Synthesis and pharmacological characterization of homo- and hetero-dimeric compounds, targeting the hH₁R and/or hH₄R

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Jianfei Wan

aus

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Board of examiners:
Apl. Prof. Dr. Rainer Müller (Chairman)
PD. Dr. Andrea Straßer (1st Referee)
Prof. Dr. Armin Buschauer (2nd Referee)
Prof. Dr. Sigurd Elz ( Examiner)
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Poster Presentations:


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Professional Training:

02/2014  Radioanalytical working methods for pharmacists. Regensburg, Germany.

12/2013-03/2017  Associated member of the Research Training Group (Graduiertenkolleg 1910) “Medicinal Chemistry of Selective GPCR Ligands” of the German Research Foundation. Regensburg, Germany.

06/2014-03/2017  Member of the Emil Fischer Graduate School of Pharmaceutical Sciences and Molecular Medicine. Regensburg, Erlangen, Germany.
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Chapter 1

Introduction
1. Introduction

1.1. G-protein coupled receptors

G-protein coupled receptors, also known as seven-transmembrane receptors, are the largest class of membrane proteins in the human genome\(^1\) and one of the most ubiquitous and versatile receptor family\(^2\). For mankind, about 800 GPCRs have been identified, and these receptors regulate a lot of physiological processes.\(^3\)

1.1.1. Classification

About 50 % of the GPCRs have sensory functions or mediate olfaction, taste, light perception and pheromone signaling.\(^4\) The remaining non-sensory GPCRs which mediate inter-signaling by ligands from small molecules to large proteins, are targets for the majority of clinical drugs.\(^5,6\) Based on sequence homology, GPCRs can be divided into: rhodopsin-like, secretin receptor family, metabotropic glutamate, fungal mating pheromone receptors, cyclic AMP receptors and frizzled/smoothened.\(^7\) As fungal mating pheromone receptors and cyclic AMP receptors are not discovered in vertebrates, the classification “GRAFS” (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2 and Secretin) was suggested based on evolutionary aspects.\(^8\)

1.1.2. Structure

The characteristic features of GPCRs (Fig. 1.1) are an extracellular amino terminus, an intracellular carboxyl terminus and seven hydrophobic transmembrane (TM) domains connected by three extracellular loops (ECL1-3) and three intracellular loops (ICL1-3).\(^9\) Extracellular and intracellular regions are involved in ligand binding and signal transduction respectively, which show the least conservation between different GPCRs.\(^10-12\) The first high-resolution 3D structure of a GPCR, the x-ray crystallography of bovine rhodopsin, was reported by Palczewski et al. in 2000.\(^9\) In 2007 the structure of the $\beta_2$ adrenergic receptor ($\beta_2$AR) was solved by Rasmussen et al.\(^13\) and Cherezov et al.\(^14\). Another milestone in crystallography of GPCR was published 2011, when Rasmussen et al. reported on the
structure of the $\beta_2$AR active state. Meanwhile, the structures of more than 30 different GPCRs are described in the literature.

**Figure. 1.1** Schematic representation of a prototypical class A GPCR (ICL = intracellular loop; ECL = extracellular loop; 1-7 = transmembrane domains)

### 1.1.3. Function

GPCRs may be considered to act as molecular switches which transmit extracellular signals to intracellular responses through conformational change. In a simplified model, a GPCR exists in an “on mode” (active, $R_a$), stabilized by an agonist, and an “off mode” (inactive, $R_i$), stabilized by an inverse agonist (Fig. 1.2). Agonists eliciting the maximal biological response are named full agonists, while ligands, which are less effective in stabilizing the active state are referred to as partial agonists, and their intrinsic activity ranges from 0 to $< 100 \%$. When the intrinsic activity of a ligand is 0 %, it does not alter the basal equilibrium between both, the active $R_a$ and the inactive $R_i$ state. Such ligands are referred as neutral antagonists. GPCRs can change their conformation from the inactive to the active state in the absence of an agonist, resulting in constitutive activity. Ligands capable of decreasing the basal activity of a receptor by stabilizing the inactive conformation are referred to as partial or full inverse agonists.
Chapter 1

1.1.4. Signaling

1.1.4.1. G-protein cycle

According to the classical model (Fig. 1.3), the GPCR active state, for example, stabilized by an agonist, is capable of interacting with the inactive form of a heterotrimeric G-protein (consisting of a Gα subunit with bound GDP and a Gβγ complex) resulting in the activation of the G-protein by evoking the exchange of GDP by GTP.\(^\text{18, 19}\) Subsequently, the Gα subunit is detached from the Gβγ subunit and both of them interact with effector proteins, resulting in changes of second messenger concentrations and various cellular responses. The G-protein returns to the inactive state upon cleavage of GTP to GDP by the intrinsic GTPase activity of the Gα subunit and re-associates with Gβγ marking the completion of the cycle.\(^\text{11}\) Regulators of G-protein signalling (RGS proteins; GTPase activating proteins, GAPs) can enhance the activity of the GTPase.\(^\text{20-22}\)

Figure. 1.2 Two state model of a GPCR. The receptor toggles between the inactive state \(R_i\) and the active state \(R_a\). (modified according to Seifert and Wenzel-Seifert\(^\text{17}\))

Figure. 1.3 Generalized diagram of the G-protein cycle. (modified according to Bridges et al.\(^\text{23}\))
1.1.4.2. G-Proteins and their pathways

Till now, 16 different Gα, 5 Gβ and 12 Gγ-subunits are known: The Gα subunits are grouped into four main families: Gαs, Gαi/o, Gαq/11 and Gα12/13 based on the differences in structure and signaling pathway.24, 25 The Gαs family can activate the adenylyl cyclases (AC 1-9), resulting in an increase in concentration of the second messenger cAMP (3',5'-cyclic adenosine monophosphate). The opposite effect on cAMP levels is caused by the Gαi/o family as a consequence of the inhibition of adenylyl cyclase activity (AC 5 and AC 6).26, 27 The second messenger cAMP activates the protein kinase A (PKA) or the mitogen-activated protein kinase (MAPK) pathway.28 The Gαq/11 family regulates the activity of phospholipase C (PLCβ), leading to the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). Both are second messengers, being responsible for various intracellular effects including the release of Ca2+ ions from intracellular stores29 and the stimulation of protein kinase C (PKC) which further modulates the function of cellular proteins by phosphorylation.30 The Gα12/13 family interferes with the cytoskeletal assembly by interaction with Rho-GEFs (Ras homology guanine nucleotide exchange factors).11, 24, 31 Besides the Gα-subunits, the Gβγ-heterodimer is also involved in effector regulation, e.g., PLCβ and K+, Ca2+ ion channels.24

1.1.5. Oligomerization

An oligomer refers to a macromolecular complex formed by non-covalent bonding of a few macromolecules. A homo-oligomer would be formed by few identical molecules and by contrast, a hetero-oligomer would be made of different macromolecules.

