (C_{quat}, Im-***C***-4), 142.86 (C_{quat}, Ph-***C***-1), 161.27 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{20}H_{26}N_6$ [$M^{+\bullet}$] 350.2219; found 350.2212. IR (cm^{-1}) = 3262 (N-H), 2930, 2864 (C-H), 2158 (C≡N), 1574 (C=N), 1452, 1426, 1362, 1104. Anal. ($C_{20}H_{26}N_6 \cdot 0.6\ CH_3OH$) C, H, N. $C_{20}H_{26}N_6$ (350.46).

(±)-2-Cyano-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(3-phenylpropyl)guanidine (5.25)

The title compound was prepared from **5.17** (0.04 g, 0.24 mmol) and **3.30** (0.068 g, 0.24 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.06 g, 72 %); mp 53 – 54 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.31 (m, 1H, C***H***$_2$), 1.49 (m, 1H, C***H***$_2$), 1.70 (m, 1H, C***H***$_2$), 1.85 (m, 1H, C***H***$_2$), 1.86 (m, 2H, C***H***$_2$-CH_2-Ph), 2.03 (m, 1H, C***H***$_2$), 2.18 (m, 1H, C***H***$_2$), 2.31 (m, 1H, C***H***), 2.64 (t, 2H, ^{3}J = 7.6 Hz, C***H***$_2$-Ph), 3.09 (m 1H, C***H***-Im), 3.16 – 3.24 (m, 4H, N-C***H***$_2$ + C***H***$_2$-N), 6.79 (s, 1H, Im-***H***-5), 7.11 – 7.27 (m, 5H, Ph-***H***), 7.58 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.10 (-, ***C***H_2), 32.31 (-, ***C***H_2), 32.93 (-, ***C***H_2), 33.99 (-, ***C***H_2), 38.84 (-, ***C***H_2), 39.05 (+, ***C***H), 40.73 (+, ***C***H), 42.38 (-, N-***C***H_2), 47.80 (-, ***C***H_2-N), 118.69 (+, Im-***C***-5), 120.98 (C_{quat}, ***C***≡N), 127.02 (+, Ph-***C***-4), 129.45 (+, 2 Ph-***C***), 129.50 (+, 2 Ph-***C***), 135.57 (+, Im-***C***-2), 140.71 (C_{quat}, Im-***C***-4), 142.86 (C_{quat}, Ph-***C***-1), 161.25 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{20}H_{26}N_6$ [$M^{+\bullet}$] 350.2219; found 350.2212. IR (cm^{-1}) = 3259 (N-H), 2939, 2864 (C-H), 2158 (C≡N), 1575 (C=N), 1451, 1426, 1362, 1104. Anal. ($C_{20}H_{26}N_6 \cdot 0.55\ CH_3OH$) C, H, N. $C_{20}H_{26}N_6$ (350.46).

(±)-2-Cyano-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-[2-(phenylthio)ethyl]guanidine (5.26)

The title compound was prepared from **5.16** (0.04 g, 0.24 mmol) and **3.31** (0.072 g, 0.24 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography

yielded a white solid (0.08 g, 90 %); mp 46 – 47 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.32 (m, 1H, C***H***$_2$), 1.73 (m, 2H, C***H***$_2$), 1.82 (m, 1H, C***H***$_2$), 1.94 (m, 1H, C***H***$_2$), 2.06 (m, 1H, C***H***$_2$), 2.35 (m, 1H, C***H***), 3.11 (m, 5H, C***H***-Im + N-C***H***$_2$ + C***H***$_2$-N), 3.41 (m, 2H, C***H***$_2$-S), 6.77 (s, 1H, Im-***H***-5), 7.17 (m, 1H, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.37 (m, 2H, Ph-***H***), 7.57 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.01 (-, ***C***H$_2$), 33.61 (-, ***C***H$_2$-S), 33.85 (-, ***C***H$_2$), 37.37 (-, ***C***H$_2$), 37.74 (+, ***C***H), 39.47 (+, ***C***H), 42.27 (-, N-***C***H$_2$), 47.82 (-, ***C***H$_2$-N), 116.69 (+, Im-***C***-5), 119.99 (C$_{quat}$, ***C***≡N), 127.34 (+, Ph-***C***-4), 130.16 (+, 2 Ph-***C***), 130.46 (+, 2 Ph-***C***), 135.92 (+, Im-***C***-2), 136.99 (C$_{quat}$, Ph-***C***-1), 141.87 (C$_{quat}$, Im-***C***-4), 161.18 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6S$ [$M^{+\bullet}$] 368.1783; found 368.1778. IR (cm^{-1}) = 3255 (N-H), 2946, 2864 (C-H), 2159 (C≡N), 1574 (C=N), 1437, 1357, 1300, 1088. Anal. ($C_{19}H_{24}N_6S \cdot 0.3\ CH_3OH$) C, H, N. $C_{19}H_{24}N_6S$ (368.50).

(±)-2-Cyano-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-[2-(phenylthio)ethyl]guanidine (5.27)

The title compound was prepared from **5.17** (0.04 g, 0.24 mmol) and **3.31** (0.072 g, 0.24 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.06 g, 68 %); mp 48 – 49 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.31 (m, 1H, C***H***$_2$), 1.47 (m, 1H, C***H***$_2$), 1.71 (m, 1H, C***H***$_2$), 1.84 (m, 1H, C***H***$_2$), 2.02 (m, 1H, C***H***$_2$), 2.20 (m, 1H, C***H***$_2$), 2.29 (m, 1H, C***H***), 3.10 (m, 5H, C***H***-Im + N-C***H***$_2$ + C***H***$_2$-N), 3.41 (t, 2H, ^{3}J = 7.3 Hz, C***H***$_2$-S), 6.78 (s, 1H, Im-***H***-5), 7.18 (t, 1H, ^{3}J = 7.6 Hz, Ph-***H***-4), 7.29 (m, 2H, Ph-***H***-3,5), 7.57 (s, 1H, Im-***H***-2), 8.08 (d, 2H, ^{3}J = 8.1 Hz, Ph-***H***-2,6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.11 (-, ***C***H$_2$), 32.92 (-, ***C***H$_2$), 33.60 (-, ***C***H$_2$-S), 38.79 (-, ***C***H$_2$), 38.98 (+, ***C***H), 40.54 (+, ***C***H), 42.28 (-, N-***C***H$_2$), 47.90 (-, ***C***H$_2$-N), 116.53 (+, Im-***C***-5), 120.00 (C$_{quat}$, ***C***≡N), 127.34 (+, Ph-***C***-4), 130.16 (+, 2 Ph-***C***), 130.44 (+, 2 Ph-***C***), 135.87 (+, Im-***C***-2), 137.01 (C$_{quat}$, Ph-***C***-1), 141.68 (C$_{quat}$, Im-***C***-4), 161.16 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6S$ [$M^{+\bullet}$] 368.1783; found 368.1774. IR (cm^{-1}) = 3243 (N-H), 2925, 2855 (C-H), 2158 (C≡N), 1572 (C=N), 1436, 1355, 1300, 1088. Anal. ($C_{19}H_{24}N_6S \cdot 0.4\ CH_3OH$) C, H, N. $C_{19}H_{24}N_6S$ (368.50).

(±)-2-Cyano-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-[2-(phenoxy)ethyl]guanidine (5.28)

The title compound was prepared from **5.16** (0.04 g, 0.24 mmol) and **5.36** (0.068 g, 0.24 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography

yielded a white solid (0.05 g, 59 %); mp 55 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.33 (m, 1H, C***H***$_2$), 1.65 (m, 1H, C***H***$_2$), 1.82 (m, 2H, C***H***$_2$), 1.93 (m, 1H, C***H***$_2$), 2.05 (m, 1H, C***H***$_2$), 2.38 (m, 1H, C***H***), 3.15 (m, 1H, C***H***-Im), 3.19 (d, 2H, ^{3}J = 7.5 Hz, C***H***$_2$-N), 3.61 (t, 2H, ^{3}J = 5.4 Hz, N-C***H***$_2$), 4.07 (t, 2H, ^{3}J = 5.4 Hz, C***H***$_2$-O), 6.75 (s, 1H, Im-***H***-5), 6.89-6.94 (m, 3H, Ph-***H***), 7.25 (m, 2H, Ph-***H***), 7.57 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.03 (-, ***C***H$_2$), 33.87 (-, ***C***H$_2$), 37.33 (-, ***C***H$_2$), 37.69 (+, ***C***H), 39.64 (+, ***C***H), 42.45 (-, N-***C***H$_2$), 47.79 (-, ***C***H$_2$-N), 67.67 (-, ***C***H$_2$-O), 115.64 (+, 2 Ph-***C***), 116.72 (+, Im-***C***-5), 120.06 (C$_{quat}$, ***C***≡N), 122.15 (+, Ph-***C***-4), 130.59 (+, 2 Ph-***C***), 135.87 (+, Im-***C***-2), 142.10 (C$_{quat}$, Im-***C***-4), 160.11 (C$_{quat}$, Ph-***C***-1), 161.54 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6O$ [$M^{+\bullet}$] 352.2012; found 352.2007. IR (cm^{-1}) = 3256 (N-H), 2971, 2901 (C-H), 2162 (C≡N), 1578 (C=N), 1406, 1241, 1071, 1049. Anal. ($C_{19}H_{24}N_6O$ · 0.5 CH_3OH) C, H, N. $C_{19}H_{24}N_6O$ (352.43).

(±)-2-Cyano-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-[2-(phenoxy)ethyl]guanidine (5.29)

The title compound was prepared from **5.17** (0.04 g, 0.24 mmol) and **5.36** (0.068 g, 0.24 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.05 g, 59 %); mp 57 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.30 (m, 1H, C***H***$_2$), 1.49 (m, 1H, C***H***$_2$), 1.70 (m, 1H, C***H***$_2$), 1.83 (m, 1H, C***H***$_2$), 2.00 (m, 1H, C***H***$_2$), 2.18 (m, 1H, C***H***$_2$), 2.31 (m, 1H, C***H***), 3.06 (m, 1H, C***H***-Im), 3.21 (d, 2H, ^{3}J = 7.0 Hz, C***H***$_2$-N), 3.61 (t, 2H, ^{3}J = 5.3 Hz, N-C***H***$_2$), 4.07 (t, 2H, ^{3}J = 5.3 Hz, C***H***$_2$-O), 6.76 (s, 1H, Im-***H***-5), 6.92 (m, 3H, Ph-***H***), 7.25 (m, 2H, Ph-***H***), 7.56 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.10 (-, ***C***H$_2$), 32.91 (-, ***C***H$_2$), 38.81 (-, ***C***H$_2$), 38.95 (+, ***C***H), 40.68 (+, ***C***H), 42.45 (-, N-***C***H$_2$), 47.87 (-, ***C***H$_2$-N), 67.66 (-, ***C***H$_2$-O), 115.64 (+, 2 Ph-***C***), 116.62 (+, Im-***C***-5), 120.23 (C$_{quat}$, ***C***≡N), 122.14 (+, Ph-***C***-4), 130.59 (+, 2 Ph-***C***), 135.88 (+, Im-***C***-2), 142.04 (C$_{quat}$, Im-***C***-4), 160.13 (C$_{quat}$, Ph-***C***-1), 161.52 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6O$ [$M^{+\bullet}$] 352.2012; found 352.2008. IR (cm^{-1}) = 3257 (N-H), 2924, 2868 (C-H), 2160 (C≡N), 1576 (C=N), 1453, 1239, 1080, 1050. Anal. ($C_{19}H_{24}N_6O$ · 0.5 CH_3OH) C, H, N. $C_{19}H_{24}N_6O$ (352.43).

(±)-2-Cyano-1-{[*trans*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-[2-(phenylamino) ethyl]guanidine (5.30)

The title compound was prepared from **5.32** (0.08 g, 0.26 mmol) and *N*^{1}-phenylethane-1,2-diamine **5.34** (0.034 mL, 0.26 mmol) in MeCN (4.5 mL) according to the general procedure.

Flash chromatography yielded a white solid (0.05 g, 55 %); mp 42 – 43 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.31 (m, 1H, C***H***$_2$), 1.58 – 1.84 (m, 3H, C***H***$_2$), 1.90 (m, 1H, C***H***$_2$), 2.05 (m, 1H, C***H***$_2$), 2.32 (m, 1H, C***H***), 3.14 (m, 3H, C***H***-Im + C***H***$_2$-N), 3.27 (t, 2H, ^{3}J = 6.2 Hz, N-C***H***$_2$), 3.41 (t, 2H, ^{3}J = 6.2 Hz, C***H***$_2$-NH-Ph), 6.63 (m, 3H, Ph-***H***), 6.79 (s, 1H, Im-***H***-5), 7.09 (m, 2H, Ph-***H***), 7.66 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.00 (-, ***C***H$_2$), 33.83 (-, ***C***H$_2$), 37.27 (-, ***C***H$_2$), 37.57 (+, ***C***H), 39.52 (+, ***C***H), 42.47 (-, N-***C***H$_2$), 44.47 (-, ***C***H$_2$-NH-Ph), 47.78 (-, ***C***H$_2$-N), 114.00 (+, 2 Ph-***C***), 116.53 (+, Im-***C***-5), 118.34 (+, Ph-***C***-4), 120.23 (C_{quat}, ***C***≡N), 130.21 (+, 2 Ph-***C***), 135.80 (+, Im-***C***-2), 141.86 (C_{quat}, Im-***C***-4), 149.85 (C_{quat}, Ph-***C***-1), 161.61 (C_{quat}, ***C***=N). HRMS (LSI-MS (MeOH/Glycerol)) calcd. for $C_{19}H_{26}N_7$ $[M + H]^+$ 352.2250; found 352.2249. IR (cm^{-1}) = 3264 (N-H), 2930, 2864 (C-H), 2159 (C≡N), 1575 (C=N), 1499, 1426, 1323, 1257. Anal. ($C_{19}H_{25}N_7 \cdot 0.75\ CH_3OH$) C, H, N. $C_{19}H_{25}N_7$ (351.45).

(±)-2-Cyano-1-{[*cis*-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-[2-(phenylamino)ethyl] guanidine (5.31)

The title compound was prepared from **5.33** (0.09 g, 0.29 mmol) and *N*^{1}-phenylethane-1,2-diamine **5.34** (0.038 mL, 0.29 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a pale yellow solid (0.07 g, 69 %); mp 55 – 57 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.30 (m, 1H, C***H***$_2$), 1.45 (m, 1H, C***H***$_2$), 1.68 (m, 1H, C***H***$_2$), 1.80 (m, 1H, C***H***$_2$), 2.01 (m, 1H, C***H***$_2$), 2.14 (m, 1H, C***H***$_2$), 2.24 (m, 1H, C***H***), 3.05 (m, 1H, C***H***-Im) 3.15 (d, 2H, ^{3}J = 8.4 Hz, C***H***$_2$-N), 3.27 (t, 2H, ^{3}J = 5.9 Hz, N-C***H***$_2$), 3.41 (t, 2H, ^{3}J = 5.9 Hz, C***H***$_2$-NH-Ph), 6.63 (m, 3H, Ph-***H***), 6.76 (s, 1H, Im-***H***-5), 7.09 (m, 2H, Ph-***H***), 7.57 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.10 (-, ***C***H$_2$), 32.90 (-, ***C***H$_2$), 38.80 (-, ***C***H$_2$), 38.92 (+, ***C***H), 40.59 (+, ***C***H), 42.46 (-, N-***C***H$_2$), 44.46 (-, ***C***H$_2$-NH-Ph), 47.90 (-, ***C***H$_2$-N), 113.98 (+, 2 Ph-***C***), 116.56 (+, Im-***C***-5), 118.34 (+, Ph-***C***-4), 120.23 (C_{quat}, ***C***≡N), 130.21 (+, 2 Ph-***C***), 135.86 (+, Im-***C***-2), 141.92 (C_{quat}, Im-***C***-4), 149.84 (C_{quat}, Ph-***C***-1), 161.58 (C_{quat}, ***C***=N). HRMS (LSI-MS (MeOH/Glycerol)) calcd. for $C_{19}H_{26}N_7$ $[M + H]^+$ 352.2250; found 352.2242. IR (cm^{-1}) = 3275 (N-H), 2971, 2901 (C-H), 2161 (C≡N), 1577 (C=N), 1499, 1406, 1251. Anal. ($C_{19}H_{25}N_7 \cdot 0.7\ CH_3OH$) C, H, N. $C_{19}H_{25}N_7$ (351.45).

5.4.1.5 Stereoselective synthesis of the cyanoguanidines 5.51-5.54

cis-Dimethyl cyclopentane-1,3-dicarboxylate (5.37)[17]

To a solution of **5.6** (41.0 g, 0.26 mol) in 200 mL methanol were added 5 g Dowex® 50x8 H^+-resin and the mixture was refluxed overnight The resin was removed by filtration and the solvent was removed *in vacuo*. Distillation under reduced pressure yielded a yellow oil (42.45 g, 88 %); bp 113 °C/3 hPa (ref.[19]: 70 °C/0.07 hPa). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.93 (m, 4H, 2 C***H***$_2$), 2.09 (m, 1H, C***H***$_2$), 2.19 (m, 1H, C***H***$_2$), 2.77 (m, 2H, 2 C***H***), 3.65 (s, 6H, 2 C***H***$_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.09 (-, 2 ***C***H_2), 33.33 (-, ***C***H_2), 43.74 (+, 2 ***C***H), 51.78 (+, 2 ***C***H_3), 175.70 (C_{quat}, 2 ***C***OOMe). CI-MS (NH_3) *m/z* (%): 187 (85) $[M + H]^+$, 204 (100) $[M + NH_4]^+$. $C_9H_{14}O_4$ (186.21).

(1*S*,3*R*)-3-(Methoxycarbonyl)cyclopentanecarboxylic acid (5.38)[17]

5.37 (20.0 g, 0.107 mol) was added to 100 mL of 0.05 M phosphate buffer, pH 7.0, containing 1 % acetonitrile at 37 °C. Under vigorous stirring, 4000 U of cholesterol esterase (porcine pancreas, 40 U/mg) were added. During the reaction the pH was kept at 7.0 by adding 1 M NaOH. After consumption of 0.9 mol equivalents base the pH was again adjusted to 7 and the mixture was extracted with diethyl ether. The organic layer was washed with water and the combined aqueous layers were acidified to pH 2.5 by adding concentrated HCl. This solution was again extracted with diethyl ether, the organic layer was dried over $MgSO_4$ and the solvent was evaporated to yield a colorless oil (16.5 g, 89 %); $[\alpha]_D^{25}$ + 0.46° (c 3.5, $CHCl_3$) (ref.[19]: + 0.2° (c 3.5, $CHCl_3$), pig liver esterase used, ee 34 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.94 (m, 4H, 2 C***H***$_2$), 2.11 (m, 1H, C***H***$_2$), 2.20 (m, 1H, C***H***$_2$), 2.78 (m, 2H, 2 C***H***), 3.64 (s, 3H, C***H***$_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.04 (-, ***C***H_2), 29.20 (-, ***C***H_2), 33.06 (-, ***C***H_2), 43.67 (+, ***C***H), 43.77 (+, ***C***H), 51.84 (+, ***C***H_3), 175.73 (C_{quat}, ***C***OOMe), 181.50 (C_{quat}, ***C***OOH). CI-MS (NH_3) *m/z* (%): 173 (25) $[M + H]^+$, 190 (100) $[M + NH_4]^+$. $C_8H_{12}O_4$ (172.18).

(1*R*,5*S*)-3-Oxabicyclo[3.2.1]octane-2,4-dione (5.39)[35]

5.6 (0.65 g, 4.1 mmol) was taken up in 5 mL acetic anhydride and heated under microwave irradiation at 150 °C for 30 min. The solvent was evaporated and the residue crystallized from diethyl ether to yield a white solid (0.41 g, 73 %); mp 157 °C (ref.[35]: 159 – 160 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.75 (dt, 1H, J_{gem} = 12.7 Hz, ^3J = 4.1 Hz, C***H***$_2$), 2.00 – 2.27 (m, 5H, 3 C***H***$_2$), 3.26 (m, 2H, 2 C***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.50 (-, 2 ***C***H_2),

31.22 (-, ***C***H_2), 41.88 (+, 2 ***C***H), 169.68 (C_{quat}, 2 ***C***=O). CI-MS (NH_3) *m/z* (%): 158 (100) $[M + NH_4]^+$, 175 (85) $[M + NH_4 + NH_3]^+$. $C_7H_8O_3$ (140.14).

(±)-3-(Methoxycarbonyl)cyclopentanecarboxylic acid (rac-5.38)[35]

5.39 (0.4 g, 2.85 mmol) was refluxed in 20 mL of absolute methanol for 4 h. Evaporation of the solvent yielded a colorless oil (0.49 g, 99 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.95 (m, 4H, 2 C***H***$_2$), 2.12 (m, 1H, C***H***$_2$), 2.22 (m, 1H, C***H***$_2$), 2.81 (m, 2H, 2 C***H***), 3.66 (s, 3H, C***H***$_3$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.07 (-, ***C***H_2), 29.23 (-, ***C***H_2), 33.08 (-, ***C***H_2), 43.65 (+, ***C***H), 43.80 (+, ***C***H), 51.87 (+, ***C***H_3), 175.75 (C_{quat}, ***C***OOMe), 181.49 (C_{quat}, ***C***OOH). CI-MS (NH_3) *m/z* (%): 173 (10) $[M + H]^+$, 190 (100) $[M + NH_4]^+$. $C_8H_{12}O_4$ (172.18).

(1*R*,3*S*)-Methyl 3-((*S*)-1-phenylethylcarbamoyl)cyclopentanecarboxylate (5.41)[17]

A mixture of **5.38** (0.15 g, 0.87 mmol), EDC (0.2 g, 1.0 mmol) and DMAP (0.17 g, 1.4 mmol) in 20 mL DCM_{abs} was stirred for 30 min at 0 °C. Subsequently (*S*)-1-phenylethanamine **5.40** (0.18 g, 1.4 mmol) was added dropwise and the mixture was stirred at room temperature overnight. The mixture was washed with 0.5 M HCl and evaporated to yield a white solid (0.22 g, 92 %); mp 133 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$), 1.89 (m, 4H, 2 C***H***$_2$), 2.09 (m, 2H, C***H***$_2$), 2.63 (m, 1H, C***H***), 2.79 (m, 1H, C***H***), 3.64 (s, 3H, COOC***H***$_3$), 5.08 (m, 1H, C***H***CH_3), 6.48 (d, 1H, ^3J = 7.5 Hz, N***H***), 7.18 – 7.33 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 21.93 (+, ***C***H_3), 29.64 (-, ***C***H_2), 30.06 (-, ***C***H_2), 33.49 (-, ***C***H_2), 43.89 (+, ***C***H-COOMe), 45.89 (+, ***C***H-CONR), 48.64 (+, ***C***H-N), 51.84 (+, COO***C***H_3), 126.10 (+, 2 Ph-***C***), 127.15 (+, Ph-***C***-4), 128.57 (+, 2 Ph-***C***), 143.57 (C_{quat}, Ph-***C***-1), 174.01 (C_{quat}, ***C***=O), 176.40 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 276 (100) $[M + H]^+$, 293 (40) $[M + NH_4]^+$. Determination of the enantiomeric purity with ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 3.62 (s, 2.58H, COOC***H***$_3$), 3.63 (s, 0.42H, COOC***H***$_3$). ee = 72 % (ref.[17]: 90 %). $C_{16}H_{21}NO_3$ (275.34).

Methyl 3-((*S*)-1-phenylethylcarbamoyl)cyclopentanecarboxylate (5.41-reference)[17]

A mixture of **rac-5.38** (0.16 g, 0.93 mmol), EDC (0.21 g, 1.1 mmol) and DMAP (0.18 g, 1.5 mmol) in 20 mL DCM_{abs} was stirred for 30 min at 0 °C. Subsequently (*S*)-1-phenylethanamine **5.40** (0.18 g, 1.4 mmol) was added dropwise and the mixture was stirred at room temperature overnight. The mixture was washed with 0.5 M HCl and evaporated to yield a white solid (0.24 g, 94 %); mp 130 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44, 1.47

(2d, 3H, ^{3}J = 6.9 Hz, C***H***$_3$), 1.92 (m, 4H, 2 C***H***$_2$), 2.13 (m, 2H, C***H***$_2$), 2.64 (m, 1H, C***H***), 2.82 (m, 1H, C***H***), 3.66, 3.67 (2s, 3H, COOC***H***$_3$), 5.10 (m, 1H, C***H***CH$_3$), 6.33 (m, 1H, N***H***), 7.20 – 7.36 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 21.91 (+, ***C***H$_3$), 29.70, 29.78 (-, ***C***H$_2$), 30.12, 30.19 (-, ***C***H$_2$), 33.33, 33.43 (-, ***C***H$_2$), 43.88 (+, ***C***H-COOMe), 46.04, 46.09 (+, ***C***H-CONR), 48.66 (+, ***C***H-N), 51.87, 51.89 (+, COO***C***H$_3$), 126.12 (+, 2 Ph-***C***), 127.12 (+, Ph-***C***-4), 128.61 (+, 2 Ph-***C***), 143.53 (C$_{quat}$, Ph-***C***-1), 173.96 (C$_{quat}$, ***C***=O), 176.43, 176.51 (C$_{quat}$, ***C***=O). CI-MS (NH_3) *m/z* (%): 276 (100) $[M + H]^+$, 293 (40) $[M + NH_4]^+$. $C_{16}H_{21}NO_3$ (275.34).

(1*S*,3*R*)-3-Carbamoylcyclopentanecarboxylic acid ammonia salt (5.42)[17]

5.38 (20.0 g, 0.116 mol) was dissolved in 150 mL 32 % $NH_{3(aq)}$ and stirred at room temperature overnight. Evaporation of the solvent and lyophilization yielded a white solid (20.9 g, 100 %); $[\alpha]_D^{25}$ - 0.71° (c 0.7, MeOH); mp 138 °C (ref.[36]: 135 °C). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.89 (m, 4H, 2 C***H***$_2$), 1.96 (m, 1H, C***H***$_2$), 2.13 (m, 1H, C***H***$_2$), 2.70 (m, 2H, 2 C***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.13 (-, ***C***H$_2$), 31.36 (-, ***C***H$_2$), 36.10 (-, ***C***H$_2$), 46.81 (+, ***C***H), 48.48 (+, ***C***H), 181.78 (C$_{quat}$, ***C***=O), 183.67 (C$_{quat}$, ***C***=O). CI-MS (NH_3) *m/z* (%): 158 (100) $[M + H]^+$, 175 (40) $[M + NH_4]^+$. $C_7H_{11}NO_3 \cdot NH_3$ (174.20).

[(1*S*,3*R*)-3-(Aminomethyl)cyclopentyl]methanol (5.43)[17]

Borane · THF (130 mL, 130 mmol, 4.5 eq) was added dropwise to a suspension of **5.42** (5.0 g, 28.7 mmol, 1 eq) in 50 mL THF_{abs} at 0 °C. After stirring at room temperature for 1 h the mixture was refluxed overnight. 100 mL 12 % HCl was slowly added under ice-cooling and stirred for 15 min. The pH was adjusted to 12 with NaOH and the mixture was extracted with 3 x 200 mL EtOAc. The organic layer was dried over $MgSO_4$ and evaporated yielding a colorless oil (10.4 g, 70 %); $[\alpha]_D^{25}$ + 1.68° (c 3.1, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.80 – 0.95 (m, 1H, C***H***$_2$), 1.17 – 1.40 (m, 2H, C***H***$_2$), 1.63 – 1.78 (m, 2H, C***H***$_2$), 1.88 – 1.99 (m, 2H, C***H*** + C***H***$_2$), 2.09 (m, 4H, N***H***$_2$ + O***H*** + C***H***$_2$), 2.60 (d, 2H, ^{3}J = 6.5 Hz, C***H***$_2$-N), 3.47 (d, 2H, ^{3}J = 6.6 Hz, C***H***$_2$-O). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.18 (-, ***C***H$_2$), 29.35 (-, ***C***H$_2$), 34.18 (-, ***C***H$_2$), 42.17 (+, ***C***H), 43.34 (+, ***C***H), 47.59 (-, ***C***H$_2$-N), 67.03 (-, ***C***H$_2$-O). CI-MS (NH_3) *m/z* (%): 130 (100) $[M + H]^+$, 147 (10) $[M + NH_4]^+$. $C_7H_{15}NO$ (129.20).

***tert*-Butyl [(1*R*,3*S*)-3-(hydroxymethyl)cyclopentyl]methylcarbamate (5.44)**

To a solution of **5.43** (8.0 g, 61.9 mmol, 1 eq) in 180 mL dioxane/water 2:1 (v/v) was added 62 mL 1 M aqueous NaOH. After cooling to 0 °C Boc_2O (14.0 g, 65.0 mmol, 1.1 eq) was added and the mixture was stirred at room temperature overnight. After removing the solvent *in vacuo* the residue was taken up in EtOAc, washed with brine and water and dried over $MgSO_4$. Evaporation of the solvent and flash chromatography (DCM/MeOH 100/0 – 90/10 v/v) yielded a colorless oil (10.5 g, 74 %); $[\alpha]_D^{25}$ + 0.73° (c 2.6, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.87 (m, 1H, C***H***$_2$), 1.25 (m, 2H, C***H*** + C***H***$_2$), 1.40 (s, 9H, Boc), 1.71 (m, 2H, C***H***$_2$), 1.86 – 2.16 (m, 3H, C***H*** + C***H***$_2$), 2.25 (brs, 1H, O***H***), 3.03 (m, 2H, C***H***$_2$-N), 3.49 (d, 2H, ^3J = 6.8 Hz, C***H***$_2$-O), 4.67 (brs, 1H, N***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.96 (-, ***C***H$_2$), 28.40 (+, C(***C***H$_3$)$_3$), 29.33 (-, ***C***H$_2$), 33.88 (-, ***C***H$_2$), 40.42 (+, ***C***H), 41.99 (+, ***C***H), 45.53 (-, ***C***H$_2$-N), 67.04 (-, ***C***H$_2$-O), 79.14 (C_{quat}, ***C***(CH$_3$)$_3$), 156.22 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 230 (95) $[M + H]^+$, 247 (60) $[M + NH_4]^+$, 191 (100) $[M – C_4H_8 + NH_4]^+$. $C_{12}H_{23}NO_3$ (229.32).

***tert*-Butyl [(1*R*,3*S*)-3-formylcyclopentyl]methylcarbamate (5.45)**

A mixture of DMSO (7.7 mL, 108.16 mmol, 4 eq) and DCM (40 mL) was added slowly to a solution of oxalyl chloride (4.7 mL, 54.08 mmol, 2 eq) in DCM (50 mL) at -78 °C over 30 min. Then a solution of **5.44** (6.2 g, 27.04 mmol, 1 eq) in DCM (50 mL) was added. The resulting mixture was stirred at the same temperature for 2 h, and then NEt_3 (30 mL, 216.32 mmol, 8 eq) was added. After the resulting mixture was stirred at the same temperature for a further 30 min it was allowed to warm to RT and stirred overnight. 120 mL water were added and the resulting layers were separated. The aqueous layer was extracted twice with 60 mL DCM and the combined organic layers washed with brine dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (PE/EtOAc 70/30 v/v) to give **5.45** as colorless oil (3.7 g, 61 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.23 (m, 1H, C***H***$_2$), 1.41 (s, 9H, Boc), 1.74 – 2.07 (m, 5H, C***H***$_2$), 2.14 (m, 1H, C***H***-CH$_2$-N), 2.80 (m, 1H, C***H***-CHO), 3.07 (m, 2H, C***H***$_2$-N), 4.66 (brs, 1H, NH), 9.60 (d, 1H, ^3J = 2.2 Hz, C***H***O). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 25.55, 25.73 (-, ***C***H$_2$), 28.39 (+, C(***C***H$_3$)$_3$), 29.64, 29.91 (-, ***C***H$_2$), 30.05, 30.34 (-, ***C***H$_2$), 40.05, 40.67 (+, ***C***H), 44.74 (-, ***C***H$_2$-N), 50.72, 51.34 (+, ***C***H-CHO), 79.20 (C_{quat}, ***C***(CH$_3$)$_3$), 156.02 (C_{quat}, ***C***=O), 203.40 (+, ***C***HO). CI-MS (NH_3) *m/z* (%): 228 (20) $[M + H]^+$, 245 (60) $[M + NH_4]^+$, 189 (100) $[M – C_4H_8 + NH_4]^+$. $C_{12}H_{21}NO_3$ (227.30).

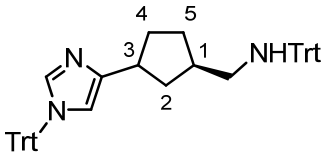
tert-Butyl [(1*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methylcarbamate (5.46)

Finely powdered sodium cyanide (0.04 g, 0.7 mmol) was added in one portion to a stirred suspension of tosylmethyl isocyanide **3.16** (0.86 g, 4.4 mmol) and **5.45** (1.0 g, 4.4 mmol) in 30 mL of absolute ethanol at 0 °C. The reaction mixture became clear, and the solution was stirred for another 30 min. The solvent was evaporated under reduced pressure. The resulting slurry is dissolved in 15 mL 7 M NH_3 in methanol and stirred under microwave irradiation at 100 °C and 12 bar for 18 h. The solvent was evaporated and the residue was purified by flash chromatography (DCM/MeOH 90/10 v/v) to yield **5.46** as brown oil (1.02 g, 87 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.27 (m, 1H, C***H***$_2$), 1.42 (s, 9H, Boc), 1.60 – 1.93 (m, 3H, C***H***$_2$), 1.98 – 2.23 (m, 3H, C***H***$_2$ + C***H***-CH_2-N), 3.08 (m, 3H, C***H***-Im + C***H***$_2$-N), 6.72, 6.73 (s, 1H, Im-***H***-5), 7.52 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.42 (+, C(***C***H_3)$_3$), 29.25, 30.01 (-, ***C***H_2), 32.24, 32.74 (-, ***C***H_2), 36.40, 37.33 (+, ***C***H), 36.47, 37.06 (-, ***C***H_2), 38.97, 39.87 (+, ***C***H-Im), 45.52, 45.64 (-, ***C***H_2-N), 79.31 (C_{quat}, ***C***$(CH_3)_3$), 116.15, 116.74 (+, Im-***C***-5), 134.57 (+, Im-***C***-2), 140.10 (C_{quat}, Im-***C***-4), 156.30, 156.49 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 266 (100) $[M + H]^+$. $C_{14}H_{23}N_3O_2$ (265.35).

[(1*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methanamine (5.47)

5.46 (2.65 g, 10.0 mmol) was dissolved in 50 mL MeOH, 5 mL HCl 37 % were added and the solution was stirred overnight at room temperature. The solvent was evaporated to yield **5.47** dihydrochloride as brown oil (2.4 g, 100 %); ^{1}H-NMR (300 MHz, $CDCl_3$, hydrochloride): δ [ppm] = 1.40 (m, 1H, C***H***$_2$), 1.62 (m, 1H, C***H***$_2$), 1.81 (m, 1H, C***H***$_2$), 2.04 (m, 3H, C***H***$_2$ + C***H***), 2.24 (m, 1H, C***H***$_2$), 2.44 (m, 2H, C***H***-Im + C***H***$_2$), 3.01 (m, 2H, C***H***$_2$-N), 7.40 (s, 1H, Im-***H***-5), 8.84 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, $CDCl_3$, hydrochloride): δ [ppm] = 30.00, 31.25 (-, ***C***H_2), 32.25, 32.41 (-, ***C***H_2), 35.79, 36.79 (+, ***C***H), 36.71, 38.34 (-, ***C***H_2), 38.15, 39.18 (+, ***C***H-Im), 45.22, 45.26 (-, ***C***H_2-N), 115.83 (+, Im-***C***-5), 134.91 (+, Im-***C***-2), 138.66, 139.02 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 166 (100) $[M + H]^+$, 145 (95) $[M + 2\ H + 3\ MeCN]^{2+}$. The hydrochloride was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). Evaporation of the solvent yielded **5.47** (1.45 g, 88 %) as yellow oil. $C_9H_{15}N_3$ (165.24).

Cis- and _trans_-1,1,1-Triphenyl-_N_-{[(1_R_)-3-(1-trityl-1_H_-imidazol-4-yl)cyclopentyl]methyl}-methanamine (_cis_-5.48, _trans_-5.48)



To a cooled solution of **5.47** (1.45 g, 8.8 mmol, 1 eq) and NEt_3 (12.2 mL, 88 mmol, 10 eq) in DCM_{abs} (60 mL), trityl chloride (15 g, 53.0 mmol, 6 eq) was slowly added. The mixture was stirred for 24 h at room temperature. The reaction was stopped by adding 80 mL water. The layers were separated and the combined organic layers were washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The products ***cis*-5.48** and ***trans*-5.48** were separated by flash-chromatography (PE/EtOAc/7 M NH_3 in MeOH 100/0/0 – 70/27/3 v/v/v) as white solids (total yield 4.58 g, 80 %);

Compound ***trans*-(1*R*,3*R*)-5.48**: $[\alpha]_D^{25}$ + 3.3° (c 3.5, $CHCl_3$); mp 159 °C. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.22 (m, 1H, C***H***$_2$-5), 1.65 (m, 2H, C***H***$_2$-2 + C***H***$_2$-4), 1.85 – 2.02 (m, 3H, C***H***$_2$-2 + C***H***$_2$-5 + C***H***$_2$-4), 2.10 (d, 2H, ^3J = 7.1 Hz, C***H***$_2$-N), 2.22 (m, 1H, C***H***-1), 3.01 (m, 1H, C***H***-Im), 6.50 (s, 1H, Im-***H***-5), 7.12 – 7.18 (m, 9H, Ph-***H***), 7.25 – 7.27 (m, 6H, Ph-***H***), 7.32 – 7.34 (m, 9H, Ph-***H***), 7.36 (s, 1H, Im-***H***-2), 7.48 – 7.50 (m, 6H, Ph-***H***). ^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 30.99 (-, ***C***H_2-5), 32.88 (-, ***C***H_2-4), 37.16 (-, ***C***H_2-2), 37.87 (+, ***C***H-Im), 39.85 (+, ***C***H-1), 48.99 (-, ***C***H_2-N), 70.75 (C_{quat}, N-***C***Ph_3), 75.11 (C_{quat}, Im-***C***Ph_3), 116.59 (+, Im-***C***-5), 126.11, 127.72, 127.98, 128.71, 129.83 (+, 30 Ph-***C***), 138.34 (+, Im-***C***-2), 142.63 (C_{quat}, 3 Ph-***C***-1), 146.03 (C_{quat}, Im-***C***-4), 146.35 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 650 (80) $[M + H]^+$, 408 (100) $[M – Trt + H]^+$, 243 (45) $[Trt]^+$. Anal. ($C_{47}H_{43}N_3 \cdot 0.1\ H_2O$) C, H, N. $C_{47}H_{43}N_3$ (649.86).

Compound ***cis*-(1*R*,3*S*)-5.48**: $[\alpha]_D^{25}$ + 17.3° (c 4.0, $CHCl_3$); mp 168 °C. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.20 (m, 1H, C***H***$_2$-2), 1.33 (m, 1H, C***H***$_2$-5), 1.62 (m, 1H, C***H***$_2$-4), 1.85 (m, 1H, C***H***$_2$-5), 1.99 (m, 1H, C***H***$_2$-4), 2.09 (m, 1H, C***H***$_2$-N), 2.17 (m, 2H, C***H***$_2$-N + C***H***-1), 2.24 (m, 1H, C***H***$_2$-2), 3.03 (m, 1H, C***H***-Im), 6.48 (s, 1H, Im-***H***-5), 7.10 – 7.17 (m, 8H, Ph-***H***), 7.18 (m, 1H, Ph-***H***), 7.22 – 7.28 (m, 6H, Ph-***H***), 7.30 – 7.35 (m, 10H, Ph-***H*** + Im-***H***-2), 7.46 – 7.50 (m, 6H, Ph-***H***). ^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 29.71 (-, ***C***H_2-5), 31.74 (-, ***C***H_2-4), 38.76 (-, ***C***H_2-2), 39.16 (+, ***C***H-Im), 41.12 (+, ***C***H-1), 49.15 (-, ***C***H_2-N), 70.77 (C_{quat}, N-***C***Ph_3), 75.08 (C_{quat}, Im-***C***Ph_3), 116.45 (+, Im-***C***-5), 126.11, 127.72, 127.94, 128.71, 129.83 (+, 30 Ph-***C***), 138.31 (+, Im-***C***-2), 142.63 (C_{quat}, 3 Ph-***C***-1), 145.57 (C_{quat}, Im-***C***-4), 146.34 (C_{quat}, 3 Ph-***C***-1). (ES-MS (DCM/MeOH +

NH_4OAc) *m/z* (%): 650 (80) $[M + H]^+$, 408 (100) $[M – Trt + H]^+$, 243 (45) $[Trt]^+$. Anal. ($C_{47}H_{43}N_3$) C, H, N. $C_{47}H_{43}N_3$ (649.86).

[(1*R*,3*R*)-3-(1*H*-Imidazol-4-yl)cyclopentyl]methanamine (5.49)

A solution of ***trans*-5.48** (1.5 g, 2.3 mmol) in 20 mL MeOH and 2 mL 37 % HCl was refluxed for 3 h. The solvent was removed *in vacuo* and the residue washed with Et_2O. Ethanol was added, and evaporation yielded the hydrochloride of **5.49** as white semisolid. The hydrochloride was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). Evaporation of the solvent yielded **5.49** as yellow oil (0.36 g, 95 %); $[\alpha]_D^{25}$ + 5.8° (c 4.2, MeOH); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.30 (m, 1H, C***H***$_2$), 1.71 (m, 2H, C***H***$_2$), 1.85 (m, 1H, C***H***$_2$), 1.96 (m, 1H, C***H***$_2$), 2.04 (m, 1H, C***H***$_2$) 2.17 (m, 1H, C***H***), 2.59 (d, 2H, ^3J = 7.0 Hz, C***H***$_2$-NH_2), 3.12 (m, 1H, C***H***-Im), 6.75 (s, 1H, Im-***H***-5), 7.54 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.38 (-, ***C***H_2), 34.07 (-, ***C***H_2), 37.63 (-, ***C***H_2), 37.86 (+, ***C***H), 42.70 (+, ***C***H), 48.26 (-, ***C***H_2-NH_2), 117.12 (+, Im-***C***-5), 135.83 (+, Im-***C***-2), 142.04 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 166 (60) $[M + H]^+$, 145 (100) [M + 2 H + 3 MeCN]$^{2+}$. Anal. ($C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.2\ H_2O$) C, H, N. $C_9H_{15}N_3$ (165.24).

[(1*R*,3*S*)-3-(1*H*-Imidazol-4-yl)cyclopentyl]methanamine (5.50)

A solution of ***cis*-5.48** (2.0 g, 3.08 mmol) in 20 mL MeOH and 2 mL 37 % HCl was refluxed for 3 h. The solvent was removed *in vacuo* and the residue washed with Et_2O. Ethanol was added and evaporation yielded the hydrochloride of **5.50** as white semisolid. The crude product was recrystallized from 2-propanol/methanol to obtain colorless crystals for analytical purposes (x-ray, see appendix). The hydrochloride was converted into the base by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). Evaporation of the solvent yielded **5.50** as yellow oil (0.5 g, 98 %); $[\alpha]_D^{25}$ + 14.7° (c 4.7, MeOH); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.29 (m, 1H, C***H***$_2$), 1.46 (m, 1H, C***H***$_2$), 1.71 (m, 1H, C***H***$_2$), 1.88 (m, 1H, C***H***$_2$), 2.06 (m, 2H, C***H***$_2$ + C***H***), 2.20 (m, 1H, C***H***$_2$), 2.62 (d, 2H, ^3J = 6.9 Hz, C***H***$_2$-NH_2), 3.08 (m, 1H, C***H***-Im), 6.75 (s, 1H, Im-***H***-5), 7.53 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.25 (-, ***C***H_2), 33.06 (-, ***C***H_2), 39.04 (+, ***C***H), 39.16 (-, ***C***H_2), 43.78 (+, ***C***H), 48.26 (-, ***C***H_2-NH_2), 117.10 (+, Im-***C***-5), 135.80 (+, Im-***C***-2), 141.78 (C_{quat}, Im-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 166 (75) $[M + H]^+$, 145 (100) [M + 2 H + 3 MeCN]$^{2+}$. Anal. ($C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.2\ H_2O$) C, H, N. $C_9H_{15}N_3$ (165.24).

General Procedure for the preparation of the cyanoguanidines 5.51 – 5.54

The isourea (1 eq) and the pertinent amine (1 eq) in MeCN were heated under microwave irradiation at 150 °C for 15 min. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (DCM/MeOH 100/0 – 80/20 v/v).

(-)-2-Cyano-1-{[*trans*-(1*R*,3*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.51)

The title compound was prepared from **5.49** (0.08 g, 0.48 mmol) and **3.27** (0.085 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.09 g, 76 %); $[\alpha]_D^{25}$ - 1.4° (c 1.8, MeOH); mp 45 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.36 (m, 1H, C***H***$_2$), 1.68 (m, 1H, C***H***$_2$), 1.80 (m, 2H, C***H***$_2$), 1.93 (m, 1H, C***H***$_2$), 2.08 (m, 1H, C***H***$_2$), 2.39 (m, 1H, C***H***), 2.79 (s, 3H, C***H***$_3$-N), 3.18 (m, 3H, C***H***-Im + C***H***$_2$-N), 6.77 (s, 1H, Im-***H***-5), 7.56 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.77 (+, ***C***H$_3$), 30.98 (-, ***C***H$_2$), 33.89 (-, ***C***H$_2$), 37.37 (-, ***C***H$_2$), 37.72 (+, ***C***H), 39.67 (+, ***C***H), 47.74 (-, ***C***H$_2$-N), 116.82 (+, Im-***C***-5), 120.33 (C$_{quat}$, ***C***≡N), 135.90 (+, Im-***C***-2), 142.12 (C$_{quat}$, Im-***C***-4), 162.04 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 247 (100) $[M + H]^+$. $C_{12}H_{18}N_6$ (246.31).

(+)-2-Cyano-1-{[*cis*-(1*R*,3*S*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.52)

The title compound was prepared from **5.50** (0.08 g, 0.48 mmol) and **3.27** (0.085 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.07 g, 59 %); $[\alpha]_D^{25}$ + 16.0° (c 2.0, MeOH); mp 63 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.34 (m, 1H, C***H***$_2$), 1.48 (m, 1H, C***H***$_2$), 1.72 (m, 1H, C***H***$_2$), 1.86 (m, 1H, C***H***$_2$), 2.03 (m, 1H, C***H***$_2$), 2.19 (m, 1H, C***H***$_2$), 2.33 (m, 1H, C***H***), 2.79 (s, 3H, C***H***$_3$-N), 3.08 (m, 1H, C***H***-Im), 3.19 (m, 2H, C***H***$_2$-N), 6.77 (s, 1H, Im-***H***-5), 7.56 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.76 (+, ***C***H$_3$), 30.08 (-, ***C***H$_2$), 32.94 (-, ***C***H$_2$), 38.79 (-, ***C***H$_2$), 39.00 (+, ***C***H), 40.71 (+, ***C***H), 47.83 (-, ***C***H$_2$-N), 116.71 (+, Im-***C***-5), 120.34 (C$_{quat}$, ***C***≡N), 135.88 (+, Im-***C***-2), 141.93 (C$_{quat}$, Im-***C***-4), 162.02 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 247 (100) $[M + H]^+$. $C_{12}H_{18}N_6$ (246.31).

(-)-2-Cyano-1-{[*trans*- (1*R*,3*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(2-(phenylthio)ethyl)guanidine (5.53)

The title compound was prepared from **5.49** (0.08 g, 0.48 mmol) and **3.31** (0.144 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.11 g, 62 %); $[\alpha]_D^{25}$ - 1.2° (c 1.2, MeOH); mp 47 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.31 (m, 1H, C***H***$_2$), 1.69 (m, 2H, C***H***$_2$), 1.81 (m, 1H, C***H***$_2$), 1.94 (m, 1H, C***H***$_2$), 2.06 (m, 1H, C***H***$_2$), 2.35 (m, 1H, C***H***), 3.09 (m, 5H, C***H***-Im + N-C***H***$_2$ + C***H***$_2$-N), 3.41 (m, 2H, C***H***$_2$-S), 6.76 (s, 1H, Im-***H***-5), 7.16 (m, 1H, Ph-***H***-4), 7.27 (m, 2H, Ph-***H***), 7.35 (m, 2H, Ph-***H***), 7.55 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 31.06 (-, ***C***H$_2$), 33.61 (-, ***C***H$_2$-S), 33.89 (-, ***C***H$_2$), 37.40 (-, ***C***H$_2$), 37.74 (+, ***C***H), 39.47 (+, ***C***H), 42.30 (-, N-***C***H$_2$), 47.87 (-, ***C***H$_2$-N), 116.80 (+, Im-***C***-5), 120.10 (C$_{quat}$, ***C***≡N), 127.35 (+, Ph-***C***-4), 130.20 (+, 2 Ph-***C***), 130.43 (+, 2 Ph-***C***), 135.93 (+, Im-***C***-2), 137.00 (C$_{quat}$, Ph-***C***-1), 142.12 (C$_{quat}$, Im-***C***-4), 161.16 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 369 (100) $[M + H]^+$. $C_{19}H_{24}N_6S$ (368.50).

(+)-2-Cyano-1-{[*cis*-(1*R*,3*S*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(2-(phenylthio)ethyl)guanidine (5.54)

The title compound was prepared from **5.50** (0.08 g, 0.48 mmol) and **3.31** (0.144 g, 0.48 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.13 g, 73 %); $[\alpha]_D^{25}$ + 9.3° (c 2.2, MeOH); mp 49 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.30 (m, 1H, C***H***$_2$), 1.45 (m, 1H, C***H***$_2$), 1.69 (m, 1H, C***H***$_2$), 1.82 (m, 1H, C***H***$_2$), 2.01 (m, 1H, C***H***$_2$), 2.17 (m, 1H, C***H***$_2$), 2.28 (m, 1H, C***H***), 3.11 (m, 5H, C***H***-Im + N-C***H***$_2$ + C***H***$_2$-N), 3.41 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-S), 6.76 (s, 1H, Im-***H***-5), 7.16 (t, 1H, ^3J = 7.2 Hz, Ph-***H***-4), 7.27 (m, 2H, Ph-***H***-3,5), 7.37 (d, 2H, ^3J = 7.8 Hz, Ph-***H***-2,6), 7.55 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.16 (-, ***C***H$_2$), 32.98 (-, ***C***H$_2$), 33.61 (-, ***C***H$_2$-S), 38.83 (-, ***C***H$_2$), 39.01 (+, ***C***H), 40.58 (+, ***C***H), 42.30 (-, N-***C***H$_2$), 47.95 (-, ***C***H$_2$-N), 116.65 (+, Im-***C***-5), 119.28 (C$_{quat}$, ***C***≡N), 127.35 (+, Ph-***C***-4), 130.20 (+, 2 Ph-***C***), 130.42 (+, 2 Ph-***C***), 135.89 (+, Im-***C***-2), 137.02 (C$_{quat}$, Ph-***C***-1), 140.35 (C$_{quat}$, Im-***C***-4), 161.15 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 369 (100) $[M + H]^+$. $C_{19}H_{24}N_6S$ (368.50).

5.4.2 Chiral separation by HPLC

5.4.2.1 Materials and methods

The preliminary screening of suitable separation conditions was performed by Sirius Fine Chemicals GmbH (Bremen, Germany). Preparative HPLC was performed in our laboratory at room temperature with a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps, a K-2001 detector (UV detection at 220 nm) and a chiral NP-column (Chiralcel OJ-H, 250 x 20 mm, 5 µm, Daicel Chemical Industries Ltd., Fort Lee, USA) at a flow rate of 18 mL/min. Mixtures of heptane and 2-propanol (85/15, v/v, isocratic elution) were used as mobile phase. Solvents were removed from the eluates under reduced pressure (final pressure: 60 mbar) at 40 °C prior to lyophilization. Chiral analytical HPLC analysis was performed on a system from Varian (Varian 920-LC, Darmstadt, Germany) and a NP-column thermostated at 30 °C (Chiralcel OJ-H, 250 x 4.6 mm, 5 µm, Daicel Chemical Industries Ltd., Fort Lee, USA; t_0 = 3.02 min) at a flow rate of 1.0 mL/min. UV-detection was done at 215 nm. Mixtures of heptane and 2-propanol (**5.18** and **5.19**: 85/15, v/v; **5.26** and **5.27**: 80/20, v/v; isocratic elution) were used as mobile phase. Helium degassing was used throughout. Compound purities were calculated as percentage peak area of the analyzed compound by UV detection at 215 nm. HPLC retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and enantiomeric purities of the synthesized compounds are listed under 5.4.2.2. Purity of tested compounds was > 95 % for all compounds as determined by high-performance liquid chromatography. Optical rotations were measured on a Perkin Elmer 141 polarimeter in the specified solvent. Concentrations are indicated in [g/100 mL]. Circular dichroism was determined with a Jasco CD1595 spectropolarimeter (Jasco GmbH, Groß-Umstadt, Germany) directly coupled to analytical HPLC at 240 nm at room temperature.

5.4.2.2 Experimental properties of the separated stereoisomers

(-)-2-Cyano-1-{[*trans*-(1*R*,3*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.18a)

Circular dichroism at 240 nm: +;

Enantiomeric excess as determined by HPLC: 98 %;

Optical rotation (c 1.0, MeOH) $[\alpha]^{25}$ (nm): - 1.9° (589), - 2.3° (546), - 4.9° (365);

HPLC (details see 5.4.2.1): t_R = 9.85 min, k' = 2.26;

(+)-2-Cyano-1-{[*trans*-(1*S*,3*S*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.18b)

Circular dichroism at 240 nm: -;

Enantiomeric excess as determined by HPLC: 96 %;

Optical rotation (c 0.75, MeOH) $[\alpha]^{25}$ (nm): + 1.7° (589), + 2.1° (546), + 6.1° (365);

HPLC (details see 5.4.2.1): t_R = 12.16 min, k' = 3.03;

(-)-2-Cyano-1-{[*cis*-(1*S*,3*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.19a)

Circular dichroism at 240 nm: -;

Enantiomeric excess as determined by HPLC: > 99 %;

Optical rotation (c 0.7, MeOH) $[\alpha]^{25}$ (nm): - 21.0° (589), - 25.0° (546), - 44.6° (365);

HPLC (details see 5.4.2.1): t_R = 9.11 min, k' = 2.01;

(+)-2-Cyano-1-{[*cis*-(1*R*,3*S*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-methylguanidine (5.19b)

Circular dichroism at 240 nm: +;

Enantiomeric excess as determined by HPLC: 97 %;

Optical rotation (c 0.66, MeOH) $[\alpha]^{25}$ (nm): + 20.9° (589), + 23.9° (546), + 39.1° (365);

HPLC (details see 5.4.2.1): t_R = 10.83 min, k' = 2.59;

(+)-2-Cyano-1-{[*trans*-(1*S*,3*S*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(2-(phenylthio)ethyl)guanidine (5.26a)

Circular dichroism at 240 nm: -;

Enantiomeric excess as determined by HPLC: > 99 %;

Optical rotation (c 2.9, MeOH) $[\alpha]^{25}$ (nm): + 0.4° (589), + 0.45° (546), + 1.2° (365);

HPLC (details see 5.4.2.1): t_R = 11.66 min, k' = 2.86;

(-)-2-Cyano-1-{[*trans*-(1*R*,3*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(2-(phenylthio)ethyl)guanidine (5.26b)

Circular dichroism at 240 nm: +;

Enantiomeric excess as determined by HPLC: 96 %;

Optical rotation (c 2.4, MeOH) $[\alpha]^{25}$ (nm): - 1.0° (589), - 1.2° (546), - 3.8° (365);

HPLC (details see 5.4.2.1): t_R = 12.76 min, k' = 3.23;

(-)-2-Cyano-1-{[*cis*-(1*S*,3*R*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(2-(phenylthio)ethyl)guanidine (5.27a)

Circular dichroism at 240 nm: -;

Enantiomeric excess as determined by HPLC: > 99 %;

Optical rotation (c 2.8, MeOH) $[\alpha]^{25}$ (nm): - 16.2° (589), - 19.3° (546), - 34.1° (365);

HPLC (details see 5.4.2.1): t_R = 11.83 min, k' = 2.92;

(+)-2-Cyano-1-{[*cis*-(1*R*,3*S*)-3-(1*H*-imidazol-4-yl)cyclopentyl]methyl}-3-(2-(phenylthio)ethyl)guanidine (5.27b)

Circular dichroism at 240 nm: +;

Enantiomeric excess as determined by HPLC: 97 %;

Optical rotation (c 4.0, MeOH) $[\alpha]^{25}$ (nm): + 15.7° (589), + 18.8° (546), + 33.5° (365);

HPLC (details see 5.4.2.1): t_R = 20.32 min, k' = 5.72;

5.4.3 Pharmacological methods

5.4.3.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide hydrochloride was synthesized according to a previously described method.[37] Iodophenpropit dihydrobromide was from Tocris Bioscience (Ellisville, USA). The H_4R antagonist JNJ-7777120 was synthesized according to ref[38]. [^{3}H]Mepyramine, [^{3}H]tiotidine, [^{3}H]N^{α}-methylhistamine and [^{3}H]histamine were from PerkinElmer Life Sciences (Boston, MA). [γ-^{32}P]GTP was synthesized according to a previously described method.[39] [^{32}P]P_i (8,500 – 9,100 Ci/mmol orthophosphoric acid) was from PerkinElmer Life Sciences (Boston, MA, USA). Guanosine diphosphate (GDP) and polyethylenimine were from Sigma-Aldrich Chemie GmbH (Munich, Germany), unlabeled GTPγS was from Roche (Mannheim, Germany). [^{35}S]GTPγS was from PerkinElmer Life Sciences (Boston, MA) or Hartmann Analytic GmbH (Braunschweig, Germany). GF/C filters were from Whatman Ltd. (Maidstone, UK). Glycerol-3-phosphate dehydrogenase, triose phosphate isomerase,

glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were from Roche (Mannheim, Germany). 3-Phosphoglycerate kinase, L-α-glycerol phosphate, tricine, ethylene glycol tetraacetic acid (EGTA), dithiothreitol (DTT), glycylglycine, forskolin, DMEM and adenosine triphosphate were from Sigma-Aldrich Chemie GmbH (Munich, Germany). HEK293 cells were from Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ) (Braunschweig, Germany) and were maintained in DMEM. Fetal bovine serum (FBS) and the antibiotic G418 were from Biochrom AG (Berlin, Germany). Hygromycin B was from MoBiTec GmbH (Göttingen, Germany). Triton-X was from Serva Electrophoresis GmbH (Heidelberg, Germany). D-luciferin potassium salt was from Synchem OHG (Felsberg, Germany). Culture flasks were from Nunc GmbH (Wiesbaden, Germany).

5.4.3.2 [^{35}S]GTPγS binding assay[40, 41]

See section 3.4.2.2.

5.4.3.3 Steady-state GTPase activity assay

GTPase activity assays were performed as previously described.[42-45] H_1R assays: Sf9 insect cell membranes coexpressing the hH_1R and RGS4 were employed, H_2R assays: Sf9 insect cell membranes expressing the hH_2R-$Gs\alpha_S$ fusion protein were used, H_3R assays: Sf9 insect cell membranes coexpressing the hH_3R, mammalian $G\alpha_{i2}$, $G\beta_1\gamma_2$ and RGS4 were employed, H_4R assays: Sf9 insect cell membranes coexpressing the hH_4R-GAIP fusion protein, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were used. The respective membranes were thawed, sedimented by centrifugation at 4 °C and 13,000 *g* for 10 min Membranes were resuspended in 10 mM Tris/HCl, pH 7.4. Each assay tube contained Sf9 membranes expressing the respective HR subtype (10 – 20 µg protein/tube), $MgCl_2$ ($H_{1,2}R$ assays: 1.0 mM, $H_{3,4}R$ assays: 5.0 mM), 100 µM EDTA, 100 µM ATP, 100 nM GTP, 100 µM adenylyl imidophosphate, 5 mM creatine phosphate, 40 µg creatine kinase and 0.2 % (w/v) bovine serum albumin in 50 mM Tris/HCl, pH 7.4 and the investigated ligands at various concentrations. All H_4R assays additionally contained 100 mM NaCl. For the determination of pK_B values (antagonist mode of the GTPase activity assay) histamine was added to the reaction mixtures (final concentrations: H_1R: 200 nM; H_2R: 1 µM; $H_{3,4}R$: 100 nM).

Reaction mixtures (80 µL) were incubated for 2 min at 25 °C. After the addition of 20 µL of [γ-^{32}P]GTP (0.1 µCi/tube), reaction mixtures were incubated for 20 min at 25 °C. Reactions were terminated by the addition of 900 µL slurry consisting of 5 % (w/v) activated charcoal and 50 mM NaH_2PO_4, pH 2.0. Charcoal absorbs nucleotides, but not P_i. Charcoal-quenched reaction mixtures were centrifuged for 7 min at room temperature at 13,000 *g*. 600 µL of the supernatant were removed and $^{32}P_i$ was determined by liquid scintillation counting. Spontaneous [γ-^{32}P]GTP degradation was determined in tubes containing all components described above, plus a high concentration of unlabeled GTP (1 mM) that due to competition with [γ-^{32}P]GTP prevents [γ-^{32}P]GTP hydrolysis by enzymatic activities present in Sf9 membranes. Spontaneous [γ-^{32}P]GTP degradation was <1 % of the total amount of radioactivity added. The experimental conditions chosen ensured that not more than 10 % of the total amount of [γ-^{32}P]GTP added was converted to $^{32}P_i$.

5.4.3.4 Radioligand binding assay[46, 47]

For the binding experiments the Sf9 insect cell membranes described above were employed. The respective membranes were thawed and sedimented by centrifugation at 4 °C and 13,000 *g* for 10 min. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4). Each tube (total volume 500 µL) contained 50 µg (hH_1R, hH_3R), 120 µg (hH_4R) or 250 µg (hH_2R) of membrane protein. Competition binding experiments were performed in the presence of 5 nM [^{3}H]mepyramine (hH_1R), 10 nM [^{3}H]tiotidine (hH_2R), 3 nM [^{3}H]N^{α}-methylhistamine (hH_3R) or 10 nM [^{3}H]histamine or 3 nM [^{3}H]UR-PI294 (hH_4R) and increasing concentrations of unlabeled ligands. Incubations were conducted for 60 min at 25 °C and shaking at 250 rpm. Bound radioligand was separated from free radioligand by filtration through 0.3 % polyethyleneimine-pretreated (PEI) GF/C filters, followed by three washes with 2 mL of cold binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting.

5.4.3.5 Luciferase reporter gene assay[48, 49]

HEK293-hH_4R-CRE-luc or HEK293-mH_4R-CRE-luc cells were cultured in DMEM supplemented with 10 % FBS and selection antibiotics (200 µg/mL of hygromycin B) in a water-saturated

atmosphere containing 5 % CO_2 at 37 °C. Cells were passaged (1:10) twice a week. For the assay approximately $2 \cdot 10^4$ cells/well were seeded in 96 well-plates in DMEM + 10 % FBS 17 h before the assay. After addition of increasing concentrations of histaminergic ligands and forskolin (400 nM (hH_4R) or 1 µM (mH_4R)) the cells were incubated for 5 h. Thereafter, the medium was discarded, the cells were washed with cold PBS and lysed in 40 µL of lysis buffer (25 mM tricine, pH 7.8, 10 % (v/v) glycerol, 2 mM EGTA, 1 % (v/v) TritonTM X-100, 5 mM $MgSO_4 \cdot 7\ H_2O$, 1 mM DTT) under shaking (180 rpm) for 30 min. Luminescence was measured with a Tecan GENios ProTM microplate reader by injection of 80 µL of D-luciferin solution (0.2 mg/mL D-luciferin potassium salt , 25 mM glycylglycine, 15 mM $MgSO_4 \cdot 7\ H_2O$, 15 mM KH_2PO_4, pH 7.8, 4 mM EGTA, pH 7.8, 2 mM ATP, 2 mM DTT) to 20 µL of the lysate.

5.4.3.6 Radioligand binding assay using HEK-293 cells expressing the mH_4R[48]

HEK-293-mH_4R cells were cultured in DMEM supplemented with 10 % FBS and the selection antibiotic G418 (600 µg/mL) in a water-saturated atmosphere containing 5 % CO_2 at 37 °C. Cells were passaged (1:10) twice a week. For the assays, approx. $5 \cdot 10^5$ cells were seeded into 175-cm^2 culture flasks and grown to approx. 70 % confluency (6 days) before the radioligand binding assays. Cells were detached with DMEM + 10 % FBS and centrifuged at 300 *g* for 5 min. Cells were washed once with Leibovitz' L-15 medium without phenol red + 1 % FBS, counted in a hemocytometer and finally suspended in Leibovitz' L-15 medium without phenol red + 1 % FBS according to a density of $1\text{-}2 \cdot 10^6$ cells/mL. For competition binding experiments, cells (160 µL of the cell suspension) were incubated with increasing concentrations of the unlabelled ligands in presence of 50 nM [^{3}H]-UR-PI294 in a 96-well plate (Greiner, Germany) (total volume 200 µL). Samples were shaken at 100 rpm for 90 min under light protection prior to harvesting. Cell-bound radioactivity was transferred to a glass fibre filter GF/C (Skatron) pre-treated with PEI (0.3 % (v/v) by a Combi Cell Harvester 11025. The filter was washed with PBS (4 °C) for approx. 10 s. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting.

5.4.3.7 Data analysis and pharmacological parameters.

See section 3.4.2.3.

5.5 References

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Chapter 6

Bioisosteric replacement of imidazole in cyanoguanidine-type hH_4R agonists - an approach to improve H_4R selectivity

6.1 Introduction

Bioisosteric approaches have been successfully applied in the search for subtype-selective histamine receptor ligands. For instance, N^G-acylated imidazolylpropylguanidines, representing highly potent H_2R agonists,[1] are also potent agonists at the hH_3R and hH_4R. This drawback could be overcome in a recent study by replacing the imidazole ring with other heterocycles. Introduction of an aminothiazole moiety, which is also present in the histamine analogue amthamine, a selective H_2R agonist,[2] resulted in highly potent and selective acylguanidine-type H_2R agonists devoid of agonistic activity at other HRs.[1] Furthermore, the acylguanidine moiety was replaced by a cyanoguanidine group yielding potent and selective H_4R agonists.[3] The selectivity for the H_4R could be improved by conformational constraints such as cyclopentane-1,3-diyl residues instead of flexible chains. However, a residual activity at H_3R was still characteristic of these compounds (cf. Chapter 5). Therefore, aiming at H_4R agonists with a further improved selectivity profile, especially compared to the H_3R, by analogy with the successful approach to H_2R agonists, the bioisosteric replacement of the 1*H*-imidazol-4-yl ring was considered a promising concept. In the present study a series of cyanoguanidine-type H_4R ligands related to UR-PI376 bearing various heterocycles (Figure 6.1) instead of the (unsubstituted) imidazol-4-yl ring were synthesized and investigated on recombinant human histamine receptor subtypes. Moreover, the respective amine precursors, which are histamine homologues related to imbutamine,[4, 5] were analyzed. The replacement of imidazole might additionally provide compounds that do not bind to the same extent to off-targets such as cytochrome P450 enzymes and have improved drug-like properties.

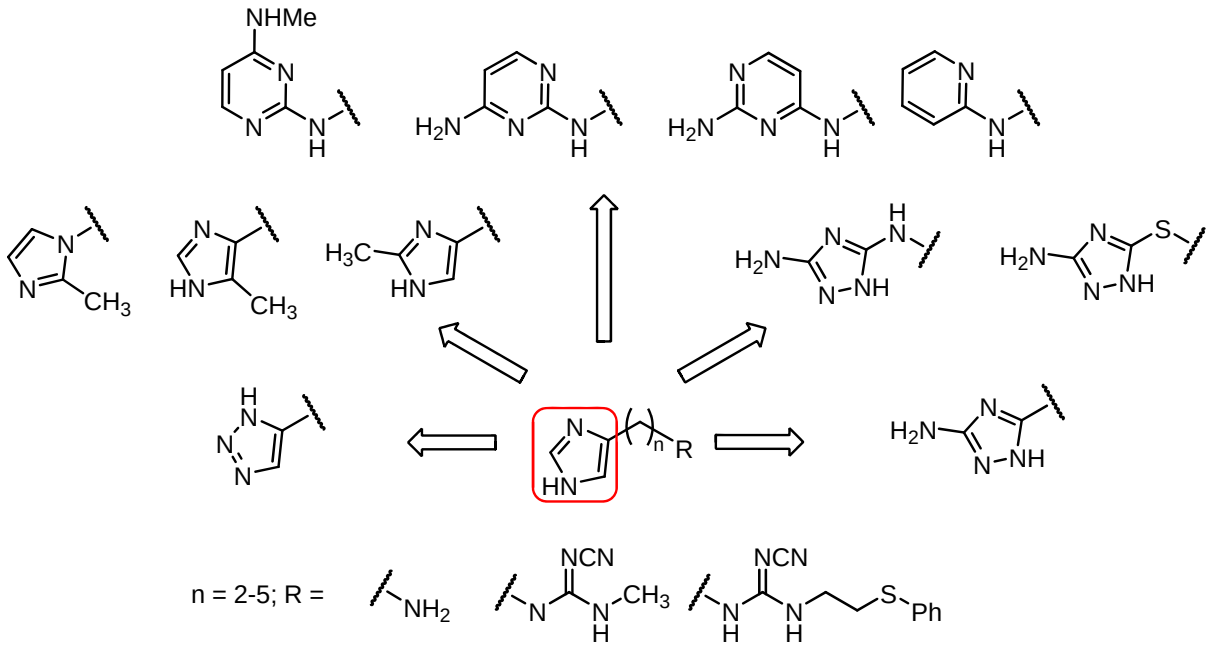


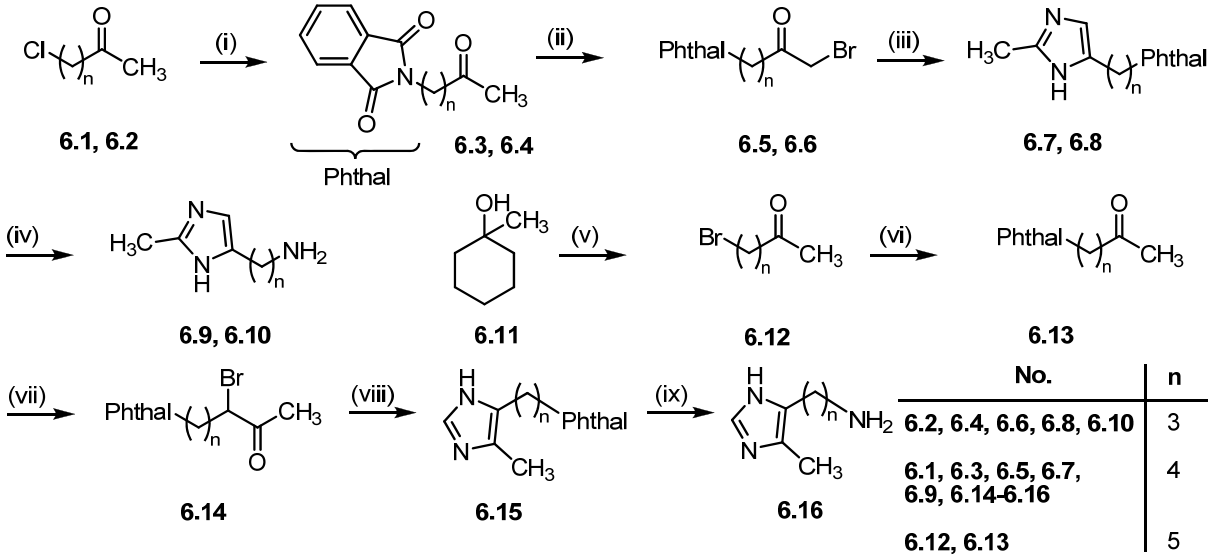
Figure 6.1 Imidazole replacement in histamine derivatives and cyanoguanidine-type H_4R ligands. Overview about the introduced heteroaromatic rings and side chains.

The attention was turned to heterocycles which had already been employed in the development of HR ligands, especially for the preparation of hetaryl analogs of histamine.[6-8] Furthermore, we tried to cover a wide range of physico-chemical properties, with emphasis on different basicity, and focused, as a first approach, on compounds that were easily accessible.

6.2 Chemistry

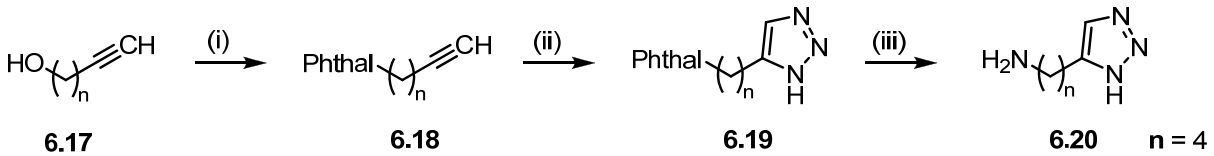
The amines required for the preparation of the cyanoguanidines **6.52-6.78** were synthesized as depicted in Scheme 6.1-Scheme 6.4. The 2- or 5-methyl-1*H*-imidazole-4-yl-substituted propanamines and butanamines **6.9, 6.10** and **6.16** were prepared by analogy with a synthetic pathway proposed by Elz and colleagues for the synthesis of homohistamine.[9] The ω-phthalimidoalkan-2-ones (**6.3**, **6.4** and **6.13**) were obtain from the reaction of the corresponding ω-chloroalkan-2-one (**6.1** and **6.2**) or 7-bromoheptan-2-one (**6.12**) with phthalimide.[10] 7-Bromoheptan-2-one (**6.12**) was prepared from 1-methylcyclohexanol **6.11** in a *Retro-Barbier* reaction.[11] Regioselective bromination of **6.3** or **6.4** was achieved by employing methanol as solvent in the presence of urea according to Zav'yalov and Kravchenko.[12] For the regioselective bromination of **6.13**, glacial acetic acid was used as solvent according to Black et al.[13] Cyclization of acetamidine with the α-bromoketone **6.5** or

6.6 in a *Bredereck* synthesis[14] gave the imidazoles **6.7** and **6.8** which were converted to **6.9** and **6.10** by hydrazinolysis of the phthalimide group. For the synthesis of **6.15** formamide was used instead of acetamidine, again followed by hydrazinolysis to yield **6.16**.



Scheme 6.1 Synthesis of the (2- or 5-methyl-1*H*-imidazole-4-yl)alkan-1-amines **6.9**, **6.10** and **6.16**. Reagents and conditions: (i) Phthalimide (1 eq), K_2CO_3 (1.5 eq), KI (cat.), DMF, 12 h, 100 °C; (ii) Br_2 (1 eq), urea (1 eq), MeOH, 24 h, rt; (iii) acetamidine hydrochloride (4 eq), K_2CO_3 (4 eq), DMF, 48 h, 75 °C; (iv) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, 2 h, reflux → overnight, rt; (v) Br_2 (5 eq), K_2CO_3 (6.1 eq), $CHCl_3$, 5 h, 0 °C; (vi) phthalimide (1 eq), K_2CO_3 (1.5 eq), KI (cat.), DMF, 12 h, 100 °C; (vii) Br_2 (1 eq), glacial acetic acid, 1.5 h, 10 °C → rt; (viii) formamide, 12 h, 170 °C; (ix) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, 2 h, reflux → overnight, rt.

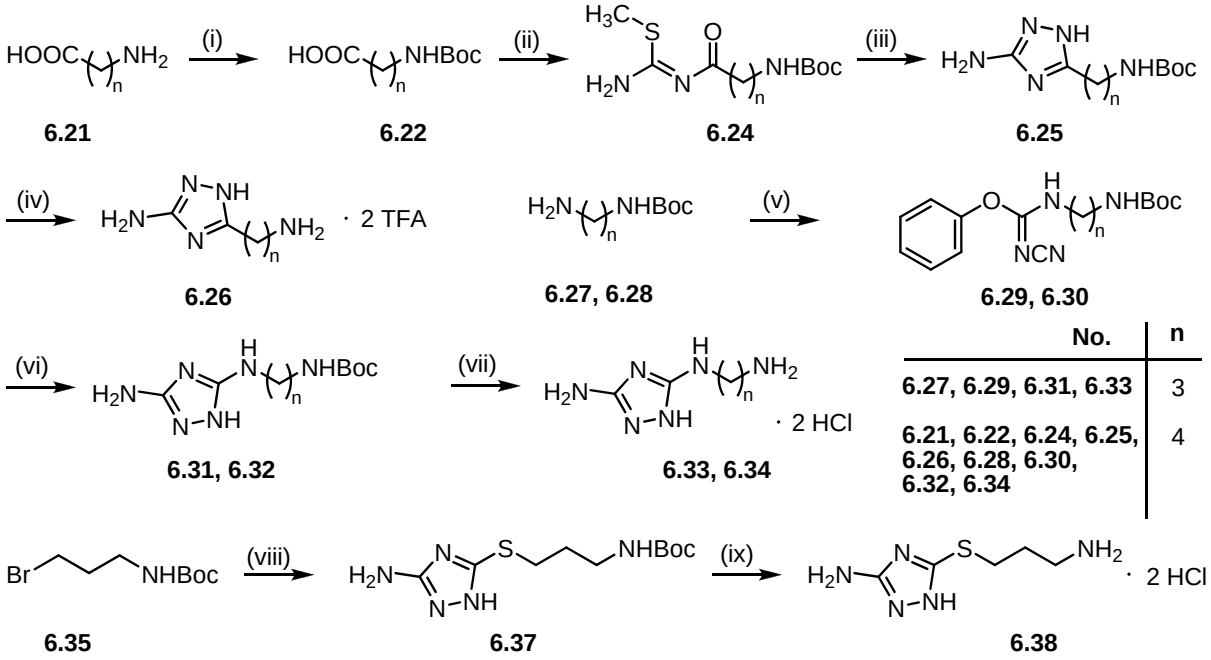
Preparation of 4-(1*H*-1,2,3-triazol-5-yl)butan-1-amine (**6.20**) started from 5-hexyn-1-ol (**6.17**), which was converted to the corresponding 2-(5-hexynyl)isoindoline-1,3-dione (**6.18**) under *Mitsunobu* conditions.[15] 2-[4-(1*H*-1,2,3-Triazol-5-yl)butyl]isoindoline-1,3-dione (**6.19**) was synthesized using c*lick-chemistry* according to Jin and co-workers,[16] and subsequently transformed to the amine **6.20** with the help of hydrazine.



Scheme 6.2 Synthesis of the 4-(1*H*-1,2,3-triazol-5-yl)butan-1-amine **6.20**. Reagents and conditions: (i) Phthalimide (1.1 eq), PPh_3 (1.1 eq), DIAD (1.1 eq), THF, overnight, 0 °C → rt; (ii) $TMSN_3$ (2 eq), CuI (cat.), DMF/MeOH 9/1 (v/v), microwave, 12 h, 100 °C; (iii) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, 2 h, reflux → overnight, rt.

The *N*-Boc protected 5-aminopentanoic acid **6.22** was coupled to **6.23** as described by Adang et al.[17] to yield **6.24**. Cyclization of **6.24** with hydrazine and deprotection under acidic

conditions gave 5-(4-aminobutyl)-1*H*-1,2,4-triazol-3-amine **6.26**. The 3,5-diamino-1,2,4-triazole derivatives (**6.33** and **6.34**) were synthesized according to Webb et al.[18] Reaction of mono-protected diaminoalkanes **6.27** and **6.28** with diphenyl cyanocarbonimidate (**3.21**) and subsequent cyclization with the help of hydrazine yielded the 3,5-diaminotriazoles **6.31** and **6.32**. Deprotection under acidic conditions gave the hydrochlorides of **6.33** and **6.34**. Substitution of *tert*-butyl 3-bromopropylcarbamate (**6.35**) with 5-amino-4*H*-1,2,4-triazole-3-thiol (**6.36**) and deprotection under acidic conditions gave **6.38.**[19]

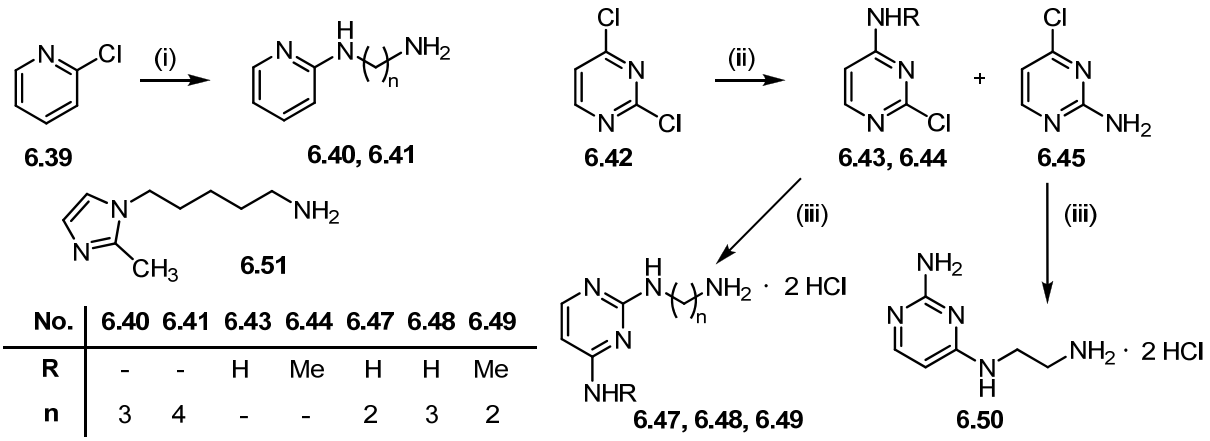


No.	n
6.27, 6.29, 6.31, 6.33	3
6.21, 6.22, 6.24, 6.25, 6.26, 6.28, 6.30, 6.32, 6.34	4

Scheme 6.3 Synthesis of the 2-amino-1,2,4-triazole derivatives **6.26**, **6.33, 6.34** and **6.38**. Reagents and conditions: (i) Boc_2O (1.1 eq), 1 M NaOH, dioxane/water 2/1 (v/v), overnight, 0 °C → rt; (ii) a) TBTU (1.2 eq), DIPEA (2 eq), DCM, 30 min, rt; b) *S*-methylisothiuronium iodide (**6.23**) (1 eq), DIPEA (3 eq), DCM, overnight, rt; (iii) $N_2H_4 \cdot H_2O$ (30 eq), NEt_3 (32.5 eq), EtOH, overnight, reflux; (iv) TFA 50 %, MeOH, overnight, rt; (v) diphenyl cyanocarbonimidate **3.21** (1 eq), 2-propanol, 2 h, rt; (vi) $N_2H_4 \cdot H_2O$ (1.5 eq), MeOH, 24 h, rt; (vii) 6 M HCl in 2-propanol, EtOAc/MeOH 6/1 (v/v), overnight, rt; (viii) 5-amino-4*H*-1,2,4-triazole-3-thiol (**6.36**) (0.8 eq), 1M NaOH, EtOH, 3 h, reflux; (ix) 6 M HCl in 2-propanol, EtOAc/MeOH 6/1 (v/v), overnight, rt.

The aminopyridine derivatives **6.40** and **6.41** were prepared via reaction of the pertinent diaminoalkane and the respective chloropyridine **6.39**[20] under microwave irradiation. Substituted diaminopyrimidines **6.47**-**6.50** were prepared starting from 2,4-dichloropyrimidine (**6.42**). Heating with ammonia under microwave irradiation yielded the isomers **6.43** and **6.45**, which can be easily separated by flash chromatography. **6.44** was synthesized from **6.42** by using methylamine instead of ammonia. The introduction of the second amino substituent was performed in a similar manner, using the respective Boc-

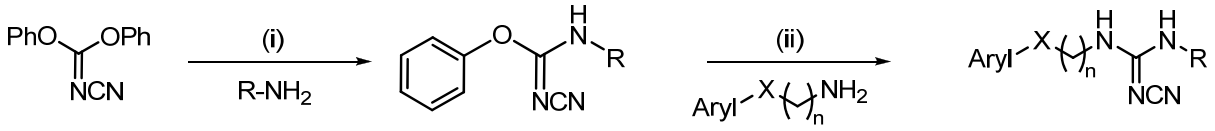
protected diaminoalkane (**6.27** or **6.46**). Subsequent deprotection under acidic conditions gave the hydrochlorides of **6.47**-**6.50**.