Over a long period of time, GPCRs were thought to exist and function exclusively as monomeric units. However, there was evidence from native cells and heterologous expression systems that GPCRs are able to form dimers or even higher-order oligomers.32-35 Trafficking, signaling and internalization have been demonstrated for GPCR monomers as well as for dimers/oligomers.33, 34, 36 Compared to GPCR monomers, GPCR dimers may provide distinct properties with respect to ligand binding37, signaling38-40, receptor trafficking,41 and it may be speculated about the clinical relevance of such differences.42, 43
1.1.6. Allosteric modulation

The term allosterism is used to describe a phenomenon which enables proteins to sense changes in their environment and to respond to them. Allosteric site is physically distinct from the orthosteric binding site, that is, the binding pocket of the endogenous ligand. Allosteric modulators cooperatively effect the binding of a ligand to the orthosteric ligand binding in a positive or a negative manner. A positive allosteric modulator (PAM) induces a receptor conformational change enhancing the binding affinity or the functional efficacy of an orthosteric agonist, whereas a negative allosteric modulator (NAM) has the opposite effect. Besides PAM and NAM, there are silent allosteric modulator (SAM) and allosteric agonists (ago-PAM). A SAM doesn’t effect orthosteric binding, and an ago-PAM can activate the receptor in the absence of an orthosteric ligand. Allosteric modulation may offer a possibility to increase the receptor subtype selectivity of ligands due to lower conservation of allosteric compared to orthosteric sites.

1.1.7. Dimeric/bivalent ligands for GPCRs

The term “dimeric/bivalent ligand” refers to molecules composed of two pharmacophoric moieties covalently linked through a spacer. The pharmacophores can be identical (homo-bivalent) or different (hetero-bivalent ligands). Such dimeric or bivalent ligands may be useful as pharmacological tools to investigate receptor dimers (Fig. 1.4 B). With a spacer of appropriate length attached in the right position, bivalent ligands might achieve higher affinity and selectivity compared to their monomeric counterparts. In principle, a bivalent or dimeric ligand with a relatively short spacer might address interactions sites on the same protomer (Fig. 1.4 A) and allow for the detection of accessory binding sites such as allosteric binding pockets.
Figure 1.4 Bivalent ligand binding to (A) a GPCR with an accessory binding site or to (B) a GPCR dimer (modified according to Portoghese et al.\textsuperscript{53} and Birnkammer et al.\textsuperscript{61}).

1.2. Histamine and its receptors

1.2.1. Histamine

Histamine, (2-(1\textit{H}-imidazol-4-yl)ethanamine), discovered more than a century ago,\textsuperscript{62} acts as an endogenous mediator, immunomodulator and neurotransmitter targeting histaminergic receptors. Histamine is a biogenic amine, synthesized from the amino acid L-histidine by decarboxylation.\textsuperscript{63}

1.2.2. Histamine receptors

1.2.2.1. The histamine H\textsubscript{1}R

The histamine H\textsubscript{1} receptor (H\textsubscript{1}R) is expressed mainly in mammalian brain, smooth muscle, endothelium cells and lymphocytes and is involved in pathophysiological processes such as allergic and inflammatory reactions.\textsuperscript{64} H\textsubscript{1}R-mediated biological effects include, for instance, vasodilatation, bronchoconstriction, increased vascular permeability, pain and itching upon insect stings.\textsuperscript{65} The H\textsubscript{1}R preferentially couples to the G\textsubscript{q} protein, resulting in an increase in intracellular Ca\textsuperscript{2+}.\textsuperscript{64, 66} Betahistine (e.g. Aequamen\textsuperscript{69}) is the only marketed H\textsubscript{1}R agonist (Fig. 1.5), approved for the treatment of Menière’s disease. H\textsubscript{1}R antagonists, also known as antihistamines, have been used to treat allergic disorders (allergic rhinitis, chronic urticarial and atopic dermatitis), nausea and vomiting, as well as to cause sedation.\textsuperscript{67, 68} The first generation antagonists like pyrilamine or diphenhydramine (Fig. 1.5) have been replaced by
the second generation antagonists, e.g., cetirizine (Fig. 1.5), in order to reduce sedation, which is a side effect of the classical antihistamines, which are capable of penetrating across the blood-brain barrier.\textsuperscript{63, 69, 70} Regardless of that, pyrilamine is still the most commonly used reference H\textsubscript{1}R antagonist and radioligand ([\textsuperscript{3}H]pyrilamine) for pharmacological studies.\textsuperscript{71} X-ray crystallography of the H\textsubscript{1}R in complex with doxepine gave insights in ligand binding on the molecular level.\textsuperscript{72} Recently, Na\textsuperscript{+} was identified as a negative allosteric regulator bound to Asp\textsuperscript{2.50} of H\textsubscript{1}R.\textsuperscript{73}

![Figure 1.5 Structures of selected H\textsubscript{1}R agonist and antagonists.](image)

**1.2.2.2. The histamine H\textsubscript{2}R**

The histamine H\textsubscript{2} receptor (H\textsubscript{2}R) is expressed in a variety of tissues including brain, gastric parietal cells, heart, airways and uterus.\textsuperscript{69} The H\textsubscript{2}R couples to the G\textsubscript{\alpha}s protein, which leads to an increase in cAMP followed by activation of PKA.\textsuperscript{74} The H\textsubscript{2}R was pharmacologically characterized in 1972 using the first H\textsubscript{2}R antagonist burimamide (Fig. 1.6), which was able to inhibit the histamine-stimulated gastric acid secretion and the positive chronotropic effect on the heart.\textsuperscript{75} Cimetidine (Tagamet\textsuperscript{®}) was the first clinically available H\textsubscript{2}R antagonist, followed by non-imidazoles such as ranitidine and famotidine (Fig. 1.6), with reduced pharmacokinetic interactions with CYP450 enzymes.\textsuperscript{63} The H\textsubscript{2}R antagonists had been very important antiulcer drugs over decades, but were replaced by the more effective proton pump inhibitors. Impromidine (Fig. 1.6) was the first highly potent H\textsubscript{2}R agonist demonstrated to be clinically effective in the treatment of severe catecholamine-refractory congestive heart failure.\textsuperscript{76, 77} In a
bivalent ligand approach, H₂R agonists such as UR-AK381 (Fig. 1.6) were developed, which are among the most potent and selective H₂R agonists known so far.⁶¹ A potential application for highly selective H₂R agonists might be the treatment of acute myeloid leukemia (AML).⁷⁸.

![Structures of selected H₂R agonists and antagonists.](image)

**Figure 1.6** Structures of selected H₂R agonists and antagonists.

### 1.2.2.3. The histamine H₃R

The histamine H₃ receptor (H₃R) was first discovered in 1983⁸⁰ and cloned in 1999.⁸¹ It is predominantly located in the CNS, acts as a presynaptic auto- and heteroreceptor and controls the release of histamine and various other neurotransmitters, including dopamine, serotonin, noradrenalin and acetylcholine.⁸²⁻⁸⁶ The H₃R couples to Goᵢ/o proteins and has been shown to interfere with various transduction pathways apart from the modulation of the AC activity, for example activation of PLA₂ and inhibition of K⁺-induced Ca²⁺ mobilization.⁸⁷ Consequently, H₃R influences the regulation of a broad variety of physiological functions like food intake, sleep-wake cycle, body temperature and blood pressure. The first approved
H₃ receptor inverse agonist, pitolisant (Fig. 1.7), used in the therapy of narcolepsy, is also considered to have potential value in the treatment of Parkinson’s disease and obstructive sleep apnoea.⁸⁸,⁸⁹ Numerous H₃R ligands, e.g. thioperamide, clobenprobit, immethridine and methimepip (Fig. 1.7) are derived from histamine. Due to the imidazole moiety, these compounds are not H₃R selective but show also H₄R affinity, and they are potential CYP450 binders, which may cause drug-drug interactions.