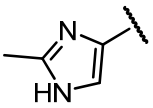
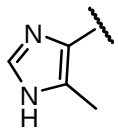
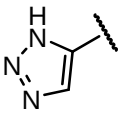
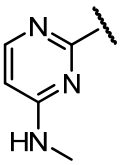
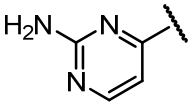
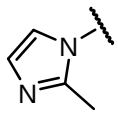
No.	6.40	6.41	6.43	6.44	6.47	6.48	6.49
R	-	-	H	Me	H	H	Me
n	3	4	-	-	2	3	2

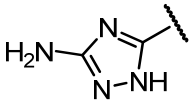
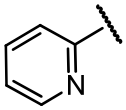
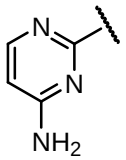
Scheme 6.4 Structure of **6.51** and synthesis of the 2-aminopyridines **6.40** and **6.41** and the diaminopyrimidines **6.47**, **6.48, 6.49** and **6.50**. Reagents and conditions: (i) Alkanediamine (6 eq), pyridine (1.3 eq), microwave, 30 min, 190 °C; (ii) for **6.43** and **6.45**: 7 M NH_3 in MeOH (1.5 eq), DIPEA (2 eq), EtOAc/THF 3/1 (v/v), microwave, 10 min, 100 °C, separation of isomers by flash chromatography; for **6.44**: 40 % Methylamine in H_2O (1 eq), DIPEA (1.5 eq), EtOAc/MeOH 1/1 (v/v), 36 h, rt (iii) a) for **6.47**, **6.49**, **6.50**: *tert*-Butyl 2-aminoethylcarbamate (**6.46**) (1 eq), DIPEA (1.5 eq), EtOH, microwave, 30 min, 120 °C, b) acetyl chloride (3 eq), MeOH, 4 h, 0 °C → rt; for **6.48**: **6.27** (1 eq), DIPEA (1.5 eq), EtOH, microwave, 30 min, 120 °C, b) acetyl chloride (3 eq), MeOH, 4 h, 0 °C → rt.

The cyanoguanidines **6.52-6.78** were synthesized by analogy with the synthetic route described in chapter 3 (Scheme 4).[18, 21, 22]



3.21 **3.22 or 3.26** **3.27 or 3.31** **6.9, 6.10, 6.16, 6.20, 6.26, 6.33, 6.34, 6.38, 6.40, 6.41, 6.47-6.51** **6.52-6.78**

No.	Aryl	n	X	R
3.22	-	-	-	$-CH_3$
3.26				$-(CH_2)_2$-S-Ph
3.27				$-CH_3$
3.31				$-(CH_2)_2$-S-Ph
6.9		3	CH_2	-
6.52				$-CH_3$
6.53				$-(CH_2)_2$-S-Ph
6.10		2	CH_2	-
6.54				$-CH_3$
6.55				$-(CH_2)_2$-S-Ph
6.16		3	CH_2	-
6.56				$-CH_3$
6.57				$-(CH_2)_2$-S-Ph
6.20		3	CH_2	-
6.58				$-CH_3$
6.59				$-(CH_2)_2$-S-Ph
6.49		2	NH	-
6.50		2	NH	-
6.76				$-(CH_2)_2$-S-Ph
6.51		4	CH_2	-
6.77				$-CH_3$
6.78				$-(CH_2)_2$-S-Ph

No.	Aryl	n	X	R
6.26	-	-	-	-
6.60		3	CH_2	$-CH_3$
6.61				$-(CH_2)_2$-S-Ph
6.33				-
6.62		3	NH	$-CH_3$
6.63				$-(CH_2)_2$-S-Ph
6.34				-
6.64		4	NH	$-CH_3$
6.65				$-(CH_2)_2$-S-Ph
6.38				-
6.66		3	S	$-CH_3$
6.67				$-(CH_2)_2$-S-Ph
6.40				-
6.68		3	NH	$-CH_3$
6.69				$-(CH_2)_2$-S-Ph
6.41				-
6.70		4	NH	$-CH_3$
6.71				$-(CH_2)_2$-S-Ph
6.47				-
6.72		2	NH	$-CH_3$
6.73				$-(CH_2)_2$-S-Ph
6.48				-
6.74		3	NH	$-CH_3$
6.75				$-(CH_2)_2$-S-Ph

Scheme 6.5 Synthesis of the cyanoguanidines **6.52-6.78**. Reagents and conditions: (i) 2-Propanol, 1 h, rt; (ii) MeCN, microwave 150 °C, 15 min.

6.3 Pharmacological results and discussion

6.3.1 Potencies and efficacies of the synthesized compounds at the HR subtypes

The synthesized amine precursors and the corresponding cyanoguanidines were investigated for agonism and antagonism at the hH_2R, hH_3R and hH_4R subtypes in [^{35}S]GTPγS binding assays using membrane preparations of Sf9 insect cells coexpressing the hH_4R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or coexpressing the hH_3R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or expressing the hH_2R-$Gs\alpha_s$ fusion protein. At the hH_1R a steady-state [^{33}P]GTPase assay with membrane preparations of Sf9 insect cells coexpressing the hH_1R plus RGS4 was used for characterization (Table 6.1 and Table 6.2).

In the following agonistic potencies are expressed as EC_{50} values. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine. Compounds identified to be inactive as agonists (α <0.1 or negative values, respectively, determined in the agonist mode) were investigated in the antagonist mode. The corresponding K_B values of neutral antagonists and inverse agonists were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding or [^{33}P]GTP hydrolysis, respectively.

In early studies on histamine analogues even minor modifications of the histamine structure led to less active compounds at the H_1R and H_2R. However, these investigations showed that it is possible to modify the ring structure and retain some histamine-like activity.[6] Recently, Lim and co-workers[4] reported that histamine analogues are potent agonists at the H_3R and the H_4R. Elongation of the spacer between imidazole and amino group from two carbon atoms (histamine) to four carbon atoms (imbutamine) resulted in a slightly higher hH_4R affinity. Based on these findings we replaced the imidazole ring in imbutamine with methyl-substituted analogues. The introduction of a 2-methyl substitution at the imidazole ring decreased potency and efficacy at both, the H_4R and the H_3R. **6.9** was a partial agonist at the H_4R with an EC_{50} of 346 nM. The same effect was observed for the 2-methyl analogue of homohistamine (**6.10**, EC_{50} = 1,700 nM). Interestingly, the decrease in potency at the H_3R was much more pronounced than at the H_4R. Hence, both compounds showed a changed selectivity profile and preferred the H_4R. This is in agreement with the findings for

2-methylhistamine.[4] Although lowering potency, substituents at position 2 of the imidazole ring should be considered with respect to selectivity in future ligand design. In agreement with the data for 5-methylhistamine, a methyl substitution in position 5 of the imidazole ring did not influence the potency at the H_4R, but slightly reduced the intrinsic activity (EC_{50} = 33 nM, α = 0.6). At the H_3R a more than 1,000-fold loss in activity and a change from full agonism to antagonistic behavior was observed. In contrast to 5-methylhistamine, **6.16** was devoid of agonistic activity at the H_3R and could therefore be an interesting alternative as pharmacological tool.

The replacement of imidazole by the less basic 1,2,3-triazole in **6.20** decreased potency and intrinsic activity in the [^{35}S]GTPγS assay and yielded a very weakly active partial agonist at both receptor subtypes. A drastic decrease in potency compared to the imidazole series was also observed for the 2-amino-1,2,4-triazole derivatives **6.26**, **6.33**, **6.34** and **6.38**. At the H_4R very poor inverse agonistic activity was found for **6.26**-**6.34**. **6.38** was inactive up to a concentration of 100 µM. These findings suggest that the basicity of the heterocycle should be comparable to that of imidazole to obtain histamine H_4 receptor activity. At the H_3R, neither agonistic nor antagonistic activity was detectable for all four derivatives in the [^{35}S]GTPγS assay. The two pyridine derivatives **6.40** and **6.41** were weak inverse agonists at both receptor subtypes. The K_B values at the H_3R were in the low micromolar range with intrinsic activities of -0.5. The activities at the H_4R were even lower. The spatial demand of the six-membered ring and again the lower basicity of the heterocycle might contribute to this dramatic loss in potency.

Recently, several studies on aminopyrimidines as H_4R antagonists were published.[8] To evaluate the effect of the replacement of imidazole by diaminopyrimidines in histamine derivatives we synthesized four N^4- or N^2-substituted aminoalkylpyrimidine-2,4-diamines. Unfortunately, the three N^2-substituted derivatives **6.47**-**6.49** were inactive or only very weakly active at the H_4R and H_3R. Interestingly, the N^4-substituted derivative **6.50** was a potent H_3R antagonist (K_B = 91 nM, α = -0.16) with some selectivity over the H_4R (EC_{50} = 500 nM, α = 0.87). For the synthesis of H_3R or H_4R ligands this compound may serve as core structure to be decorated with various substituents.

Finally, the impentamine derivative **6.51** was available in our compound collection and investigated in the [^{35}S]GTPγS assay. At the H_4R no activity was detected, at the H_3R inverse agonistic activity (α = -0.97) with a K_B value of 2,460 nM was determined. According to

Yao et al. a hydrogen bridge with the imidazole N-H is necessary for H_3R and H_4R activation.[23, 24] This is not possible in the case of compound **6.51**. Another explanation could be the spacer length of five carbon atoms that seems to be too long for optimal orientation of the ligand in the binding pocket of the H_4R. At the H_1R and the H_2R for some compounds very weak partial agonistic or antagonistic activity was detected. Most of the amines proved to be inactive at both receptor subtypes up to a concentration of 10 µM.

The investigation of the synthesized cyanoguanidines for H_4R activity revealed a high sensitivity even towards minor structural modifications and confirmed in this respect the findings published by Igel et al.[3] Unfortunately, all cyanoguanidines bearing heterocycles other than imidazole showed only negligible partial agonistic or antagonistic behavior, or were even inactive at all four histamine receptor subtypes. The phenylthioethyl substituted aminopyrimidine derivative **6.76** was the only compound with inverse agonistic activity in the lower micromolar range (K_B ~ 3 µM) at both, the H_4R and the H_3R.

As expected, the compounds bearing a methyl substituted imidazole ring showed some activity at the H_4R. The 2-methyl imidazole derivatives **6.52** and **6.53** with a butyl chain connecting imidazole and cyanoguanidine were weak inverse agonists at the H_4R and showed no noteworthy activity at the other HR subtypes. As found previously, the phenylthioethyl residue results in higher potency at the H_4R compared to a methyl substitution. Reducing the spacer chain length to three carbon atoms provides the partial H_4R agonists **6.54** and **6.55** with EC_{50} values around 500 nM and no agonistic activity at the other three histamine receptors. This is in agreement with the results for the amines **6.9** and **6.10**. Compound **6.56**, the carba analogue of cimetidine, a blockbuster drug for the treatment of gastric ulcer,[25] was a weak partial agonist at the H_4R (EC_{50} = 3.6 µM) and the H_3R (EC_{50} = 1 µM) and showed only very weak antagonistic properties at the H_1R and at the H_2R. Interestingly the exchange of the sulfur atom in the spacer between imidazole and cyanoguanidine with a methylene group drastically reduced the antagonistic potency at the H_2R. The phenylthioethyl-substituted cyanoguanidine **6.57** was by a factor of 15 more potent at the H_4R than the methyl-substituted cyanoguanidine **6.56**. With an EC_{50} value of 247 nM at the H_4R, the 5-methyl analogue of UR-PI376, **6.57**, showed a more than 10-fold selectivity over the H_3R and the other HR subtypes. Nevertheless, none of the investigated hetarylalkylcyanoguanidines was superior to UR-PI376 in terms of H_4R agonistic potency or receptor subtype selectivity.

Table 6.1 Potencies and efficacies of the synthesized amines and selected reference compounds at the hHR subtypes in the [^{35}S]GTPγS assay[a] or the GTPase assay.[b]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
Histamine	190 ± 8[c]	1.00		1,200 ± 300[c]	1.00		13 ± 2	1.00	3	11± 3	1.00	5
Homohistamine	n.d.	n.d.		n.d.	n.d.		40 ± 15[d]	0.90		200 ± 50[d]	0.80	
Imbutamine	n.d.	n.d.		n.d.	n.d.		0.6 ± 0.2[d]	1.00		32 ± 8[d]	0.80	
Impentamine	n.d.	n.d.		n.d.	n.d.		4 ± 1[d]	0.90		n.d.	0[d]	
5-Methylhistamine	16,000 ± 540[d]	0.90		n.d.	n.d.		>1 mM[d]	0.12		70 ± 18[d]	0.9	
2-Methylhistamine	837 ± 110[d]	0.98		n.d.	n.d.		n.d.	n.d.		1,300 ± 300[d]	0.8	
6.9	(> 10,000)	0.03 ± 0.02	2	inactive	n.d.	2	> 10,000	0.45 ± 0.04	2	346 ± 7	0.39 ± 0.05	2
6.10	(> 10,000)	0.02 ± 0.0	2	> 10,000	0.15 ± 0.04	2	(> 10,000)	0.09 ± 0.04	2	1,700 ± 186	0.39 ± 0.01	2
6.16	(> 10,000)	0.04 ± 0.02	2	> 10,000	0.14 ± 0.09	2	(890 ± 44)	-0.02 ± 0.04	2	33 ± 3	0.60 ± 0.05	2
6.20	inactive	n.d.	2	inactive	n.d.	2	> 10,000	0.36 ± 0.04	3	> 10,000	0.20 ± 0.04	3
6.26	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2	(> 10,000)	-0.15 ± 0.03	2
6.33	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2	(> 10,000)	-0.30 ± 0.1	2
6.34	(> 10,000)	0.05 ± 0.02	2	inactive	n.d.	2	inactive	n.d.	2	(> 10,000)	-0.11 ± 0.09	2
6.38	(> 10,000)	0.04 ± 0.01	2	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2

Table 6.1 (continued)

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
6.40	> 10,000	0.12 ± 0.01	2	(> 10,000)	-0.01 ± 0.02	2	(2,790 ± 164)	-0.51 ± 0.09	2	(> 10,000)	-0.22 ± 0.08	2
6.41	> 10,000	0.16 ± 0.02	2	(> 10,000)	-0.01 ± 0.0	2	(1,430 ± 164)	-0.50 ± 0.05	2	(> 10,000)	-0.22 ± 0.09	2
6.47	> 10,000	0.19 ± 0.01	2	> 10,000	0.18 ± 0.1	**2**	inactive	n.d.	2	inactive	n.d.	3
6.48	> 10,000	0.12 ± 0.01	2	inactive	n.d.	**2**	(> 10,000)	-0.28 ± 0.14	2	inactive	n.d.	2
6.49	inactive	n.d.	2	inactive	n.d.	2	(> 10,000)	-0.13 ± 0.11	2	inactive	n.d.	2
6.50	inactive	n.d.	2	> 10,000	0.21 ± 0.02	2	(91 ± 1)	-0.16 ± 0.03	2	500 ± 150	0.87 ± 0.04	3
6.51	(> 10,000)	0.02 ± 0.03	2	inactive	n.d.	2	(2,460 ± 58)	-0.97 ± 0.12	3	inactive	n.d.	2

[a] Functional [^{35}S]GTPγS binding assays with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_2R-$Gs\alpha_s$ fusion protein were performed as described in section *Pharmacological methods*.[b] Steady-state GTPase activity in Sf9 cell membranes expressing the hH_1R + RGS4 was determined as described in section *Pharmacological methods*.[a,b] Reaction mixtures contained ligands at a concentration from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. N gives the number of independent experiments performed in duplicate each. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. The α values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM. The K_B values of neutral antagonists and inverse agonists were determined in the antagonist mode versus histamine as the agonist.[c] Data taken from Xie et al.[26] [d] Data taken from Lim et al.[4]

Table 6.2 Potencies and efficacies of the synthesized cyanoguanidines at the hHR subtypes in the [^{35}S]GTPγS assay[a] or the GTPase assay.[b]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
Histamine	190 ± 8[c]	1.00		1,200 ± 300[c]	1.00		13 ± 2	1.00	3	11± 3	1.00	5
UR-PI376	>10,000[d]	0.07		(>10,000)[d]	0.08		(720 ± 38)	-0.52 ± 0.05	2	37± 3	0.88 ± 0.08	3
Cimetidine	n.d.	n.d.		(1,700 ± 430)[e]	-0.08 ± 0.01		n.d.	n.d.		(> 10,000)[f]	n.d.	
6.52	(> 10,000)	0.01 ± 0.03	2	(> 10,000)	0.04 ± 0.02	2	(> 10,000)	-0.13 ± 0.08	2	(> 10,000)	-0.23 ± 0.03	2
6.53	(> 5,000)	-0.01 ± 0.03	2	(16,375 ± 45)	-0.05 ± 0.02	2	(> 10,000)	-0.47 ± 0.04	2	(620 ± 63)	-0.24 ± 0.11	2
6.54	inactive	n.d.	2	(> 10,000)	-0.02 ± 0.01	2	(> 10,000)	-0.52 ± 0.04	2	550 ± 27	0.74 ± 0.02	2
6.55	(> 5,000)	0.03 ± 0.05	2	(> 10,000)	-0.05 ± 0.02	2	(> 10,000)	-1.11 ± 0.03	2	470 ± 28	0.40 ± 0.07	2
6.56	(> 10,000)	-0.03 ± 0.02	2	(> 10,000)	0.04 ± 0.01	2	1,080 ± 110	0.23 ± 0.03	2	3,650 ± 375	0.60 ± 0.17	2
6.57	(> 5,000)	-0.03 ± 0.01	2	(4,400 ± 430)	-0.06 ± 0.02	2	(3,175 ± 123)	-0.65 ± 0.02	2	247 ± 2	0.37 ± 0.1	2
6.58	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2
6.59	(> 10,000)	-0.02 ± 0.01	2	(> 10,000)	-0.04 ± 0.0	2	inactive	n.d.	2	inactive	n.d.	2
6.60	inactive	n.d.	2	> 10,000	0.31 ± 0.02	2	inactive	n.d.	2	inactive	n.d.	2
6.61	(> 10,000)	0.01 ± 0.04	2	(> 10,000)	0.03 ± 0.02	2	inactive	n.d.	2	inactive	n.d.	2
6.62	inactive	n.d.	2	(> 10,000)	-0.03 ± 0.0	2	inactive	n.d.	2	inactive	n.d.	2
6.63	inactive	n.d.	2	(> 10,000)	-0.01 ± 0.04	2	inactive	n.d.	2	inactive	n.d.	2
6.64	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2	inactive	n.d.	2
6.65	inactive	n.d.	2	(> 10,000)	-0.09 ± 0.01	2	inactive	n.d.	2	inactive	n.d.	2
6.66	inactive	n.d.	2	(> 10,000)	-0.02 ± 0.0	2	inactive	n.d.	2	inactive	n.d.	2

Table 6.2 (continued)

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N	EC_{50} or (K_B) (nM)	α	N
6.67	inactive	n.d.	2	(> 10,000)	-0.05 ± 0.00	2	inactive	n.d.	2	inactive	n.d.	2
6.68	inactive	n.d.	2	inactive	n.d.	2	(> 10,000)	-0.03 ± 0.04	2	(> 10,000)	-0.13 ± 0.05	2
6.69	(> 10,000)	0.02 ± 0.03	2	(> 10,000)	-0.12 ± 0.02	2	(> 10,000)	-0.21 ± 0.02	2	(> 10,000)	-0.10 ± 0.01	2
6.70	(> 10,000)	0.03 ± 0.03	2	inactive	n.d.	2	(> 10,000)	-0.10 ± 0.03	2	(> 10,000)	-0.11 ± 0.03	2
6.71	(> 10,000)	-0.01 ± 0.01	2	(> 10,000)	-0.13 ± 0.01	2	(> 10,000)	-0.36 ± 0.04	2	(> 10,000)	-0.34 ± 0.01	2
6.72	inactive	n.d.	2	inactive	n.d.	2	(> 10,000)	-0.16 ± 0.02	2	(> 10,000)	-0.11 ± 0.03	2
6.73	(> 10,000)	0.01 ± 0.03	2	(> 10,000)	-0.08 ± 0.02	2	(> 10,000)	-1.43 ± 0.14	2	inactive	n.d.	2
6.74	(> 10,000)	0.03 ± 0.02	2	inactive	n.d.	2	(> 10,000)	-0.24 ± 0.05	2	inactive	n.d.	2
6.75	(> 10,000)	0.01 ± 0.02	2	(> 10,000)	-0.11 ± 0.01	2	(7,320 ± 60)	-0.71 ± 0.03	2	(> 10,000)	-0.09 ± 0.05	2
6.76	(> 10,000)	-0.02 ± 0.04	2	(> 10,000)	-0.07 ± 0.01	2	(2,910 ± 27)	-1.03 ± 0.01	2	(3,700 ± 790)	-0.83 ± 0.06	2
6.77	(> 10,000)	-0.01 ± 0.03	2	(> 10,000)	-0.07 ± 0.01	2	(> 10,000)	-0.26 ± 0.03	2	inactive	0.06 ± 0.03	2
6.78	(> 10,000)	0.03 ± 0.01	2	(> 10,000)	-0.08 ± 0.00	2	(> 10,000)	-0.42 ± 0.06	2	(> 10,000)	-0.05 ± 0.04	2

[a] Functional [^{35}S]GTPγS binding assays with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_2R-$Gs\alpha_s$ fusion protein were performed as described in section *Pharmacological methods*.[b] Steady-state GTPase activity in Sf9 cell membranes expressing the hH_1R + RGS4 was determined as described in section *Pharmacological methods*.[a,b] Reaction mixtures contained ligands at a concentration from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. N gives the number of independent experiments performed in duplicate each. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. The α values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM. The K_B values of neutral antagonists and inverse agonists were determined in the antagonist mode versus histamine as the agonist.[c] Data taken from Xie et al.[26] [d] Data taken from Igel.[27] [e] Data (GTPase assay) taken from Preuss.[28] [f] Data (K_i) taken from Lim et al.[4]

6.3.2 Summary and conclusion

Essentially, the results are in agreement with data from previous studies on histamine derivatives, suggesting that both ethylamine and butylamine side chains are equivalent substructures in H_4R ligand binding. The bioisosteric replacement of the imidazole ring in imbutamine and analogues by various heterocycles has not been successful in terms of H_4R activity so far. Obviously, the basicity of the heterocycle, the H-bond donor and acceptor properties and the spatial demand should be comparable to that of imidazole. The introduction of substituents at the 4-imidazolyl ring was most promising with regard to H_4R selectivity. This holds for methyl substitution in position 2 and especially in position 5. 5-methylimbutamine (**6.16**) was the most potent and selective H_4R agonist in this series. This compound might be useful as a pharmacological tool. In contrast to 5-methylhistamine, **6.16** shows no agonistic activity at the H_3R.

The cyanoguanidines derived from OUP-16 and UR-PI376 revealed high sensitivity of this compound class against both, replacement of the heterocycle and minor structural modifications such as methyl-substitution of the imidazole ring. None of the analogues improved potency and/or H_4R selectivity compared to UR-PI376. Except for the heterocycle there is no basic group in the cyanoguanidine-type compounds. As previous studies revealed higher potency of H_4R agonists with retained basicity in the central structural motif, e. g. acylguanidines, a combination of bioisosteric replacements of both imidazole ring and cyanoguanidine moiety should be considered in future ligand design.

In conclusion, the presented data suggest alternative bioisosteric approaches, including the synthesis and pharmacological evaluation of additional heterocyclic analogues of known histamine receptor ligands, with respect to retained/increased potency, improved receptor subtype selectivity and drug-like properties.

6.4 Experimental Section

6.4.1 Chemistry

6.4.1.1 General Conditions

See section 3.4.1.1.

5-(2-methyl-1*H*-imidazol-1-yl)pentan-1-amine (**6.51**) was a gift from Dr. Birgit Striegl, Department of Pharmaceutical/medicinal Chemistry I, University of Regensburg. N^2-(3-aminopropyl)pyrimidine-2,4-diamine dihydrochloride (**6.48**), N^2-(2-aminoethyl)pyrimidine-2,4-diamine dihydrochloride (**6.47**), N^2-(3-aminopropyl)-N^4-methylpyrimidine-2,4-diamine dihydrochloride (**6.49**) and N^4-(2-aminoethyl)pyrimidine-2,4-diamine dihydrochloride (**6.50**) were a gift from Melanie Kaske, Department of Pharmaceutical/medicinal Chemistry II, University of Regensburg.

Preparative HPLC was performed at room temperature with a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps, a K-2001 detector (UV detection at 220 nm) and a RP-column (VP Nucleodur 100-5 C18 ec, 250 x 21 mm, 5 µm, Macherey Nagel, Düren, Germany) at a flow rate of 18 mL/min. Mixtures of acetonitrile and millipore water were used as mobile phase. Acetonitrile was removed from the eluates under reduced pressure (final pressure: 60 mbar) at 40 °C prior to lyophilization.

6.4.1.2 Preparation of 4-(2-methyl-1H-imidazol-4-yl)butan-1-amine 6.9

2-(5-Oxohexyl)isoindoline-1,3-dione (6.3)[29]

A mixture of 6-chlorohexan-2-one (**6.1**) (25 mL, 185.7 mmol), phthalimide (27.3 g, 185.7 mmol), K_2CO_3 (38.5 g, 278.5 mmol) and KI (0.1 g, 0.6 mmol, catalytic amounts) in 200 mL dimethylformamide was stirred for 12 h at 100 °C. The cooled mixture was poured in 500 mL of ice-cold water and extracted with $CHCl_3$. The combined organic layers were washed with saturated $NaHCO_3$, 2 % HCl and brine and dried over $MgSO_4$. Evaporation yielded a yellowish oil that crystallized from EtOH/H_2O as white solid (40.5 g, 89 %); mp 58 °C (ref.[29]: 57 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.58 (m, 4H, 2 C***H***$_2$), 2.07 (s, 3H, C***H***$_3$), 2.42 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$-$COCH_3$), 3.62 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-Phthal), 7.64 (m, 2H, Phthal-***H***), 7.77 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 20.76 (-, ***C***H_2), 27.91 (-, ***C***H_2), 29.95 (+, ***C***H_3), 37.47 (-, ***C***H_2), 42.85 (-, ***C***H_2), 123.24 (+, Phthal-***C***-4,7), 132.06 (C_{quat}, Phthal-***C***-3a,7a), 133.94 (+, Phthal-***C***-5,6), 168.42 (C_{quat}, 2 Phthal-***C***=O), 208.47 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 246 (20) $[M + H]^+$, 263 (100) $[M + NH_4]^+$. Anal. ($C_{14}H_{15}NO_3$) C, H, N. $C_{14}H_{15}NO_3$ (245.27).

2-(6-Bromo-5-oxohexyl)isoindoline-1,3-dione (6.5)[30]

To a solution of **6.3** (33.0 g, 134.5 mmol) and urea (8.1 g, 134.5 mmol) in MeOH (200 mL), bromine (21.5 g, 134.5 mmol) was rapidly added and stirred for 24 h. The precipitated

product was filtered, washed with MeOH (10 mL) and dried *in vacuo* to yield a white solid (22.4 g, 51 %); mp 105 °C (ref.[27]: 112 – 113 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.68 (m, 4H, Phthal-CH_2-C***H***$_2$-C***H***$_2$), 2.72 (t, 2H, ^{3}J = 7.1 Hz, Phthal-$(CH_2)_3$-C***H***$_2$), 3.69 (t, 2H, ^{3}J = 6.8 Hz, Phthal-C***H***$_2$), 3.87 (s, 2H, C***H***$_2$-Br), 7.69 – 7.72 (m, 2H, Phthal-***H***), 7.81 – 7.84 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 20.84 (-, ***C***H_2), 27.77 (-, ***C***H_2), 34.20 (-, ***C***H_2), 37.33 (-, ***C***H_2), 38.95 (-, ***C***H_2), 123.26 (+, Phthal-***C***-4,7), 132.05 (C_{quat}, Phthal-***C***-3a,7a), 133.99 (+, Phthal-***C***-5,6), 168.43 (C_{quat}, 2 Phthal-***C***=O), 201.58 (C_{quat}, ***C***OCH_2Br). CI-MS (NH_3) *m/z* (%): 341 (40) $[M + NH_4]^+$, 263 (100) $[M – Br + H + NH_4]^+$. Anal. ($C_{14}H_{14}BrNO_3$) C, H, N. $C_{14}H_{14}BrNO_3$ (324.17).

2-[4-(2-Methyl-1*H*-imidazol-4-yl)butyl]isoindoline-1,3-dione (6.7)

A mixture of **6.5** (5 g, 15.4 mmol), acetamidine hydrochloride (5.8 g, 61.7 mmol) and K_2CO_3 (8.5 g, 61.7 mmol) in 50 mL dimethylformamide was stirred for 72 h at 50 °C. The solvent was evaporated and the residue taken up in DCM and extracted with saturated $NaHCO_3$. Evaporation of the solvent and flash chromatography (DCM/MeOH 95/5 – 85/15 v/v) yielded a yellow oil (0.84 g, 20 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.60 (m, 4H, Phthal-CH_2-C***H***$_2$-C***H***$_2$), 2.27 (s, 3H, C***H***$_3$), 2.50 (t, 2H, ^{3}J = 7.1 Hz, Phthal-$(CH_2)_3$-C***H***$_2$), 3.58 (t, 2H, ^{3}J = 6.7 Hz, Phthal-C***H***$_2$), 6.58 (s, 1H, Im-***H***-5), 7.67 (m, 4H, Phthal-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.68 (+, ***C***H_3), 27.08 (-, ***C***H_2), 27.81 (-, ***C***H_2), 29.21 (-, ***C***H_2), 38.68 (-, ***C***H_2), 117.45 (+, Im-***C***-5), 124.10 (+, Phthal-***C***-4,7), 133.26 (C_{quat}, Phthal-***C***-3a,7a), 135.33 (+, Phthal-***C***-5,6), 137.13 (C_{quat}, Im-***C***-4), 145.18 (C_{quat}, Im-***C***-2), 169.68 (C_{quat}, 2 Phthal-***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 284 (100) $[M + H]^+$. $C_{16}H_{17}N_3O_2$ (283.33).

4-(2-Methyl-1*H*-imidazol-4-yl)butan-1-amine (6.9)

A solution of **6.7** (0.8 g, 2.8 mmol) and hydrazine monohydrate (0.7 mL, 14.1 mmol, 5 eq) in EtOH (50 mL) was refluxed for 2 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and flash chromatography (DCM/MeOH/32 % $NH_{3(aq)}$ 80/18/2 v/v/v) yielded a yellow oil (0.31 g, 72 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.53 (m, 2H, C***H***$_2$), 1.62 (m, 2H, C***H***$_2$), 2.29 (s, 3H, C***H***$_3$), 2.52 (t, 2H, ^{3}J = 7.6 Hz, C***H***$_2$), 2.68 (t, 2H, ^{3}J = 7.0 Hz, C***H***$_2$), 6.58 (s, 1H, Im-***H***-5). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.51 (+, ***C***H_3), 27.35 (-, ***C***H_2), 27.82 (-, ***C***H_2), 32.47 (-, ***C***H_2), 42.05 (-, ***C***H_2-NH_2), 117.25 (+, Im-***C***-5), 137.59 (C_{quat}, Im-***C***-4), 145.10 (C_{quat}, Im-

C-2). HRMS (EI-MS) calcd. for $C_8H_{15}N_3$ [$M^{+\bullet}$] 153.1266; found 153.1262. IR (cm^{-1}) = 2933, 2859, 2669 (C-H), 1390, 1020. Anal. ($C_8H_{15}N_3 \cdot 0.85\ H_2O$) C, H, N. $C_8H_{15}N_3$ (153.22).

6.4.1.3 Preparation of 3-(2-methyl-1H-imidazol-4-yl)propan-1-amine 6.10

2-(5-Oxopentyl)isoindoline-1,3-dione (6.4)[31]

A mixture of 5-chloropentan-2-one (**6.2**) (25 mL, 207.3 mmol), phthalimide (30.5 g, 207.3 mmol), K_2CO_3 (43.0 g, 311.0 mmol) and KI (0.1 g, 0.6 mmol, catalytic amounts) in 200 mL dimethylformamide was stirred for 12 h at 100 °C. The cooled mixture was poured in 500 mL of ice-cold water and extracted with $CHCl_3$. The combined organic layers were washed with saturated $NaHCO_3$, 2 % HCl and brine and dried over $MgSO_4$. Evaporation yielded a yellowish oil that crystallized from EtOH/H_2O as white solid (23.0 g, 48 %); mp 68°C (ref.[31]: 73 – 74 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.92 (m, 2H, C***H***$_2$), 2.11 (s, 3H, C***H***$_3$), 2.47 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$-$COCH_3$), 3.67 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$-Phthal), 7.69 (m, 2H, Phthal-***H***), 7.80 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] =22.35 (-, ***C***H_2), 29.91 (+, ***C***H_3), 37.18 (-, ***C***H_2), 40.53 (-, ***C***H_2), 123.23 (+, Phthal-***C***-4,7), 132.02 (C_{quat}, Phthal-***C***-3a,7a), 133.98 (+, Phthal-***C***-5,6), 168.46 (C_{quat}, 2 Phthal-***C***=O), 207.44 (C_{quat}, ***C***=O). EI-MS (70 eV) *m/z* (%): 231 (20) [$M^{+\bullet}$], 174 (100) [M – CH_2COCH_3] $^{+\bullet}$, 160 (60) [M – $CH_2CH_2COCH_3$]$^{+\bullet}$. $C_{13}H_{13}NO_3$ (231.25).

2-(5-Bromo-4-oxopentyl)isoindoline-1,3-dione (6.6)[21]

To a solution of **6.3** (23.0 g, 99.5 mmol) and urea (6.0 g, 99.5 mmol) in MeOH (200 mL), bromine (15.9 g, 99.5 mmol) was rapidly added and stirred for 24 h. The solvent was evaporated and the residue taken up in $CHCl_3$ and extracted with water. The organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (PE/EtOAc 90/10 – 70/30 v/v) yielded a white solid (8.5 g, 28 %); mp 120 °C (ref.[32]: 128 – 129 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.98 (m, 2H, Phthal-CH_2-C***H***$_2$), 2.69 (t, 2H, ^3J = 7.0 Hz, Phthal-$(CH_2)_2$-C***H***$_2$), 3.69 (t, 2H, ^3J = 6.8 Hz, Phthal-C***H***$_2$), 3.90 (s, 2H, C***H***$_2$-Br), 7.69 (m, 2H, Phthal-***H***), 7.81 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 22.85 (-, ***C***H_2), 34.38 (-, ***C***H_2), 36.80 (-, ***C***H_2), 36.88 (-, ***C***H_2), 123.28 (+, Phthal-***C***-4,7), 131.96 (C_{quat}, Phthal-***C***-3a,7a), 134.06 (+, Phthal-***C***-5,6), 168.48 (C_{quat}, 2 Phthal-***C***=O), 200.98 (C_{quat}, ***C***OCH_2Br). CI-MS (NH_3) *m/z* (%): 329 (30) [M + NH_4]$^+$, 249 (100) [M – Br + H + NH_4]$^+$. Anal. ($C_{13}H_{12}BrNO_3$) C, H, N. $C_{13}H_{12}BrNO_3$ (310.14).

2-[3-(2-Methyl-1*H*-imidazol-4-yl)propyl]isoindoline-1,3-dione (6.8)[33]

A mixture of **6.6** (8.0 g, 25.8 mmol), acetamidine hydrochloride (9.8 g, 103.2 mmol) and K_2CO_3 (14.3 g, 103.2 mmol) in 50 mL dimethylformamide was stirred for 48 h at 75 °C. The solvent was evaporated and the residue taken up in DCM and extracted with saturated $NaHCO_3$. Evaporation of the solvent and flash chromatography (DCM/MeOH 100/0 – 90/10 v/v) yielded a yellow oil (1.0 g, 15 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.93 (m, 2H, Phthal-CH_2-C***H***$_2$), 1.98 (s, 3H, C***H***$_3$), 2.56 (t, 2H, ^3J = 7.2 Hz, Phthal-$(CH_2)_2$-C***H***$_2$), 3.70 (t, 2H, ^3J = 6.9 Hz, Phthal-C***H***$_2$), 6.66 (s, 1H, Im-***H***-5), 7.75 – 7.99 (m, 4H, Phthal-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.21 (+, ***C***H_3), 25.11 (-, ***C***H_2), 29.02 (-, ***C***H_2), 38.49 (-, ***C***H_2), 116.98 (+, Im-***C***-5), 124.05 (+, Phthal-***C***-4,7), 133.39 (C_{quat}, Phthal-***C***-3a,7a), 135.33 (+, Phthal-***C***-5,6), 136.75 (C_{quat}, Im-***C***-4), 145.26 (C_{quat}, Im-***C***-2), 169.87 (C_{quat}, 2 Phthal-***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 270 (100) $[M + H]^+$. $C_{15}H_{15}N_3O_2$ (269.30).

3-(2-Methyl-1*H*-imidazol-4-yl)propan-1-amine (6.10)[34]

A solution of **6.8** (0.5 g, 1.9 mmol) and hydrazine monohydrate (0.54 mL, 11.1 mmol, 5 eq) in EtOH (20 mL) was refluxed for 2 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo*, and flash chromatography (DCM/7 M NH_3 in MeOH 90/10 – 70/30 v/v) yielded a yellow oil (0.21 g, 79 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.75 (m, 2H, C***H***$_2$), 2.29 (s, 3H, C***H***$_3$), 2.54 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$), 2.64 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$), 6.59 (s, 1H, Im-***H***-5). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.54 (+, ***C***H_3), 25.02 (-, ***C***H_2), 33.43 (-, ***C***H_2), 41.99 (-, ***C***H_2-NH_2), 117.19 (+, Im-***C***-5), 137.43 (C_{quat}, Im-***C***-4), 145.20 (C_{quat}, Im-***C***-2). HRMS (EI-MS) calcd. for $C_7H_{13}N_3$ $[M^{+\bullet}]$ 139.1109; found 139.1113. IR (cm^{-1}) = 3200, 2931, 2872 (C-H), 1645, 1570, 1485, 1433, 1323, 1014. Anal. ($C_7H_{13}N_3 \cdot 0.5\ H_2O \cdot 0.4\ CH_3OH$) C, H, N. $C_7H_{13}N_3$ (139.20).

6.4.1.4 Preparation of 4-(5-methyl-1H-imidazol-4-yl)butan-1-amine 6.16

7-Bromoheptan-2-one (6.12)[11]

A mixture of 1-methylcyclohexanol (**6.11**) (10 g, 88.0 mmol) and K_2CO_3 (72.97 g, 540 mmol) in 300 mL $CHCl_3$ was cooled to 0 °C. Under stirring bromine (22.5 mL, 440 mmol) was added and the mixture was stirred at 0 °C for 5 h. The suspension was washed with 300 mL $Na_2SO_{3(aq)}$ and water, dried over $MgSO_4$ and evaporated to yield a yellow oil (16.97 g, 99 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (m, 2H, C***H***$_2$), 1.57 (m, 2H, C***H***$_2$), 1.84 (m, 2H, C***H***$_2$),

2.11 (s, 3H, C***H***$_3$), 2.42 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$-Br), 3.38 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$-CO). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 22.80 (-, ***C***H$_2$), 27.64 (-, ***C***H$_2$), 29.93 (+, ***C***H$_3$), 32.51 (-, ***C***H$_2$), 33.58 (-, ***C***H$_2$), 43.38 (-, ***C***H$_2$), 208.67 (C$_{quat}$, ***C***=O). EI-MS (70 eV) *m/z* (%): 194 (10) [$M^{+\bullet}$], 113 (30) [M – Br]$^{+\bullet}$, 58 (90) $[CH_3COCH_3]^+$, 43 (100) $[CH_3CO]^+$. $C_7H_{13}BrO$ (193.08).

2-(6-Oxoheptyl)isoindoline-1,3-dione (6.13)[35]

A mixture of **6.12** (16.5 g, 85.8 mmol), phthalimide (12.6 g, 85.5 mmol), K_2CO_3 (17.7 g, 128.2 mmol) and KI (0.1 g, 0.6 mmol, catalytic amounts) in 100 mL dimethylformamide was stirred for 12 h at 100 °C. The cooled mixture was poured in 500 mL of ice-cold water and extracted with $CHCl_3$. The combined organic layers were washed with saturated $NaHCO_3$, 2 % HCl and brine and dried over $MgSO_4$. Evaporation yielded a yellowish oil that crystallized from EtOH/H_2O as white solid (14.7 g, 66 %); mp 71 °C (ref.[35]: 71.5 – 74 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.32 (m, 2H, C***H***$_2$), 1.64 (m, 4H, 2 C***H***$_2$), 2.11 (s, 3H, C***H***$_3$), 2.41 (t, 2H, ^3J = 7.4 Hz, C***H***$_2$-$COCH_3$), 3.66 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$-Phthal), 7.69 (m, 2H, Phthal-***H***), 7.82 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 23.23 (-, ***C***H$_2$), 26.36 (-, ***C***H$_2$), 28.40 (-, ***C***H$_2$), 29.91 (+, ***C***H$_3$), 37.78 (-, ***C***H$_2$), 43.46 (-, ***C***H$_2$), 123.19 (+, Phthal-***C***-4,7), 132.11 (C$_{quat}$, Phthal-***C***-3a,7a), 133.91 (+, Phthal-***C***-5,6), 168.46 (C$_{quat}$, 2 Phthal-***C***=O), 208.88 (C$_{quat}$, ***C***=O). EI-MS (70 eV) *m/z* (%): 259 (10) [$M^{+\bullet}$], 202 (30) [M – CH_2COCH_3]$^{+\bullet}$, 160 (100) [M – PhthalCH_2]$^{+\bullet}$. $C_{15}H_{17}NO_3$ (259.30).

2-(5-Bromo-6-oxoheptyl)isoindoline-1,3-dione (6.14)

6.13 (14.0 g, 54.0 mmol) was dissolved in 150 mL glacial acetic acid. Bromine (2.7 g, 52.0 mmol) was added at a temperature of about 10 °C, the mixture was allowed to warm to room temperature and stirred for 1.5 h. Subsequently the solution was poured into 1 L of ice-cold water and was extracted with $CHCl_3$. The organic layer was evaporated and the residue was crystallized from 100 mL MeOH. The precipitated product was filtered, washed with MeOH (10 mL) and dried *in vacuo* to yield a white solid (11.3 g, 62 %); mp 82 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.51 (m, 2H, C***H***$_2$), 1.71 (m, 2H, C***H***$_2$), 2.00 (m, 2H, C***H***$_2$), 2.34 (s, 2H, C***H***$_3$), 3.68 (t, 2H, ^3J = 7.1 Hz, Phthal-C***H***$_2$), 4.21 (t, 1H, ^3J = 6.9 Hz, C***H***-Br), 7.70 (m, 2H, Phthal-***H***), 7.82 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.55 (-, ***C***H$_2$), 26.36 (+, ***C***H$_3$), 27.92 (-, ***C***H$_2$), 32.79 (-, ***C***H$_2$), 37.47 (-, ***C***H$_2$), 53.86 (+, ***C***H-Br), 123.25 (+, Phthal-***C***-4,7), 132.05 (C$_{quat}$, Phthal-***C***-3a,7a), 133.99 (+, Phthal-***C***-5,6), 168.39 (C$_{quat}$, 2 Phthal-

C=O), 201.81 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 355, 357 (50) $[M + NH_4]^+$, 277 (100) $[M – Br + H + NH_4]^+$. Anal. ($C_{15}H_{16}BrNO_3$) C, H, N. $C_{15}H_{16}BrNO_3$ (338.20).