**Figure 1.7** Structures of selected H₃R agonists and antagonists.

### 1.2.2.4. The histamine H₄R

The histamine H₄ receptor (H₄R) is the latest member of histamine receptor family. An “eosinophil” histamine receptor was already postulated more than 40 years ago.⁹⁰,⁹¹ In 2000 and 2001, the H₄R was independently identified and cloned by six research groups,⁹²-⁹⁸ and the H₄R was reported to be expressed in bone marrow and immunocytes.⁹⁹ Like the H₃R, the H₄R interacts with the Goᵢᵥ₀ protein, leading to an inhibition of the AC and an activation of the phospholipase C-β (PLCᵢ) via Gᵢᵥ complexes.⁹⁹-¹⁰¹ As a major player in immunological and inflammatory reactions, the H₄R was suggested to be a potential drug target for the treatment of asthma, pruritus, and rheumatoid arthritis.¹⁰⁰,¹⁰²-¹⁰⁹ The indole derivative JNJ-7777120 (Fig. 1.8) was the first selective high-affinity non-imidazole H₄R antagonist and has been widely used as a reference compound in vitro and in vivo.¹¹⁰ However, the pharmacological action of JNJ-7777120 is species-dependent, for example, to a rather low sequence identity comparing human and rodent H₄Rs and to a different extent of constitutive activity of the H₄R orthologues.¹¹¹-¹¹³ Furthermore, JNJ-7777120 was reported to be a biased agonist,
stimulating H₄R mediated β-arrestin recruitment. Several H₄R antagonists, e.g. PF-3893787, JNJ-39758979 and Toreforant (Fig. 1.8), entered clinical trials for the treatment of allergic rhinitis, allergic asthma, atopic dermatitis and rheumatoid arthritis. Compared with antagonists, H₄R agonists (Fig. 1.8) are important as pharmacological tools rather than as drugs.

Figure 1.8 Structures of selected H₄R agonists and antagonists.

1.2.3. Dual ligands of histamine receptors

Ligands with moderate to high affinity for two histamine receptor subtypes are referred as dual histamine receptor ligands. Theoretically, a single ligand which can address multiple desired receptor targets may enhance affinity, potency, efficacy and safety, e.g. lower the risk of drug-drug interactions compared to drug cocktails. In the histamine receptor field, a
synergistic effect of H1R and H4R antagonism was observed in various models of inflammation.\textsuperscript{119-121} Some ligands exhibiting affinity to both, H1R and H4R, were discovered (Fig. 1.9). These results suggest that despite the low sequence homology between both receptors it should be feasible to obtain H1R/H4R ligands with balanced receptor subtype affinity.\textsuperscript{122-125} Another combination is H1R and H3R antagonists: series of H1R/H3R antagonists were developed by GSK. GSK835726 and GSK1004723 (Fig. 1.9) entered clinical trials to treat allergic rhinitis, but both compounds did not show a differentiation from H1R antagonist treatment.\textsuperscript{126, 127} Considering the high sequence similarity between the transmembrane domains (TMs) of the human H3R and H4R, it is not surprising that many H4R ligands containing imidazole moieties show also H3R affinity.\textsuperscript{128} Dual H3R/H4R antagonists (Fig. 1.9) may have a potential in the treatment of pain and cancer since it is likely these two targets contribute to the development of pain sensation and itching as well as cell-proliferation-associated effects\textsuperscript{129}. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.9.png}
\caption{Structures of selected dual HxR ligand structures.}
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Chapter 2

Scope and objectives
2. Scope and objectives

To reach multiple molecular targets simultaneously, a prevailing way is to use cocktails of drugs. But this approach is sometimes hampered due to poor patient compliance. A single ligand addressing multiple desired receptor targets (multitarget ligands) might be of advantage to enhance affinity, potency, efficacy and safety, e.g., due to a lower risk of drug-drug interactions, compared to drug cocktails. Besides their potential clinical value, such hybrid compounds may also be useful pharmacological tools for in vitro investigations.

Among the four histamine receptor subtypes (H₁R, H₂R, H₃R, and H₄R) both, the H₁R and H₄R, were suggested to be involved in type-I allergic reactions. A synergistic effect of co-administered H₁R and H₄R antagonists was observed, e.g., regarding inhibition of pruritus and skin inflammation from chronic dermatitis, acute hapten-induced scratching, and peanut-induced intestinal allergy. All these results suggest that dual H₁R/H₄R antagonists may have a potential clinical value.

The aim of this work is to synthesize and characterize potential dual H₁R/H₄R antagonists. Homo- and hetero-dimeric (or bivalent) compounds will be constructed from crucial structural features of various H₁R and H₄R ligands (Fig. 2.1), which will be combined in different ways, e.g., by merging pharmacophoric moieties or separating the respective substructures by spacers of different chemical nature and appropriate length. The resulting compounds should be useful to explore the interactions with the H₁R and the H₄R on the molecular level and, possibly, to search for additional binding sites on H₁R and H₄R, respectively.
Chapter 2

Fig. 2.1 Structures and affinities of selected H₁R and/or H₄R ligands as pharmacophores. Pharmacological data were taken from a) Strasser et al.⁹ b) Igel et al.¹⁰ c) Smits et al.¹¹ d) Wagner et al.¹² e) Wittmann et al.¹³ f) Deml et al.¹⁴ g) Baumeister ¹⁵ h) Geyer et al.¹⁶

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Chapter 3

Homo-dimeric ligands as human histamine $H_1$ receptor ligands or dual $H_1/H_4$ receptor ligands
3. Homo-dimeric ligands as human histamine $H_1$ receptor ligands or dual $H_1/H_4$ receptor ligands

3.1. Introduction

Over the past decades, our knowledge of GPCR has been changed revolutionarily with the discovery of receptor oligomerization\textsuperscript{1-4} and allosteric modulation\textsuperscript{5, 6}. The existence of oligomers in class A GPCRs including opioid receptors\textsuperscript{7-9}, adrenergic receptors\textsuperscript{10}, somatostatin receptors\textsuperscript{11, 12}, dopamine receptors\textsuperscript{13-15}, muscarinic receptors\textsuperscript{16, 17} and all histamine receptor subtypes\textsuperscript{18-21} has been demonstrated. It is supposed that potentially all GPCRs possess drugable allosteric sites.\textsuperscript{22} Consequently, suitable pharmacological tools for investigating GPCR dimers or high order oligomers and putative allosteric binding sites are required. One possible tool are dimeric ligands, which refer to molecules composed of two pharmacophoric moieties covalently linked through a spacer.\textsuperscript{23-25} Homo- and hetero-dimeric ligands have emerged in the GPCR field in recent years, e.g., histamine\textsuperscript{26, 27}, dopamine\textsuperscript{28-30}, and adenosine\textsuperscript{31, 32} and NPY\textsuperscript{33, 34} receptors.