2-[4-(5-Methyl-1*H*-imidazol-4-yl)butyl]isoindoline-1,3-dione (6.15)

6.14 (1.0 g, 3.0 mmol) was dissolved in formamide (30 mL) and the mixture was stirred for 12 h at 170 °C. The mixture was taken up in 100 mL saturated $NaHCO_3$ and extracted with 3x 80 mL $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (DCM/MeOH 95/5 v/v) yielded a yellow solid (0.58 g, 68 %); mp 115 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.62 (m, 4H, Phthal-CH_2-C***H***$_2$-C***H***$_2$), 2.13 (s, 3H, C***H***$_3$), 2.55 (t, 2H, ^3J = 6.9 Hz, Phthal-$(CH_2)_3$-C***H***$_2$), 3.66 (t, 2H, ^3J = 6.7 Hz, Phthal-C***H***$_2$), 7.44 (s, 1H, Im-***H***-2), 7.80 – 8.04 (m, 4H, Phthal-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 10.42 (+, ***C***H_3), 25.53 (-, ***C***H_2), 28.09 (-, ***C***H_2), 28.96 (-, ***C***H_2), 38.57 (-, ***C***H_2), 124.10 (+, Phthal-***C***-4,7), 127.32 (C_{quat}, Im-***C***), 131.71 (C_{quat}, Im-***C***), 133.40 (C_{quat}, Phthal-***C***-3a,7a), 133.94 (+, Im-***C***-2), 135.37 (+, Phthal-***C***-5,6), 169.90 (C_{quat}, 2 Phthal-***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 284 (100) $[M + H]^+$. $C_{16}H_{17}N_3O_2$ (283.33).

4-(5-Methyl-1*H*-imidazol-4-yl)butan-1-amine (6.16)[36]

A solution of **6.15** (2.5 g, 8.8 mmol) and hydrazine monohydrate (2.14 mL, 44.1 mmol, 5 eq) in EtOH (50 mL) was refluxed for 2 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and flash chromatography (Superflash SF15-30g, RP C18 pre-packed column, H_2O/MeCN 100/0 – 80/20 v/v) yielded a yellow oil (1.1 g, 82 %); mp (hydrochloride) 184 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.71 (m, 4H, C***H***$_2$), 2.29 (s, 3H, C***H***$_3$), 2.72 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$), 2.97 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$), 8.53 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 9.16 (+, ***C***H_3), 24.07 (-, ***C***H_2), 27.12 (-, ***C***H_2), 27.86 (-, ***C***H_2), 40.45 (-, ***C***H_2-NH_2), 126.63 (C_{quat}, Im-***C***), 130.08 (C_{quat}, Im-***C***-4), 133.19 (+, Im-***C***-2). HRMS (EI-MS) calcd. for $C_8H_{15}N_3$ $[M^{+\bullet}]$ 153.1266; found 153.1265. IR (cm^{-1}) = 2983, 2741, 2660, 2536, 1486 (C-H), 1631, 1607, 1521, 1386. Anal. ($C_8H_{15}N_3 \cdot 0.6\ N_2H_4 \cdot 3\ HCl$) C, H, N. $C_8H_{15}N_3$ (153.22).

6.4.1.5 Preparation of 4-(1H-1,2,3-triazol-5-yl)butan-1-amine 6.20

2-(5-Hexynyl)isoindoline-1,3-dione (6.18)[37]

To a stirred solution of 5-hexyn-1-ol **6.17** (3.4 mL, 30.6 mmol) in THF_{abs} (100 mL), phthalimide (6.8 g, 45.9 mmol) and triphenylphosphine (12.0 g, 45.9 mmol) was added at 0 °C. Under ice cooling DIAD (13.0 mL, 65.6 mmol) in 50 mL THF_{abs} was added dropwise over a period of 1.5 h. The mixture was allowed to warm to ambient temperature, stirred overnight and concentrated *in vacuo*. Flash chromatography (PE/EtOAc 80/20 – 60/40 v/v) yielded a white solid that was recrystallized in PE (5.35 g, 77 %); mp 68 °C (ref.[38]: 74 – 75 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.56 (m, 2H, C***H***$_2$), 1.78 (m, 2H, C***H***$_2$), 1.93 (t, 1H, ^3J = 2.6 Hz, C***H***), 2.23 (m, 2H, C***H***$_2$CCH), 3.70 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-Phthal), 7.70 (m, 2H, Phthal-***H***), 7.82 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 17.99 (-, ***C***H_2CCH), 25.63 (-, ***C***H_2), 27.64 (-, ***C***H_2), 37.42 (-, ***C***H_2-Phthal), 68.80 (+, ***C***H), 83.77 (C_{quat}, ***C***CH), 123.21 (+, Phthal-***C***-4,7), 132.09 (C_{quat}, Phthal-***C***-3a,7a), 133.93 (+, Phthal-***C***-5,6), 168.42 (C_{quat}, 2 ***C***=O). EI-MS (70 eV) *m/z* (%): 227 (15) [$M^{+\bullet}$], 160 (100) [M – C_5H_7]$^{+\bullet}$. Anal. ($C_{14}H_{13}NO_2$) C, H, N. $C_{14}H_{13}NO_2$ (227.26).

2-[4-(1*H*-1,2,3-Triazol-5-yl)butyl]isoindoline-1,3-dione (6.19)

To a solution of **6.18** (1.5 g, 6.6 mmol) in DMF/MeOH (10 mL, 9/1 v/v), azidotrimethylsilane (1.73 mL, 13.2 mmol) and CuI (0.63 g, 0.33 mmol) was added.[16] The mixture was stirred under microwave irradiation at 100 °C for 12 h and then cooled to room temperature. The solvents were evaporated, and the residue was taken up in 50 mL EtOAc. The solution was filtered through a short pad of silicagel and washed with 3 x 30 mL water. Flash chromatography (DCM/MeOH 95/5 v/v) yielded a yellow solid (1.5 g, 84 %); mp 113 – 114 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.73 (m, 4H, 2 C***H***$_2$), 2.79 (m, 2H, C***H***$_2$-Triazol), 3.71 (t, 2H, ^3J = 6.4 Hz, C***H***$_2$-Phthal), 7.51 (s, 1H, Triazol-***H***), 7.70 (m, 2H, Phthal-***H***), 7.80 (m, 2H, Phthal-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.38 (-, ***C***H_2), 26.44 (-, ***C***H_2), 28.04 (-, ***C***H_2), 37.52 (-, ***C***H_2-Phthal), 123.26 (+, Phthal-***C***-4,7), 132.04 (C_{quat}, Phthal-***C***-3a,7a), 133.99 (+, Phthal-***C***-5,6), 134.29 (+, Triazol-***C***), 141.76 (C_{quat}, Triazol-***C***), 168.55 (C_{quat}, 2 ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 271 (90) [M + H]$^+$, 312 (100) [M + H + MeCN]$^+$. $C_{14}H_{14}N_4O_2$ (270.29).

4-(1*H*-1,2,3-Triazol-5-yl)butan-1-amine (6.20)

A solution of **6.19** (4.0 g, 14.8 mmol) and hydrazine monohydrate (3.6 mL, 75 mmol, 5 eq) in EtOH (50 mL) was refluxed for 1.5 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and the residue taken up in 30 mL water and lyophilized to yield a white solid (2.0 g, 97 %); mp 119 – 120 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.58 (m, 2H, C***H***$_2$), 1.72 (m, 2H, C***H***$_2$), 2.74 (m, 4H, 2 C***H***$_2$), 7.52 (s, 1H, Triazol-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 25.13 (-, ***C***H$_2$), 27.54 (-, ***C***H$_2$), 30.25 (-, ***C***H$_2$), 41.18 (-, ***C***H$_2$-NH$_2$), 129.14 (+, Triazol-***C***), 145.47 (C$_{quat}$, Triazol-***C***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 141 (100) $[M + H]^+$, 182 (25) $[M + H + MeCN]^+$. HRMS (EI-MS) calcd. for $C_6H_{12}N_4$ $[M^{+\bullet}]$ 140.1062; found 140.1058. IR (cm^{-1}) = 2558, 2255 (C-H), 1536, 1150, 1086, 992, 849. Anal. ($C_6H_{12}N_4$) C, H, N. $C_6H_{12}N_4$ (140.19).

6.4.1.6 Preparation of 5-(4-aminobutyl)-1H-1,2,4-triazol-3-amine 6.26

5-(*tert*-Butoxycarbonylamino)pentanoic acid (6.22)[39]

To a solution of 5-aminopentanoic acid **6.21** (5.4 g, 46.2 mmol, 1 eq) in 120 mL dioxane/water 2:1 (v/v) was added 50 mL 1 M aqueous NaOH. After cooling to 0 °C Boc_2O (11.1 g, 50.8 mmol, 1.1 eq) was added and the mixture was stirred at room temperature overnight. After removing the solvent *in vacuo* the residue was taken up in EtOAc, washed with citric acid 5 % and brine and dried over $MgSO_4$. Evaporation of the solvent yielded a white solid that was used without further purification. (9.1 g, 90 %); mp 39 °C (ref.[39]: 44.5 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44 (s, 9H, Boc), 1.55 (m, 2H, C***H***$_2$), 1.66 (m, 2H, C***H***$_2$), 2.37 (t, 2H, ^{3}J = 7.4 Hz, C***H***$_2$-COOH), 3.12 (m, 2H, C***H***$_2$-NHBoc). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 218 (30) $[M + H]^+$, 235 (40) $[M + NH_4]^+$, 259 (15) $[M + H + MeCN]^+$, 435 (100) $[2(M + H)]^+$. $C_{10}H_{19}NO_4$ (217.26).

***S*-Methylisothiuronium iodide (6.23)[40]**

Thiourea (10 g, 131 mmol) and methyl iodide (18.6 g, 131 mmol) in MeOH (100 mL) were refluxed for 1 h. After evaporation, the crude product was taken up in diethyl ether, sucked off and washed twice with diethyl ether to yield a white solid (27.96 g, 98 %); mp 108 °C (ref.[41]: 113.5 – 115 °C). The crude product was used in the next step without further purification. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.57 (s, 3H, C***H***$_3$), 8.88 (brs, 4H, N***H***$_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 91 (100) $[M]^+$. $C_2H_7IN_2S$ (218.06)

tert-Butyl 5-[Amino(methylthio)methyleneamino]-5-oxopentylcarbamate (6.24)

TBTU (8.8 g, 28 mmol, 1.2 eq) was added to a solution of **6.22** (5.0 g, 23 mmol, 1 eq) and DIPEA (8.1 mL, 46 mmol, 2 eq) in DCM (50 mL) and the mixture was stirred for 30 min. Subsequently, a solution of **6.23** (5.0 g, 23 mmol, 1 eq) and DIPEA (12.2 mL, 69 mmol, 3 eq) in DCM (20 mL) was slowly added and the mixture was stirred overnight. After adding 50 mL ice-cold water the mixture was extracted with 3 x 30 mL DCM. The combined organic layers were dried over $MgSO_4$ and the solvents removed *in vacuo*. Flash chromatography (PE/EtOAc 60/40 v/v) yielded a yellow oil (3.0 g, 45 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44 (s, 9H, Boc), 1.52 (m, 2H, C***H***$_2$), 1.68 (m, 2H, C***H***$_2$), 2.45 (m, 2H, C***H***$_2$), 2.48 (s, 3H, S-C***H***$_3$), 3.12 (m, 2H, C***H***$_2$-NHBoc). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 13.26 (+, ***C***H$_3$), 22.45 (-, ***C***H$_2$), 28.39 (+, C(***C***H$_3$)$_3$), 29.50 (-, ***C***H$_2$), 39.23 (-, ***C***H$_2$), 40.12 (-, ***C***H$_2$), 78.37 (C_{quat}, ***C***(CH$_3$)$_3$), 156.08 (C_{quat}, ***C***=O), 165.72 (C_{quat}, C-S), 182.49 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 290 (100) $[M + H]^+$. $C_{12}H_{23}N_3O_3S$ (289.39).

tert-Butyl 4-(3-amino-1*H*-1,2,4-triazol-5-yl)butylcarbamate (6.25)

A solution of **6.24** (3.6 g, 12.4 mmol), hydrazine monohydrate (18 mL, 374 mmol) and NEt_3 (56 mL, 404 mmol) in ethanol (150 mL) was heated at reflux overnight. The solvent was evaporated and flash chromatography (DCM/MeOH 95/5 v/v) yielded a white semisolid (0.7 g, 22 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44 (s, 9H, Boc), 1.54 (m, 2H, C***H***$_2$), 1.72 (m, 2H, C***H***$_2$), 2.69 (m, 2H, C***H***$_2$), 3.14 (m, 2H, C***H***$_2$-NHBoc). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.71 (-, ***C***H$_2$), 30.66 (-, ***C***H$_2$), 32.12 (+, C(***C***H$_3$)$_3$), 32.98 (-, ***C***H$_2$), 43.74 (-, ***C***H$_2$), 83.10 (C_{quat}, ***C***(CH$_3$)$_3$), 160.70 (C_{quat}, ***C***=O), 162.91 (C_{quat}, Triazol-***C***), 171.27 (C_{quat}, Triazol-***C***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 256 (100) $[M + H]^+$. $C_{11}H_{21}N_5O_2$ (255.32).

5-(4-Aminobutyl)-1*H*-1,2,4-triazol-3-amine (6.26)[42]

6.25 (0.7 g, 2.9 mmol) was dissolved in 5 mL MeOH and 5 mL TFA were added. The mixture was stirred overnight and the solvents removed *in vacuo*. The crude product was purified by preparative HPLC to yield a white solid (0.59 g, 53 %, trifluoroacetate); mp 135 – 137 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.77 (m, 2H, 2 C***H***$_2$), 2.73 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$), 2.96 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 24.40 (-, ***C***H$_2$), 25.81 (-, ***C***H$_2$), 27.74 (-, ***C***H$_2$), 40.21 (-, ***C***H$_2$-NH$_2$), 152.25 (C_{quat}, Triazol-***C***), 153.33 (C_{quat}, Triazol-***C***), 163.32 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 156 (45)

$[M + H]^+$, 161 (100) $[M + 2\ H + 4\ MeCN]^{2+}$. HRMS (EI-MS) calcd. for $C_6H_{13}N_5$ $[M^{+\bullet}]$ 155.1171; found 155.1172. IR (cm^{-1}) = 3074 (N-H), 2880, 2805 (C-H), 1676 (C=O), 1633, 1434, 1180, 1127. Anal. ($C_6H_{13}N_5 \cdot 1.9\ TFA \cdot 0.4\ H_2O$) C, H, N. $C_6H_{13}N_5 \cdot 2\ TFA$ (383.25).

6.4.1.7 Preparation of the N^3-aminoalkyl-1H-1,2,4-triazole-3,5-diamines 6.33 and 6.34

General procedure for the protection of diaminoalkanes[43]

A solution of Boc_2O (1 eq) in 50 mL dioxane was added over a period of 2.5 h to a solution of the pertinent diamine (7.5 eq) in 50 mL dioxane and the mixture was stirred at room temperature overnight. After evaporation of the solvent water was added and the insoluble bis-protected side product removed by filtration. The filtrate was extracted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*.

tert-Butyl 3-aminopropylcarbamate (6.27)[43]

The title compound was prepared from 1,3-diaminopropane (25 mL, 300 mmol) and Boc_2O (8.7 g, 40 mmol) according to the general procedure yielding a yellow oil (5.4 g, 77 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (s, 9H, Boc), 1.57 (m, 2H, C***H***$_2$), 2.72 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$-NH_2), 3.17 (m, 2H, C***H***$_2$-NHBoc). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.40 (+, C(***C***$H_3)_3$), 33.43 (-, ***C***H_2), 38.21 (-, ***C***H_2-N), 39.70 (-, ***C***H_2-N), 78.99 (C_{quat}, ***C***$(CH_3)_3$), 156.14 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 175 (100) $[M + H]^+$, 216 (40) $[M + H + MeCN]^+$, 349 (30) $[2\ M + H]^+$. $C_8H_{18}N_2O_2$ (174.24).

tert-Butyl 4-aminobutylcarbamate (6.28)[43]

The title compound was prepared from 1,4-diaminobutane (30 mL, 300 mmol) and Boc_2O (8.7 g, 40 mmol) according to the general procedure yielding a yellow oil (5.4 g, 72 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (s, 9H, Boc), 1.41 (m, 4H, C***H***$_2$), 2.65 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$-NH_2), 3.06 (m, 2H, C***H***$_2$-NHBoc). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.46 (-, ***C***H_2), 28.39 (+, C(***C***$H_3)_3$), 30.92 (-, ***C***H_2), 40.39 (-, ***C***H_2-N), 41.82 (-, ***C***H_2-N), 78.90 (C_{quat}, ***C***$(CH_3)_3$), 156.01 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 189 (100) $[M + H]^+$, 230 (30) $[M + H + MeCN]^+$, 377 (30) $[2\ M + H]^+$. $C_9H_{20}N_2O_2$ (188.23).

General procedure for the preparation of the isoureas 6.29 and 6.30[18, 22]

A solution of the pertinent amine (1 eq) and diphenyl cyanocarbonimidate (**3.21**, 1 eq) in 2-propanol was stirred for 2 h. After evaporation of the solvent, the product was crystallized from Et_2O.

***tert*-Butyl 3-[cyanoimino(phenoxy)methyl]aminopropylcarbamate (6.29)**

The title compound was prepared from **6.27** (1.5 g, 8.4 mmol) and **3.21** (2.0 g, 8.4 mmol) in 2-propanol (50 mL) according to the general procedure. Flash chromatography (PE/EtOAc 60/40 – 40/60 v/v) yielded a white solid (2.26 g, 85 %); mp 113 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 9H, Boc), 1.80 (m, 2H, C***H***$_2$), 3.24 (m, 2H, C***H***$_2$), 3.46 (m, 2H, C***H***$_2$), 7.08 (m, 2H, Ph-***H***), 7.26 (m, 1H, Ph-***H***-4), 7.40 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.39 (+, C(***C***H$_3$)$_3$), 29.87 (-, ***C***H$_2$), 37.33 (-, ***C***H$_2$-N), 39.65 (-, ***C***H$_2$-N), 79.48 (C$_{quat}$, ***C***(CH$_3$)$_3$), 115.88 (C$_{quat}$, ***C***≡N), 121.49 (+, 2 Ph-***C***), 126.62 (+, Ph-***C***-4), 129.52 (+, 2 Ph-***C***), 151.07 (C$_{quat}$, Ph-***C***-1), 156.33 (C$_{quat}$, ***C***=O), 163.90 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 319 (65) [M + H]$^+$, 637 (100) [2 M +]$^+$, 654 (90) [2 M + NH_4]$^+$. Anal. ($C_{16}H_{22}N_4O_3$) C, H, N. $C_{16}H_{22}N_4O_3$ (318.37).

***tert*-Butyl 4-[cyanoimino(phenoxy)methyl]aminobutylcarbamate (6.30)**

The title compound was prepared from **6.28** (1.58 g, 8.4 mmol) and **3.21** (2.0 g, 8.4 mmol) in 2-propanol (50 mL) according to the general procedure. Flash chromatography (PE/EtOAc 60/40 – 40/60 v/v) yielded a white solid (2.24 g, 80 %); mp 99 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9H, Boc), 1.56 (m, 2H, C***H***$_2$), 1.65 (m, 2H, C***H***$_2$), 3.14 (m, 2H, C***H***$_2$), 3.41 (m, 2H, C***H***$_2$), 7.07 (m, 2H, Ph-***H***), 7.26 (m, 1H, Ph-***H***-4), 7.40 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.71 (-, ***C***H$_2$), 27.21 (-, ***C***H$_2$), 28.41 (+, C(***C***H$_3$)$_3$), 39.98 (-, ***C***H$_2$-N), 42.22 (-, ***C***H$_2$-N), 79.26 (C$_{quat}$, ***C***(CH$_3$)$_3$), 115.87 (C$_{quat}$, ***C***≡N), 121.50 (+, 2 Ph-***C***), 126.57 (+, Ph-***C***-4), 129.55 (+, 2 Ph-***C***), 151.09 (C$_{quat}$, Ph-***C***-1), 156.07 (C$_{quat}$, ***C***=O), 163.94 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 333 (70) [M + H]$^+$, 665 (80) [2 M +]$^+$, 682 (100) [2 M + NH_4]$^+$. Anal. ($C_{17}H_{24}N_4O_3$) C, H, N. $C_{17}H_{24}N_4O_3$ (332.40).

General procedure for the preparation of the 3,5-diaminotriazoles 6.31 and 6.32[18]

Hydrazine monohydrate was added to a solution of the isourea in 40 mL MeOH and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, the residue

taken up in 30 mL water and 50 mL DCM were added. The precipitated product was filtered off and washed with water and DCM.

tert-Butyl 3-(5-amino-1*H*-1,2,4-triazol-3-ylamino)propylcarbamate (6.31)

The title compound was prepared from **6.29** (2.2 g, 6.9 mmol) and hydrazine monohydrate (0.5 mL, 10 mmol) in MeOH (40 mL) according to the general procedure yielding a white solid (1.25 g, 71 %); mp 84 – 85 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.43 (s, 9H, Boc), 1.70 (m, 2H, C***H***$_2$), 3.11 (t, 2H, ^{3}J = 6.7 Hz, C***H***$_2$-NHBoc), 3.16 (t, 2H, ^{3}J = 6.8 Hz, C***H***$_2$-NH). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.80 (+, C(***C***H$_3$)$_3$), 30.97 (-, ***C***H$_2$), 38.77 (-, ***C***H$_2$-N), 41.39 (-, ***C***H$_2$-N), 79.97 (C_{quat}, ***C***(CH$_3$)$_3$), 158.68 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 257 (100) $[M + H]^+$. Anal. ($C_{10}H_{20}N_6O_2$) C, H, N. $C_{10}H_{20}N_6O_2$ (256.30).

tert-Butyl 4-(5-amino-1*H*-1,2,4-triazol-3-ylamino)butylcarbamate (6.32)

The title compound was prepared from **6.30** (2.3 g, 6.9 mmol) and hydrazine monohydrate (0.5 mL, 10 mmol) in MeOH (40 mL) according to the general procedure yielding a white solid (1.6 g, 86 %); mp 138 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.42 (s, 9H, Boc), 1.53 (m, 4H, C***H***$_2$), 3.05 (t, 2H, ^{3}J = 6.6 Hz, C***H***$_2$-NHBoc), 3.13 (t, 2H, ^{3}J = 6.4 Hz, C***H***$_2$-NH). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.08 (-, ***C***H$_2$), 28.40 (-, ***C***H$_2$), 28.83 (+, C(***C***H$_3$)$_3$), 41.09 (-, ***C***H$_2$-N), 43.80 (-, ***C***H$_2$-N), 79.88 (C_{quat}, ***C***(CH$_3$)$_3$), 158.59 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 271 (100) $[M + H]^+$. Anal. ($C_{11}H_{22}N_6O_2$) C, H, N. $C_{11}H_{22}N_6O_2$ (270.33).

General procedure for the preparation of N^3-aminoalkyl-1*H*-1,2,4-triazole-3,5-diamines 6.33 and 6.34

30 mL 6 M HCl in 2-propanol were added to a solution of the Boc-protected precursor in 30 mL EtOAc and 5 mL MeOH. The mixture was stirred overnight and the precipitate was filtered off, washed and dried *in vacuo*.

N^3-(3-Aminopropyl)-1*H*-1,2,4-triazole-3,5-diamine hydrochloride (6.33)

The title compound was prepared from **6.31** (1.2 g, 4.7 mmol) according to the general procedure yielding a white solid (0.95 g, 72 %); mp 117 – 118 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.83 (m, 2H, C***H***$_2$), 2.81 (m, 2H, C***H***$_2$), 3.23 (t, 2H, ^{3}J = 6.6 Hz, C***H***$_2$), 7.68 (brs, 1H, N***H***), 8.22 (brs, 3H, N***H***), 10.58 (brs, 4H, N***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] =

26.17 (-, ***C***H_2), 36.31 (-, ***C***H_2-N), 39.15 (-, ***C***H_2-N), 151.55 (C_{quat}, 2 Triazol-***C***). HRMS (EI-MS) calcd. for $C_5H_{12}N_6$ [$M^{+\bullet}$] 156.1123; found 156.1126. IR (cm^{-1}) = 3398, 3281, 3023, 2984, 2906 (C-H), 1684, 1646, 1487, 1160. Anal. ($C_5H_{12}N_6 \cdot 2.5\ HCl \cdot 0.3\ H_2O$) C, H, N. $C_5H_{12}N_6 \cdot 3\ HCl$ (265.57).

N^3-(4-Aminobutyl)-1*H*-1,2,4-triazole-3,5-diamine hydrochloride (6.34)

The title compound was prepared from **6.32** (1.6 g, 5.9 mmol) according to the general procedure yielding a white solid (1.6 g, 96 %); mp 120 – 121 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.57 (m, 4H, 2 C***H***$_2$), 2.75 (m, 2H, C***H***$_2$), 3.14 (t, 2H, ^3J = 6.4 Hz, C***H***$_2$), 7.72 (brs, 1H, N***H***), 8.19 (brs, 3H, N***H***), 11.31 (brs, 4H, N***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 24.09 (-, ***C***H_2), 25.52 (-, ***C***H_2), 38.14 (-, ***C***H_2-N), 41.50 (-, ***C***H_2-N), 151.70 (C_{quat}, 2 Triazol-***C***). HRMS (EI-MS) calcd. for $C_6H_{14}N_6$ [$M^{+\bullet}$] 170.1280; found 170.1279. IR (cm^{-1}) = 3219, 3004, 2870, 2690 (C-H), 1713, 1652, 1589, 1473, 1025. Anal. ($C_6H_{14}N_6 \cdot 2.5\ HCl \cdot 0.5\ H_2O$) C, H, N. $C_6H_{14}N_6 \cdot 3\ HCl$ (279.60).

6.4.1.8 Preparation of 3-(3-aminopropylthio)-1H-1,2,4-triazol-5-amine 6.38

***tert*-Butyl 3-bromopropylcarbamate (6.35)[44]**

A solution of Boc_2O (6.55 g, 30 mmol) in 20 mL DCM was slowly added to a solution of 3-bromopropan-1-amine hydrobromide (5.47 g, 25 mmol) and DIPEA (10.2 mL, 60 mmol) in 40 mL DCM and the mixture was stirred at room temperature overnight. The mixture was washed with 0.1 M HCl, saturated $NaCl_{(aq.)}$ and water. The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. Flash chromatography (DCM/MeOH 95/5 v/v) yielded a colorless oil (5.2 g, 87 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 9H, Boc), 2.03 (m, 2H, C***H***$_2$), 3.26 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$), 3.43 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.39 (+, C(***C***$H_3)_3$), 30.82 (-, ***C***H_2), 32.71 (-, ***C***H_2), 39.05 (-, ***C***H_2-N), 79.49 (C_{quat}, ***C***$(CH_3)_3$), 155.97 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 238 (20) $[M + H]^+$, 255 (70) $[M + NH_4]^+$, 199 (100) $[M + NH_4 – C_4H_8]^+$. $C_8H_{16}BrNO_2$ (238.12).

***tert*-Butyl 3-(5-amino-1*H*-1,2,4-triazol-3-ylthio)propylcarbamate (6.37)**

A solution of **6.35** (3.0 g, 12.6 mmol) in 50 mL EtOH was added to a mixture of 12.6 mL 1 M NaOH and 5-amino-4*H*-1,2,4-triazole-3-thiol (**6.36**) (1.22 g, 10.5 mmol). The solution was refluxed for 3 h, concentrated *in vacuo*, diluted with 10 mL water and extracted with

3 x 100 mL DCM. The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. Flash chromatography (DCM/MeOH 95/5 v/v) yielded a colorless foam-like solid (2.25 g, 78 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9H, Boc), 1.84 (m, 2H, C***H***$_2$), 3.04 (t, 2H, ^3J = 6.0 Hz, C***H***$_2$-S), 3.19 (m, 2H, C***H***$_2$-N). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.47 (+, C(***C***H_3)$_3$), 29.55 (-, ***C***H_2), 30.08 (-, ***C***H_2-S), 39.03 (-, ***C***H_2-N), 79.48 (C_{quat}, ***C***$(CH_3)_3$), 155.50 (C_{quat}, Triazol-***C***), 156.46 (C_{quat}, ***C***=O), 158.24 (C_{quat}, Triazol-***C***-3). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 271 (100) $[M + H]^+$. Anal. ($C_{10}H_{19}N_5O_2S$) C, H, N. $C_{10}H_{19}N_5O_2S$ (273.36).

3-(3-Aminopropylthio)-1*H*-1,2,4-triazol-5-amine hydrochloride (6.38)

30 mL 6 M HCl in 2-propanol were added to a solution of **6.37** (2.2 g, 8 mmol) in 30 mL EtOAc and 5 mL MeOH. The mixture was stirred overnight and the precipitate was filtered off, washed and dried *in vacuo*. The crude product was recrystallized in EtOH to yield a white solid (1.67 g, 85 %); mp 137 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.96 (m, 2H, C***H***$_2$), 2.85 (m, 2H, C***H***$_2$), 3.19 (t, 2H, ^3J = 7.0 Hz, S-C***H***$_2$), 8.28 (brs, 5H, N***H***), 13.22 (brs, 2H, N***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 26.60 (-, ***C***H_2), 28.33 (-, ***C***H_2-S), 37.08 (-, ***C***H_2-N), 146.91 (C_{quat}, Triazol-***C***-5), 152.19 (C_{quat}, Triazol-***C***-3). HRMS (EI-MS) calcd. for $C_5H_{11}N_5S$ $[M^{+\bullet}]$ 173.0735; found 173.0734. IR (cm^{-1}) = 3246, 2989, 2932, 2742 (C-H), 1680, 1533, 1479, 1257, 1007. Anal. ($C_5H_{11}N_5S \cdot 1.9$ HCl) C, H, N. $C_5H_{11}N_5S \cdot 2$ HCl (246.16).

6.4.1.9 Preparation of the N^1-(pyridin-2-yl)alkyldiamines 6.40 and 6.41

General procedure[20]

A mixture of the pertinent diamine (6 eq), 2-chloropyridine (**6.39**, 1 eq) and pyridine (1.3 eq) was heated under microwave irradiation at 190 °C for 30 min. After adding 30 mL of water the pH was adjusted to 6 and the solution was extracted with DCM. The aqueous layer was basified and again extracted with DCM. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure.

***N*^{1}-(Pyridin-2-yl)propane-1,3-diamine (6.40)[20]**

The title compound was prepared from 1,3-diaminopropane (7.6 mL, 90 mmol), **6.39** (1.4 mL, 15 mmol) and pyridine (1.6 mL, 20 mmol) according to the general procedure yielding a yellow oil (1.2 g, 52 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.74 (m, 2H, C***H***$_2$), 2.71 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-NH_2), 3.31 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-NH-Py), 6.50 (m, 2H, Py-***H***-3 +

Py-***H***-5), 7.39 (m, 1H, Py-***H***-4), 7.89 (m, 1H, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 33.64 (-, ***C***H_2), 40.01 (-, ***C***H_2-N), 40.17 (-, ***C***H_2-N), 109.83 (+, Py-***C***-3), 113.01 (+, Py-***C***-5), 138.70 (+, Py-***C***-4), 147.94 (+, Py-***C***-6), 160.50 (C_{quat}, Py-***C***-2). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 152 (100) $[M + H]^+$, 138 (90) $[M + 2 H + 3 MeCN]^{2+}$. HRMS (EI-MS) calcd. for $C_8H_{13}N_3$ $[M^{+\bullet}]$ 151.1109; found 151.1113. IR (cm^{-1}) = 3260 (N-H), 2928, 2856 (C-H), 1577, 1507, 1314, 1138. Anal. ($C_8H_{13}N_3 \cdot 0.65\ H_2O$) C, H, N. $C_8H_{13}N_3$ (151.21).

N^1-(Pyridin-2-yl)butane-1,4-diamine (6.41)[20]

The title compound was prepared from 1,4-diaminobutane (9.0 mL, 90 mmol), **6.39**(1.4 mL, 15 mmol) and pyridine (1.6 mL, 20 mmol) according to the general procedure yielding a yellow oil (1.2 g, 47 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.32 (brs, 2H, N***H***$_2$), 1.49 (m, 2H, C***H***$_2$), 1.58 (m, 2H, C***H***$_2$), 2.67 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-NH_2), 3.20 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$-NH-Py), 4.62 (brs, 1H, N***H***), 6.29 (m, 1H, Py-***H***-3), 6.47 (m, 1H, Py-***H***-5), 7.33 (m, 1H, Py-***H***-4), 7.99 (m, 1H, Py-***H***-6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.94 (-, ***C***H_2), 31.14 (-, ***C***H_2), 41.91 (-, ***C***H_2-N), 42.05 (-, ***C***H_2-N), 106.53 (+, Py-***C***-3), 112.61 (+, Py-***C***-5), 137.36 (+, Py-***C***-4), 148.18 (+, Py-***C***-6), 158.86 (C_{quat}, Py-***C***-2). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 166 (70) $[M + H]^+$, 145 (100) $[M + 2 H + 3 MeCN]^{2+}$. HRMS (EI-MS) calcd. for $C_9H_{15}N_3$ $[M^{+\bullet}]$ 165.1266; found 165.1270. IR (cm^{-1}) = 3269 (N-H), 2924, 2856 (C-H), 1599, 1519, 1332. Anal. ($C_9H_{15}N_3 \cdot 0.6\ H_2O$) C, H, N. $C_9H_{15}N_3$ (165.24).

6.4.1.10 Preparation of the N-(ω-aminoalkyl)pyrimidine-2,4-diamines 6.47-6.50

***tert*-Butyl 2-aminoethylcarbamate (6.46)[43]**

A solution of Boc_2O (9.82 g, 45 mmol, 1 eq) in 50 mL dioxane was added over a period of 2.5 h to a solution of 1,2-diaminoethane (30 mL, 450 mmol, 10 eq) in 50 mL dioxane and the mixture was stirred at room temperature overnight. After evaporation of the solvent water was added and the insoluble bis-protected side product removed by filtration. The filtrate was extracted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* yielding a yellow oil (5.0 g, 70 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.45 (s, 9H, Boc), 2.78 (t, 2H, ^3J = 6.2 Hz, C***H***$_2$-NH_2), 3.17 (t, 2H, ^3J = 6.2 Hz, C***H***$_2$-NHBoc). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.77 (+, C(***C***H_3)$_3$), 41.91 (-, ***C***H_2-N), 42.39 (-, ***C***H_2-N), 80.32 (C_{quat}, ***C***$(CH_3)_3$), 158.72 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 161 (100) $[M + H]^+$, 202 (45) $[M + H + MeCN]^+$, 321 (30) $[2 M + H]^+$. $C_7H_{16}N_2O_2$ (160.21).

2-Chloro-*N*-methylpyrimidin-4-amine (6.44)[45]

2,4-Dichloropyrimidine (**6.42**) (1.1 g, 7.5 mmol) was dissolved in 10 mL EtOAc/MeOH 1:1 (v/v). A 40 % solution of methylamine in water (0.7 mL, 7.5 mmol) and DIPEA (2 mL, 11.5 mmol) was added and the mixture was stirred at room temperature for 36 h. The reaction mixture was diluted with EtOAc, washed with water and brine and the organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (DCM/MeOH 100/0 – 99/1 v/v) yielded a white solid (0.85 g, 79 %); mp 123-125 °C (ref.[45]: 128 – 129 °C). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.89 (s, 3H, C***H***$_3$), 6.38 (d, 1H, ^3J = 5.8 Hz, Py-***H***-5), 7.79 (d, 1H, ^3J = 5.2 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.40 (+, ***C***H$_3$), 105.93 (+, Py-***C***-5), 155.26 (+, Py-***C***-6), 161.66 (C_{quat}, Py-***C***), 165.79 (C_{quat}, Py-***C***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 144 (100) $[M + H]^+$, 185 (30) $[M + H + MeCN]^+$. $C_5H_6ClN_3$ (143.57).

2-Chloropyrimidin-4-amine (6.43) and 4-chloropyrimidin-2-amine (6.45)[46]

2,4-Dichloropyrimidine (**6.42**) (2.0 g, 13.4 mmol) was dissolved in 10 mL EtOAc/THF 3:1 (v/v). A 7 M solution of ammonia in methanol (2.9 mL, 20 mmol) and DIPEA (4.7 mL, 26.9 mmol) were added and the mixture was heated under microwave irradiation at 100 °C for 10 min. The reaction mixture was diluted with EtOAc, washed with water and brine and the organic layer was dried over $MgSO_4$ and evaporated. The isomers, formed in a ratio of 2:1 (**6.43** : **6.45**), were separated by flash chromatography (DCM/MeOH 100/0 – 99/1 v/v).

2-Chloropyrimidin-4-amine (**6.43**): white solid (0.98 g, 56 %); mp 178-180 °C (dec.) (ref.[46]: 206 – 208 °C). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 6.42 (d, 1H, ^3J = 6.0 Hz, Py-***H***-5), 7.90 (d, 1H, ^3J = 6.0 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 104.90 (+, Py-***C***-5), 157.09 (+, Py-***C***-6), 161.43 (C_{quat}, Py-***C***), 167.01 (C_{quat}, Py-***C***). CI-MS (NH_3) *m/z* (%): 130 (100) $[M + H]^+$, 147 (30) $[M + NH_4]^+$. $C_4H_4ClN_3$ (129.55).

4-Chloropyrimidin-2-amine (**6.45**): white solid (0.49 g, 28 %); mp 154-56 °C (dec.) (ref.[46]: 155 – 156 °C). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 6.65 (d, 1H, ^3J = 5.3 Hz, Py-***H***-5), 8.12 (d, 1H, ^3J = 5.3 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 110.88 (+, Py-***C***-5), 160.65 (+, Py-***C***-6), 162.79 (C_{quat}, Py-***C***), 163.41 (C_{quat}, Py-***C***). CI-MS (NH_3) *m/z* (%): 130 (100) $[M + H]^+$, 147 (30) $[M + NH_4]^+$. $C_4H_4ClN_3$ (129.55).

General Procedure for the preparation of the *N*-(ω-aminoalkyl)pyrimidine-2,4-diamines

The respective chloropyrimidine (1 eq) was dissolved in 10 mL ethanol. The pertinent diamine (1 eq) and DIPEA (1.5 eq) were added and the mixture was heated under microwave irradiation at 120 °C for 30 min. The reaction mixture was diluted with EtOAc, washed with water and brine and the organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (DCM/MeOH 90/10 v/v) gave the Boc-protected precursors which were dissolved in 10 mL methanol. Acetyl chloride (3 eq) was slowly added at 0 °C and the mixture was stirred at room temperature for 4 h. Evaporation of the solvent and crystallization from MeOH/EtOAc yielded the hydrochlorides as white solids.

N^2-(2-Aminoethyl)pyrimidine-2,4-diamine dihydrochloride (6.47)[47]

The title compound was prepared from **6.43** (0.155 g, 1.2 mmol) and **6.46** (0.192 g, 1.2 mmol) according to the general procedure. Crystallization yielded a white solid (0.13 g, 49 %); mp 228 – 230 °C (dec.) ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.22 (t, 2H, ^3J = 5.7 Hz, C***H***$_2$-N), 3.74 (t, 2H, ^3J = 5.7 Hz, C***H***$_2$-N), 6.17 (d, 1H, ^3J = 7.2 Hz, Py-***H***-5), 7.69 (d, 1H, ^3J = 7.2 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 39.70 (-, ***C***H$_2$-N), 40.48 (-, ***C***H$_2$-N), 98.67 (+, Py-***C***-5), 143.08 (+, Py-***C***-6), 155.81 (C_{quat}, Py-***C***), 166.88 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_6H_{11}N_5$ [$M^{+\bullet}$] 153.1014; found 153.1016. Anal. ($C_6H_{11}N_5 \cdot 2$ HCl) C, H, N. $C_6H_{11}N_5 \cdot 2$ HCl (226.11)

N^2-(3-Aminopropyl)pyrimidine-2,4-diamine (6.48)

The title compound was prepared from **6.43** (0.155 g, 1.2 mmol) and **6.27** (0.209 g, 1.2 mmol) according to the general procedure. Crystallization yielded a white solid (0.13 g, 44 %); mp 193 – 195 °C (dec.) ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.99 (m, 2H, C***H***$_2$), 3.02 (m, 2H, C***H***$_2$-N), 3.53 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$-N), 6.12 (d, 1H, ^3J = 7.2 Hz, Py-***H***-5), 7.64 (d, 1H, ^3J = 7.3 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.36 (-, ***C***H$_2$), 38.35 (-, ***C***H$_2$-N), 38.81 (-, ***C***H$_2$-N), 98.33 (+, Py-***C***-5), 142.89 (+, Py-***C***-6), 155.50 (C_{quat}, Py-***C***), 166.85 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_7H_{13}N_5$ [$M^{+\bullet}$] 167.1171; found 167.1172. Anal. ($C_7H_{13}N_5 \cdot 2.5$ HCl $\cdot$ 0.8 H_2O) C, H, N. $C_7H_{13}N_5 \cdot 2$ HCl (240.13)

N^2-(2-Aminoethyl)-N^4-methylpyrimidine-2,4-diamine (6.49)

The title compound was prepared from **6.44** (0.215 g, 1.5 mmol) and **6.46** (0.24 g, 1.5 mmol) according to the general procedure. Crystallization yielded a pale green solid (0.2 g, 55 %); mp 217 – 219 °C (dec.) ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.03 (s, 3H, C***H***$_3$), 3.25 (t, 2H, ^3J = 6.0 Hz, C***H***$_2$-N), 3.79 (t, 2H, ^3J = 6.0 Hz, C***H***$_2$-N), 6.14 (d, 1H, ^3J = 7.3 Hz, Py-***H***-5), 7.58 (d, 1H, ^3J = 7.3 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.21 (+, ***C***H_3), 39.68 (-, ***C***H_2-N), 40.01 (-, ***C***H_2-N), 99.59 (+, Py-***C***-5), 141.19 (+, Py-***C***-6), 155.73 (C_{quat}, Py-***C***), 164.77 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_7H_{13}N_5$ [$M^{+\bullet}$] 167.1171; found 167.1170. Anal. ($C_7H_{13}N_5 \cdot 2$ HCl) C, H, N. $C_7H_{13}N_5 \cdot 2$ HCl (240.13)

N^4-(2-Aminoethyl)pyrimidine-2,4-diamine (6.50)[48]

The title compound was prepared from **6.45** (0.155 g, 1.2 mmol) and **6.46** (0.192 g, 1.2 mmol) according to the general procedure. Crystallization yielded a white solid (0.16 g, 60 %); mp 239 – 241 °C (dec.) ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.22 (t, 2H, ^3J = 5.9 Hz, C***H***$_2$-N), 3.77 (t, 2H, ^3J = 5.9 Hz, C***H***$_2$-N), 6.20 (d, 1H, ^3J = 7.3 Hz, Py-***H***-5), 7.64 (d, 1H, ^3J = 7.3 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 39.54 (-, ***C***H_2-N), 40.22 (-, ***C***H_2-N), 99.83 (+, Py-***C***-5), 141.85 (+, Py-***C***-6), 157.05 (C_{quat}, Py-***C***), 165.71 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_6H_{11}N_5$ [$M^{+\bullet}$] 153.1014; found 153.1016. Anal. ($C_6H_{11}N_5 \cdot 2$ HCl) C, H, N. $C_6H_{11}N_5 \cdot 2$ HCl (226.11)

6.4.1.11 Preparation of the cyanoguanidines 6.52-6.78

General Procedure[21, 49]

Hydrochlorides of **6.33, 6.34** and **6.38** were converted into the bases by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). The isourea (1 eq) and the pertinent amine (1 eq) in MeCN were heated under microwave irradiation at 150 °C for 15 min. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (DCM/MeOH 98/2 – 80/20 v/v).