On one hand, dimeric ligands with a long spacer, e.g., a molecular modeling study suggests that the distance between two recognition sites of a contact opioid receptor dimer with a TM5/TM6 interface is about 22–27 Å\textsuperscript{23}, can be designed to probe dimerized receptors. On the other hand, a relatively short spacer allows that the duplication of pharmacophores addresses to two neighboring interaction sites on the same protein.\textsuperscript{26, 35} Theoretically, bivalent ligands were postulated to increase the affinity compared to their constituent mono-valent ligands because the binding of one of the constituent pharmacophore proceeds through a univalent bound state and therefore allows the unbound partner to be in closer proximity to neighboring binding sites.\textsuperscript{36}

The present study was aiming at high affinity dimeric/bivalent $H_1$R and/or $H_4$R ligands as pharmacological tools. Small series of homo-dimeric/homo-bivalent ligands were synthesized using building blocks derived from the well-known $H_1$R antagonists\textsuperscript{37} diphenhydramine (3.1) and pyrilamine (3.2) and from a dual $H_1$R/$H_4$R antagonist, the
quinazoline derivative $\text{(3.3)}$. Alkyl chains with/without amide groups, and various lengths (6-18 atoms) were employed as spacers (Figure 1). Such compounds should be helpful in searching for putative accessory binding sites on the receptors of interest.

Figure 1 Selected H1R and H1R/H4R dual ligands, and general structures of synthesized homo-dimeric ligands. Pharmacological data were taken from a) Wittmann et al.\textsuperscript{39} b) Deml et al.\textsuperscript{40} c) Smits et al.\textsuperscript{38} d) Wagner et al.\textsuperscript{41}
3.2. Results and discussion

3.2.1. Chemistry

The bivalent ligands 3.6a-d, 3.7a-d, 3.9a-d, 3.13a, 3.13b, 3.15a-d, 3.16a-d were according to the synthetic pathway shown in Schemes 1 and 2.

Scheme 1 Synthesis of compounds 3.6a-d, 3.7a-d and 3.9a-d.
Scheme 2 Synthesis of compounds $3.13a$, $3.13b$, $3.15a$-$d$ and $3.16a$-$d$. 
3.2.2. Results and discussion

3.2.2.1. Competition binding studies

Twenty-two novel homo-dimeric ligands were synthesized and routinely analyzed by radioligand competition binding assays. All assays were performed using membrane preparations of Sf9 insect cells expressing the hH₁R + RGS4 (regulator of G-protein signaling 4)\textsuperscript{42} in presence of 5 nM $[^3]$Hpyrilamine, or the hH₂R + $G_{αi2}$ + $G_{βγ12}$\textsuperscript{43} in presence of 10 nM $[^3]$Hhistamine. The resulting affinity data are summarized in Tables 1-3.

**Table 1** hH₁R affinities of compound 3.6a-d and 3.9a-d

<table>
<thead>
<tr>
<th>cpd.</th>
<th>spacer length</th>
<th>spacer type (X)</th>
<th>(pKᵢ) hH₁R</th>
</tr>
</thead>
<tbody>
<tr>
<td>diphenhydramine (3.1)</td>
<td></td>
<td></td>
<td>7.82 ± 0.03</td>
</tr>
<tr>
<td>3.6a</td>
<td>6</td>
<td>(CH₂)₆</td>
<td>6.94 ± 0.15</td>
</tr>
<tr>
<td>3.6b</td>
<td>8</td>
<td>(CH₂)₈</td>
<td>7.04 ± 0.10</td>
</tr>
<tr>
<td>3.6c</td>
<td>10</td>
<td>(CH₂)₁₀</td>
<td>6.95 ± 0.13</td>
</tr>
<tr>
<td>3.6d</td>
<td>12</td>
<td>(CH₂)₁₂</td>
<td>6.24 ± 0.19</td>
</tr>
<tr>
<td>3.9a</td>
<td>10</td>
<td>(H₂C)₂HN(C(H₂)₂CNH(C(H₂)₂)</td>
<td>7.82 ± 0.08</td>
</tr>
<tr>
<td>3.9b</td>
<td>12</td>
<td>(H₂C)₂HN(C(H₂)₄CNH(C(H₂)₂)</td>
<td>7.84 ± 0.12</td>
</tr>
<tr>
<td>3.9c</td>
<td>14</td>
<td>(H₂C)₂HN(C(H₂)₆CNH(C(H₂)₂)</td>
<td>7.61 ± 0.06</td>
</tr>
<tr>
<td>3.9d</td>
<td>16</td>
<td>(H₂C)₂HN(C(H₂)₈CNH(C(H₂)₂)</td>
<td>7.58 ± 0.08</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM of at least three independent experiments each performed in triplicate.
Two types of spacers, alkyl chains with/without amide groups, with different length were used to bridge two identical pharmacophores. However, both spacer types failed to improve affinities of all homo-dimeric ligand series at hH₁R and hH₄R in an obvious way. Thus, introducing amide bearing alkyl spacers by analogy with a report by McRobb et al. on diphenhydramine (3.1), pyrilamine (3.2) and quinazoline derivative (3.3) proved unsuccessful. The spacer length from this given study was 9-20Å, shorter than a suggested receptor dimer interface (22–27 Å), were more suitable for probing potential accessory binding sites on the receptors of interest rather than receptor dimers. At hH₁R, all homo-dimeric ligands with spacers containing amide groups (3.9a-d, 3.15a-d and 3.16a-d) showed similar affinities compared to corresponding monomeric parent leads (Tables 1-3) and no significant influence of spacer length variation on binding affinities was observed. The homo-dimeric ligands with amide-free-spacers present much lower affinities at hH₁R compared to the amide-containing ones. A reason for that may be the introducing of the pure alkyl spacers raised up the lipophilicity of the compound resulted a solubility problem and high non-specific binding in assays. At hH₄R, it may propose the introducing of spacers to the piperazine moiety of quinazoline derivative hindered the pharmacophore from optimally bound to orthosteric
binding site, leading to a considerable affinity drop for all quinazoline type homo-dimeric ligands.

**Table 3** hH₁R and hH₄R affinities of compound 3.13a, 3.13b, 3.15a-d and 3.16a-d

![Chemical structure of the compounds](image)

<table>
<thead>
<tr>
<th>cpd.</th>
<th>spacer length</th>
<th>spacer type (X)</th>
<th>(pKᵢ) hH₁R</th>
<th>(pKᵢ) hH₄R</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td></td>
<td></td>
<td>6.26 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.37 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.13a</td>
<td>10</td>
<td>(CH₂)₆</td>
<td>5.14 ± 0.05</td>
<td>5.19 ± 0.19</td>
</tr>
<tr>
<td>3.13b</td>
<td>12</td>
<td>(CH₂)₈</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.15a</td>
<td>10</td>
<td>(H₂C)₂HNC(CH₂)₂CNH(CH₂)₂</td>
<td>6.30 ± 0.23</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.15b</td>
<td>12</td>
<td>(H₂C)₈HNC(CH₂)₄CNH(CH₂)₂</td>
<td>5.77 ± 0.10</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.15c</td>
<td>14</td>
<td>(H₂C)₆HNC(CH₂)₆CNH(CH₂)₂</td>
<td>6.13 ± 0.13</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.15d</td>
<td>16</td>
<td>(H₂C)₈HNC(CH₂)₈CNH(CH₂)₂</td>
<td>6.82 ± 0.09</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.16a</td>
<td>12</td>
<td>(H₂C)₃HNC(CH₂)₂CNH(CH₂)₃</td>
<td>6.06 ± 0.04</td>
<td>5.60 ± 0.23</td>
</tr>
<tr>
<td>3.16b</td>
<td>14</td>
<td>(H₂C)₃HNC(CH₂)₄CNH(CH₂)₃</td>
<td>6.48 ± 0.07</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.16c</td>
<td>16</td>
<td>(H₂C)₆HNC(CH₂)₆CNH(CH₂)₃</td>
<td>6.55 ± 0.07</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3.16d</td>
<td>18</td>
<td>(H₂C)₃HNC(CH₂)₈CNH(CH₂)₃</td>
<td>6.26 ± 0.04</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM of at least three independent experiments each performed in triplicate. <sup>a</sup>(pKᵢ) hH₁R = 7.70, hH₄R = 8.12 (Smits et al.<sup>38</sup>)
3.2.2.2. Conclusion

Aiming at H₁R and/or H₄R pharmacological tools with high affinities, H₁R antagonists or dual H₁R/H₄R antagonists were employed as building blocks to generate series of homo-dimeric ligands. Based on the competition binding study results, it was speculated while one of the pharmacophore (diphenhydramine, pyrilamine or quinazoline moiety) of homo-dimeric ligand bound to receptor’s orthosteric binding site, the other pharmacophore did not contribute to the binding of receptor. Later, a computational MD simulation of compound 3.15d together with hH₁R (crystal structure 3RZE⁴⁴) confirmed this proposed binding mode (Figure 2). This suggested either there were no accessory binding sites, or diphenhydramine, pyrilamine and quinazoline derivative have no enough affinities to bind to the putative accessory binding sites.

![flexibly waving around on the receptor surface](image)

Figure 2 An overlay of two snapshots of 3.15d bound to hH₁R. (MD simulation, performed by PD Dr. Andrea Strasser)

To sum up, no concrete proof of an additional binding site at the extracellular surface of hH₁R or hH₄R can be found from given competition binding results. In order to prove or disprove the existence of accessory binding sites on hH₁R and hH₄R, different linkage point for spacer or a hetero-dimeric ligand approach should be considered in future studies.
3.3. Experimental section

3.3.1. Chemistry

3.3.1.1. General conditions

Commercially available reagents were from the following suppliers: Merck (Darmstadt, Germany), Acros Organics (Geel, Belgium), Sigma Aldrich (Munich, Germany) and TCI Europe (Eschborn, Germany). All solvents were of analytical grade or distilled prior to use and stored under protective gas. Deuterated solvents for NMR spectroscopy were from Deutero (Kastellaun, Germany). 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 argon. Column chromatography was carried out using Merck silica gel Geduran 60 (0.063-0.200 mm) and Merck silica gel 60 (0.040-0.063 mm) for flash-column chromatography. In certain cases, flash-chromatography was performed on an Intelli Flash-310 Flash-Chromatography Workstation from Varian Deutschland GmbH (Darmstadt, Germany). Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F254 aluminium sheets and spots were visualized with UV light at 254 nm and/or iodine vapor, ninhydrine spray. Nuclear Magnetic Resonance (\(^1\)H NMR and \(^{13}\)C NMR) spectra were recorded on an Avance-300 (\(^1\)H: 300 MHz, \(^{13}\)C: 75 MHz), Avance-400 (\(^1\)H: 400 MHz, \(^{13}\)C: 101 MHz), or Avance-600 (\(^1\)H: 600 MHz, \(^{13}\)C: 151 MHz) NMR spectrometer from Bruker BioSpin (Karlsruhe, Germany). Multicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), bs (for broad singulet), as well as 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), “quat” quaternary carbon atom. In certain cases, 2D-NMR techniques were used to assign \(^1\)H and \(^{13}\)C chemical shifts. All spectra were analyzed using the program MestReNova (Mestrelab Research, Santiago de Compostela, Spain). High-resolution mass spectrometry (HRMS) was performed on an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS system (Agilent Technologies, Santa Clara, CA) using an ESI source. Melting points (mp) were measured on a Büchi 530 (Büchi GmbH, Essen, Germany)
and are uncorrected. Lyophilisation of the products was done with a Christ alpha 2-4 LD, equipped with a Vacuubrand RZ 6 rotary vane vacuum pump. Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was a Eurospher-100 (250 x 32 mm) (Knauer, Berlin, Germany) or a Nucleodur 100-5 C18 ec (250 x 21 mm, 5 μm (Macherey-Nagel, Düren, Germany), which was attached to the UV-detector model K-2000 (Knauer, Berlin, Germany). UV-detection was done at 220 nm. The temperature was between rt and 30 °C and the flow rate was 15 mL/min. All compounds were filtered through PTFE-filters (25 mm, 0.2 μm, Phenomenex, Aschaffenburg, Germany) prior to preparative HPLC. Analytical HPLC analysis was performed on a system from Merck, composed of an L-5000 controller, a 655A-12 pump, a 655A-40 auto sampler, a L-4250 UV-VIS detector, and a RP column Kinetex-XB C18, 5 μm, 250 mm x 4.6 mm (Phenomenex, Aschaffenburg, Germany) at a flow rate of 0.8 mL/min. Mixtures of acetonitrile (A) and 0.1% aqueous TFA solution (B) were used as mobile phase. Detection was performed at 220 nm, the oven temperature was 30 °C. Helium degassing prior to HPLC analysis was performed. Compound purities were calculated as percentage peak area of the analyzed compound by UV detection at 220 nm. HPLC conditions, retention times (tR), the capacity (retention) factors were calculated according to k = (tR-t0)/t0, and purities of the synthesized compounds are listed in the appendix. Purity of tested compounds was >95% as determined by high-performance liquid chromatography. For all compounds, which were obtained as TFA salts, the number of respective hydrotrifluoroacetate were calculated by ACD/I-lab (ACD/Labs, Toronto, Ontario, Canada).

3.3.1.2. Synthesis

**Benzyl (2-bromoethyl)carbamate.**

Benzyl (2-bromoethyl)carbamate was synthesized according to Aissaoui et al.  

**tert-Butyl(3-bromopropyl)carbamate.**

*tert*-butyl(3-bromopropyl)carbamate were synthesized according to Jörg et al.  

**tert-Butyl piperazine-1-carboxylate.**
A solution of piperazine (8.6 g, 100 mmol) in DCM (100 mL) was cooled to 0 °C. Di-tert-butyl dicarbonate (10.9 g, 50 mmol) dissolved in DCM (100 mL) was added dropwise. The resulting mixture was stirred at rt overnight and concentrated under reduced pressure. The crude product was dissolved in brine (100 mL) and extracted with diethyl ether (3 x 50 mL). The organic layer was dried over Na₂SO₄, and organic solvent was evaporated, then subjected to silica gel column chromatography (EtOAc/MeOH, 9/1) to yield a white solid (6.67 g, 35.9 mmol, 71.8%). ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 3.40-3.36 (m, 4H), 2.83-2.78 (m, 4H), 1.77 (s, 1H), 1.45 (s, 9H).