2-Cyano-1-methyl-3-[4-(2-methyl-1*H*-imidazol-4-yl)butyl]guanidine (6.52)

The title compound was prepared from **6.9** (0.08 g, 0.52 mmol) and **3.27** (0.092 g, 0.52 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.08 g, 66 %); mp 50 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.59

(m, 4H, 2 C***H***$_2$), 2.31 (s, 3H, C***H***$_3$), 2.54 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-Im), 2.77 (s, 3H, C***H***$_3$-N), 3.20 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-N), 6.62 (s, 1H, Im-***H***-5). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.36 (+, Im-***C***H$_3$), 27.09 (-, ***C***H$_2$), 27.61 (-, ***C***H$_2$), 28.70 (+, ***C***H$_3$), 30.02 (-, ***C***H$_2$), 42.46 (-, ***C***H$_2$-N), 117.08 (+, Im-***C***-5), 120.29 (C_{quat}, ***C***≡N), 137.44 (C_{quat}, Im-***C***-4), 145.14 (C_{quat}, Im-***C***-2), 161.97 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{11}H_{18}N_6$ [$M^{+\bullet}$] 234.1597; found 234.1593. IR (cm^{-1}) = 3289 (N-H), 2938, 2861 (C-H), 2164 (C≡N), 1580 (C=N), 1422, 1369, 1177, 1060. Anal. ($C_{11}H_{18}N_6 \cdot 0.95\ H_2O$) C, H, N. $C_{11}H_{18}N_6$ (234.30).

2-Cyano-1-[4-(2-methyl-1*H*-imidazol-4-yl)butyl]-3-[2-(phenylthio)ethyl]guanidine (6.53)

The title compound was prepared from **6.9** (0.08 g, 0.52 mmol) and **3.31** (0.156 g, 0.52 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.1 g, 54 %); mp 60 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.58 (m, 4H, 2 C***H***$_2$), 2.30 (s, 3H, C***H***$_3$), 2.52 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-Im), 3.11 (m, 4H, 2 C***H***$_2$-N), 3.39 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-S), 6.60 (s, 1H, Im-***H***-5), 7.17 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.38 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.44 (+, Im-***C***H$_3$), 27.19 (-, ***C***H$_2$), 27.62 (-, ***C***H$_2$), 29.80 (-, ***C***H$_2$), 33.56 (-, ***C***H$_2$-S), 42.26 (-, ***C***H$_2$-N), 42.57 (-, ***C***H$_2$-N), 116.98 (+, Im-***C***-5), 119.99 (C_{quat}, ***C***≡N), 127.33 (+, Ph-***C***-4), 130.15 (+, 2 Ph-***C***), 130.45 (+, 2 Ph-***C***), 136.99 (C_{quat}, Ph-***C***-1), 137.62 (C_{quat}, Im-***C***-4), 145.17 (C_{quat}, Im-***C***-2), 161.11 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{18}H_{24}N_6S$ [$M^{+\bullet}$] 356.1783; found 356.1787. IR (cm^{-1}) = 3253 (N-H), 3150, 2928, 2865 (C-H), 2163 (C≡N), 1572 (C=N), 1423, 1351, 1179, 1025. Anal. ($C_{18}H_{24}N_6S \cdot 0.5\ H_2O$) C, H, N. $C_{18}H_{24}N_6S$ (356.49)

2-Cyano-1-methyl-3-[3-(2-methyl-1*H*-imidazol-4-yl)propyl]guanidine (6.54)

The title compound was prepared from **6.10** (0.05 g, 0.36 mmol) and **3.27** (0.063 g, 0.36 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.04 g, 50 %); mp 48 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.84 (m, 2H, C***H***$_2$), 2.34 (s, 3H, C***H***$_3$), 2.56 (t, 2H, ^3J = 7.4 Hz, C***H***$_2$-Im), 2.78 (s, 3H, C***H***$_3$-N), 3.22 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-N), 6.69 (s, 1H, Im-***H***-5). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.25 (+, Im-***C***H$_3$), 24.67 (-, ***C***H$_2$), 28.72 (+, ***C***H$_3$), 30.21 (-, ***C***H$_2$), 42.17 (-, ***C***H$_2$-N), 116.59 (+, Im-***C***-5), 120.26 (C_{quat}, ***C***≡N), 137.17 (C_{quat}, Im-***C***-4), 145.31 (C_{quat}, Im-***C***-2), 162.00 (C_{quat}, ***C***=N). HRMS (ES-MS) calcd. for $C_{10}H_{16}N_6$ [M+H]$^+$ 221.1515; found 221.1509. IR (cm^{-1}) = 3277 (N-H), 2938, 2881 (C-H),

2162 (C≡N), 1572 (C=N), 1421, 1366, 1174, 1051. Anal. ($C_{10}H_{16}N_6 \cdot 0.5\ H_2O \cdot 0.2\ CH_3OH$) C, H, N. $C_{10}H_{16}N_6$ (220.27).

2-Cyano-1-[3-(2-methyl-1*H*-imidazol-4-yl)propyl]-3-[2-(phenylthio)ethyl]guanidine (6.55)

The title compound was prepared from **6.10** (0.05 g, 0.36 mmol) and **3.31** (0.11 g, 0.36 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a yellow solid (0.05 g, 41 %); mp 52 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.81 (m, 2H, C***H***$_2$), 2.30 (s, 3H, C***H***$_3$), 2.53 (t, 2H, ^3J = 7.4 Hz, C***H***$_2$-Im), 3.09 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-N), 3.15 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-N), 3.39 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-S), 6.64 (s, 1H, Im-***H***-5), 7.16 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.27 (m, 2H, Ph-***H***), 7.38 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 13.46 (+, Im-***C***H$_3$), 24.82 (-, ***C***H$_2$), 30.05 (-, ***C***H$_2$), 33.55 (-, ***C***H$_2$-S), 42.28 (-, ***C***H$_2$-N), 42.29 (-, ***C***H$_2$-N), 116.39 (+, Im-***C***-5), 119.99 (C$_{quat}$, ***C***≡N), 127.33 (+, Ph-***C***-4), 130.16 (+, 2 Ph-***C***), 130.44 (+, 2 Ph-***C***), 136.98 (C$_{quat}$, Ph-***C***-1), 137.54 (C$_{quat}$, Im-***C***-4), 145.37 (C$_{quat}$, Im-***C***-2), 161.16 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{17}H_{22}N_6S$ [$M^{+\bullet}$] 342.1627; found 342.1624. IR (cm^{-1}) = 3254 (N-H), 3122, 2927 (C-H), 2156 (C≡N), 1572 (C=N), 1425, 1355, 1183, 1025. Anal. ($C_{17}H_{22}N_6S \cdot 0.4\ CH_3OH$) C, H, N. $C_{17}H_{22}N_6S$ (342.46)

2-Cyano-1-methyl-3-[4-(5-methyl-1*H*-imidazol-4-yl)butyl]guanidine (6.56)[36]

The title compound was prepared from **6.16** (0.08 g, 0.52 mmol) and **3.27** (0.092 g, 0.52 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.07 g, 57 %); mp 48 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.56 (m, 4H, 2 C***H***$_2$), 2.14 (s, 3H, C***H***$_3$), 2.53 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-Im), 2.77 (s, 3H, C***H***$_3$-N), 3.19 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$-N), 7.45 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 10.46 (+, Im-***C***H$_3$), 25.79 (-, ***C***H$_2$), 28.01 (-, ***C***H$_2$), 28.33 (+, ***C***H$_3$), 29.99 (-, ***C***H$_2$), 42.50 (-, ***C***H$_2$-N), 120.36 (C$_{quat}$, ***C***≡N), 127.04 (C$_{quat}$, Im-***C***-5), 132.18 (C$_{quat}$, Im-***C***-4), 133.93 (+, Im-***C***-2), 161.94 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{11}H_{18}N_6$ [$M^{+\bullet}$] 234.1597; found 234.1596. IR (cm^{-1}) = 3233 (N-H), 3149, 2936, 2859 (C-H), 2163 (C≡N), 1586 (C=N), 1452, 1418, 1369, 1175. Anal. ($C_{11}H_{18}N_6 \cdot 0.9\ H_2O$) C, H, N. $C_{11}H_{18}N_6$ (234.30).

2-Cyano-1-[4-(5-methyl-1*H*-imidazol-4-yl)butyl]-3-[2-(phenylthio)ethyl]guanidine (6.57)

The title compound was prepared from **6.16** (0.08 g, 0.52 mmol) and **3.31** (0.156 g, 0.52 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded

a white solid (0.15 g, 81 %); mp 56 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.55 (m, 4H, 2 C***H***$_2$), 2.14 (s, 3H, C***H***$_3$), 2.53 (t, 2H, ^{3}J = 7.0 Hz, C***H***$_2$-Im), 3.11 (m, 4H, 2 C***H***$_2$-N), 3.39 (t, 2H, ^{3}J = 7.0 Hz, C***H***$_2$-S), 7.17 (t, 1H, ^{3}J = 7.3 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.39 (m, 2H, Ph-***H***), 7.43 (s, 1H, Im-***H***-2). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 10.38 (+, Im-***C***H$_3$), 25.82 (-, ***C***H$_2$), 28.01 (-, ***C***H$_2$), 29.73 (-, ***C***H$_2$), 33.57 (-, ***C***H$_2$-S), 42.25 (-, ***C***H$_2$-N), 42.59 (-, ***C***H$_2$-N), 119.99 (C$_{quat}$, ***C***≡N), 127.34 (+, Ph-***C***-4), 130.15 (+, 2 Ph-***C***), 130.21 (C$_{quat}$, Im-***C***-5), 130.47 (+, 2 Ph-***C***), 130.85 (C$_{quat}$, Im-***C***-4), 133.94 (+, Im-***C***-2), 136.99 (C$_{quat}$, Ph-***C***-1), 161.10 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_{18}H_{24}N_6S$ [$M^{+\bullet}$] 356.1783; found 356.1790. IR (cm^{-1}) = 3249 (N-H), 2922, 2864 (C-H), 2159 (C≡N), 1573 (C=N), 1437, 1350, 1232, 1088. Anal. ($C_{18}H_{24}N_6S \cdot 0.4\ H_2O$) C, H, N. $C_{18}H_{24}N_6S$ (356.49)

2-Cyano-1-[4-(1*H*-1,2,3-triazol-5-yl)butyl]-3-methylguanidine (6.58)

The title compound was prepared from **6.20** (0.05 g, 0.36 mmol) and **3.27** (0.063 g, 0.36 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a colorless oil (0.07 g, 88 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.58 (m, 2H, C***H***$_2$), 1.70 (m, 2H, C***H***$_2$), 2.76 (t, 2H, ^{3}J = 7.6 Hz, C***H***$_2$-Triazol), 2.78 (s, 3H, C***H***$_3$-N), 3.22 (t, 2H, ^{3}J = 7.0 Hz, C***H***$_2$-N), 7.58 (s, 1H, Triazol-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 24.97 (-, ***C***H$_2$), 27.43 (-, ***C***H$_2$), 28.65 (+, ***C***H$_3$), 29.91 (-, ***C***H$_2$), 42.26 (-, ***C***H$_2$-N), 120.23 (C$_{quat}$, ***C***≡N), 139.98 (+, Triazol-***C***-5), 141.59 (C$_{quat}$, Triazol-***C***-4), 161.98 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for $C_9H_{15}N_7$ [$M^{+\bullet}$] 221.1389; found 221.1389. IR (cm^{-1}) = 3290 (N-H), 3136, 2933, 2860 (C-H), 2159 (C≡N), 1574 (C=N), 1367. Anal. ($C_9H_{15}N_7 \cdot 0.3\ H_2O$) C, H, N. $C_9H_{15}N_7$ (221.26).

2-Cyano-1-[4-(1*H*-1,2,3-triazol-5-yl)butyl]-3-[2-(phenylthio)ethyl]guanidine (6.59)

The title compound was prepared from **6.20** (0.05 g, 0.36 mmol) and **3.31** (0.106 g, 0.36 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a colorless oil (0.12 g, 97 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.55 (m, 2H, C***H***$_2$), 1.69 (m, 2H, C***H***$_2$), 2.75 (t, 2H, ^{3}J = 7.5 Hz, C***H***$_2$-Triazol), 3.09 (t, 2H, ^{3}J = 6.6 Hz, C***H***$_2$-N), 3.15 (t, 2H, ^{3}J = 6.9 Hz, C***H***$_2$-N), 3.40 (t, 2H, ^{3}J = 7.0 Hz, C***H***$_2$-S), 7.17 (m, 1H, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.37 (m, 2H, Ph-***H***), 7.58 (s, 1H, Triazol-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 24.99 (-, ***C***H$_2$), 27.46 (-, ***C***H$_2$), 29.72 (-, ***C***H$_2$), 33.53 (-, ***C***H$_2$-S), 42.27 (-, ***C***H$_2$-N), 42.40 (-, ***C***H$_2$-N), 119.95 (C$_{quat}$, ***C***≡N), 127.32 (+, Ph-***C***-4), 130.15 (+, 2 Ph-***C***), 130.43 (+, 2 Ph-***C***), 137.00 (C$_{quat}$, Ph-***C***-1), 138.03 (+, Triazol-***C***-5), 140.13 (C$_{quat}$, Triazol-***C***-4), 161.12 (C$_{quat}$, ***C***=N). HRMS (EI-MS) calcd. for

$C_{16}H_{21}N_7S$ [$M^{+\bullet}$] 343.1579; found 343.1573. IR (cm^{-1}) = 3272 (N-H), 3137, 2931, 2860 (C-H), 2160 (C≡N), 1571 (C=N), 1438, 1356. Anal. ($C_{16}H_{21}N_7S \cdot 0.2\ CH_3OH$) C, H, N. $C_{16}H_{21}N_7S$ (343.45)

1-[4-(3-Amino-1*H*-1,2,4-triazol-5-yl)butyl]-2-cyano-3-methylguanidine (6.60)

The title compound was prepared from **6.26** (0.12 g, 0.31 mmol) and **3.27** (0.06 g, 0.34 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.29 mL DIPEA were added to the mixture. Flash chromatography and subsequent preparative HPLC (millipore water without TFA was used as mobile phase) yielded a white semisolid (0.06 g, 82 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.56 (m, 2H, C***H***$_2$), 1.68 (m, 2H, C***H***$_2$), 2.66 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$-Triazol), 2.78 (s, 3H, C***H***$_3$-N), 3.20 (t, 2H, ^3J = 7.1 Hz, C***H***$_2$-N). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 26.07 (-, ***C***H$_2$), 27.96 (-, ***C***H$_2$), 28.77 (+, ***C***H$_3$), 29.91 (-, ***C***H$_2$), 42.31 (-, ***C***H$_2$-N), 120.35 (C_{quat}, ***C***≡N), 160.00 (C_{quat}, Triazol-***C***), 160.92 (C_{quat}, Triazol-***C***), 161.96 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_9H_{16}N_8$ [$M^{+\bullet}$] 236.1498; found 236.1503. IR (cm^{-1}) = 3168 (N-H), 3094, 2940 (C-H), 2167 (C≡N), 1576 (C=N), 1366, 1201, 1131, 1060. Anal. ($C_9H_{16}N_8 \cdot 0.7\ H_2O$) C, H, N. $C_9H_{16}N_8$ (236.28).

1-[4-(3-Amino-1*H*-1,2,4-triazol-5-yl)butyl]-2-cyano-3-[2-(phenylthio)ethyl]guanidine (6.61)

The title compound was prepared from **6.26** (0.1 g, 0.26 mmol) and **3.31** (0.09 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.17 mL DIPEA were added to the mixture. Flash chromatography yielded a white semisolid (0.06 g, 64 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.55 (m, 2H, C***H***$_2$), 1.68 (m, 2H, C***H***$_2$), 2.56 (t, 2H, ^3J = 7.1 Hz, C***H***$_2$-Triazol), 3.11 (m, 4H, 2 C***H***$_2$-N), 3.40 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$-S), 7.18 (t, 1H, ^3J = 7.2 Hz, Ph-***H***-4), 7.29 (m, 2H, Ph-***H***), 7.39 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 25.95 (-, ***C***H$_2$), 27.87 (-, ***C***H$_2$), 29.64 (-, ***C***H$_2$), 33.52 (-, ***C***H$_2$-S), 42.31 (-, ***C***H$_2$-N), 42.39 (-, ***C***H$_2$-N), 120.01 (C_{quat}, ***C***≡N), 127.31 (+, Ph-***C***-4), 130.15 (+, 2 Ph-***C***), 130.43 (+, 2 Ph-***C***), 137.01 (C_{quat}, Ph-***C***-1), 160.73 (C_{quat}, Triazol-***C***), 160.91 (C_{quat}, Triazol-***C***), 161.10 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{16}H_{22}N_8S$ [$M^{+\bullet}$] 358.1688; found 358.1690. IR (cm^{-1}) = 3246 (N-H), 2928, 2857 (C-H), 2159 (C≡N), 1569 (C=N), 1453, 1346, 1087, 1068. Anal. ($C_{16}H_{22}N_8S \cdot 0.6\ H_2O$) C, H, N. $C_{16}H_{22}N_8S$ (358.46)

1-[3-(3-Amino-1*H*-1,2,4-triazol-5-ylamino)propyl]-2-cyano-3-methylguanidine (6.62)

The title compound was prepared from **6.33** (0.063 g, 0.4 mmol) and **3.27** (0.07 g, 0.4 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.05 g, 53 %); mp 154 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.75 (m, 2H, C***H***$_2$), 2.79 (s, 3H, C***H***$_3$-N), 3.20 (t, 2H, ^{3}J = 6.5 Hz, C***H***$_2$-N), 3.27 (t, 2H, ^{3}J = 6.6 Hz, C***H***$_2$-N). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.75 (+, ***C***H$_3$), 30.79 (-, ***C***H$_2$), 39.88 (-, ***C***H$_2$), 41.09 (-, ***C***H$_2$), 120.46 (C_{quat}, ***C***≡N), 162.06 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_8H_{15}N_9$ [$M^{+\bullet}$] 237.1450; found 237.1450. IR (cm^{-1}) = 3283 (N-H), 2945, 2864 (C-H), 2155 (C≡N), 1561 (C=N), 1420, 1342. Anal. ($C_8H_{15}N_9 \cdot 1.1\ H_2O$) C, H, N. $C_8H_{15}N_9$ (237.27).

1-[3-(3-Amino-1*H*-1,2,4-triazol-5-ylamino)propyl]-2-cyano-3-[2-(phenylthio)ethyl] guanidine (6.63)

The title compound was prepared from **6.33** (0.047 g, 0.3 mmol) and **3.31** (0.09 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.05 g, 46 %); mp 142 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.71 (m, 2H, C***H***$_2$), 3.09 (t, 2H, ^{3}J = 7.4 Hz, C***H***$_2$-N), 3.18 (m, 4H, 2 C***H***$_2$-N), 3.41 (t, 2H, ^{3}J = 7.4 Hz, C***H***$_2$-S), 7.17 (t, 1H, ^{3}J = 7.3 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.38 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.73 (-, ***C***H$_2$), 33.48 (-, ***C***H$_2$-S), 39.85 (-, ***C***H$_2$), 41.02 (-, ***C***H$_2$), 42.39 (-, ***C***H$_2$), 120.42 (C_{quat}, ***C***≡N), 127.30 (+, Ph-***C***-4), 130.17 (+, 2 Ph-***C***), 130.36 (+, 2 Ph-***C***), 137.03 (C_{quat}, Ph-***C***-1), 161.32 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{15}H_{21}N_9S$ [$M^{+\bullet}$] 359.1641; found 359.1630. IR (cm^{-1}) = 3283 (N-H), 3016, 2946, 2864 (C-H), 2155 (C≡N), 1561 (C=N), 1420, 1342, 1083. Anal. ($C_{15}H_{21}N_9S \cdot 0.2\ CH_3OH \cdot 0.7\ H_2O$) C, H, N. $C_{15}H_{21}N_9S$ (359.45)

1-[4-(3-Amino-1*H*-1,2,4-triazol-5-ylamino)butyl]-2-cyano-3-methylguanidine (6.64)

The title compound was prepared from **6.34** (0.068 g, 0.4 mmol) and **3.27** (0.07 g, 0.4 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.06 g, 60 %); mp 192 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.45 (m, 4H, 2 C***H***$_2$), 2.65 (d, 3H, ^{3}J = 4.3 Hz, C***H***$_3$-N), 2.98 (m, 2H, C***H***$_2$-N), 3.07 (m, 2H, C***H***$_2$-N). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 26.50 (-, ***C***H$_2$), 26.58 (-, ***C***H$_2$), 28.16 (+, ***C***H$_3$), 40.77 (-, ***C***H$_2$), 42.33 (-, ***C***H$_2$), 118.22 (C_{quat}, ***C***≡N), 159.87 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_9H_{17}N_9$ [$M^{+\bullet}$] 251.1607; found 251.1613. IR (cm^{-1}) = 3321 (N-H), 2944, 2870 (C-H), 2151 (C≡N), 1566 (C=N), 1531, 1138, 941. Anal. ($C_9H_{17}N_9 \cdot 0.5\ H_2O$) C, H, N. $C_9H_{17}N_9$ (251.29).

1-[4-(3-Amino-1*H*-1,2,4-triazol-5-ylamino)butyl]-2-cyano-3-[2-(phenylthio)ethyl]guanidine (6.65)

The title compound was prepared from **6.34** (0.052 g, 0.3 mmol) and **3.31** (0.09 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white solid (0.08 g, 71 %); mp 230 °C dec.. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.57 (m, 4H, 2 C***H***$_2$), 3.07 (t, 2H, ^{3}J = 7.4 Hz, C***H***$_2$-N), 3.14 (m, 4H, 2 C***H***$_2$-N), 3.39 (t, 2H, ^{3}J = 7.3 Hz, C***H***$_2$-S), 7.16 (m, 1H, Ph-***H***-4), 7.27 (m, 2H, Ph-***H***), 7.37 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 26.33 (-, ***C***H$_2$), 26.54 (-, ***C***H$_2$), 30.94 (-, ***C***H$_2$-S), 40.45 (-, ***C***H$_2$), 40.86 (-, ***C***H$_2$), 42.31 (-, ***C***H$_2$), 117.90 (C_{quat}, ***C***≡N), 125.55 (+, Ph-***C***-4), 127.67 (+, 2 Ph-***C***), 128.97 (+, 2 Ph-***C***), 135.56 (C_{quat}, Ph-***C***-1), 159.00 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{16}H_{23}N_9S$ [$M^{+\bullet}$] 373.1797; found 373.1789. IR (cm^{-1}) = 3153 (N-H), 2939, 2863 (C-H), 2151 (C≡N), 1557 (C=N), 1417, 1354, 1300, 1091. Anal. ($C_{16}H_{23}N_9S \cdot 1.3\ H_2O$) C, H, N. $C_{16}H_{23}N_9S$ (373.48)

1-[3-(3-Amino-1*H*-1,2,4-triazol-5-ylthio)propyl]-2-cyano-3-methylguanidine (6.66)

The title compound was prepared from **6.38** (0.12 g, 0.69 mmol) and **3.27** (0.12 g, 0.69 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography and subsequent preparative HPLC (millipore water without TFA was used as mobile phase) yielded a white semisolid (0.04 g, 23 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.89 (m, 2H, C***H***$_2$), 2.79 (s, 3H, C***H***$_3$-N), 3.05 (t, 2H, ^{3}J = 6.9 Hz, C***H***$_2$-N), 3.32 (t, 2H, ^{3}J = 6.7 Hz, C***H***$_2$-S). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.75 (+, ***C***H$_3$), 29.71 (-, ***C***H$_2$), 30.92 (-, ***C***H$_2$), 40.81 (-, ***C***H$_2$), 120.59 (C_{quat}, ***C***≡N), 135.88 (C_{quat}, Triazol-***C***-5), 146.31 (C_{quat}, Triazol-***C***-2), 162.00 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_8H_{14}N_8S$ [$M^{+\bullet}$] 254.1062; found 254.1059. IR (cm^{-1}) = 3311 (N-H), 2936 (C-H), 2154 (C≡N), 1568 (C=N), 1494, 1368, 1268, 1093. Anal. ($C_8H_{14}N_8S \cdot 0.8\ CH_3OH$) C, H, N. $C_8H_{14}N_8S$ (254.32).

1-[3-(3-Amino-1*H*-1,2,4-triazol-5-ylthio)propyl]-2-cyano-3-[2-(phenylthio)ethyl]guanidine (6.67)

The title compound was prepared from **6.38** (0.07 g, 0.4 mmol) and **3.31** (0.12 g, 0.4 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white semisolid (0.03 g, 20 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.84 (m, 2H, C***H***$_2$), 3.03 (t, 2H, ^{3}J = 6.7 Hz, C***H***$_2$), 3.11 (t, 2H, ^{3}J = 7.3 Hz, C***H***$_2$), 3.25 (t, 2H, ^{3}J = 6.3 Hz, C***H***$_2$), 3.42 (t, 2H, ^{3}J = 7.3 Hz, C***H***$_2$-S-Ph), 7.17 (m, 1H, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.39 (m, 2H, Ph-***H***). ^{13}C-NMR

(75 MHz, CD_3OD): δ [ppm] = 29.52 (-, ***C***H_2), 30.85 (-, ***C***H_2-S), 33.46 (-, ***C***H_2-S), 40.63 (-, ***C***H_2-N), 42.43 (-, ***C***H_2-N), 119.62 (C_{quat}, ***C***≡N), 127.27 (+, Ph-***C***-4), 130.13 (+, 2 Ph-***C***), 130.34 (+, 2 Ph-***C***), 137.08 (C_{quat}, Ph-***C***-1), 139.91 (C_{quat}, Triazol-***C***-5), 147.29 (C_{quat}, Triazol-***C***-2), 161.20 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{15}H_{20}N_8S_2$ [$M^{+\bullet}$] 376.1232; found 376.1242. IR (cm^{-1}) = 3311 (N-H), 3199, 2936 (C-H), 2154 (C≡N), 1568 (C=N), 1368, 1268, 1094. Anal. ($C_{15}H_{20}N_8S_2 \cdot CH_3OH$) C, H, N. $C_{15}H_{20}N_8S_2$ (376.50)

2-Cyano-1-methyl-3-[3-(pyridin-2-ylamino)propyl]guanidine (6.68)

The title compound was prepared from **6.40** (0.05 g, 0.33 mmol) and **3.27** (0.057 g, 0.33 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white semisolid (0.07 g, 91 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.74 (m, 2H, C***H***$_2$), 2.92 (d, 3H, ^3J = 4.8 Hz, C***H***$_3$-N), 3.33 (m, 2H, C***H***$_2$), 3.46 (m, 2H, C***H***$_2$), 5.06 (brs, 1H, N***H***), 5.91 (brs, 1H, N***H***), 6.45 (d, 1H, ^3J = 8.4 Hz, Py-***H***-3), 6.55 (m, H, Py-***H***-5), 7.05 (brs, 1H, N***H***), 7.39 (m, 1H, Py-***H***-4), 7.97 (m, 1H, Py-***H***-6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.42 (+, ***C***H_3), 30.00 (-, ***C***H_2), 38.05 (-, ***C***H_2-N), 38.15 (-, ***C***H_2-N), 109.16 (+, Py-***C***-3), 112.81 (+, Py-***C***-5), 120.39 (C_{quat}, ***C***≡N), 137.91 (+, Py-***C***-4), 146.48 (+, Py-***C***-6), 158.68 (C_{quat}, ***C***=N), 160.61 (C_{quat}, Py-***C***-2). HRMS (EI-MS) calcd. for $C_{11}H_{16}N_6$ [$M^{+\bullet}$] 232.1436; found 232.1437. IR (cm^{-1}) = 3270 (N-H), 2929, 2861 (C-H), 2158 (C≡N), 1568 (C=N), 1511, 1417, 1359, 1152. Anal. ($C_{11}H_{16}N_6 \cdot 0.3\ H_2O$) C, H, N. $C_{11}H_{16}N_6$ (232.28)

2-Cyano-1-[2-(phenylthio)ethyl]-3-[3-(pyridin-2-ylamino)propyl]guanidine (6.69)

The title compound was prepared from **6.40** (0.05 g, 0.33 mmol) and **3.31** (0.098 g, 0.33 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white semisolid (0.1 g, 85 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.73 (m, 2H, C***H***$_2$), 3.11 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$-N), 3.24 (m, 2H, C***H***$_2$), 3.45 (m, 4H, 2 C***H***$_2$), 4.95 (brs, 1H, N***H***), 5.99 (brs, 1H, N***H***), 6.42 (d, 1H, ^3J = 8.4 Hz, Py-***H***-3), 6.54 (m, H, Py-***H***-5), 7.04 (brs, 1H, N***H***), 7.19 (m, 1H, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.37 (m, 3H, Ph-***H*** + Py-***H***-4), 8.02 (m, 1H, Py-***H***-6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.01 (-, ***C***H_2), 33.03 (-, ***C***H_2-S), 38.18 (-, ***C***H_2-N), 38.55 (-, ***C***H_2-N), 41.02 (-, ***C***H_2-N), 108.96 (+, Py-***C***-3), 112.98 (+, Py-***C***-5), 118.85 (C_{quat}, ***C***≡N), 126.70 (+, Ph-***C***-4), 129.22 (+, 2 Ph-***C***), 129.77 (+, 2 Ph-***C***), 134.21 (C_{quat}, Ph-***C***-1), 137.76 (+, Py-***C***-4), 147.02 (+, Py-***C***-6), 158.64 (C_{quat}, ***C***=N), 159.69 (C_{quat}, Py-***C***-2). HRMS (EI-MS) calcd. for $C_{18}H_{22}N_6S$ [$M^{+\bullet}$]

354.1627; found 354.1627. IR (cm^{-1}) = 3263 (N-H), 3053, 2949 (C-H), 2159 (C≡N), 1567 (C=N), 1509, 1481, 1300. Anal. ($C_{18}H_{22}N_6S \cdot 0.2\ CH_3OH$) C, H, N. $C_{18}H_{22}N_6S$ (354.47)

2-Cyano-1-methyl-3-[4-(pyridin-2-ylamino)butyl]guanidine (6.70)

The title compound was prepared from **6.41** (0.05 g, 0.3 mmol) and **3.27** (0.053 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white semisolid (0.06 g, 81 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.63 (m, 4H, 2 C***H***$_2$), 2.79 (d, 3H, ^3J = 4.7 Hz, C***H***$_3$-N), 3.25 (m, 4H, 2 C***H***$_2$), 5.04 (brs, 1H, N***H***), 5.83 (brs, 1H, N***H***), 6.00 (brs, 1H, N***H***), 6.40 (d, 1H, ^3J = 8.4 Hz, Py-***H***-3), 6.53 (m, H, Py-***H***-5), 7.39 (m, 1H, Py-***H***-4), 7.98 (d, 1H, ^3J = 4.1 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.58 (-, 2 ***C***H$_2$), 28.49 (+, ***C***H$_3$), 41.41 (-, ***C***H$_2$-N), 41.61 (-, ***C***H$_2$-N), 107.56 (+, Py-***C***-3), 112.69 (+, Py-***C***-5), 119.97 (C_{quat}, ***C***≡N), 137.79 (+, Py-***C***-4), 147.22 (+, Py-***C***-6), 158.58 (C_{quat}, ***C***=N), 160.61 (C_{quat}, Py-***C***-2). HRMS (EI-MS) calcd. for $C_{12}H_{18}N_6$ [$M^{+\bullet}$] 246.1593; found 246.1593. IR (cm^{-1}) = 3288 (N-H), 2987, 2970, 2940 (C-H), 2158 (C≡N), 1569 (C=N), 1513, 1443, 1370, 1027. Anal. ($C_{12}H_{18}N_6 \cdot 0.4\ CH_3OH$) C, H, N. $C_{12}H_{18}N_6$ (246.31)

2-Cyano-1-[2-(phenylthio)ethyl]-3-[4-(pyridin-2-ylamino)butyl]guanidine (6.71)

The title compound was prepared from **6.41** (0.05 g, 0.3 mmol) and **3.31** (0.09 g, 0.3 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a white semisolid (0.08 g, 72 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.65 (m, 4H, 2 C***H***$_2$), 3.09 (t, 2H, ^3J = 6.4 Hz, C***H***$_2$-N), 3.14 (m, 2H, C***H***$_2$), 3.32 (m, 2H, C***H***$_2$), 3.44 (m, 2H, C***H***$_2$), 4.96 (brs, 1H, N***H***), 5.69 (brs, 2H, 2 N***H***), 6.42 (d, 1H, ^3J = 8.4 Hz, Py-***H***-3), 6.56 (m, H, Py-***H***-5), 7.20 (m, 1H, Ph-***H***-4), 7.29 (m, 2H, Ph-***H***), 7.34 (m, 2H, Ph-***H***), 7.42 (m, 1H, Py-***H***-4), 8.01 (m, 1H, Py-***H***-6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.26 (-, ***C***H$_2$), 26.60 (-, ***C***H$_2$), 33.16 (-, ***C***H$_2$-S), 41.02 (-, ***C***H$_2$-N), 41.26 (-, ***C***H$_2$-N), 41.65 (-, ***C***H$_2$-N), 107.83 (+, Py-***C***-3), 112.86 (+, Py-***C***-5), 118.41 (C_{quat}, ***C***≡N), 126.73 (+, Ph-***C***-4), 129.25 (+, 2 Ph-***C***), 129.67 (+, 2 Ph-***C***), 134.63 (C_{quat}, Ph-***C***-1), 138.05 (+, Py-***C***-4), 146.85 (+, Py-***C***-6), 158.25 (C_{quat}, ***C***=N), 159.77 (C_{quat}, Py-***C***-2). HRMS (EI-MS) calcd. for $C_{19}H_{24}N_6S$ [$M^{+\bullet}$] 368.1783; found 368.1788. IR (cm^{-1}) = 3270 (N-H), 2987, 2901 (C-H), 2158 (C≡N), 1567 (C=N), 1508, 1437, 1329. Anal. ($C_{19}H_{24}N_6S \cdot 0.35\ CH_3OH$) C, H, N. $C_{19}H_{24}N_6S$ (368.50)

1-[2-(4-Aminopyrimidin-2-ylamino)ethyl]-2-cyano-3-methylguanidine (6.72)

The title compound was prepared from N^2-(2-aminoethyl)pyrimidine-2,4-diamine dihydrochloride **6.47** (0.034 g, 0.15 mmol) and **3.27** (0.027 g, 0.15 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.13 mL DIPEA were added to the mixture. Flash chromatography yielded a colorless oil (0.03 g, 85 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.78 (s, 3H, C***H***$_3$-N), 3.35 (t, 2H, ^3J = 5.6 Hz, C***H***$_2$-N), 3.48 (t, 2H, ^3J = 5.6 Hz, C***H***$_2$-N), 5.90 (d, 1H, ^3J = 6.2 Hz, Py-***H***-5), 7.69 (d, 1H, ^3J = 6.1 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.75 (+, ***C***H_3), 41.37 (-, ***C***H_2-N), 43.55 (-, ***C***H_2-N), 97.20 (+, Py-***C***-5), 120.48 (C_{quat}, ***C***≡N), 153.75 (+, Py-***C***-6), 162.14 (C_{quat}, ***C***=N), 162.25 (C_{quat}, Py-***C***), 166.54 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_9H_{14}N_8$ [$M^{+\bullet}$] 234.1341; found 234.1337. IR (cm^{-1}) = 3064 (N-H), 2876 (C-H), 2168 (C≡N), 1655, 1566 (C=N), 1421, 1360, 1231, 1175. $C_9H_{14}N_8$ (234.26).

1-[2-(4-Aminopyrimidin-2-ylamino)ethyl]-2-cyano-3-[2-(phenylthio)ethyl]guanidine (6.73)

The title compound was prepared from N^2-(2-aminoethyl)pyrimidine-2,4-diamine dihydrochloride **6.47** (0.039 g, 0.17 mmol) and **3.31** (0.051 g, 0.17 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.14 mL DIPEA were added to the mixture. Flash chromatography yielded a colorless oil (0.05 g, 83 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.10 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-N), 3.25 (m, 2H, C***H***$_2$-N), 3.43 (m, 4H, 2 C***H***$_2$), 5.91 (d, 1H, ^3J = 6.2 Hz, Py-***H***-5), 7.16 (t, 1H, ^3J = 8.5 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.37 (m, 2H, Ph-***H***), 7.66 (d, 1H, ^3J = 6.2 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 33.45 (-, ***C***H_2-S), 41.23 (-, ***C***H_2-N), 42.54 (-, ***C***H_2-N), 43.25 (-, ***C***H_2-N), 97.42 (+, Py-***C***-5), 120.31 (C_{quat}, ***C***≡N), 127.33 (+, Ph-***C***-4), 130.20 (+, 2 Ph-***C***), 130.75 (+, 2 Ph-***C***), 137.04 (C_{quat}, Ph-***C***-1), 153.41 (+, Py-***C***-6), 161.40 (C_{quat}, ***C***=N), 162.07 (C_{quat}, Py-***C***), 166.19 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_{16}H_{20}N_8S$ [$M^{+\bullet}$] 356.1532; found 356.1524. IR (cm^{-1}) = 3225 (N-H), 3064, 2962, 2876 (C-H), 2168 (C≡N), 1655, 1566 (C=N), 1421, 1360, 1175. $C_{16}H_{20}N_8S$ (356.45)

1-[3-(4-Aminopyrimidin-2-ylamino)propyl]-2-cyano-3-methylguanidine (6.74)

The title compound was prepared from N^2-(3-aminopropyl)pyrimidine-2,4-diamine dihydrochloride **6.48** (0.05 g, 0.2 mmol) and **3.27** (0.036 g, 0.2 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.17 mL DIPEA were added to the mixture. Flash chromatography yielded a colorless oil (0.04 g, 81 %); ^{1}H-NMR

(300 MHz, CD_3OD): δ [ppm] = 1.76 (m, 2H, C***H***$_2$), 2.79 (s, 3H, C***H***$_3$-N), 3.26 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$-N), 3.39 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$-N), 5.90 (d, 1H, ^3J = 6.4 Hz, Py-***H***-5), 7.65 (d, 1H, ^3J = 6.3 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.76 (+, ***C***H$_3$), 30.62 (-, ***C***H$_2$), 38.79 (-, ***C***H$_2$-N), 39.69 (-, ***C***H$_2$-N), 97.06 (+, Py-***C***-5), 120.65 (C$_{quat}$, ***C***≡N), 151.54 (+, Py-***C***-6), 160.83 (C$_{quat}$, ***C***=N), 162.05 (C$_{quat}$, Py-***C***), 166.33 (C$_{quat}$, Py-***C***). HRMS (EI-MS) calcd. for $C_{10}H_{16}N_8$ [$M^{+\bullet}$] 248.1498; found 248.1493. IR (cm^{-1}) = 3064 (N-H), 2962, 2876 (C-H), 2168 (C≡N), 1655, 1566 (C=N), 1421, 1360, 1334. $C_{10}H_{16}N_8$ (248.29).

1-[3-(4-Aminopyrimidin-2-ylamino)propyl]-2-cyano-3-[2-(phenylthio)ethyl]guanidine (6.75)

The title compound was prepared from *N*^{2}-(3-aminopropyl)pyrimidine-2,4-diamine dihydrochloride **6.48** (0.03 g, 0.12 mmol) and **3.31** (0.037 g, 0.12 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.17 mL DIPEA were added to the mixture. Flash chromatography yielded a yellow oil (0.04 g, 90 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.71 (m, 2H, C***H***$_2$), 3.11 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-N), 3.18 (t, 2H, ^3J = 6.3 Hz, C***H***$_2$-N), 3.37 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$-N), 3.42 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$-S), 5.90 (d, 1H, ^3J = 6.3 Hz, Py-***H***-5), 7.17 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.39 (m, 2H, Ph-***H***), 7.66 (d, 1H, ^3J = 6.3 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 30.60 (-, ***C***H$_2$), 33.46 (-, ***C***H$_2$-S), 38.56 (-, ***C***H$_2$-N), 39.58 (-, ***C***H$_2$-N), 42.37 (-, ***C***H$_2$-N), 97.00 (+, Py-***C***-5), 120.66 (C$_{quat}$, ***C***≡N), 127.29 (+, Ph-***C***-4), 130.17 (+, 2 Ph-***C***), 130.33 (+, 2 Ph-***C***), 137.06 (C$_{quat}$, Ph-***C***-1), 152.31 (+, Py-***C***-6), 161.23 (C$_{quat}$, ***C***=N), 162.11 (C$_{quat}$, Py-***C***), 166.32 (C$_{quat}$, Py-***C***). HRMS (EI-MS) calcd. for $C_{17}H_{22}N_8S$ [$M^{+\bullet}$] 370.1688; found 370.1692. IR (cm^{-1}) = 3173 (N-H), 3064, 2962, 2876 (C-H), 2168 (C≡N), 1655, 1566 (C=N), 1421, 1360, 1175. $C_{17}H_{22}N_8S$ (370.48)

1-[2-(2-Aminopyrimidin-4-ylamino)ethyl]-2-cyano-3-[2-(phenylthio)ethyl]guanidine (6.76)

The title compound was prepared from *N*^{4}-(2-aminoethyl)pyrimidine-2,4-diamine dihydrochloride **6.50** (0.03 g, 0.14 mmol) and **3.31** (0.04 g, 0.14 mmol) in MeCN (4.5 mL) according to the general procedure. To deprotonate the amine 0.12 mL DIPEA were added to the mixture. Flash chromatography yielded a colorless oil (0.045 g, 90 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 3.08 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-N), 3.33 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-N), 3.40 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-S), 3.54 (m, 2H, C***H***$_2$-N), 5.99 (d, 1H, ^3J = 6.7 Hz, Py-***H***-5), 7.17 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.38 (m, 2H, Ph-***H***), 7.57 (d, 1H, ^3J = 6.6 Hz, Py-***H***-6). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 33.47 (-, ***C***H$_2$-S), 40.91 (-, ***C***H$_2$-N), 42.41 (-,

CH_2-N), 43.02 (-, ***C***H_2-N), 95.02 (+, Py-***C***-5), 120.02 (C_{quat}, ***C***≡N), 127.38 (+, Ph-***C***-4), 130.18 (+, 2 Ph-***C***), 130.47 (+, 2 Ph-***C***), 136.92 (C_{quat}, Ph-***C***-1), 147.10 (+, Py-***C***-6), 160.16 (C_{quat}, ***C***=N), 161.35 (C_{quat}, Py-***C***), 165.36 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_{16}H_{20}N_8S$ [$M^{+\bullet}$] 356.1532; found 356.1539. IR (cm^{-1}) = 3305 (N-H), 3053, 2986 (C-H), 2162 (C≡N), 1654, 1564 (C=N), 1530, 1433, 1362, 1199. $C_{16}H_{20}N_8S$ (356.45)

2-Cyano-1-[5-(2-methyl-1*H*-imidazol-1-yl)pentyl]-3-[2-(phenylthio)ethyl]guanidine (6.77)

The title compound was prepared from **6.51** (0.1 g, 0.6 mmol) and **3.27** (0.1 g, 0.6 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a colorless oil (0.07 g, 47 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.32 (m, 2H, C***H***$_2$), 1.57 (m, 2H, C***H***$_2$), 1.76 (m, 2H, C***H***$_2$), 2.35 (s, 3H, Im-C***H***$_3$), 2.77 (s, 3H, C***H***$_3$-N), 3.18 (t, 2H, ^3J = 7.1 Hz, C***H***$_2$-N), 3.92 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$-Im), 6.79 (d, 1H, ^3J = 1.4 Hz, Im-***H***), 6.99 (d, 1H, ^3J = 1.4 Hz, Im-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 12.59 (+, Im-***C***H_3), 24.65 (-, ***C***H_2), 28.73 (+, ***C***H_3), 30.09 (-, ***C***H_2), 31.38 (-, ***C***H_2), 42.38 (-, ***C***H_2-N), 46.90 (-, ***C***H_2-Im), 120.26 (C_{quat}, ***C***≡N), 120.84 (+, Im-***C***-5), 126.75 (+, Im-***C***-4), 145.75 (C_{quat}, Im-***C***-2), 161.98 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{12}H_{20}N_6$ [$M^{+\bullet}$] 248.1749; found 248.1745. IR (cm^{-1}) = 3226 (N-H), 2948, 2857 (C-H), 2168 (C≡N), 1584 (C=N), 1498, 1369, 1280, 1107. Anal. ($C_{12}H_{20}N_6 \cdot 0.2\ H_2O$) C, H, N. $C_{12}H_{20}N_6$ (248.33).