2-(Benzhydryloxy)-N-methylethanamine (3.4).⁴⁸

Diphenhydramine hydrochloride (15 g, 51.4 mmol) was dissolved in a solution of K₂CO₃ (10.5 g, 76 mmol) in 100 mL water. The mixture was extracted three times with 150 mL (50 x 3) DCM. After removal of the solvent, the remaining crystal oil was dissolved in a solution of 150 mL of 1,2-dichloroethane, Na₂CO₃ (6 g, 56.61 mmol) was added. The mixture was cooled to -10 °C, and 1-chloroethyl chloroformate (11.13 mL, 104 mmol) was added dropwise. After stirring for 10 min at -10 °C, the solution was heated under reflux for 2 h (94 °C). The solvent was removed, the remaining oily residue was dissolved in 150 mL methanol and heated at 50 °C for 2 h. The solvent was removed and the resulting pale brown crystalline residue was dissolved in a solution of Na₂CO₃ (8 g, 75.48 mmol) in 150 mL of water in order to get the free base of the product, which was extracted with DCM and purified by column chromatography (EtOAc/MeOH containing 1% NH₃, 4/1). Yellow oil (4.51 g, 18.69 mmol, 35.9%). ¹H-NMR (300 MHz, DMSO-d₆) δ [ppm]: 7.43-7.18 (m, 10H), 5.43 (s, 1H), 3.45 (t, J = 5.6 Hz, 2H), 2.69 (t, J = 5.6 Hz, 2H), 2.28 (s, 3H). ¹³C-NMR (75 MHz, DMSO-d₆) δ [ppm]: 142.59 (C₂quat), 128.18 (+, Ar-CH), 127.08 (+, Ar-CH), 126.42 (+, Ar-CH), 82.46 (+, CH), 67.64 (-, CH₂), 50.77 (-, CH₂), 35.92 (+, CH₃). HRMS (EI-MS) calcd for C₁₆H₁₉NO [MH⁺] 242.1539, found 242.1549.

N¹-(4-Methoxybenzyl)-N²-methyl-N¹-(pyridin-2-yl)ethane-1,2-diamine (3.5).

Compound 3.5 was prepared from pyrilamine maleate (20 g, 49 mmol) by analogy with the procedure for the preparation of compound 3.4. Yellow oil was obtained. (910 mg, 3.35 mmol, 6.7%). ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 8.16-8.12 (m, 1H), 7.41-7.34 (m, 1H), 7.16-7.09
(m, 2H), 6.85-6.81 (m, 2H), 6.57-6.52 (m, 1H), 6.52-6.48 (m, 1H), 4.69 (s, 2H), 3.77 (s, 3H), 3.70 (t, J = 6.3 Hz, 2H), 2.85 (t, J = 6.4 Hz, 3H), 2.45 (s, 3H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 158.66 (C$_{quat}$), 158.43 (C$_{quat}$), 147.88 (+, Ar-CH), 137.41 (+, Ar-CH), 130.37 (C$_{quat}$), 128.01 (+, 2Ar-CH), 114.00 (+, 2Ar-CH), 112.08 (+, Ar-CH), 106.16 (+, Ar-CH), 55.28 (+, CH$_3$), 51.61 (-, CH$_2$), 49.90 (-, CH$_2$), 48.11 (-, CH$_2$), 36.32 (+, CH$_3$).

$N^1,N^6$-Bis[2-(benzhydryloxy)ethyl]-$N^1,N^6$-dimethylhexane-1,6-diamine (3.6a).

To a solution of compound 3.4 (241 mg, 1 mmol) in 5 mL acetone, Cs$_2$CO$_3$ (325 mg, 1 mmol) was added, and 1,6-diiodohexane (169 mg, 0.5 mmol) was added dropwise. The mixture was stirred at room temperature for 8 h (control by TLC). The solvent was removed under reduced pressure and the remaining mixture was subjected to preparative TLC over silica gel (EtOAc/MeOH containing 1% NH$_3$, 50/1). Yellow oil (62.8 mg, 0.11 mmol, 22.2%).

$^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.36-7.22 (m, 20H), 5.37 (s, 2H), 3.61 (t, J = 5.9 Hz, 4H), 2.74 (t, J = 5.9 Hz, 4H), 2.44 (t, J = 5.9 Hz, 4H), 2.32 (s, 6H), 1.55-1.42 (m, 4H), 1.32-1.21 (m, 4H).

$^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 142.14 (4C$_{quat}$), 128.39 (+, 8Ar-CH), 127.47 (+, 4Ar-CH), 126.96 (+, 8Ar-CH), 84.06 (+, 2CH), 66.86 (-, 2CH$_2$), 57.89 (-, 2CH$_2$), 56.63 (-, 2CH$_2$), 42.65 (+, 2CH$_3$), 27.28 (-, 2CH$_2$), 26.77 (-, 2CH$_2$). HRMS (EI-MS) calcd for C$_{38}$H$_{48}$N$_2$O$_2$ [M+H] $^+$ 565.3789, found 565.3790.

$N^1,N^{10}$-Bis[2-(benzhydryloxy)ethyl]-$N^1,N^{10}$-dimethyloctane-1,8-diamine (3.6b).

Compound 3.6b was prepared from compound 3.4 (241 mg, 1 mmol) and 1,8-diiodooctane (183 mg, 0.5 mmol) by analogy with the procedure for the preparation of compound 3.6a. Yellow oil (37.7 mg, 0.06 mmol, 12.8%).

$^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.37-7.20 (m, 20H), 5.37 (s, 2H), 3.58 (t, J = 6.1 Hz, 4H), 2.70 (t, J = 6.1 Hz, 4H), 2.32 (s, 6H), 1.55-1.42 (m, 4H), 1.32-1.21 (m, 4H).

$^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 142.27 (4C$_{quat}$), 128.36 (+, 8Ar-CH), 127.42 (+, 4Ar-CH), 126.98 (+, 8Ar-CH), 84.02 (+, 2CH), 67.20 (-, 2CH$_2$), 58.15 (-, 2CH$_2$), 56.77 (-, 2CH$_2$), 42.84 (+, 2CH$_3$), 29.57 (-, 2CH$_2$), 27.46 (-, 2CH$_2$), 27.08 (-, 2CH$_2$). HRMS (EI-MS) calcd for C$_{40}$H$_{52}$N$_2$O$_2$ [M+H] $^+$ 593.4102, found 593.4101.

$N^1,N^{10}$-Bis[2-(benzhydryloxy)ethyl]-$N^1,N^{10}$-dimethyldecane-1,10-diamine (3.6c).

$N^1,N^{10}$-Bis[2-(benzhydryloxy)ethyl]-$N^1,N^{10}$-dimethyldecane-1,10-diamine (3.6c).
Compound 3.6c was prepared from compound 3.4 (241 mg, 1 mmol) and 1,10-diiodododecane (197 mg, 0.5 mmol) by analogy with the procedure for the preparation of compound 3.6a. Yellow oil (136.8 mg, 0.22 mmol, 44.0%). \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.37-7.20 (m, 20H), 5.37 (s, 2H), 3.59 (t, \(J = 6.1\) Hz, 4H), 2.71 (t, \(J = 6.1\) Hz, 4H), 2.41 (t, \(J = 6.1\) Hz, 4H), 2.29 (s, 6H), 1.54-1.39 (d, \(J = 6.6\) Hz, 4H), 1.29-1.21 (m, 12H).