2-Cyano-1-[5-(2-methyl-1*H*-imidazol-1-yl)pentyl]-3-[2-(phenylthio)ethyl]guanidine (6.78)

The title compound was prepared from **6.51** (0.1 g, 0.6 mmol) and **3.31** (0.178 g, 0.6 mmol) in MeCN (4.5 mL) according to the general procedure. Flash chromatography yielded a colorless oil (0.1 g, 45 %); ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.31 (m, 2H, C***H***$_2$), 1.55 (m, 2H, C***H***$_2$), 1.74 (m, 2H, C***H***$_2$), 2.33 (s, 3H, Im-C***H***$_3$), 3.10 (m, 4H, 2 C***H***$_2$), 3.39 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-S), 3.90 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$-Im), 6.79 (d, 1H, ^3J = 1.4 Hz, Im-***H***), 6.97 (d, 1H, ^3J = 1.4 Hz, Im-***H***), 7.18 (t, 1H, ^3J = 7.3 Hz, Ph-***H***-4), 7.28 (m, 2H, Ph-***H***), 7.38 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 12.56 (+, Im-***C***H_3), 24.72 (-, ***C***H_2), 29.90 (-, ***C***H_2), 31.38 (-, ***C***H_2), 33.57 (-, ***C***H_2-S), 42.25 (-, ***C***H_2-N), 42.53 (-, ***C***H_2-N), 46.96 (-, ***C***H_2-Im), 119.98 (C_{quat}, ***C***≡N), 120.91 (+, Im-***C***-5), 126.53 (+, Im-***C***-4), 127.35 (+, Ph-***C***-4), 130.21 (+, 2 Ph-***C***), 130.37 (+, 2 Ph-***C***), 137.02 (C_{quat}, Ph-***C***-1), 145.72 (C_{quat}, Im-***C***-2), 161.10 (C_{quat}, ***C***=N). HRMS (EI-MS) calcd. for $C_{19}H_{26}N_6S$ [$M^{+\bullet}$] 370.1940; found 370.1935. IR (cm^{-1}) = 3250 (N-H), 2933, 2858 (C-H), 2160

(C≡N), 1578 (C=N), 1480, 1356, 1179, 1088. Anal. ($C_{19}H_{26}N_6S \cdot 0.7\ H_2O$) C, H, N. $C_{19}H_{26}N_6S$ (370.57).

6.4.2 Pharmacological methods

6.4.2.1 Materials

See section 5.4.3.1.

6.4.2.2 [^{35}S]GTPγS binding assay[50, 51]

See section 3.4.2.2.

6.4.2.3 Steady-state GTPase activity assay.

See section 5.4.3.3.

6.4.2.4 Data analysis and pharmacological parameters.

See section 3.4.2.3.

6.5 References

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Chapter 7

Towards fluorescently labeled H_4 receptor ligands

7.1 Introduction

Fluorescent and radiolabeled ligands are valuable pharmacological tools for the detection and molecular analysis of receptors and for the study of ligand-receptor interactions. The currently known radioligands used for the characterization of H_4 receptor agonists and antagonists are either not receptor subtype-selective or not easily accessible. Recently, Igel et al. described [^{3}H]UR-PI294 as a high affinity radioligand for both the H_3 and H_4 receptor.[1] This also holds for the commercially available [^{3}H]histamine, the tritiated form of the non-selective natural histamine receptor agonist, which binds weakly to moderately to H_1R and H_2R (µM range), but has high affinity for H_3R and H_4R. Radiometric and fluorescence-based techniques are complementary to each other, and in some respect the latter offer highly attractive alternatives. Fluorescence-labeled compounds have considerable advantages compared to radioligands in terms of safety precautions and waste disposal. Furthermore, fluorescent ligands are applicable to fluorescence microscopy and flow cytometry, powerful techniques, which are routinely used in many laboratories.[2, 3]

Compared to radioligands, the preparation of fluorescent ligands is more challenging, because coupling of an affinity-conferring moiety, in particular in the case of small molecules, with bulky fluorescent dyes often results in loss of binding affinity. Nevertheless, a growing number of low molecular weight fluorescent GPCR ligands, particularly in the field of aminergic GPCRs, is reported in the literature.[4-8] However, no fluorescent H_4 receptor ligand has been reported so far. This prompted us to search for fluorescent ligands starting from 2-arylbenzimidazoles, recently reported as potent and selective H_4R ligands, e.g. **7.1** (Figure 7.1).[9, 10] This compound class shows high affinity for the H_4R and provides a primary amine as a building block that can easily be labeled with fluorescent dyes (Figure 7.1). As a first approach we decided to synthesize and evaluate a series of four compounds, bearing the same benzimidazole moiety (**7.2**), coupled to different fluorescent dyes.

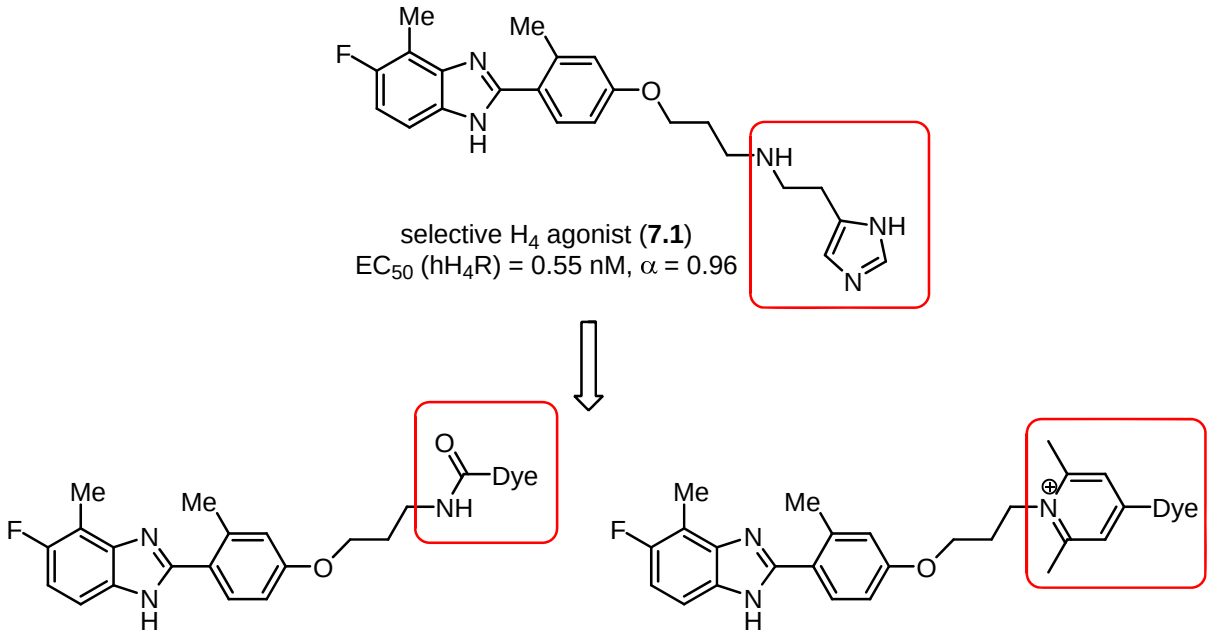


Figure 7.1 Fluorescent labeling of the 2-arylbenzimidazole building block.

7.2 Chemistry

The 2-arylbenzimidazole building block **7.2** was labeled with four different dyes. By analogy with previously optimized strategies for the preparation of fluorescent GPCR ligands,[11, 12] red emitting fluorophores (emission wavelength >590 nm) were preferred to improve the signal-to-noise ratio in cellular assays. We used pyrylium dyes[13, 14] (**Py-1** and **Py-5**), as well as the Cy5-related cyanine dye **S0436** and a bodipy ("boron-dipyrromethene") dye, **Bodipy** 650/665-X (Figure 7.2). The cyanine- and bodipy fluorophores are excitable with a red diode laser (633 nm), the emission is detectable at wavelengths >650 nm. The pyrylium dyes are excitable with a 488 nm (Argon) laser and their emission can be detected above 600 nm. Both excitation wavelengths are standard components of flow cytometers and confocal microscopes. **Py-1** and **Py-5** belong to the class of "chameleon labels", which has been developed for labeling and quantification of very low concentrations of proteins.[14] Primary amines react very rapidly with pyrylium dyes under basic conditions at room temperature to form positively charged *N*-substituted pyridinium adducts.[15] The ring transformation is accompanied with a change in color from blue to red, thus, the reaction can be followed easily. The dyes **S0436** and **Bodipy** contain a carboxylate side chain, which enables coupling to amines, preferably by aminolysis of the corresponding succinimidyl esters.

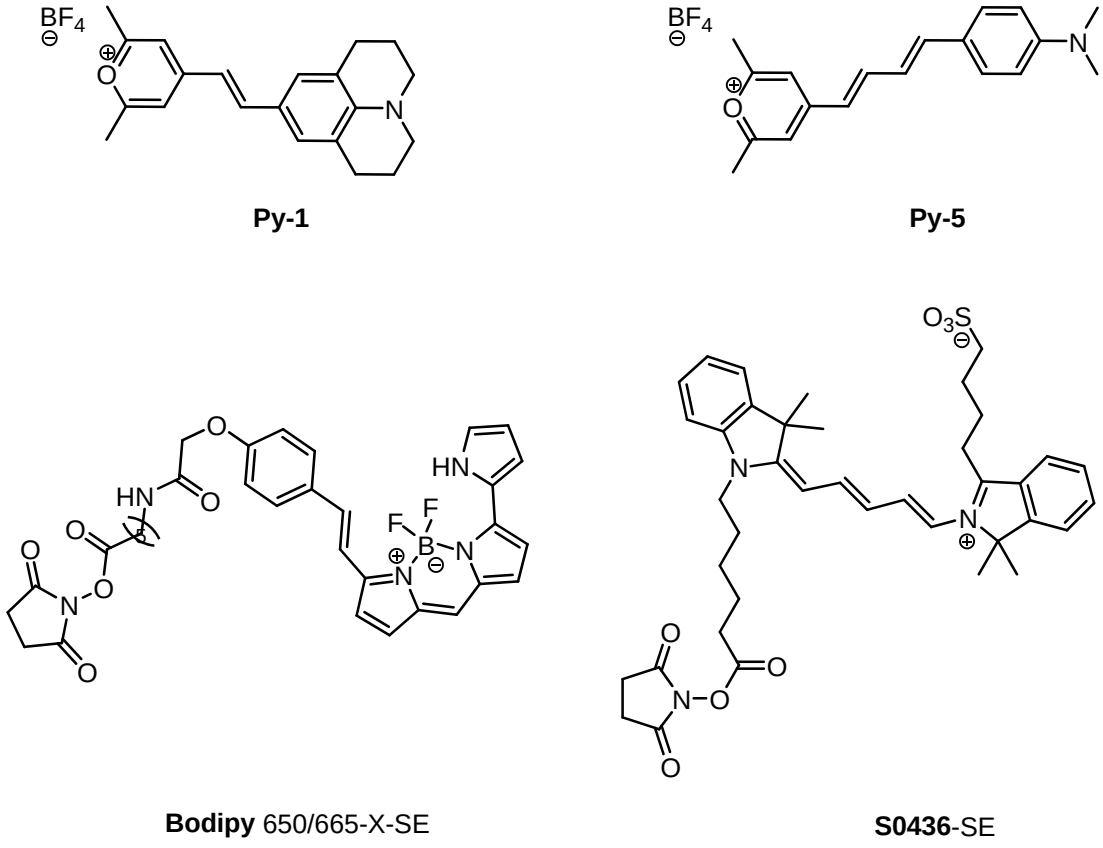
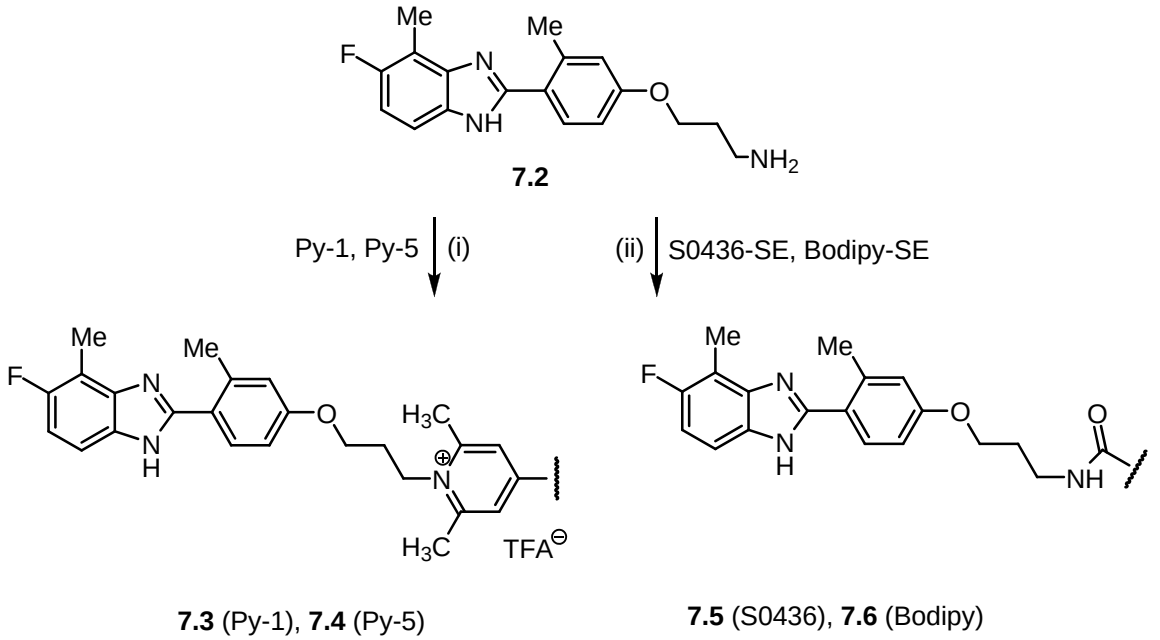


Figure 7.2 Structures of fluorescent dyes used for labeling of **7.2.**

The benzimidazole building block **7.2** was synthesized by Paul Baumeister in our research group as part of his dissertation project (to be reported elsewhere) according to a previously described method.[16]



Scheme 7.1 Synthesis of the fluorescent H_4R ligands **7.3**-**7.6**. Reagents and conditions: (i) NEt_3, MeCN, DMF, 2 h, rt, 18–64 %; (ii) NEt_3, MeCN, 12 h, rt, 81–86 %.

7.3 Fluorescence properties of the labeled H_4R ligands

The fluorescence properties of the labeled H_4R ligands are summarized in Table 7.1. The fluorescence quantum yield ϕ, which is defined as the ratio of the number of photons emitted to the number of photons absorbed, was determined in three different solvents. Cresyl violet perchlorate was used as reference, according to previously described procedures.[17, 18] For reasons of comparison and to simulate assay conditions phosphate buffered saline (PBS) at pH 7.0 and PBS with 1 % bovine serum albumin (BSA) were used as solvents. Additionally, the quantum yield was also determined in ethanol to examine the influence of the polarity of the solvent.

Table 7.1 Spectroscopic properties of the fluorescent H_4R ligands **7.3**-**7.6**: Influence of the polarity of the solvent (PBS pH 7 vs. ethanol) and protein (BSA) on the quantum yield ϕ (reference: cresyl violet perchlorate) as well as excitation maxima, λ_{ex} /emission maxima, λ_{em}.

		PBS		**PBS + 1 % BSA**		**EtOH**	
Cmpd.	Dye	λ_{ex} / λ_{em}	ϕ [%]	λ_{ex} / λ_{em}	ϕ [%]	λ_{ex} / λ_{em}	ϕ [%]
7.3	Py-1	543 / 650	0.1	519 / 611	47	514 / 634	1.5
7.4	Py-5	495 / 710	0.6	489 / 641	26	495 / 708	18
7.5	S0436	648 / 664	9.6	660 / 679	39	648 / 673	32
7.6	Bodipy	681 / -	0.1	672 / -	0.6	650 / 668	35

All fluorescent ligands showed very low quantum yield in pure PBS, and strongly increased quantum yield in the presence of 1 % BSA (e.g., 0.1 vs. 47 % in case of compound **7.3**) except for the **Bodipy**-labeled compound **7.6**. The increase in fluorescence efficiency in BSA-containing solvents is in agreement with previous studies and can be explained by intermolecular interactions, particularly hydrophobic and electrostatic interactions, of the fluorophores with the protein.[12] The quantum yields in ethanol were also quite high (≈ 30 %) for the **S0436**, **Bodipy** and **Py-5** labeled ligands **7.4 – 7.6** but much lower for the **Py-1**-labeled compound **7.3**. Obviously, ligands labeled with **Py-1** are less sensitive to hydrophobic interactions than those labeled with the other fluorophores. The excitation and corrected emission spectra of the fluorescence labeled ligands in PBS containing 1 % of BSA, in case of **7.6** in EtOH, are depicted in Figure 7.3. The largest Stoke's shift was observed for the compounds labeled with pyrylium dyes whereas only a small shift was detected for **S0436-** and **Bodipy**-labeled compounds. In summary, the fluorescence properties of all synthesized fluorescent ligands were in agreement with previously reported values and suitable for flow cytometry and confocal microscopy.

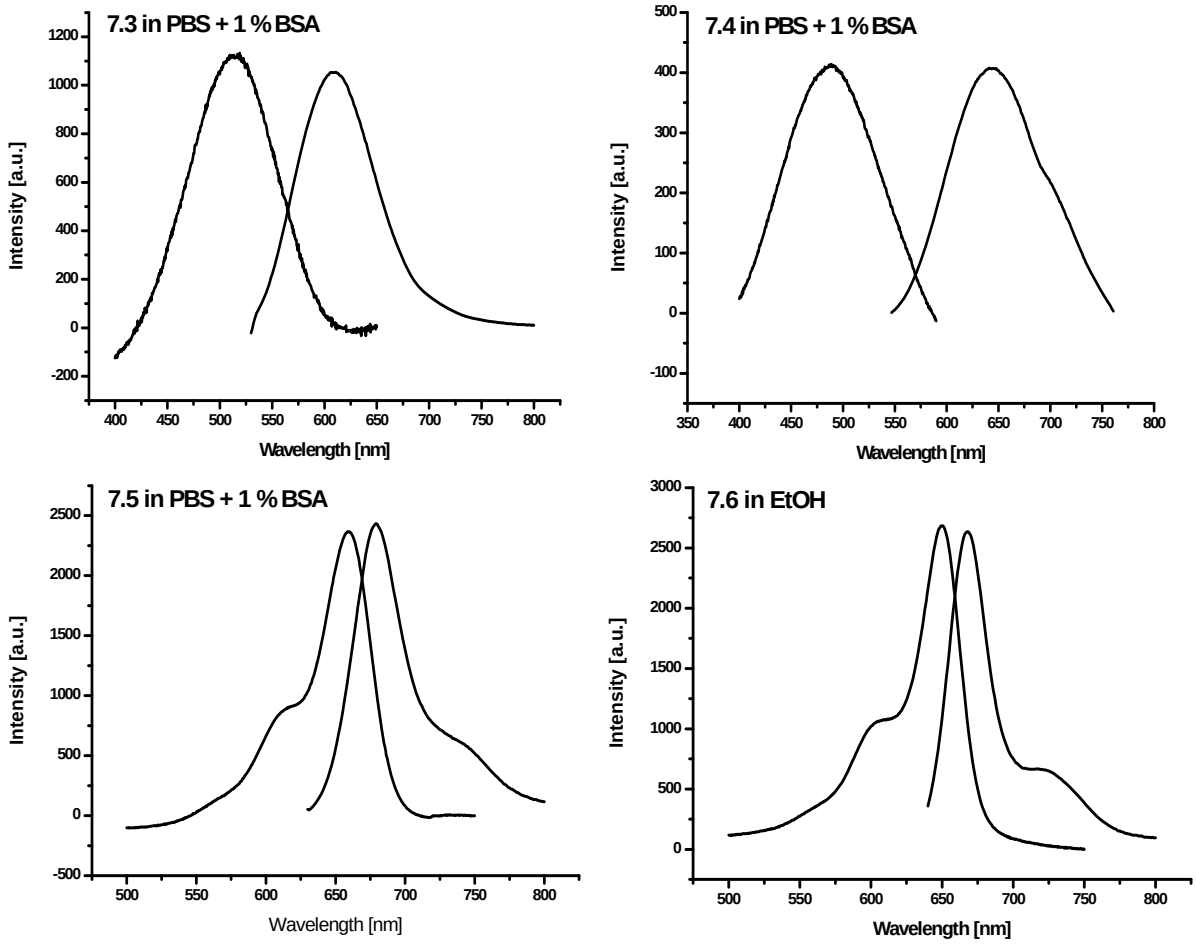


Figure 7.3 Excitation and corrected emission spectra of **7.3**, **7.4**, **7.5** in PBS + 1 % BSA and of **7.6** in EtOH (recorded at 22 °C).

7.4 Pharmacological results and discussion

7.4.1 Binding data of the fluorescent compounds at the human histamine receptor subtypes and efficacies at the hH_3R and hH_4R

In the reported benzimidazole series various residues were coupled to the primary amine in the side chain, resulting in highly potent H_4R ligands.[10] This prompted us to investigate the derivatization of the amine with fluorescent dyes, especially considering the pyrylium dyes, which are relatively low molecular weight compounds (about 300 - 400 g/mol), and are therefore expected to be well suited for coupling to small molecules. In addition, compared to structurally related benzimidazole-typ H_4R ligands, a positive charge is conserved due to the resonance stabilized pyridinium fluorophore. Previous studies suggest that such fluorescence labels are capable of contributing additional receptor affinity.[12, 19] Furthermore, to cover a broader range of spectral properties and fluorophore structures, a cyanine dye

and a bodipy dye were considered. The synthesized fluorescent compounds were investigated in radioligand binding studies at the four human histamine receptor subtypes. In addition, the intrinsic activities at the hH_3R and the hH_4R were determined in a functional [^{35}S]GTPγS binding assay.

All fluorescent ligands bound to the H_4R with K_i values in the micromolar range (1.4 - 30 µM) (Table 7.2). The two pyrylium labeled compounds **7.3** and **7.4** were both inverse agonists at the H_4R, but their K_i values differ by a factor of 25, with a lower K_i for **7.4**, labeled with the smaller **Py-5** dye. **7.5** showed inverse agonistic activity and a K_i of 7 µM. Interestingly, **7.6**, labeled with the bulky **Bodipy** dye, was comparable in affinity with the **Py-5** labeled **7.4**, but, unlike **7.4**, had a weak agonistic effect (**7.6**: K_i = 1.8 µM, α = 0.25). At the H_3R the K_i values were similar to the values determined at the H_4R. Only **7.6** showed an affinity for the H_3R in the sub-micromolar range. **7.3**, **7.4** and **7.5** were inverse agonists with an intrinsic activity from -0.27 up to -0.96. Again the **Bodipy** labeled compound **7.6** differed and showed neutral antagonistic activity. The affinities for the H_1R and the H_2R were in the range between 1 and 50 µM. In summary, the synthesized fluorescence labeled compounds showed affinities in the micromolar range at the four histamine receptor subtypes. There was no obvious relation between structure and affinity or selectivity. Bulky fluorophores replacing small heterocyclylalkyl substituents at the primary amine in **7.2** were not tolerated and even the relatively small pyrylium dyes drastically decreased the affinity for the H_4R.

Table 7.2 Binding data of the synthesized fluorescent compounds **7.3**-**7.6** at the four human histamine receptor subtypes and efficacies at the hH_3R and hH_4R in the [^{35}S]GTPγS assay.[a]

No.	Dye	**hH_1R**		**hH_2R**		**hH_3R**			**hH_4R**		
		K_i (µM)	N	K_i (µM)	N	K_i (µM)	α	N	K_i (µM)	α	N
7.3	Py-1	3.7 ± 0.03	2	20 ± 1.3	2	20 ± 2	-0.60	2	28 ± 3.4	-0.07	2
7.4	Py-5	5.1 ± 0.4	2	20 ± 1	2	1.6 ± 0.08	-0.96	2	1.4 ± 0.05	-0.38	2
7.5	S0436	2.0 ± 0.3	2	0.9 ± 0.06	2	2.4 ± 0.3	-0.27	2	7.0 ± 0.1	-0.11	2
7.6	Bodipy	48.2 ± 0.6	2	5.4 ± 0.04	2	0.5 ± 0.04	0.01	2	1.8 ± 0.03	0.25	2

[a] Displacement of [^{3}H]mepyramine (5 nM), [^{3}H]tiotidine (10 nM), [^{3}H]N^{α}-methylhistamine (3 nM) and [^{3}H]histamine (10 nM) from Sf9 insect cell membranes expressing the hH_1R + RGS4, hH_2R-$G\alpha_s$ fusion protein, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$. Radioligand binding was determined as described in section *Pharmacological methods*. Data were analyzed for best fit to one site (monophasic) competition curves. Functional [^{35}S]GTPγS binding assays using the above mentioned membrane preparations were performed as described in section *Pharmacological methods*. N gives the number of independent experiments performed in duplicate each. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. α values of neutral antagonists and inverse agonists were determined at 10 µM.

7.4.2 Summary and conclusion

Based on recently reported 2-arylbenzimidazoles, providing a primary amine in the side chain and showing high affinity and selectivity for the H_4R, four fluorescence labeled analogs were synthesized and investigated in radioligand binding studies. Aiming at fluorescent pharmacological tools for the H_4R, the different heterocyclylalkyl sidechains used in the published study[10] were replaced by four different fluorescent dyes. Two pyrylium dyes, Py-1 and Py-5, the cyanine dye S0436 and a bodipy dye were used. All synthesized compounds showed fluorescence properties suitable for flow cytometry or confocal microscopy. With respect to detection and investigation of GPCRs low nanomolar affinities are desired. Unfortunately, the K_i values determined in radioligand binding experiments, were above 1 µM at all four histamine receptor subtypes, i. e., neither the affinity nor the receptor subtype selectivity were sufficient to justify more detailed investigations. Obviously, bulky fluorophores combined with benzimidazole-type building blocks are incompatible with high affinity of H_4R ligands. However, regardless of spatial demand, such fluorophore are not necessarily detrimental to GPCR binding, as demonstrated previously for NPY Y_1,[17] Y_2,[20] histamine H_1,[6] H_2[8] and H_3[11] receptors. Therefore, unsatisfactory activities at the H_4R may depend on the selected affinity-conferring building block as well. The pharmacological properties of a labeled compound as a new chemical entity are difficult to predict. As there is a growing demand for fluorescent pharmacological tools for the histamine H_4 receptor, the results of this study suggest to explore the labeling of a set of structurally diverse precursors with high H_4R affinity, using various fluorescent dyes.

7.5 Experimental Section

7.5.1 Chemistry

7.5.1.1 General Conditions

See section 3.4.1.1.

The tetrafluoroborate salts of the pyrylium dyes Py-1 and Py-5 were synthesized in the Institute of Analytical Chemistry, Chemo- and Biosensors at the University of Regensburg (note: these dyes are commercially available from Active Motif Chromeon, www.activemotif.com). The activated (NHS ester) fluorescent dyes S0536 (S0436-NHS) and

Bodipy650/665-X (SE) were obtained from FEW Chemicals (Bitterfeld-Wolfen, Germany) and Molecular Probes (now Invitrogen; Darmstadt, Germany), respectively. The amine **7.2** was synthesized by Paul Baumeister in our laboratories and kindly provided for labeling.
For preparative HPLC see section 6.4.1.1.

7.5.1.2 Preparation of fluorescent ligands 7.3-7.6

General procedure for the coupling of primary amines with pyrylium dyes

The amine precursor **7.2** (3 eq) and NEt_3 (7-8 eq) was dissolved in 500 µL acetonitrile followed by the addition of the pyrylium dye × BF_4^- (1 eq) in a mixture of acetonitrile and DMF (4/1 v/v, total volume 250 µL). The reaction was stopped by addition of 10 % aq. TFA (corresponding to 6-10 eq TFA) after an incubation period of 2 h at room temperature. The product was purified by preparative HPLC.

(*E*)-1-{3-[4-(5-Fluoro-4-methyl-1*H*-benzo[d]imidazol-2-yl)-3-methylphenoxy]propyl}-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium trifluoroacetate; Compound 7.2 labeled with Py-1 (7.3)

Red solid; yield: 64 % (1.41 mg); RP-HPLC (220 nm, gradient and system b, see Appendix): 97.2 % (t_R = 12.0 min, k = 3.49); ES-MS (DCM/MeOH + 10 mM NH_4OAc) *m/z* (%): 601 (60) $[M]^+$, 301 (100) $[M + H]^{2+}$. $C_{39}H_{42}FN_4O^+ \times C_2F_3O_2$ (714.79).

4-{(1*E*,3*E*)-4-[4-(Dimethylamino)phenyl]buta-1,3-dienyl}-1-{3-[4-(5-fluoro-4-methyl-1*H*-benzo[d]imidazol-2-yl)-3-methylphenoxy)propyl)-2,6-dimethylpyridinium trifluoroacetate; Compound 7.2 labeled with Py-5 (7.4)

Red solid; yield: 18 % (0.78 mg); RP-HPLC (220 nm, gradient and system b, see Appendix): 98.1 % (t_R = 10.2 min, k = 2.82); ES-MS (DCM/MeOH + 10 mM NH_4OAc) *m/z* (%): 575 (60) $[M]^+$, 288 (100) $[M + H]^{2+}$. $C_{37}H_{40}FN_4O^+ \times C_2F_3O_2$ (688.75).

General procedure for the coupling of primary amines with succinimidyl esters of fluorescent dyes

The amine precursor **7.2** (3-4 eq) and NEt_3 (7-10 eq) was dissolved in 500 µL acetonitrile followed by the addition of the succinimidyl ester of the dye (1 eq) in a mixture of acetonitrile and DMF (4/1 v/v, total volume 250 µL). The reaction was stopped by addition of

10 % aq. TFA (corresponding to 6-10 eq TFA) after stirring overnight in the dark at room temperature. The product was purified with preparative HPLC.

4-(2-{(1*E*,3*E*,5*E*)-5-[1-(6-{3-[4-(5-Fluoro-4-methyl-1*H*-benzo[d]imidazol-2-yl)-3-methylphenoxy]propylamino}-6-oxohexyl)-3,3-dimethylindolinium-2-ylidene]penta-1,3-dienyl}-1,1-dimethyl-1*H*-isoindolium-3-yl)butane-1-sulfonate trifluoroacetate; Compound 7.2 labeled with S0436 (7.5)

Blue solid; yield: 81 % (1.16 mg); RP-HPLC (220 nm, gradient and system b, see Appendix): 95.2 % (t_R = 12.8 min, k = 3.80); ES-MS (DCM/MeOH + 10 mM NH_4OAc) *m/z* (%): 901 (60) $[M + H]^+$, 451 (100) $[M + 2H]^{2+}$. $C_{53}H_{62}FN_5O_5S \times C_2F_3O_2$ (1014.18).

(*E*)-5,5-Difluoro-3-{4-[2-(6-{3-[4-(5-fluoro-4-methyl-*1H*-benzo[d]imidazol-2-yl)-3-methylphenoxy]propylamino}-6-oxohexylamino)-2-oxoethoxy]styryl}-7-(1*H*-pyrrol-2-yl)-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide; Compound 7.2 labeled with Bodipy (7.6)

Blue solid; yield: 86 % (1.35 mg); RP-HPLC (220 nm, gradient and system b, see Appendix): 95.9 % (t_R = 14.3 min, k = 4.36); ES-MS (DCM/MeOH + 10 mM NH_4OAc) *m/z* (%): 842 (100) $[M + H]^+$. $C_{47}H_{47}BF_3N_7O_4$ (841.73).

7.5.2 Determination of Quantum Yields

Determination of quantum yields was performed with a Cary Eclipse spectrofluorimeter and a Cary 100 UV/VIS photometer (Varian Inc., Mulgrave, Victoria, Australia). The photomultiplier voltage of the Cary Eclipse spectrofluorimeter was set to 400 V throughout. Recording of excitation spectra was performed with an excitation slit of 5 nm and an emission slit of 10 nm. Emission spectra depicted in Figure 7.3 were recorded with an excitation slit of 10 nm and an emission slit of 10 nm. For the determination of quantum yields cresyl violet perchlorate (Acros Organics, Geel, Belgium) was used as a red fluorescent standard, for which a quantum yield of 54 % in ethanol was reported in the literature.[18] It is important to use the perchlorate, because only this salt is provided with sufficiently high purity. Spectra were recorded in acryl cuvettes (10 × 10 mm, Ref. 67.755, Sarstedt, Nümbrecht, Germany). The use of disposable acryl cuvettes instead of glass cuvettes was less time consuming as solutions had not to be transferred when recording fluorescence and

absorption spectra. Quantum yields proved to be nearly unaffected by the cuvette material (glass/acryl). First of all, the concentrations of the fluorescent ligands for the determination of the quantum yields were determined. For this purpose an absorption spectrum was recorded at concentrations in the range of 3-7 µM. For the determination of quantum yields solutions with absorbances between 0.1 and 0.2 at the excitation wavelength were used. The excitation wavelength was chosen as close to the absorption maximum as possible or at a plateau of the absorption spectrum (e.g. in case of S0436 and Bodipy labeled compounds). It was strictly avoided to excite the fluorescent compounds in a flank of the excitation spectrum. Solutions of the fluorescent ligands from 10 mM stock solutions (DMSO) of the compounds were freshly prepared in PBS, PBS + 1 % BSA or ethanol and immediately protected from light. Fluorescence spectra were recorded at two different slit adjustments (excitation/emission): 10/5 nm and 10/10 nm. Spectra of the cresyl violet standard were only recorded in ethanol. For the determination of reference spectra, the pure solvents with the same DMSO content, but without fluorescent compound, were used. The solutions were always maintained in the dark. The emission spectra were recorded within 15 - 20 min at a temperature of 22 °C using the medium scan rate. The filter settings were “auto” for the excitation and “open” for the emission filter. The emission starting point was set 10 nm above the excitation wavelength. From every emission spectrum the corresponding reference spectrum was subtracted, yielding the net spectra, which were multiplied with the corresponding lamp correction spectra. The resulting corrected net spectra were integrated up to 800 nm. The absorbance at the excitation wavelength was determined by recording absorption spectra immediately after the recording of the emission spectra (within 30 min after preparation of test solutions). Baselines were stored using reference solutions and subtracted from the raw spectra. The quantum yield was calculated according to the following equation:

$$\Phi_{F(X)} = (A_s/A_x)\,(F_x/F_s)\,(n_x/n_s)^2\,\Phi_{F(S)}$$

where A_s is the absorbance and F_s the integral of the corrected emission spectrum of the cresyl violet standard solution. A_x and F_x stand for the absorbance and the integral of the corrected emission spectrum of the fluorescent ligand. The refraction indices of the solvents for the fluorescent ligands and the cresyl violet standard are denoted n_x and n_s, respectively. $\Phi_{F(S)}$ is the reported quantum yield of cresyl violet, in this case 54 %.

7.5.3 Pharmacological methods

7.5.3.1 Materials

See section 5.4.3.1.

7.5.3.2 [^{35}S]GTPγS binding assay[21, 22]

See section 3.4.2.2.

7.5.3.3 Radioligand binding assays

See section 5.4.3.4.

7.5.3.4 Data analysis and pharmacological parameters.

See section 5.4.3.7.

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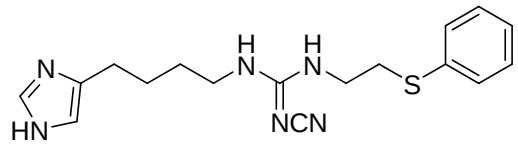
Chapter 8

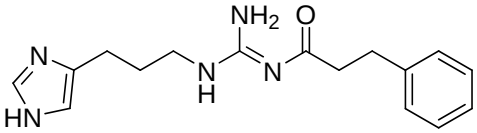
Biopharmaceutical and toxicological investigations on representative histamine H_4 receptor ligands

8.1 Introduction

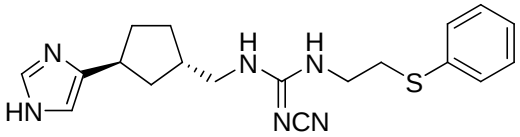
In preliminary studies on the influence of H_4R agonists on antigen presenting cells, performed by Gutzmer and coworkers,[1] inconsistencies were observed concerning potencies of several H_4R ligands. The pEC_{50} values were too low compared to data from functional assays and radioligand binding studies using membrane preparations of Sf9 insect cells. Therefore, and with respect to future investigations of the synthesized compounds in pharmacological test systems with higher complexity and the use of selected ligands as pharmacological tools *in vitro* and *in vivo*, eight representative ligands were evaluated in biopharmaceutical and toxicological studies. The structures of the selected compounds are shown in Figure 8.1. Three potent and selective cyanoguanidine-type agonists, UR-PI376, *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) and *trans*-(+)-(1*S*,3*S*)-UR-RG94 (**5.18b**) were chosen as representatives of the compounds presented in this thesis. Furthermore, two acylguanidine-type non-selective H_4R agonists (UR-AK51[2] and UR-PI294[3]), the non-imidazole agonist VUF-8430[4] and two aminopyrimidines, the partial agonist ST-1006 and the inverse agonist ST-1012 (synthesized in the group of Prof. Holger Stark, Frankfurt am Main, Germany) were included in this study. Most of these molecules comprise a lipophilic fragment (alkyl, aryl residues) and a polar motive (e.g. imidazole, piperazine). Due to these structural features, an interaction with cell membranes or plasma proteins must be considered. Therefore, selected H_4R ligands were evaluated with respect to the induction of hemolysis, the toxicity of selected substances was investigated in a standard chemosensitivity assay, and the plasma protein binding of the compounds was studied.



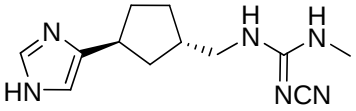
UR-PI376



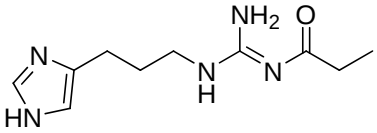
UR-AK51



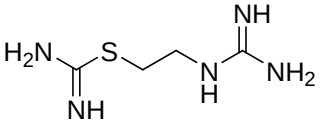
trans-(+)-(1*S*,3*S*)-UR-RG98, **5.26a**



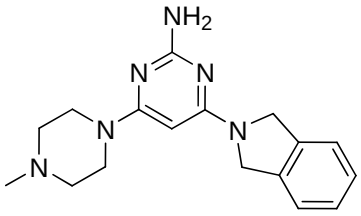
trans-(+)-(1*S*,3*S*)-UR-RG94, **5.18b**



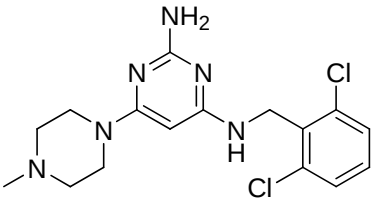
UR-PI294



VUF-8430



ST-1012



ST-1006

Figure 8.1 Structures of the investigated H_4R ligands.

8.2 Materials and methods

8.2.1 Determination of hemolytic properties using human erythrocytes

The determination of hemolytic properties of the compounds was performed as previously described.[5, 6]

8.2.1.1 Isolation and purification of erythrocytes

Isotonic NaCl solution (4 ml) was added to fresh human heparinized blood (8 ml), and the suspension was centrifuged at 4 °C (2,000 *g*, 10 min). After removal of the supernatant plasma and the thin colorless leukocyte-layer using a Pasteur pipette, the erythrocytes were re-suspended in isotonic NaCl solution (8 ml) and centrifuged again (2,000 *g*, 10 min, 4 °C). After removal of the supernatant, the washing procedure was repeated twice. Finally, the

supernatant was discarded and the erythrocytes were stored on ice before use on the same day.