\(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 142.30 (4C \(\text{quat}\)), 128.36 (+, 8Ar-CH), 127.41 (+, 4Ar-CH), 126.98 (+, 8Ar-CH), 84.01 (+, 2CH), 67.26 (-, 2CH\(_2\)), 58.19 (-, 2CH\(_2\)), 56.81 (-, 2CH\(_2\)), 42.89 (+, 2CH\(_3\)), 29.61 (-, 4CH\(_2\)), 27.51 (-, 2CH\(_2\)), 27.17 (-, 2CH\(_2\)). HRMS (EI-MS) calcd for C\(_{42}\)H\(_{56}\)N\(_2\)O\(_2\) [M+H\(^+\)] 621.4415, found 621.4419.

\(N^1,N^2\)-Bis[2-(benzhydryloxy)ethyl]-\(N^1',N^2\)-dimethylidodecane-1,12-diamine (3.6d).

Compound 3.6d was prepared from compound 3.4 (241 mg, 1 mmol) and 1,12-dibromododecane (164 mg, 0.5 mmol) by analogy with the procedure for the preparation of compound 3.6a. Yellow oil (270 mg, 0.42 mmol, 84.0%). \(^1\)H-NMR (300 MHz, CH\(_3\)OD) \(\delta\) [ppm]: 7.39-7.12 (m, 20H), 5.36 (s, 2H), 3.53 (t, \(J = 5.7\) Hz, 4H), 2.64 (t, \(J = 5.7\) Hz, 4H), 2.44-2.30 (m, 4H), 2.22 (s, 6H), 1.52-1.38 (m, 4H), 1.31-1.19 (m, 16H). \(^{13}\)C-NMR (75 MHz, CH\(_3\)OD) \(\delta\) [ppm]: 143.88 (4C \(\text{quat}\)), 129.43 (+, 8Ar-CH), 127.41 (+, 4Ar-CH), 126.98 (+, 8Ar-CH), 84.01 (+, 2CH), 67.94 (-, 2CH\(_2\)), 59.01 (-, 2CH\(_2\)), 57.68 (-, 2CH\(_2\)), 43.30 (+, 2CH\(_3\)), 30.85 (-, 2CH\(_2\)), 30.82 (-, 2CH\(_2\)), 30.78 (-, 2CH\(_2\)), 28.75 (-, 2CH\(_2\)), 27.81 (-, 2CH\(_2\)). HRMS (EI-MS) calcd for C\(_{44}\)H\(_{60}\)N\(_2\)O\(_2\) [M+H\(^+\)] 649.4728, found 649.4728.

\(N^1,N^1\)-(Hexane-1,6-diyl)bis[\(N^2\)-(4-methoxybenzyl)-\(N^1\)-methyl-\(N^2\)-(pyridin-2-yl)ethane-1,2-diamine] (3.7a).

To a solution of compound 3.5 (350 mg, 1.29 mmol) in 5 mL MeCN, K\(_2\)CO\(_3\) (178 mg, 1.29 mmol) was added, and 1,6-diiodohexane (216 mg, 0.64 mmol) was added dropwise. The mixtures were stirred at room temperature for 8 h (control by TLC). The solvent was removed under reduced pressure, and the remaining mixtures were subjected to column chromatography over silica gel (EtOAc/MeOH containing 1% NH\(_3\), 20/1). Yellow oil (58 mg, 0.09 mmol, 14.5%). \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 8.16-8.13 (m, 2H), 7.40-7.37 (m, 2H), 7.18-7.11 (m, 4H), 6.86-6.79 (m, 4H), 6.56-6.44 (m, 4H), 4.69 (s, 4H), 3.77 (s, 6H), 3.74-3.64
(m, 4H), 2.72-2.56 (m, 4H), 2.51-2.38 (m, 4H), 2.34 (s, 6H), 1.55-1.43 (m, 4H), 1.33-1.22 (m, 6H). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.66 (2C\(_{\text{quat}}\)), 158.06 (2C\(_{\text{quat}}\)), 148.02 (+, 2Ar-CH), 137.28 (+, 2Ar-CH), 130.59 (2C\(_{\text{quat}}\)), 128.16 (+, 4Ar-CH), 113.97 (+, 4Ar-CH), 111.88 (+, 2Ar-CH), 105.90 (+, 2Ar-CH), 57.75 (-, 2CH\(_2\)), 55.28 (+, 2CH\(_3\)), 54.34 (-, 2CH\(_2\)), 51.49 (-, 2CH\(_2\)), 45.92 (-, 2CH\(_2\)), 42.27 (+, 2CH\(_3\)), 27.23 (-, 4CH\(_2\)). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.62 (2C\(_{\text{quat}}\)), 158.12 (2C\(_{\text{quat}}\)), 148.04 (+, 2Ar-CH), 137.19 (+, 2Ar-CH), 130.77 (2C\(_{\text{quat}}\)), 128.19 (+, 4Ar-CH), 113.92 (+, 4Ar-CH), 111.71 (+, 2Ar-CH), 105.78 (+, 2Ar-CH), 58.05 (-, 2CH\(_2\)), 55.26 (+, 2CH\(_3\)), 54.48 (-, 2CH\(_2\)), 51.35 (-, 2CH\(_2\)), 46.20 (-, 2CH\(_2\)), 42.52 (+, 2CH\(_3\)), 29.55 (-, 2CH\(_2\)), 27.41 (-, 2CH\(_2\)), 27.09 (-, 2CH\(_2\)). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.60 (2C\(_{\text{quat}}\)), 158.17 (2C\(_{\text{quat}}\)), 148.07 (+, 2Ar-CH), 137.17 (+, 2Ar-CH), 130.89 (2C\(_{\text{quat}}\)), 128.22 (+, 2CH\(_2\)), 126.09 (2C\(_{\text{quat}}\)), 119.21 (2C\(_{\text{quat}}\)), 59.05 (-, 2CH\(_2\)), 57.75 (-, 2CH\(_2\)), 51.49 (-, 2CH\(_2\)), 27.23 (-, 4CH\(_2\)). HRMS (EI-MS) calcd for C\(_{38}\)H\(_{52}\)N\(_6\)O\(_2\) [M\(^{+}\)] 625.4225, found 625.4227.

\(N',N''\)-(Octane-1,8-diyl)bis[\(N^2\)-(4-methoxybenzyl)-\(N^1\)-methyl-\(N^6\)-(pyridin-2-yl)ethane-1,2-diamine] (3.7b).

Compound 3.7b was prepared from compound 3.5 (271 mg, 1 mmol) and 1,8-diiodooctane (183 mg, 0.5 mmol) by analogy with the procedure for the preparation of compound 3.7a. Yellow oil (121 mg, 0.19 mmol, 37.1%). \(^1\text{H-NMR}\) (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 8.16-8.13 (m, 2H), 7.40-7.34 (m, 2H), 7.15 (d, \(J = 8.7\) Hz, 4H), 6.86-6.79 (m, 2H), 6.55-6.49 (m, 2H), 6.46 (d, \(J = 8.6\) Hz, 2H), 4.70 (s, 4H), 3.77 (s, 6H), 3.68-3.62 (m, 4H), 2.61-2.56 (m, 4H), 2.43-2.33 (m, 4H), 2.28 (s, 6H), 1.53-1.37 (m, 4H), 1.27-1.24 (m, 8H). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.62 (2C\(_{\text{quat}}\)), 158.12 (2C\(_{\text{quat}}\)), 148.04 (+, 2Ar-CH), 137.20 (+, 2Ar-CH), 130.77 (2C\(_{\text{quat}}\)), 128.19 (+, 4Ar-CH), 113.92 (+, 4Ar-CH), 111.71 (+, 2Ar-CH), 105.78 (+, 2Ar-CH), 58.05 (-, 2CH\(_2\)), 55.26 (+, 2CH\(_3\)), 54.48 (-, 2CH\(_2\)), 51.35 (-, 2CH\(_2\)), 46.20 (-, 2CH\(_2\)), 42.52 (+, 2CH\(_3\)), 29.55 (-, 2CH\(_2\)), 27.41 (-, 2CH\(_2\)), 27.09 (-, 2CH\(_2\)). HRMS (EI-MS) calcd for C\(_{40}\)H\(_{56}\)N\(_6\)O\(_2\) [M\(^{+}\)] 653.4538, found 653.4535.