8.2.1.2 Determination of hemolysis

Stock solutions (10 mM) of H_4R ligand dissolved in millipore water or a mixture (v/v) of 50 % DMSO and 50 % millipore water were prepared and diluted to concentrations of 0.5, 1.5 and 5 mM with 70 % EtOH in millipore water. Freshly prepared erythrocytes (500 µl) were suspended in isotonic NaCl solution (9.5 ml) and stored on ice. 50 µl of this suspension and an appropriate volume of the solutions of the compounds were added to a 96-well flat-bottomed microtitration plate (Greiner, Frickenhausen, Germany) to obtain the final concentrations of the test compounds (10, 30 and 100 µM). To achieve 100 % hemolysis, a solution of digitonin in H_2O (0.2 %, w/v) and a solution of Triton X-100 in H_2O (10 %, w/v) were used as reference. For the 0 % value solvents without ligands were added. After careful mixing, the microtitration plate was incubated at 37 °C for 1 h and vortexed periodically every 15 min. The suspensions were centrifuged at 4 °C (2,000 *g*, 10 min), and 30 µl of the supernatant were transferred to a new transparent microtitration plate and diluted with isotonic NaCl solution (100 µl). Absorbance of the samples was measured at 485 nm and 580 nm in a microplate reader (GeniosPro, Tecan Group Ltd., Männedorf, Switzerland). The hemolytic activity (percentage) was calculated according to the following equation:

$$\% \text{ Hemolysis} = 100 \cdot (A_{580/A485} - A0\ \%) / (A100\ \% - A0\ \%)$$

whereas A580 and A485 are the measured absorbances of the sample at the respective wavelength, A0 % is the absorbance of the references with solvent only and A100 % is the absorption of the sample with digitonin.

8.2.2 Chemosensitivity assay

The assay was performed as previously described by Bernhardt et al.[7]: Tumor cell suspensions (100 µl/well) were seeded into 96-well flat-bottomed microtitration plates (Greiner, Frickenhausen, Germany) at a density of ca. 15 cells/microscopic field (magnification 320x). After 2 days of incubation, the culture medium was removed by

suction and replaced by fresh medium (200 µl/well) containing varying H_4R ligand concentrations. On every plate 16 wells served as controls and 16 wells were used per compound concentration. After various periods of incubation the cells were fixed with glutaraldehyde (Merck, Darmstadt, Germany) and stored at 4 °C. At the end of the experiment all plates were stained with crystal violet (Serva, Heidelberg, Germany) simultaneously. Absorbance was measured at 578 nm using a Tecan microplate reader (GeniosPro, Tecan Group Ltd., Männedorf, Switzerland). Growth curves were constructed using SigmaPlot analysis software (Systat Software GmBH, Erkrath,Germany).

8.2.3 Investigations on serum protein binding using HPLC

The HPLC based assay was performed as previously described by Spickenreither.[8] A mixture of binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4), human plasma, DMSO and test compound (500 µL total volume, 2 % DMSO by analogy with the [^{35}S]GTPγS binding assays, 200 µM ligand, 100, 200 or 500 µM serum protein) was incubated for 30 min at 37 °C. 400 µl of the incubation mixture were filtered using Microcon centrifugal filter devices (Microcon YM-10, 10000 MWCO from Micon® Bioseparations, Millipore, Eschborn, Germany). After approximately half of the solution was filtered (13,000 rpm, 13,000 *g*), 100 µl samples of the filtrate, the supernatant and the unfiltered sample were taken and diluted with 100 µl of ice-cold acetonitrile. To complete deproteinisation, the solutions were stored on ice for 30 min and centrifuged again (13,000 rpm, 13,000 *g*, 4 °C, 15 min). 175 µl of the supernatants were diluted with aqueous TFA (0.05 %). The solutions were used for HPLC analysis immediately. As a reference, the same procedure was repeated without human serum (replaced by water). Analytical HPLC analysis was performed on a system from Thermo Separation Products (TSP, Egelsbach, Germany) composed of a SN400 controller, a P4000 pump, an AS3000 autosampler, a degasser (Degassex DG-4400, Phenomenex), a Spectra Focus UV-VIS detector and a RP-column thermostated at 30 °C (Eurosphere-100 C18, 250 × 4.0 mm, 5 µm; Knauer, Berlin, Germany; t_0 = 3.32 min). UV-detection was done at 220 nm. Mixtures of acetonitrile and 0.05 % aq. TFA were used as mobile phase. Helium degassing was used throughout. The relative amount of compound in the sample was calculated as percentage peak area of the analyzed compound by UV detection at 220 nm. 50 µl of each sample were injected.

8.3 Results and discussion

8.3.1 Hemolytic properties of selected H_4R ligands

Amphiphilic compounds may induce hemolysis, by disruption of red blood cells, leading to a release of hemoglobin into the surrounding fluid. As mentioned in the introduction, at least some of the selected compounds possess structural features that may induce hemolysis. Thus, hemolytic properties were investigated as a prerequisite for further investigations. Figure 8.2 shows the percentage of hemolysis compared to the reference compound digitonin, which is known to be strongly hemolytic[9] (see Figure 8.1 for structures of investigated H_4R ligands). The detergent Triton X-100 was also investigated for comparative reasons.

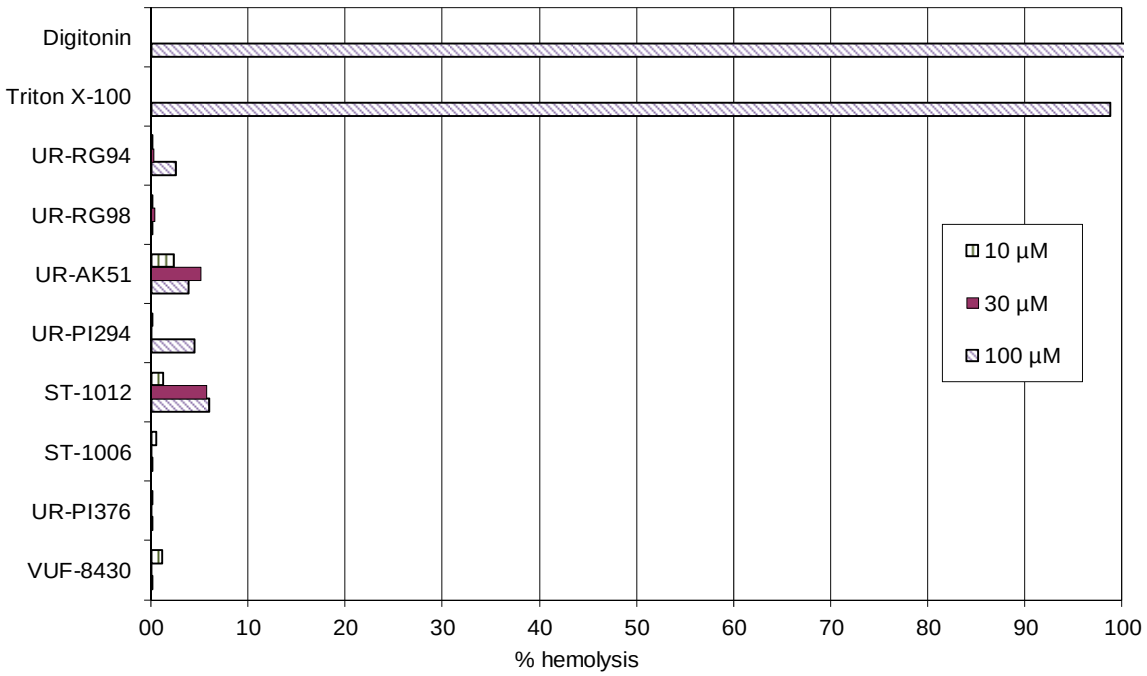


Figure 8.2 Hemolysis induced by H_4R ligands at a concentration of 10, 30 and 100 µM and Triton X-100 in % values at various concentrations compared to digitonin.

Compared to digitonin and Triton X-100, all investigated histamine receptor ligands did not induce significant hemolysis, even at the highest concentrations tested (100 µM). For the acylguanidines UR-AK51 and UR-PI294, and for the aminopyrimidine ST-1012 hemolysis was observed in the range of 5 %. For the cyanoguanidine *trans*-(+)-(1*S*,3*S*)-UR-RG94 (**5.18b**) only in the highest concentration a weak effect was detected. The other ligands did not induce hemolysis at all. A clear structure-hemolysis relationship was not observed. Taken together,

with respect to *in vivo* experiments, hemolysis should not influence the investigations of histamine H_4 receptor ligands up to a concentration of 100 µM.

8.3.2 Cytotoxicity of representative histamine receptor ligands

As a prerequisite for application as pharmacological tools *in vivo*, several H_4R ligands were tested with respect to cytotoxicity in the crystal violet based chemosensitivity assay.[7] The colon carcinoma cell line HT-29 was used for these investigations. Cisplatin was taken as reference compound. Structures of investigated compounds are shown in Figure 8.1. Exemplary data is depicted in Figure 8.3.

The reference compound cisplatin clearly caused a concentration dependent anti-proliferative effect (Figure 8.3), whereas cell proliferation was not affected by all of the investigated H_4R ligands up to a concentration of 10 µM (see Figure 8.3 for exemplary data). Hence, cytotoxic effects of the investigated ligands will not hamper future investigations in biological test systems and their use as pharmacological tools.

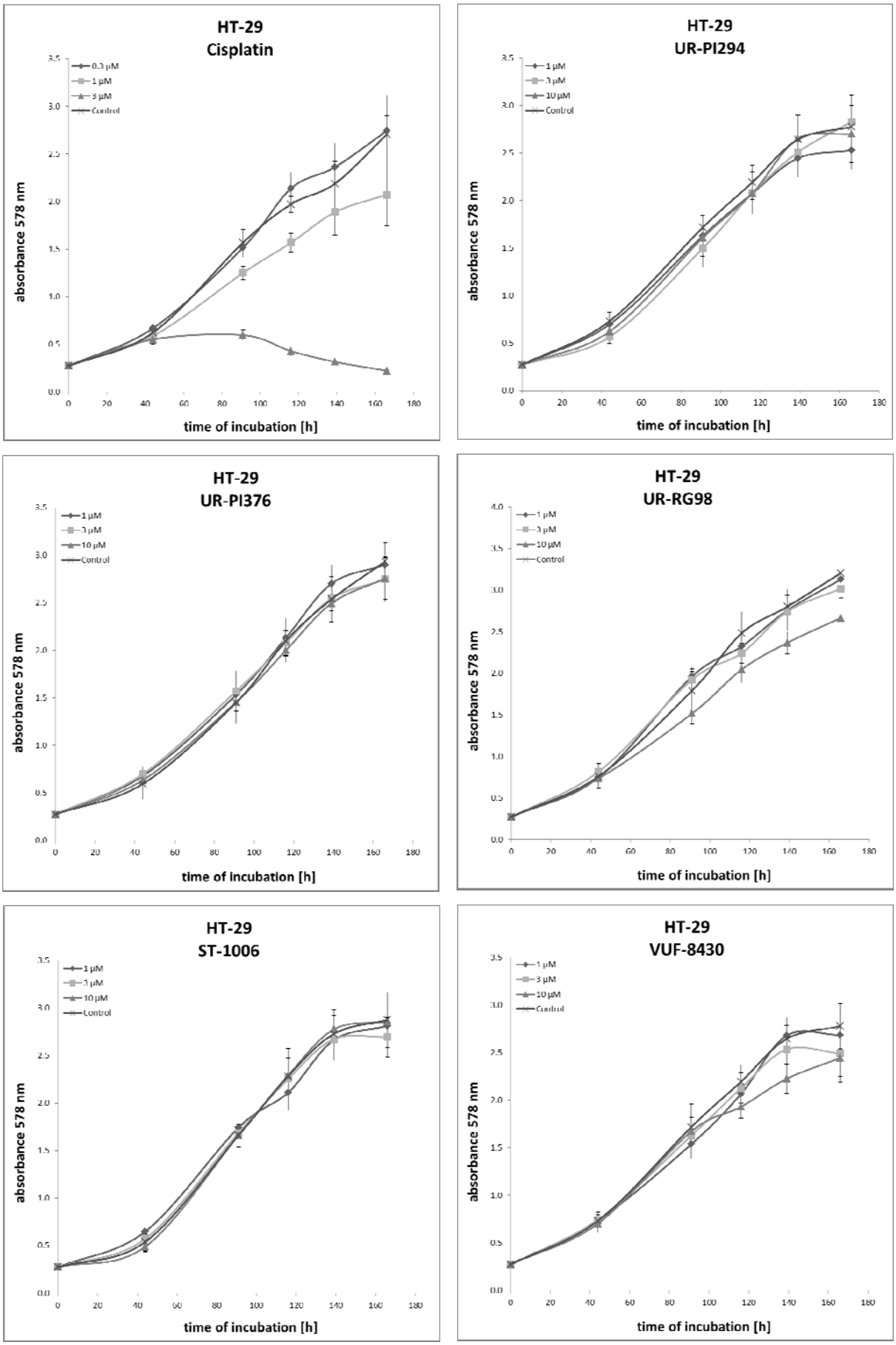


Figure 8.3 Proliferation of HT-29 cells in the presence of various concentrations of cisplatin or selected H_4R ligands.

8.3.3 Determination of the plasma-protein binding

Extensive investigations on the binding properties of low molecular weight compounds to serum albumin proved that especially anionic and lipophilic drugs show high binding to serum albumin.[10] As the investigated H_4R ligands, in particular the cyanoguanidines, possess lipophilic motives, and are in general rather unpolar compounds, the protein binding was investigated. An HPLC based approach was carried out: a mixture containing binding buffer, human plasma (which contains human serum albumin as major protein component), DMSO and ligand (see Figure 8.1) was incubated at 37 °C and then filtered using a cutoff of at 10 kDa to remove serum albumin. The concentration of serum proteins was increased from approximately 100 to 200 and to 500 µM in the incubation mixture; the inhibitor concentration was 200 µM. As a control experiment, the same procedure was carried out with solutions containing water instead of plasma. After preparation for analytical HPLC, samples before filtration, and samples from the filtrate as well as from the supernatant were analyzed. In the control experiment without serum, the inhibitor was able to pass the membrane. The correlation between plasma concentration and plasma-protein binding is shown in Figure 8.4. The results are summarized in Table 8.1. Representative HPLC-traces for the plasma samples are depicted in Figure 8.5 and Figure 8.6.

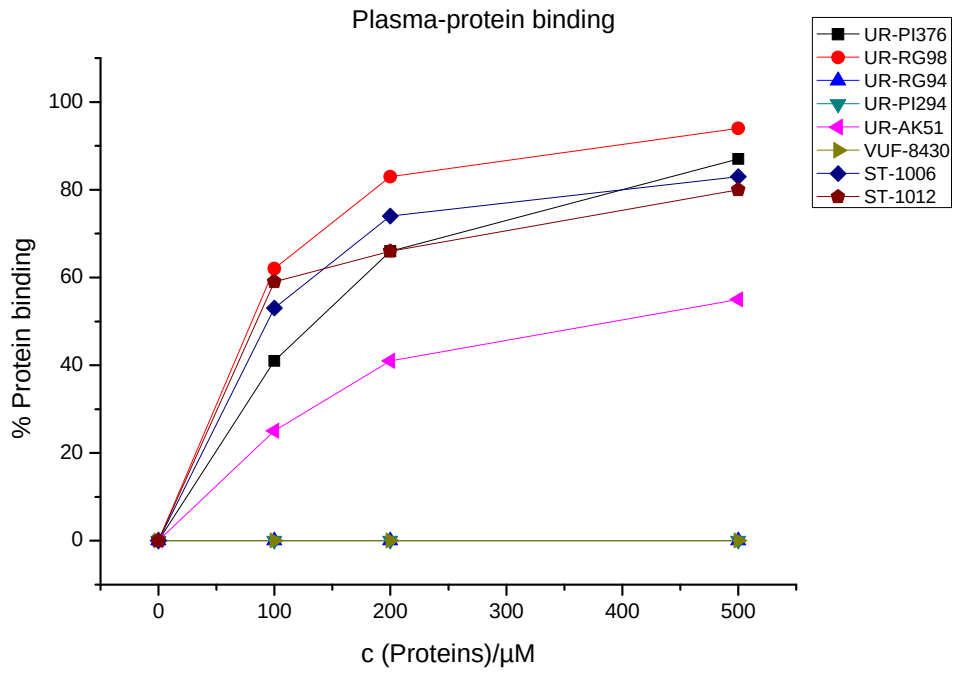
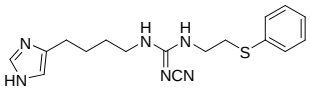
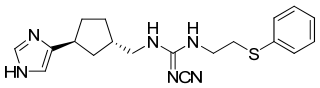
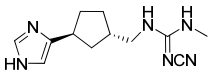
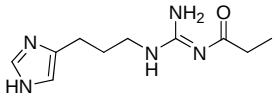
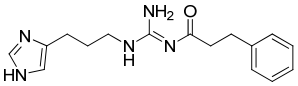
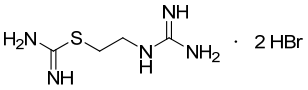
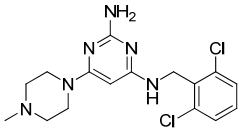
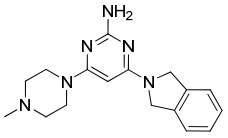


Figure 8.4 Correlation between the amount of compound bound to plasma protein and protein concentration in the incubation mixture.

Table 8.1 Structures of the investigated compounds and the percentage of plasma-protein binding.

	Structure	Plasma-protein binding ± 5 %
UR-PI376		87 %
UR-RG98 (5.26a)		94 %
UR-RG94 (5.18b)		-
UR-PI294		-
UR-AK51		55 %
VUF-8430		-
ST-1006		83 %
ST-1012		80 %

As shown in Figure 8.6 a higher concentration of *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) was found in the supernatant when compared to the sample without filtration. In the filtrate only a very small amount of the agonist was detectable, indicating that a part of the compound was bound to serum proteins and therefore did not pass the membrane in the filtration step. To evaluate the percentage of bound ligand under physiological conditions the whole procedure was repeated with higher concentrations of human plasma. The plasma-protein binding was calculated to be 94 % for compound **5.26a** which was the

highest value identified in the investigated set of ligands. As expected, similar amounts of the flexible analogue of **5.26a**, UR-PI376 were bound to serum protein. For the acylguanidine UR-AK51, bearing an aromatic moiety in the eastern part of the molecule, a value of 55 % was determined. The two aminopyrimidines ST-1006 and ST-1012 exhibit comparable results and approx. 80 % of the compounds bind to serum protein. The three most polar compounds, the ionic VUF-8430 and the two guanidine derivatives with small alkyl substituents did not bind to serum protein. Due to the fact that the binding sites of serum albumin, the main protein in human plasma, are mostly formed by hydrophobic and positively charged residues,[10] it was expected that unpolar compounds bind more efficiently than polar, positively charged compounds. The results are in full agreement with this assumption and with data from previous studies.[8] Taken together, the experiments show that the investigated ligands share the fate of many other drugs and bind to serum proteins. Surely, these results must be considered, when the ligands are used in *in vivo* experiments, but the amount of compound bound to serum protein is in a range, that makes it unlikely that the aforementioned effects (see introduction) are caused by plasma-protein binding. Due to the high potency of these drugs, the amount of unbound drug should still be sufficient to generate the desired effect. However, especially in the case of the investigated cyanoguanidines and aminopyrimidines, the effect of plasma-protein binding might influence the potencies, efficacies and the quality of action in various pharmacological experiments. For further ligand development, and particularly when searching for future drugs, improved drug-like properties should be considered.

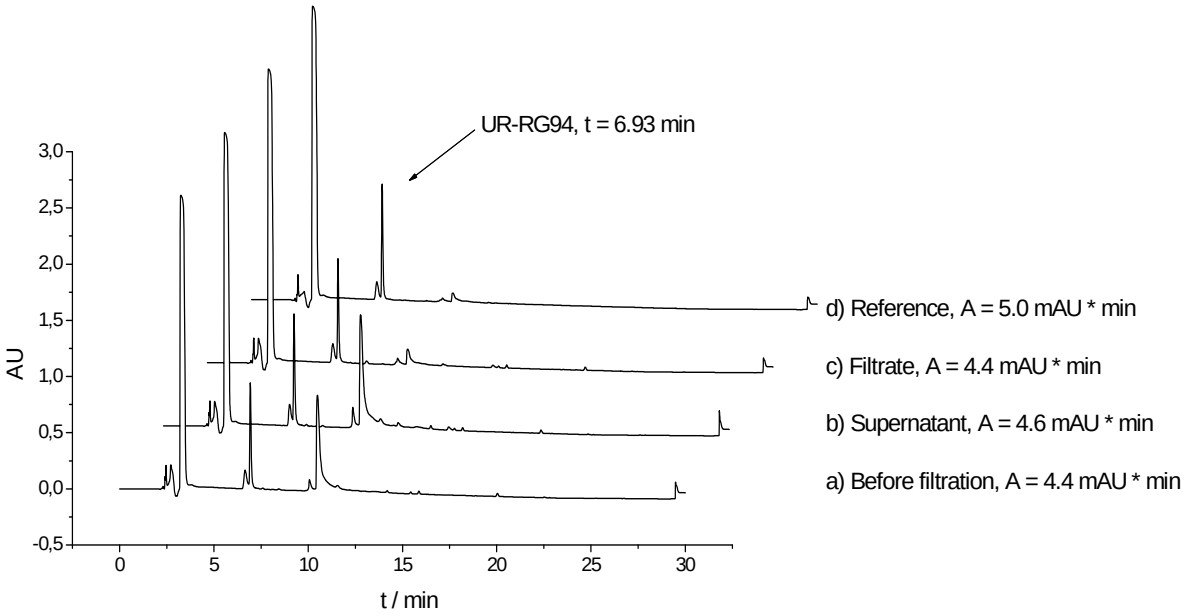


Figure 8.5 HPLC traces of samples containing *trans*-(+)-(1*S*,3*S*)-UR-RG94 (**5.18b**) in presence of human plasma taken a) before filtration, b) from the supernatant and c) from the filtrate. d) Reference run without human plasma. The area under the curve (A) is given for the ligand peak.

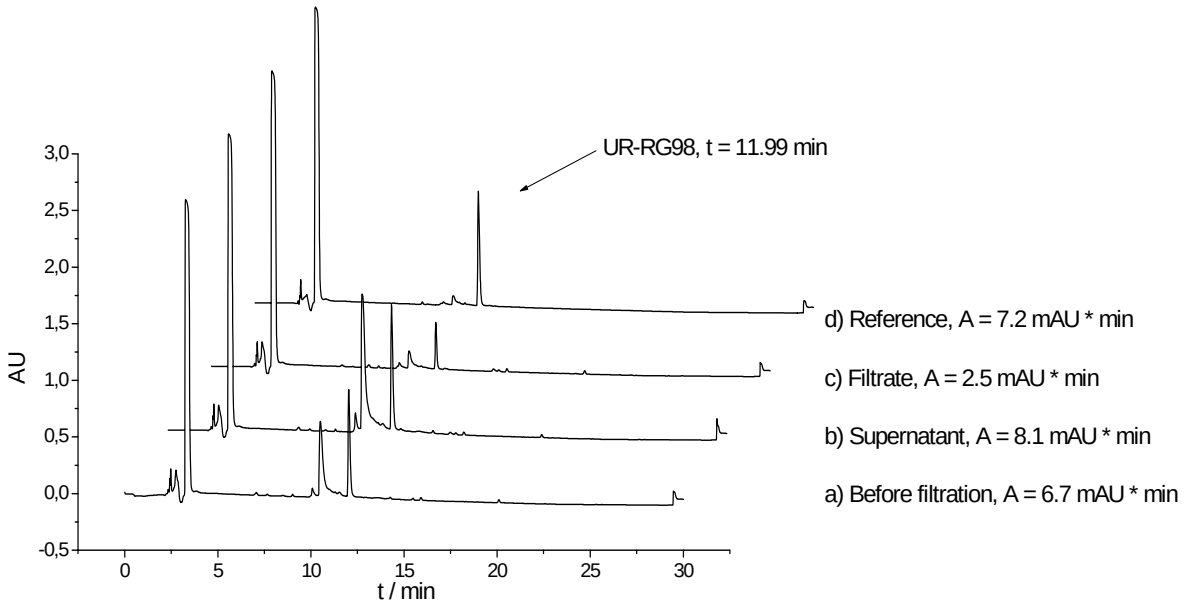


Figure 8.6 HPLC traces of samples containing *trans*-(+)-(1*S*,3*S*)-UR-RG98 (**5.26a**) in presence of human plasma taken a) before filtration, b) from the supernatant and c) from the filtrate. d) Reference run without human plasma. The area under the curve (A) is given for the ligand peak.

8.3.4 Summary and conclusion

In view of planned *in vivo* experiments using the synthesized ligands, the hemolytic properties and the toxicity of selected compounds were determined: some of the compounds induced very weak hemolytic effects at higher concentrations. Nevertheless, the compounds were generally inconspicuous at concentrations up to 100 µM. In the chemosensitivity assay using HT-29 cells the investigated compounds did not show any cytotoxic effects. Thus, the presented ligands should be applicable *in vivo*. The investigation of protein binding revealed that the synthesized H_4R ligands as well as the selected reference compounds bind to plasma proteins and thus share the fate of well-known drugs having a high affinity to various proteins. The determined values were between 55 and 94 %, depending on the lipophilicity of the compounds. The three most polar compounds did not bind to serum protein. When the ligands are used in the presence of high amounts of protein, especially *in vivo*, it is recommended to take these findings into account.

8.4 References

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Chapter 9

Summary

The recently discovered histamine H_4 receptor (H_4R) is reported to be involved in immunological processes and inflammatory diseases. However, the (patho)physiological role of the H_4R is far from being fully understood. Controversial data on H_4R agonists as well as reports on β-arrestin-mediated signaling and partial agonistic effects of the standard H_4R antagonist JNJ-7777120 at certain H_4R species orthologs complicate the interpretation of *in vivo* studies. Therefore, additional potent and receptor subtype selective H_4R ligands, antagonists as well as agonists, are required as pharmacological tools.

Discrimination between the closely related H_3 and H_4 receptors turned out to be a critical issue in the development of selective H_4R agonists. Starting from UR-PI376, a lead from our laboratory, the major objective of this thesis was the design, synthesis and pharmacological characterization of bioisosteric and conformationally constrained cyanoguanidine-type H_4R agonist to evaluate structure-activity and –selectivity relationships. The prepared compounds were investigated for agonism and antagonism at the human (h) H_1R, H_2R, H_3R and H_4R in functional [^{35}S]GTPyS binding assays or steady-state GTPase assays, respectively, using Sf9 cell membranes expressing the HR subtype of interest. Selected compounds were evaluated in radioligand binding studies at the human HR subtypes (Sf9 cell membranes) and mouse H_4R (mH_4R expressed in HEK293 cells). In addition, representative compounds were investigated in a luciferase gene reporter assay at mH_4R and hH_4R using genetically engineered HEK293 cells.

In a first approach, based on a previously suggested model of UR-PI376 binding to the hH_4R, the tetramethylene chain in UR-PI376 was replaced by conformationally restricted spacers connecting imidazole ring with the cyanoguanidine moiety. Phenylene linkers proved to be inappropriate: None of the prepared compounds or amine precursor showed relevant activity at the H_3R and H_4R. To retain some flexibility, in a second series the phenylene was replaced by a cyclohexylene linker. Compounds with a *cis*-configured 1,4-cyclohexylene spacer, turned out to be moderately potent and selective hH_4R agonists. The same holds for the building block, *cis*-4-(1*H*-imidazol-4-yl)cyclohexylamine. In case of the *trans*-configured analogues, there was a tendency toward preference for the hH_3R.

To optimize the spacer regarding ring size, balance between rigidification and flexibility, regioisomers and stereochemical properties a cyclopentane-1,3-diyl moiety and an

additional exocyclic methylene group were introduced. The most potent H_4R agonists identified among a set of 14 racemic compounds were separated by chiral HPLC to yield eight enantiomerically pure compounds. *trans*-(+)-(1*S*,3*S*)-UR-RG98 was the most potent and selective H_4R agonist in this series with an EC_{50} of 11 nM, a more than 100-fold selectivity for the H_4R over the H_3R and negligible activities at the other HR subtypes. By contrast, the optical antipode, *trans*-(-)-(1*R*,3*R*)-UR-RG98, proved to be an H_4R antagonist in the [^{35}S]GTPyS assay. The absolute configuration of the stereoisomers was determined subsequent to an enzyme-assisted stereoselective synthesis.

In search for bioisosteric replacements of the imidazole ring in cyanoguanidine-type H_4R agonists, ten different heterocycles were combined with linkers of various chain lengths and substitution patterns. A total of 42 compounds, cyanoguanidines and amine precursor, were synthesized and evaluated at the HR subtypes. The exchange of the 4-imidazolyl ring considerably affected the activities at all HRs. Only the 2- and 5-methyl substituted imidazoles showed H_4R agonistic potency. None of the investigated compounds was superior to UR-PI376.

In an attempt to synthesize fluorescent H_4R ligands several fluorophores (pyrylium, bodipy and cyanine dyes) were coupled to a benzimidazole building block, reported to have high H_4R affinity. The K_i-values of the labeled compounds were only in the micromolar range (1.3 - 30 µM) at the hH_4R and the other HR subtypes as well, suggesting that a different affinity-conferring moiety should be selected.

The investigation of selected H_4R agonists revealed neither cytotoxicity nor hemolytic activity but remarkable plasma protein binding (up to 94 %), which has to be taken into account in future *in vivo* investigations.

In summary, the structural variations of the cyanoguanidine-type H_4R ligands produced valuable information regarding structure-activity and structure-selectivity relationships and led to several promising pharmacological tools. The identified hH_4R agonist *trans*-(+)-(1*S*, 3*S*)-UR-RG98 is one of the most potent and selective H_4R ligands identified so far. The optimized stereochemical properties of the ligands provide valuable information with respect to future ligand design and refined ligand-receptor models.

Chapter 10

Appendix

10.1 Data analysis and pharmacological parameters

All data are presented as mean of N independent experiments ± SEM. Agonist potencies were given as EC_{50} values (molar concentration of the agonist causing 50 % of the maximal response) or as pEC_{50} values (negative decadic logarithm of the EC_{50} value). Maximal responses (intrinsic activities) were expressed as α values. The α value of histamine was set to 1.00, α values of other compounds were referred to this value.

IC_{50} values were converted to K_i and K_B values using the Cheng-Prussoff equation[1]. K_i values were analyzed by nonlinear regression and best fit to one-site (monophasic) competition curves. EC_{50}/K_B values from the functional [^{35}S]GTPγS and GTPase assays were analyzed by nonlinear regression and best fit to sigmoidal dose-response curves (GraphPad Prism 5.0 software, San Diego, CA).

10.2 Elemental analysis data

No.	formula	calculated (%)			found (%)		
		C	H	N	C	H	N
JNJ-7777120	$C_{14}H_{16}ClN_3O$	60.54	5.81	15.13	60.47	5.93	15.24
VUF-8430	$C_4H_{11}N_5S \cdot 2\,HBr$	14.87	4.06	21.68	14.5	4.24	21.29
Thioperamide	$C_{15}H_{24}N_4S \cdot HCl \cdot 0.7\,H_2O$	52.75	7.79	16.41	52.54	7.74	16.73
3.5	$C_9H_9N_3 \cdot 1.9\,HCl$	47.31	4.81	18.39	47.80	4.83	18.04
3.19	$C_{10}H_{11}N_3 \cdot 2\,HCl$	48.80	5.32	17.07	48.73	5.44	16.89
3.20	$C_9H_9N_3 \cdot 2\,HCl$	46.57	4.78	18.10	46.34	4.68	18.02
3.26	$C_8H_{11}NS \cdot HCl \cdot 0.2\,H_2O$	49.71	6.47	7.25	49.70	6.47	7.17
3.32	$C_{13}H_{14}N_6 \cdot 0.75\,CH_3OH$	59.34	6.16	30.20	60.50	5.79	29.81
3.33	$C_{15}H_{16}N_6 \cdot 1.5\,CH_3OH$	60.35	6.75	26.59	60.26	6.42	25.64
3.34	$C_{16}H_{20}N_6$	64.61	7.18	28.47	64.61	7.18	28.47
3.35	$C_{21}H_{22}N_6 \cdot 0.3\,CH_3OH$	69.51	6.35	22.83	69.67	6.47	22.70

Elemental analysis data (continued)

3.36	$C_{20}H_{20}N_6S \cdot 0.5\,CH_3OH \cdot 0.8\,H_2O$	60.51	5.85	20.65	60.49	6.24	20.58
3.37	$C_{12}H_{12}N_6 \cdot 0.4\,CH_3OH \cdot 0.6\,H_2O$	56.44	5.65	31.85	56.20	5.80	32.17
3.38	$C_{14}H_{14}N_6 \cdot 0.6\,CH_3OH$	61.42	5.79	29.43	61.24	5.90	29.34
3.39	$C_{15}H_{18}N_6 \cdot 0.4\,CH_3OH$	62.67	6.69	28.47	62.41	6.78	28.79
3.40	$C_{20}H_{20}N_6 \cdot 0.5\,H_2O$	67.97	5.99	23.78	67.76	5.98	24.05
3.41	$C_{19}H_{18}N_6S \cdot 0.1\,CH_3OH$	62.74	5.07	22.98	62.34	5.18	23.14
3.42	$C_{12}H_{12}N_6 \cdot 0.5\,CH_3OH \cdot 0.3\,H_2O$	57.37	5.62	32.11	57.44	5.81	32.05
3.43	$C_{14}H_{14}N_6 \cdot 0.9\,CH_3OH$	60.64	6.01	28.74	60.79	6.05	28.14
3.44	$C_{15}H_{18}N_6 \cdot 0.75\,CH_3OH$	61.74	6.91	27.43	63.01	6.82	27.08
3.45	$C_{20}H_{20}N_6 \cdot 0.6\,CH_3OH$	68.04	6.21	23.11	68.5	6.31	22.75
4.2	$C_9H_{15}N_3 \cdot 2\,HCl \cdot H_2O$	42.2	7.48	16.4	42.63	7.65	16.09
4.3	$C_9H_{15}N_3 \cdot 2\,HCl \cdot 0.1\,H_2O$	45.05	7.22	17.51	44.88	7.73	17.13
4.10	$C_{12}H_{18}N_6 \cdot 0.55\,H_2O$	56.25	7.51	32.8	55.89	7.27	33.18
4.11	$C_{12}H_{18}N_6 \cdot 0.65\,H_2O$	55.86	7.54	32.57	55.70	7.03	32.92
4.12	$C_{14}H_{24}N_6S \cdot 0.25\,H_2O$	61.18	6.62	22.53	60.92	6.21	22.48
4.13	$C_{14}H_{24}N_6S \cdot 0.25\,H_2O$	61.18	6.62	22.53	60.73	6.56	22.96
5.16	$C_9H_{15}N_3 \cdot 2\,HCl \cdot 0.2\,H_2O$	44.71	7.25	17.38	44.82	7.33	17.46
5.17	$C_9H_{15}N_3 \cdot 2\,HCl \cdot 0.2\,H_2O$	44.71	7.25	17.38	44.73	7.10	17.22
5.18	$C_{12}H_{18}N_6 \cdot CH_3OH \cdot 0.5\,H_2O$	54.34	8.07	29.25	54.39	7.67	28.93
5.19	$C_{12}H_{18}N_6 \cdot 0.2\,CH_3OH \cdot 1.2\,H_2O$	53.41	7.79	30.63	53.41	7.42	30.28
5.20	$C_{14}H_{20}N_6 \cdot 0.4\,CH_3OH$	60.65	7.63	29.47	60.87	7.46	29.33
5.21	$C_{14}H_{20}N_6 \cdot 0.35\,CH_3OH$	60.78	7.61	29.64	61.48	7.53	29.35

Elemental analysis data (continued)

5.22	$C_{15}H_{24}N_6 \cdot 0.5\ CH_3OH$	61.16	8.61	27.61	62.57	8.54	27.22
5.23	$C_{15}H_{24}N_6 \cdot 0.5\ CH_3OH$	61.16	8.61	27.61	62.60	8.46	27.11
5.24	$C_{20}H_{26}N_6 \cdot 0.6\ CH_3OH$	66.93	7.74	22.73	66.89	7.59	22.62
5.25	$C_{20}H_{26}N_6 \cdot 0.55\ CH_3OH$	67.06	7.72	22.83	67.45	7.63	22.43
5.26	$C_{19}H_{24}N_6S \cdot 0.3\ CH_3OH$	61.31	6.72	22.23	61.41	6.58	21.95
5.27	$C_{19}H_{24}N_6S \cdot 0.4\ CH_3OH$	61.11	6.77	22.04	61.10	6.57	21.90
5.28	$C_{19}H_{24}N_6O \cdot 0.5\ CH_3OH$	63.57	7.11	22.81	63.42	7.08	22.73
5.29	$C_{19}H_{24}N_6O \cdot 0.5\ CH_3OH$	63.57	7.11	22.81	63.73	7.05	22.50
5.30	$C_{19}H_{25}N_7 \cdot 0.75\ CH_3OH$	63.18	7.52	26.11	63.79	7.47	25.77
5.31	$C_{19}H_{25}N_7 \cdot 0.7\ CH_3OH$	63.29	7.49	26.22	63.33	7.55	25.94
5.48 *trans*	$C_{47}H_{43}N_3 \cdot 0.1\ H_2O$	86.62	6.68	6.45	86.3	6.8	6.41
5.48 *cis*	$C_{47}H_{43}N_3$	86.86	6.67	6.47	86.78	6.92	6.36
5.49	$C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.2\ H_2O$	44.71	7.25	17.38	44.82	7.33	17.46
5.50	$C_9H_{15}N_3 \cdot 2\ HCl \cdot 0.2\ H_2O$	44.71	7.25	17.38	44.73	7.10	17.22
6.3	$C_{14}H_{15}NO_3$	68.56	6.16	5.71	68.74	6.33	5.7
6.5	$C_{14}H_{14}BrNO_3$	51.87	4.35	4.32	51.86	4.36	4.32
6.6	$C_{13}H_{12}BrNO_3$	50.34	3.9	4.52	51.09	3.84	4.48
6.9	$C_8H_{15}N_3 \cdot 0.85\ H_2O$	57.01	9.99	24.93	57.22	9.62	24.48
6.10	$C_7H_{13}N_3 \cdot 0.5\ H_2O \cdot 0.4\ CH_3OH$	55.2	9.77	26.1	55.03	10.02	26.2
6.14	$C_{15}H_{16}BrNO_3$	53.27	4.77	4.14	53.57	4.76	4.03
6.15	$C_{16}H_{17}N_3O_2$	67.83	6.05	14.83	67.56	6.38	14.9
6.16	$C_8H_{15}N_3 \cdot 0.6\ N_2H_4 \cdot 3\ HCl$	34.09	7.3	20.87	33.9	7.31	20.75

Elemental analysis data (continued)

6.18	$C_{14}H_{13}NO_2$	73.99	5.77	6.16	74.09	5.86	6.17
6.20	$C_6H_{12}N_4$	51.41	8.63	39.97	51.06	8.57	39.91
6.26	$C_6H_{13}N_5 \cdot 1.9\ TFA \cdot 0.4\ H_2O$	31.05	4.2	18.48	30.66	4.6	18.88
6.29	$C_{16}H_{22}N_4O_3$	60.36	6.97	17.6	60.19	7.06	17.69
6.30	$C_{17}H_{24}N_4O_3$	61.43	7.28	16.86	61.15	7.46	16.89
6.31	$C_{10}H_{20}N_6O_2$	46.86	7.87	32.79	46.44	8.5	33.09
6.32	$C_{11}H_{22}N_6O_2$	48.87	8.2	31.09	48.62	8.49	31.65
6.33	$C_5H_{12}N_6 \cdot 2.5\ HCl \cdot 0.3\ H_2O$	23.76	6.02	33.25	23.44	6.44	33.62
6.34	$C_6H_{14}N_6 \cdot 2.5\ HCl \cdot 0.5\ H_2O$	26.65	6.52	31.08	26.6	6.91	31.24
6.37	$C_{10}H_{19}N_5O_2S$	43.94	7.01	25.62	43.59	7.5	25.39
6.38	$C_5H_{11}N_5S \cdot 1.9\ HCl$	24.76	5.36	28.88	24.48	6.05	29.01
6.40	$C_8H_{13}N_3 \cdot 0.65\ H_2O$	58.98	8.85	25.79	59.33	8.93	25.48
6.41	$C_9H_{15}N_3 \cdot 0.6\ H_2O$	61.4	9.28	23.87	61.46	9.35	23.87
6.47	$C_6H_{11}N_5 \cdot 2\ HCl$	31.87	5.8	31.36	31.71	6.04	31.53
6.48	$C_7H_{13}N_5 \cdot 2.5\ HCl \cdot 0.8\ H_2O$	30.73	6.36	25.75	30.82	6.32	25.67
6.49	$C_7H_{13}N_5 \cdot 2\ HCl$	35.01	6.3	29.16	34.71	6.34	29.26
6.50	$C_6H_{11}N_5 \cdot 2\ HCl$	31.87	5.8	31.36	32.19	5.84	30.32
6.52	$C_{11}H_{18}N_6 \cdot 0.95\ H_2O$	52.55	7.98	33.43	52.25	7.83	33.74
6.53	$C_{18}H_{24}N_6S \cdot 0.5\ H_2O$	59.15	6.89	22.99	58.94	6.65	23.22
6.54	$C_{10}H_{16}N_6 \cdot 0.5\ H_2O \cdot 0.2\ CH_3OH$	51.98	7.61	35.66	52.01	7.46	35.66
6.55	$C_{17}H_{22}N_6S \cdot 0.4\ CH_3OH$	58.82	6.7	23.65	58.49	6.67	23.97
6.56	$C_{11}H_{18}N_6 \cdot 0.9\ H_2O$	52.74	7.97	33.55	52.67	7.7	33.91

Elemental analysis data (continued)

6.57	$C_{18}H_{24}N_6S \cdot 0.4\ H_2O$	59.44	6.87	23.11	59.44	6.47	23.15
6.58	$C_9H_{15}N_7 \cdot 0.3\ CH_3OH$	48.38	7.07	42.47	48.23	7.31	42.66
6.59	$C_{16}H_{21}N_7S \cdot 0.2\ CH_3OH$	55.61	6.28	28.02	55.8	6.48	27.9
6.60	$C_9H_{16}N_8 \cdot 0.7\ H_2O$	43.43	7.05	n.d.	43.34	6.95	n.d.
6.61	$C_{16}H_{22}N_8S \cdot 0.6\ H_2O$	52.04	6.33	30.34	51.99	6.34	30.36
6.62	$C_8H_{15}N_9 \cdot 1.1\ H_2O$	37.38	6.74	49.04	37.74	6.43	48.63
6.63	$C_{15}H_{21}N_9S \cdot 0.2\ CH_3OH \cdot 0.7\ H_2O$	48.24	6.18	33.31	48.53	6.1	33.02
6.64	$C_9H_{17}N_9 \cdot 0.5\ H_2O$	41.53	6.97	48.43	41.07	6.77	48.82
6.65	$C_{16}H_{23}N_9S \cdot 1.3\ H_2O$	48.42	6.5	31.76	48.57	6.33	31.65
6.66	$C_8H_{14}N_8S \cdot 0.8\ CH_3OH$	37.75	6.19	40.03	37.64	5.89	39.98
6.67	$C_{15}H_{20}N_8S_2 \cdot CH_3OH$	47.04	n.d.	27.43	47.21	n.d.	27.13
6.68	$C_{11}H_{16}N_6 \cdot 0.3\ H_2O$	55.58	7.04	35.36	55.67	7.18	35.15
6.69	$C_{18}H_{22}N_6S \cdot 0.2\ CH_3OH$	60.57	6.37	23.29	60.31	6.48	23.57
6.70	$C_{12}H_{18}N_6 \cdot 0.4\ CH_3OH$	57.47	7.62	32.43	57.24	7.74	32.55
6.71	$C_{19}H_{24}N_6S \cdot 0.35\ CH_3OH$	61.21	6.74	22.13	60.82	6.93	22.52
6.77	$C_{12}H_{20}N_6 \cdot 0.2\ H_2O$	57.21	8.16	33.36	56.82	8.53	33.64
6.78	$C_{19}H_{26}N_6S \cdot 0.7\ H_2O$	59.56	7.21	21.94	59.58	7.26	22.15

10.3 HPLC purity data

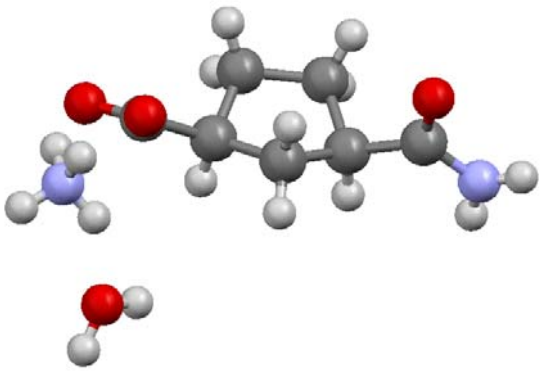
No.	t_R (min)	k'	purity (%)	No.	t_R (min)	k'	purity (%)
JNJ-7777120[c]	12.52	2.27	100	**5.31**[a]	9.27	1.78	99.4
Thioperamide[a]	11.35	2.42	98.6	**6.9**[b]	6.80	1.54	96.5
3.5[b]	3.66	0.37	97.2	**6.10**[b]	3.65	0.36	96.1
3.19[b]	3.67	0.37	98.6	**6.16**[b]	6.74	1.52	95.3
3.20[b]	3.67	0.37	97.4	**6.20**[a]	3.98	0.25	97.4
3.32[a]	7.49	1.26	96.6	**6.26**[a]	5.14	0.55	95.9
3.33[a]	8.43	1.54	99.4	**6.33**[a]	3.33	0.10	100
3.34[a]	10.04	2.03	99.3	**6.34**[a]	3.69	0.11	100
3.35[a]	12.06	2.63	99.5	**6.38**[a]	3.87	0.17	100
3.36[a]	12.02	2.62	99.2	**6.40**[a]	3.87	0.14	98.7
3.37[a]	6.67	1.01	100	**6.41**[a]	3.90	0.18	98.5
3.38[a]	7.72	1.33	100	**6.52**[b]	4.90	0.83	98.8
3.39[a]	9.71	1.93	99.7	**6.53**[b]	9.31	2.48	96.1
3.40[a]	12.28	2.63	98.0	**6.54**[b]	4.06	0.52	97.9
3.41[a]	11.85	2.57	100	**6.55**[b]	8.99	2.36	95.3
3.42[a]	6.83	1.06	97.5	**6.56**[b]	5.22	0.95	98.7
3.43[a]	7.85	1.37	97.8	**6.57**[b]	9.39	2.51	95.9
3.44[a]	9.86	1.97	90.2	**6.58**[a]	7.47	1.35	100
3.45[a]	12.11	2.65	90.8	**6.59**[a]	13.28	3.17	100
3.46[a]	12.00	2.62	82.8	**6.60**[a]	5.87	0.77	94.8
4.2[b]	5.25	0.96	100	**6.61**[a]	11.11	2.35	96.9
4.3[b]	4.78	0.79	100	**6.62**[a]	5.34	0.61	96.4
4.10[b]	5.31	0.98	97.2	**6.63**[a]	10.79	2.25	99.2
4.11[b]	5.10	0.91	99.2	**6.64**[a]	5.85	0.76	98.6
4.12[b]	9.59	2.59	97.1	**6.65**[a]	11.06	3.33	98.1

4.13[b]	9.56	2.57	99.3	**6.66**[b]	5.07	0.90	95.4
5.14/5.15[d]	38.03	8.93	95.9	**6.67**[a]	11.97	2.61	95.0
5.16[b]	3.67	0.37	95.1	**6.68**[a]	6.24	0.88	98.6
5.17[b]	3.67	0.37	96.1	**6.69**[a]	11.61	2.50	97.5
5.18[a]	6.99	1.11	98.8	**6.70**[a]	6.83	1.06	100
5.19[a]	6.99	1.11	98.4	**6.71**[a]	12.09	2.64	99.0
5.20[a]	8.00	1.41	99.2	**6.72**[b]	3.82	0.43	98.2
5.21[a]	8.02	1.41	98.3	**6.73**[b]	8.84	2.31	95.7
5.22[a]	9.91	1.99	100	**6.74**[b]	4.67	0.74	99.5
5.23[a]	9.92	1.99	97.9	**6.75**[b]	9.40	2.51	98.7
5.24[a]	12.11	2.65	100	**6.76**[b]	8.78	2.28	99.0
5.25[a]	12.13	2.65	98.7	**6.77**[b]	5.04	0.88	99.1
5.26[a]	11.99	2.61	99.4	**6.78**[b]	9.47	2.54	98.0
5.27[a]	12.00	2.61	97.3	**7.3**[b]	12.01	3.49	97.2
5.28[a]	11.22	2.38	100	**7.4**[b]	10.22	2.82	98.1
5.29[a]	11.19	2.38	97.1	**7.6**[b]	14.33	4.36	95.9
5.30[a]	9.24	1.78	97.8	**7.5**[b]	12.84	3.80	95.2

[a] Eurosphere-100 C18, 250 × 4.0 mm, 5 µm; Knauer, Berlin, Germany; t_0 = 3.32 min; gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 10/90, 20 min: 90/10, 21 min: 95/5, 30 min: 95/5, 31 min: 10/90, 40 min: 10/90; [b] MN Nucleodur 100-5 C18 ec, 250 x 4.0 mm, 5 µm; Macherey Nagel, Düren, Germany; t_0 = 2.68 min; gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 10/90, 20 min: 90/10, 21 min: 95/5, 30 min: 95/5, 31 min: 10/90, 40 min: 10/90; [c] Gemini NX C18, 250 x 4.6 mm, 5 µm; Phenomenex, Aschaffenburg, Germany; t_0 = 3.83 min; gradient mode: MeCN/0.1 % formic acid (aq.): 0 min: 5/95, 30 min: 95/5, 40 min: 95/5, 41 min: 5/95, 49 min: 5/95; [d] Luna C18-2, 150 x 4.6 mm, 4 µm; Phenomenex, Aschaffenburg, Germany; t_0 = 2.88 min; gradient mode: MeCN/H_2O: 0 min: 5/95, 20 min: 98/2, 35 min: 98/2, 36 min: 5/95, 45 min: 5/95.

10.4 X-ray crystallographic data

10.4.1 *cis*-(-)-(1*S*,3*R*)-3-Carbamoylcyclopentanecarboxylic acid ammonia salt (5.42)



Crystal data and structure refinement for 5.42

Empirical formula	$C_7H_{10}NO_3 \cdot NH_4 \cdot H_2O$
Formula weight	366.42
Crystal size	0.2064 x 0.1418 x 0.0135 mm
Crystal description	plate
Crystal colour	colourless
Crystal system	Monoclinic
Space group	C 2
Unit cell dimensions	a = 11.7993(4) Å, alpha = 90 deg.
	b = 4.9571(2) Å, beta = 94.432(4) deg.
	c = 15.8915(6) Å, gamma = 90 deg.
Volume	926.72(6) $Å^3$
Z, Calculated density	2, 1.313 Mg/m^3
Absorption coefficient	0.884 mm^{-1}
F(000)	396
Measurement device type	SuperNova, Single source at offset), Atlas
Measurement method	\w scans
Temperature	123 K
Wavelength	1.54184 A

Monochromator	graphite
Theta range for data collection	5.58 to 70.88 deg.
Index ranges	-14<=h<=14, -5<=k<=5, -19<=l<=18
Reflections collected / unique	3549 / 1604 [R(int) = 0.0273]
Reflections greater I>2\s(I)	1522
Absorption correction	Analytical
Max. and min. transmission	0.988 and 0.885
Refinement method	Full-matrix least-squares on F^2
Hydrogen treatment	mixed
Data / restraints / parameters	1604 / 1 / 129
Goodness-of-fit on F^2	1.073
Final R indices [I>2sigma(I)]	R1 = 0.0350, wR2 = 0.0942
R indices (all data	R1 = 0.0372, wR2 = 0.0963
Absolute structure parameter	0.3(2)
Largest diff. peak and hole	0.206 and -0.169 e. $Å^{-3}$

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 5.42. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(1)	-1602(1)	-912(3)	786(1)	38(1)
O(2)	182(1)	-4053(3)	3705(1)	27(1)
O(3)	1953(1)	-2534(3)	3938(1)	26(1)
N(1)	-1592(1)	3566(3)	610(1)	28(1)
C(1)	-263(2)	1622(4)	1676(1)	27(1)
C(2)	-438(2)	0(4)	2475(1)	26(1)
C(3)	758(1)	-253(4)	2903(1)	24(1)
C(4)	1516(2)	-701(5)	2160(1)	38(1)
C(5)	864(2)	531(6)	1387(1)	44(1)
C(6)	-1218(2)	1336(4)	990(1)	25(1)
C(7)	963(1)	-2434(4)	3560(1)	22(1)
N(2)	3098(1)	2363(4)	4268(1)	26(1)
O(4)	0	1923(5)	5000	59(1)

Bond lengths [Å] and angles (deg) for 5.42

O(1)-C(6)	1.237(2)	H(2N)-N(2)-H(2P)	113(2)
O(2)-C(7)	1.257(2)	C(5)-C(1)-C(6)	111.37(15)
O(3)-C(7)	1.273(2)	C(2)-C(1)-C(5)	103.82(16)
O(4)-H(4O)	0.87(4)	C(2)-C(1)-C(6)	114.07(15)
O(4)-H(4O)#1	0.87(4)	C(1)-C(2)-C(3)	103.49(14)
N(1)-C(6)	1.320(2)	C(2)-C(3)-C(4)	103.95(14)
N(1)-H(1O)	0.87	C(2)-C(3)-C(7)	117.44(15)
N(1)-H(1N)	0.82	C(4)-C(3)-C(7)	110.67(16)
N(2)-H(2P)	0.96(3)	C(3)-C(4)-C(5)	105.73(16)
N(2)-H(2N)	0.86(3)	C(1)-C(5)-C(4)	107.07(16)
N(2)-H(2O)	0.86(3)	O(1)-C(6)-C(1)	120.74(17)
N(2)-H(2Q)	0.93(2)	N(1)-C(6)-C(1)	117.12(17)
C(1)-C(2)	1.531(3)	O(1)-C(6)-N(1)	122.11(17)
C(1)-C(5)	1.538(3)	O(2)-C(7)-C(3)	120.27(15)
C(1)-C(6)	1.513(2)	O(3)-C(7)-C(3)	116.54(16)
C(2)-C(3)	1.523(2)	O(2)-C(7)-O(3)	123.19(17)
C(3)-C(4)	1.551(3)	C(2)-C(1)-H(1)	109
C(3)-C(7)	1.510(3)	C(5)-C(1)-H(1)	109
C(4)-C(5)	1.526(3)	C(6)-C(1)-H(1)	109
C(1)-H(1)	1	C(1)-C(2)-H(2A)	111
C(2)-H(2A)	0.99	C(1)-C(2)-H(2B)	111
C(2)-H(2B)	0.99	C(3)-C(2)-H(2A)	111
C(3)-H(3)	1	C(3)-C(2)-H(2B)	111
C(4)-H(4A)	0.99	H(2A)-C(2)-H(2B)	109
C(4)-H(4B)	0.99	C(2)-C(3)-H(3)	108
C(5)-H(5B)	0.99	C(4)-C(3)-H(3)	108
C(5)-H(5A)	0.99	C(7)-C(3)-H(3)	108
		C(5)-C(4)-H(4A)	111
H(4O)-O(4)-H(4O)#1	107(4)	C(3)-C(4)-H(4A)	111
C(6)-N(1)-H(1O)	121	C(3)-C(4)-H(4B)	111
H(1N)-N(1)-H(1O)	120	C(5)-C(4)-H(4B)	111
C(6)-N(1)-H(1N)	119	H(4A)-C(4)-H(4B)	109
H(2N)-N(2)-H(2Q)	110(2)	C(1)-C(5)-H(5A)	110
H(2O)-N(2)-H(2Q)	108(2)	C(1)-C(5)-H(5B)	110
H(2P)-N(2)-H(2Q)	109(2)	C(4)-C(5)-H(5A)	110
H(2O)-N(2)-H(2P)	113(2)	C(4)-C(5)-H(5B)	110
H(2N)-N(2)-H(2O)	105(3)	H(5A)-C(5)-H(5B)	109

Anisotropic displacement parameters (A^2 x 10^3) for 5.42. 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)	49(1)	20(1)	42(1)	1(1)	-18(1)	-4(1)
O(2)	25(1)	24(1)	33(1)	2(1)	4(1)	-1(1)
O(3)	24(1)	27(1)	28(1)	1(1)	-2(1)	1(1)
N(1)	32(1)	21(1)	29(1)	-1(1)	-6(1)	0(1)
C(1)	28(1)	24(1)	28(1)	2(1)	-3(1)	-2(1)
C(2)	23(1)	26(1)	27(1)	1(1)	1(1)	3(1)
C(3)	23(1)	22(1)	26(1)	-1(1)	-1(1)	-1(1)
C(4)	28(1)	54(2)	34(1)	13(1)	9(1)	7(1)
C(5)	31(1)	68(2)	32(1)	12(1)	5(1)	7(1)
C(6)	25(1)	22(1)	27(1)	0(1)	-1(1)	1(1)
C(7)	24(1)	20(1)	22(1)	-3(1)	2(1)	2(1)
N(2)	23(1)	27(1)	27(1)	0(1)	0(1)	1(1)
O(4)	108(2)	31(2)	37(1)	0	7(1)	0

Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for 5.42.

	x	y	z	U(eq)
H(1)	-167	3571	1828	32
H(1N)	-2099	3461	232	33
H(1O)	-1349	5141	782	33
H(2A)	-945	968	2841	31
H(2B)	-766	-1797	2334	31
H(3)	970	1518	3172	28
H(4A)	2261	201	2274	46
H(4B)	1646	-2650	2072	46
H(5A)	715	-853	943	52
H(5B)	1310	2010	1155	52
H(2N)	3190(20)	2430(60)	4809(17)	39
H(2O)	2800(20)	3880(60)	4122(17)	39
H(2P)	2640(20)	860(60)	4066(16)	39
H(2Q)	3800(20)	2250(60)	4041(14)	39
H(4O)	130(30)	2970(90)	4580(20)	88

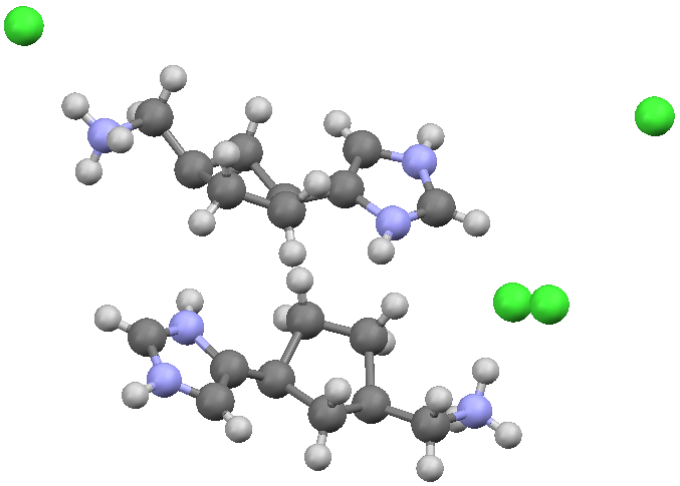
Torsion angles [deg] for 5.42.

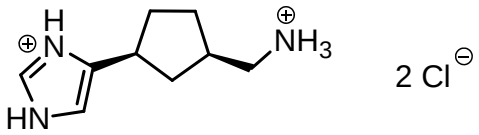
C(5)-C(1)-C(2)-C(3)	38.05(19)
C(6)-C(1)-C(2)-C(3)	159.44(16)
C(2)-C(1)-C(5)-C(4)	-22.7(2)
C(6)-C(1)-C(5)-C(4)	-145.87(18)
C(2)-C(1)-C(6)-O(1)	-47.1(2)
C(2)-C(1)-C(6)-N(1)	134.78(18)
C(5)-C(1)-C(6)-O(1)	70.0(2)
C(5)-C(1)-C(6)-N(1)	-108.1(2)
C(1)-C(2)-C(3)-C(4)	-38.81(19)
C(1)-C(2)-C(3)-C(7)	-161.42(16)
C(2)-C(3)-C(4)-C(5)	24.6(2)
C(7)-C(3)-C(4)-C(5)	151.56(18)
C(2)-C(3)-C(7)-O(2)	3.9(3)
C(2)-C(3)-C(7)-O(3)	-176.82(16)
C(4)-C(3)-C(7)-O(2)	-115.25(18)
C(4)-C(3)-C(7)-O(3)	64.1(2)
C(3)-C(4)-C(5)-C(1)	-1.1(2)

Hydrogen-bonds for 5.42 [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1N)...O(1)#2	0.82	2.16	2.966(2)	168
N(1)-H(1O)...O(1)#3	0.87	1.98	2.752(2)	147
N(2)-H(2N)...O(3)#4	0.86(3)	2.01(3)	2.857(2)	168(2)
N(2)-H(2O)...O(3)#3	0.86(3)	2.05(3)	2.896(2)	171(2)
N(2)-H(2P)...O(3)	0.96(3)	1.87(3)	2.808(2)	164(2)
N(2)-H(2Q)...O(2)#5	0.93(2)	1.87(2)	2.770(2)	163(3)
O(4)-H(4O)...O(2)#3	0.87(4)	2.03(4)	2.886(2)	168(4)
C(3)-H(3)...O(2)#3	1	2.55	3.416(2)	144

10.4.2 *cis*-(+)-(1*R*,3*S*)-3-(1*H*-Imidazol-4-yl)cyclopentyl)methanamine dihydrochloride (5.50)





Crystal data and structure refinement for 5.50

Empirical formula	$C_9H_{15}N_3 \cdot 2\ HCl$
Formula weight	236.14
Crystal size	0.1461 x 0.0880 x 0.0710 mm
Crystal description	prism
Crystal colour	colourless
Crystal system	Triclinic
Space group	P 1
Unit cell dimensions	a = 7.8481(19) Å, alpha = 90.11(2) deg.
	b = 8.982(2) Å, beta = 96.41(2) deg.
	c = 9.093(2) Å, gamma = 97.66(2) deg.
Volume	631.2(3) $Å^3$
Z, Calculated density	2, 1.242 Mg/m^3
Absorption coefficient	4.378 mm^{-1}
F(000)	248
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	4.89 to 89.27 deg.
Index ranges	-6<=h<=9, -11<=k<=10, -11<=l<=9
Reflections collected / unique	3886 / 2627 [R(int) = 0.0393]
Reflections greater I>2\s(I)	2356
Absorption correction	Analytical
Max. and min. transmission	0.743 and 0.616
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2627 / 7 / 246
Goodness-of-fit on F^2	1.074
Final R indices [I>2sigma(I)]	R1 = 0.0688, wR2 = 0.1850
R indices (all data)	R1 = 0.0749, wR2 = 0.1955
Absolute structure parameter	-0.06(4)
Largest diff. peak and hole	0.653 and -0.830 e. $Å^{-3}$

Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 5.50. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
N(1)	12379(3)	5040(3)	12392(3)	36(1)
N(2)	5757(3)	5367(3)	5934(2)	33(1)
N(3)	8274(3)	4054(3)	5627(3)	38(1)
C(1)	10607(4)	4511(3)	10391(3)	40(1)
C(2)	12043(4)	4370(5)	9100(3)	62(1)
C(3)	11155(4)	4111(5)	7686(3)	59(1)
C(4)	9264(4)	4079(3)	8227(3)	40(1)
C(5)	8970	5001	9636	39
C(6)	10849(3)	5557(3)	11636(3)	36(1)
C(7)	8043(4)	4573(3)	7095(3)	40(1)
C(8)	6473(3)	5431(3)	7269(3)	29(1)
C(9)	6886(4)	4498(3)	4968(3)	38(1)
N(4)	4546	-384	3379	35
N(5)	11283(3)	-564(3)	9742(3)	40(1)
N(6)	8656(3)	500(3)	10113(3)	37(1)
C(10)	6076(4)	-615(4)	5646(3)	40(1)
C(11)	5256(5)	879(4)	6306(4)	56(1)
C(12)	6025(4)	1021(4)	7808(4)	49(1)

C(13)	7290(4)	-394(3)	7899(3)	39(1)
C(14)	7862(3)	-870(3)	6264(3)	39(1)
C(15)	6270(4)	-740(4)	3991(3)	42(1)
C(16)	8828(3)	-231(3)	8754(3)	33(1)
C(17)	10516(4)	-886(3)	8511(3)	37(1)
C(18)	10151(3)	226(3)	10692(3)	34(1)
Cl(1)	5814(1)	2693(1)	1818(1)	37(1)
Cl(2)	1178(1)	2019(1)	3858(1)	37(1)
Cl(3)	4639(1)	7596(1)	554(1)	35(1)
Cl(4)	12395(1)	7198(1)	15073(1)	37(1)

Bond lengths [Å] and angles (deg) for 5.50

N(1)-C(6)	1.448(4)	C(16)-N(6)-H(6N)	131.1(10)
N(2)-C(8)	1.277(3)	C(2)-C(1)-C(5)	109.4
N(2)-C(9)	1.588(4)	C(2)-C(1)-C(6)	126.3(3)
N(3)-C(7)	1.451(4)	C(5)-C(1)-C(6)	97.39
N(3)-C(9)	1.295(4)	C(1)-C(2)-C(3)	110.5(3)
N(1)-H(1P)	0.91	C(2)-C(3)-C(4)	94.7(2)
N(1)-H(1N)	0.91	C(5)-C(4)-C(7)	102.71
N(1)-H(1O)	0.91	C(3)-C(4)-C(7)	112.0(2)
N(2)-H(2N)	0.961(12)	C(3)-C(4)-C(5)	121.67
N(3)-H(3N)	0.974(12)	C(1)-C(5)-C(4)	87.52
N(4)-C(15)	1.48	N(1)-C(6)-C(1)	97.3(2)
N(5)-C(17)	1.225(4)	N(3)-C(7)-C(8)	118.3(2)
N(5)-C(18)	1.541(4)	C(4)-C(7)-C(8)	128.9(2)
N(6)-C(18)	1.286(4)	N(3)-C(7)-C(4)	112.6(3)
N(6)-C(16)	1.426(4)	N(2)-C(8)-C(7)	100.1(2)
N(4)-H(4O)	0.91	N(2)-C(9)-N(3)	117.8(2)
N(4)-H(4P)	0.91	C(1)-C(2)-H(2A)	109
N(4)-H(4N)	0.91	C(1)-C(2)-H(2B)	110
N(5)-H(5N)	0.956(15)	C(3)-C(2)-H(2A)	110
N(6)-H(6N)	0.977(13)	H(2A)-C(2)-H(2B)	108
C(1)-C(5)	1.5068	C(3)-C(2)-H(2B)	110
C(1)-C(2)	1.731(4)	C(4)-C(3)-H(3A)	113
C(1)-C(6)	1.449(4)	C(4)-C(3)-H(3B)	113
C(2)-C(3)	1.396(4)	C(2)-C(3)-H(3B)	113
C(3)-C(4)	1.612(4)	H(3A)-C(3)-H(3B)	110
C(4)-C(7)	1.439(4)	C(2)-C(3)-H(3A)	113
C(4)-C(5)	1.5791	C(1)-C(5)-H(5B)	114
C(7)-C(8)	1.560(4)	H(5A)-C(5)-H(5B)	111
C(2)-H(2A)	0.99	C(4)-C(5)-H(5A)	114
C(2)-H(2B)	0.99	C(4)-C(5)-H(5B)	114
C(3)-H(3A)	0.99	C(1)-C(5)-H(5A)	114
C(3)-H(3B)	0.99	H(6A)-C(6)-H(6B)	110
C(5)-H(5A)	0.99	N(1)-C(6)-H(6A)	112
C(5)-H(5B)	0.99	N(1)-C(6)-H(6B)	112

C(6)-H(6B)	0.99	C(1)-C(6)-H(6A)	112
C(6)-H(6A)	0.99	C(1)-C(6)-H(6B)	112
C(8)-H(8A)	0.95	N(2)-C(8)-H(8A)	130
C(9)-H(9A)	0.95	C(7)-C(8)-H(8A)	130
C(10)-C(11)	1.700(5)	N(3)-C(9)-H(9A)	121
C(10)-C(15)	1.535(4)	N(2)-C(9)-H(9A)	121
C(10)-C(14)	1.498(4)	C(14)-C(10)-C(15)	98.9(2)
C(11)-C(12)	1.429(5)	C(11)-C(10)-C(14)	118.1(3)
C(12)-C(13)	1.711(4)	C(11)-C(10)-C(15)	120.0(3)
C(13)-C(16)	1.351(4)	C(10)-C(11)-C(12)	103.3(3)
C(13)-C(14)	1.671(4)	C(11)-C(12)-C(13)	101.4(3)
C(16)-C(17)	1.557(4)	C(12)-C(13)-C(16)	120.3(2)
C(11)-H(11A)	0.99	C(12)-C(13)-C(14)	113.9(2)
C(11)-H(11B)	0.99	C(14)-C(13)-C(16)	101.6(2)
C(12)-H(12A)	0.99	C(10)-C(14)-C(13)	85.7(2)
C(12)-H(12B)	0.99	N(4)-C(15)-C(10)	98.91
C(14)-H(14A)	0.99	N(6)-C(16)-C(17)	121.2(2)
C(14)-H(14B)	0.99	N(6)-C(16)-C(13)	109.2(2)
C(15)-H(15A)	0.99	C(13)-C(16)-C(17)	129.1(2)
C(15)-H(15B)	0.99	N(5)-C(17)-C(16)	96.8(2)
C(17)-H(17A)	0.95	N(5)-C(18)-N(6)	119.1(2)
C(18)-H(18A)	0.95	C(10)-C(11)-H(11A)	111
		C(10)-C(11)-H(11B)	111
C(8)-N(2)-C(9)	107.9(2)	C(12)-C(11)-H(11A)	111
C(7)-N(3)-C(9)	95.8(2)	C(12)-C(11)-H(11B)	111
C(6)-N(1)-H(1N)	109	H(11A)-C(11)-H(11B)	109
C(6)-N(1)-H(1O)	109	C(11)-C(12)-H(12A)	111
C(6)-N(1)-H(1P)	109	C(11)-C(12)-H(12B)	112
H(1N)-N(1)-H(1O)	110	C(13)-C(12)-H(12A)	111
H(1N)-N(1)-H(1P)	110	C(13)-C(12)-H(12B)	111
H(1O)-N(1)-H(1P)	110	H(12A)-C(12)-H(12B)	109
C(8)-N(2)-H(2N)	101.1(16)	C(10)-C(14)-H(14A)	114
C(9)-N(2)-H(2N)	151.0(16)	C(10)-C(14)-H(14B)	114
C(7)-N(3)-H(3N)	117.3(16)	C(13)-C(14)-H(14A)	114
C(9)-N(3)-H(3N)	145.8(15)	C(13)-C(14)-H(14B)	114
C(17)-N(5)-C(18)	110.6(2)	H(14A)-C(14)-H(14B)	112
C(16)-N(6)-C(18)	92.2(2)	N(4)-C(15)-H(15A)	112
C(15)-N(4)-H(4N)	110	N(4)-C(15)-H(15B)	112
H(4O)-N(4)-H(4P)	109	C(10)-C(15)-H(15A)	112
H(4N)-N(4)-H(4O)	109	C(10)-C(15)-H(15B)	112
H(4N)-N(4)-H(4P)	109	H(15A)-C(15)-H(15B)	110
C(15)-N(4)-H(4O)	109	N(5)-C(17)-H(17A)	132
C(15)-N(4)-H(4P)	110	C(16)-C(17)-H(17A)	132
C(18)-N(5)-H(5N)	124.1(15)	N(5)-C(18)-H(18A)	120
C(17)-N(5)-H(5N)	123.5(15)	N(6)-C(18)-H(18A)	120
C(18)-N(6)-H(6N)	136.7(10)		

Anisotropic displacement parameters (A^2 x 10^3) for 5.50. 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
N(2)	25(1)	36(1)	34(1)	-12(1)	-16(1)	2(1)
N(3)	33(1)	51(1)	27(1)	-13(1)	-11(1)	1(1)
C(1)	40(1)	37(1)	34(1)	-5(1)	-17(1)	-8(1)
C(2)	30(1)	117(3)	36(1)	-44(2)	-14(1)	18(2)
C(3)	24(1)	114(3)	36(1)	-37(2)	-19(1)	12(2)
C(4)	40(1)	35(1)	37(1)	1(1)	-20(1)	-3(1)
C(5)	39	39	39	1	4	5
C(6)	32(1)	40(1)	28(1)	-9(1)	-17(1)	-8(1)
C(7)	46(1)	42(1)	25(1)	-9(1)	-12(1)	-7(1)
C(8)	27(1)	35(1)	22(1)	-10(1)	-4(1)	-1(1)
C(9)	38(1)	46(1)	23(1)	-8(1)	-8(1)	-6(1)
N(4)	35	35	35	1	4	5
N(5)	38(1)	42(1)	33(1)	-7(1)	-13(1)	-5(1)
N(6)	35(1)	37(1)	35(1)	-15(1)	-9(1)	4(1)
C(10)	31(1)	48(1)	38(1)	-11(1)	-10(1)	2(1)
C(11)	58(2)	49(2)	52(2)	-12(1)	-33(1)	10(1)
C(12)	50(2)	47(2)	42(2)	-14(1)	-19(1)	2(1)
C(13)	33(1)	38(1)	39(1)	-5(1)	-12(1)	-7(1)
C(14)	32(1)	45(1)	34(1)	-18(1)	-24(1)	11(1)
C(15)	36(1)	51(2)	33(1)	-3(1)	-12(1)	1(1)
C(16)	26(1)	41(1)	28(1)	-10(1)	-11(1)	4(1)
C(17)	36(1)	39(1)	29(1)	-14(1)	-13(1)	-8(1)
C(18)	36(1)	29(1)	31(1)	-9(1)	-11(1)	-6(1)
Cl(1)	34(1)	39(1)	35(1)	-13(1)	-9(1)	5(1)
Cl(2)	34(1)	43(1)	33(1)	-13(1)	-8(1)	9(1)
Cl(3)	31(1)	44(1)	28(1)	-13(1)	-7(1)	1(1)
Cl(4)	30(1)	46(1)	32(1)	-15(1)	-11(1)	6(1)

Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for 5.50.

	x	y	z	U(eq)
H(1N)	13226	5115	11779	43
H(1O)	12121	4065	12651	43
H(1P)	12750	5617	13219	43
H(2A)	12860	5312	9111	74
H(2B)	12720	3536	9363	74
H(2N)	4796(12)	5910(16)	6020(30)	40
H(3A)	11433	4942	7002	71
H(3B)	11327	3142	7243	71

H(3N)	9189(13)	3434(15)	5550(30)	46
H(5A)	9039	6098	9485	47
H(5B)	7914	4607	10090	47
H(6A)	11070	6615	11327	43
H(6B)	9862	5427	12234	43
H(8A)	6144	5875	8125	35
H(9A)	6554	4311	3941	45
H(4N)	4571	630	3325	42
H(4O)	3744	-761	3978	42
H(4P)	4261	-803	2457	42
H(5N)	12303(13)	-970(19)	10130(30)	47
H(6N)	7733(13)	1025(16)	10410(20)	44
H(11A)	5588	1797	5748	67
H(11B)	3979	682	6249	67
H(12A)	5139	864	8508	58
H(12B)	6728	2014	8011	58
H(14A)	8800	-162	5909	46
H(14B)	8089	-1924	6190	46
H(15A)	6456	-1764	3701	50
H(15B)	7217	2	3697	50
H(17A)	10844	-1375	7681	45
H(18A)	10553	508	11691	41

Torsion angles [deg] for 5.50.

C(9)-N(2)-C(8)-C(7)	-0.5(3)
C(8)-N(2)-C(9)-N(3)	-1.5(4)
C(9)-N(3)-C(7)-C(4)	171.7(3)
C(7)-N(3)-C(9)-N(2)	2.5(3)
C(9)-N(3)-C(7)-C(8)	-3.0(3)
C(17)-N(5)-C(18)-N(6)	-2.7(4)
C(18)-N(5)-C(17)-C(16)	0.4(3)
C(18)-N(6)-C(16)-C(17)	-3.0(3)
C(18)-N(6)-C(16)-C(13)	169.5(2)
C(16)-N(6)-C(18)-N(5)	3.1(3)
C(6)-C(1)-C(5)-C(4)	166.32
C(2)-C(1)-C(5)-C(4)	33.53
C(6)-C(1)-C(2)-C(3)	-140.4(3)
C(5)-C(1)-C(6)-N(1)	177.65
C(5)-C(1)-C(2)-C(3)	-24.99
C(2)-C(1)-C(6)-N(1)	-61.6(3)
C(1)-C(2)-C(3)-C(4)	-1.2(4)
C(2)-C(3)-C(4)-C(5)	28.98
C(2)-C(3)-C(4)-C(7)	150.9(3)
C(5)-C(4)-C(7)-N(3)	170.51
C(5)-C(4)-C(7)-C(8)	-15.51
C(3)-C(4)-C(5)-C(1)	-41.78

C(3)-C(4)-C(7)-N(3)	38.3(3)
C(7)-C(4)-C(5)-C(1)	-167.97
C(3)-C(4)-C(7)-C(8)	-147.7(3)
C(4)-C(7)-C(8)-N(2)	-171.4(3)
N(3)-C(7)-C(8)-N(2)	2.3(3)
C(11)-C(10)-C(14)-C(13)	40.4(3)
C(15)-C(10)-C(14)-C(13)	171.4(2)
C(14)-C(10)-C(15)-N(4)	-176.29
C(14)-C(10)-C(11)-C(12)	-31.9(4)
C(15)-C(10)-C(11)-C(12)	-152.6(3)
C(11)-C(10)-C(15)-N(4)	-46.51
C(10)-C(11)-C(12)-C(13)	1.3(3)
C(11)-C(12)-C(13)-C(14)	24.7(3)
C(11)-C(12)-C(13)-C(16)	145.6(3)
C(16)-C(13)-C(14)-C(10)	-168.7(2)
C(12)-C(13)-C(16)-N(6)	40.4(3)
C(12)-C(13)-C(14)-C(10)	-37.9(3)
C(14)-C(13)-C(16)-N(6)	167.1(2)
C(14)-C(13)-C(16)-C(17)	-21.2(4)
C(12)-C(13)-C(16)-C(17)	-147.9(3)
N(6)-C(16)-C(17)-N(5)	1.7(3)
C(13)-C(16)-C(17)-N(5)	-169.1(3)

Hydrogen-bonds for 5.50 [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1N)...Cl(3)#1	0.91	2.66	3.287(3)	127
N(1)-H(1O)...Cl(2)#1	0.91	2.23	3.105(3)	162
N(1)-H(1P)...Cl(4)	0.91	2.27	3.110(3)	154
N(2)-H(2N)...Cl(4)#2	0.961(12)	2.420(14)	3.314(3)	155(2)
N(3)-H(3N)...Cl(2)#3	0.974(12)	2.734(18)	3.611(3)	150(2)
N(4)-H(4N)...Cl(1)	0.91	2.47	3.2003	138
N(4)-H(4O)...Cl(4)#4	0.91	2.28	3.0913	148
N(4)-H(4P)...Cl(3)#5	0.91	2.32	3.1555	153
N(5)-H(5N)...Cl(3)#6	0.956(15)	2.376(14)	3.308(3)	165(2)
N(6)-H(6N)...Cl(1)#7	0.977(13)	2.679(14)	3.632(3)	165.2(13)
C(2)-H(2A)...Cl(3)#1	0.99	2.59	3.479(4)	150
C(3)-H(3A)...Cl(4)#8	0.99	2.77	3.755(4)	177
C(5)-H(5B)...Cl(1)#7	0.99	2.82	3.7567	157
C(9)-H(9A)...Cl(1)	0.95	2.39	3.259(3)	153
C(18)-H(18A)...Cl(2)#1	0.95	2.36	3.264(3)	159

10.5 Abbreviations

α	intrinsic activity or selectivity factor
A	agonist
abs	absolute
AC	adenylyl cyclase
Anal.	analysis
aq	aqueous
Ar	aromatic
Asp	aspartate
ATP	adenosine triphosphate
ATR	attenuated total reflection
Boc	*tert*-butoxycarbonyl
B_{max}	the maximal specific binding of a ligand
bp	boiling point
brs	broad singlet
BSA	bovine serum albumin
calcd.	calculated
cAMP	cyclic 3', 5'-adenosine monophosphate
cat.	catalytical amounts
CD	circular dichroism
Ci	Curie
CI	chemical ionization
CNS	central nervous system
COSY	correlated spectroscopy
cPr	cyclopropyl
C_{quat}	quaternary carbon atom
CRE	cAMP response element
CREB	cAMP response element binding protein
d	day(s) or doublet
DAG	diacylglycerol
DCM	dichloromethane
dec.	decomposition
DEPT	distortionless enhancement by polarization transfer
DIAD	diisopropyl azodicarboxylate
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMEM	Dulbecco's modified eagle medium
DMF	*N*, *N*-dimethylformamide
DMSO	dimethylsulfoxide
DMSO-*d*6	per-deuterated DMSO

DNABP	DNA binding protein
DTT	dithiothreitol
ee	enantiomeric excess
E_1, E_2	1st and 2nd extracellular loop of a GPCR
ECL	enterochromaffin-like
EDC	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide
EC_{50}	molar concentration of the agonist causing 50 % of the maximal response
EDTA	ethylendiaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
EI	electron impact ionization
E_{max}	maximal response relative to histamine (1.00)
eq	equivalents
ES	electrospray ionization
Et_2O	diethylether
EtOAc	ethylacetate
EtOH	ethanol
FBS	fetal bovine serum
G	G-protein
GDP	guanosine diphosphate
GF/C	glass microfibre , grade c (fine)
Glu	glutamate
GPCR	G-protein coupled receptor
GTP	guanosine triphosphate
GTPγS	guanosine 5'-thiotriphosphate
h	hour(s) or human
HDC	L-histidine-decarboxylase
HEK	human embryonic kidney
HR	histamine receptor
hH_1R	human histamine H_1 receptor
hH_2R	human histamine H_2 receptor
hH_2R-$Gs\alpha_S$	fusion protein between the hH_2R and short splice variant of Gsα
hH_3R	human histamine H_3 receptor
hH_4R	human histamine H_4 receptor
hH_4R-GAIP	fusion protein between the hH_4R and RGS19
HMBC	heteronulcear multiple bond correlation
HMQC	heteronuclear multiple quantum correlation
HNMT	histamine *N*-methyltransferase
HPLC	high performance (pressure) liquid chromatography
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum correlation

H_1R, H_2R, H_3R, H_4R	histamine receptor subtypes
HSQC	heteronuclear single quantum coherence
HT-29	human colon adenocarcinoma cell line
IC_{50}	functional assay: antagonist (inverse agonist) concentration suppressing 50 % of an agonist induced effect radioligand binding assay: ligand concentration inhibiting the binding of a radioligand by 50 %
IgE	immunoglobuline E
Im	imidazolyl
IP_3	inositol-1,4,5-trisphosphate
IP_3R	inositol trisphosphate receptor
IR	infrared spectroscopy
J	coupling constant
k’	capacity factor
K_B	dissociation constant (functional assay)
K_D	dissociation constant (saturation binding)
K_I	dissociation constant (competition binding)
K^tBuO	potassium *tert*-butanolate
LSI	liquid secondary ion
LUC	luciferase
m	multiplet or milli or mouse
µ	micro
MAPK	mitogen-activated protein kinase
MeCN	acetonitrile
MeOH	methanol
mH_4R	mouse histamine H_4 receptor
min	minute(s)
mp	melting point
MS	mass spectrometry
n	nano
NEt_3	triethylamine
NHS	*N*-hydroxysuccinimide
N^G	guanidino-nitrogen
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
NP	normal phase
OAc	acetate
OR	optical rotation
PBS	phoshpate buffered saline
PE	petroleum ether
PEI	polyethyleneimine

Ph	phenyl
P_i	inorganic phosphate
PIP_2	phosphatidylinositol-4,5-bisphosphate
PKA	protein kinase A
PKC	protein kinase C
PLA_2	phospholipase A_2
PLC_β	phospholipase C_β
ppm	part per million
Py	pyridyl or pyrimidyl or pyrylium
Phthal	phthalimide
pEC_{50}	negative decadic logarithm of the molar concentration of the agonist causing 50 % of the maximal response
q	quartet
R	inactive state of a GPCR
R*	active state of a GPCR
R_s	resolution of HPLC peaks
ref	reference
RGS	regulator of G-protein signaling
RP	reversed phase
rt	room temperature
rpm	revolutions per minute
s	singlet
sat.	saturated
SEM	standard error of the mean
Sf9	*Spodoptera frugiperda* insect cell line
SK-N-MC cells	human neuroblastoma cell line established from the supraorbital metastasis of a neuroblastoma of a 14-year old girl in 1971
t	triplet
t_0	dead time
TBDPS	*tert*-butyldiphenylsily
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TFA	trifluoroacetic acid
Th2	T-helper cell type 2
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	transmembrane
TMEDA	N^1,N^1,N^2,N^2-tetramethylethane-1,2-diamine
TMS	trimethylsilyl
Tos	tosyl

TosMIC	tosylmethyl isocyanide
t_R	retention time
Tris	tris(hydroxymethyl)aminomethane
Trt	trityl, triphenylmethyl
UV	ultraviolet

10.6 References

1. Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973,** 22, 3099-3108.

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Regensburg,

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