\(N',N''\)-(Decane-1,10-diyl)bis[\(N^2\)-(4-methoxybenzyl)-\(N^1\)-methyl-\(N^6\)-(pyridin-2-yl)ethane-1,2-diamine] (3.7c).

Compound 3.7c was prepared from compound 3.5 (200 mg, 0.74 mmol) and 1,10-diiododecane (146 mg, 0.37 mmol) by analogy with the procedure for the preparation of compound 3.7a. Yellow oil (110 mg, 0.16 mmol, 43.0%). \(^1\text{H-NMR}\) (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 8.17-8.12 (m, 2H), 7.40-7.34 (m, 2H), 7.19-7.12 (m, 4H), 6.86-6.79 (m, 4H), 6.55-6.41 (m, 4H), 4.71 (s, 4H), 3.78 (s, 6H), 3.66-3.58 (m, 4H), 2.58-2.50 (m, 4H), 2.39-2.30 (m, 4H), 2.26 (s, 6H), 1.49-1.35 (m, 4H), 1.29-1.21 (m, 12H). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.60 (2C\(_{\text{quat}}\)), 158.17 (2C\(_{\text{quat}}\)), 148.07 (+, 2Ar-CH), 137.17 (+, 2Ar-CH), 130.89 (2C\(_{\text{quat}}\)), 128.22 (+,
4Ar-CH), 113.91 (+, 4Ar-CH), 111.64 (+, 2Ar-CH), 105.71 (+, 2Ar-CH), 58.21 (+, 2CH₃), 55.27 (+, 2CH₂), 54.60 (+, 2CH₂), 51.27 (-, 2CH₂), 46.37 (-, 2CH₂), 42.69 (+, 2CH₃), 29.64 (-, 4CH₂), 27.52 (-, 2CH₂), 27.35 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₄H₆₀N₆O₂ [M+H⁺] 681.4851, found 681.4850.

\(N',N''-\) (Dodecane-1, 12-diyl)bis[\(N''-\) (4-methoxybenzyl)- \(N'\)-methyl- \(N''\)- (pyridin-2-y lethane-1, 2-diamine] (3.7d).

Compound 3.7d was prepared from compound 3.5 (542 mg, 2 mmol) and 1,12-dibromododecane (328 mg, 1 mmol) by analogy with the procedure for the preparation of compound 3.7a. Yellow oil (410 mg, 0.58 mmol, 57.9%). \(^1\)H-NMR (300 MHz, CDCl₃) δ [ppm]: 8.17-8.13 (m, 2H), 7.40-7.34 (m, 2H), 7.19-7.12 (m, 4H), 6.86-6.80 (m, 2H), 6.55-6.49 (m, 2H), 6.48-6.43 (m, 2H), 4.70 (s, 4H), 3.77 (s, 6H), 3.67-3.62 (m, 4H), 2.61-2.55 (m, 4H), 2.42-2.33 (m, 4H), 2.28 (s, 6H), 1.50-1.37 (m, 4H), 1.30-1.20 (m, 16H). \(^{13}\)C-NMR (75 MHz, CDCl₃) δ [ppm]: 158.61 (2C quat), 158.12 (2C quat), 148.08 (+, 2Ar-CH), 137.21 (+, 2Ar-CH), 130.78 (2C quat), 128.20 (+, 4Ar-CH), 113.92 (+, 4Ar-CH), 111.71 (+, 2Ar-CH), 105.77 (+, 2Ar-CH), 58.09 (-, 2CH₂), 55.27 (+, 2CH₃), 54.47 (-, 2CH₂), 51.33 (-, 2CH₂), 46.22 (-, 2CH₂), 42.55 (+, 2CH₃), 29.65 (-, 6CH₂), 27.48 (-, 2CH₂), 27.14 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₄H₆₀N₆O₂ [M+H⁺] 709.5164, found 709.5167.

\(N'\)-[2-(Benzhydryloxy)ethyl]-\(N'\)-methylethane-1,2-diamine (3.8).

To a mixture of compound 3.4 (3.31 g, 13.73 mmol) and K₂CO₃ (5.68 g, 41.19 mmol) in 50 mL of MeCN benzyl (2-bromoethyl)carbamate (4.24 g, 16.48 mmol) was added. The reaction mixture was stirred at 100 °C for 2 h. The reaction mixture was cooled to rt, concentrated, and the residue dissolved in 15 mL MeOH. After addition of 5% palladium-on-charcoal catalyst (450 mg) a slow stream of hydrogen was passed via a glass tube into the vigorously stirred suspension at 1 atm H₂ for 17 h. After depletion of the starting material (control by TLC) the catalyst was removed by filtration through Celite. The filtrate was concentrated in vacuo to give the product as a white solid (2.0 g, 7.04 mmol, 98%). \(^1\)H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.38-7.19 (m, 10H), 5.37 (s, 1H), 3.56 (t, \(J = 5.9\) Hz, 2H), 2.77-2.65 (m, 4H), 2.47 (t, \(J = 6.0\) Hz, 2H), 2.27 (s, 3H).
\(N^1,N^6\text{-Bis}(2\text{-[(2\text{-benzhydryloxy}ethyl)(methyl)amino]ethyl})\text{succindiamide (3.9a).}\)

To a solution of compound 3.8 (284 mg, 1 mmol) and TEA (303 mg, 3 mmol) in 3 mL of anhydrous THF, succinyl dichloride (101 mg, 0.65 mmol) were added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The mixture was allowed to warm to room temperature with stirring for 2 h (control by TLC). The solvent was evaporated, and the remaining mixture was poured into water. The aqueous layer was extracted with DCM, and the combined organic layers were dried over Na\(_2\)SO\(_4\). After removal of the solvent under reduced pressure the product was purified by column chromatography (silica gel, DCM/MeOH/25% aqueous ammonia, 100/2.5/1). Colorless oil (170 mg, 0.26 mmol, 52.3%).

\(^1\text{H-NMR}\) (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.38-7.18 (m, 20H), 5.36 (s, 2H), 3.54 (t, \(J\) = 5.6 Hz, 4H), 3.32-3.25 (m, 4H), 2.70-2.64 (m, 4H), 2.52 (t, \(J\) = 6.0 Hz, 4H), 2.26 (s, 6H), 2.24-2.22 (m, 4H).

\(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 172.05 (2C \(\text{quat}\)), 142.19 (4C \(\text{quat}\)), 128.45 (+, 8Ar-CH), 127.95 (+, 4Ar-CH), 126.60 (+, 8Ar-CH), 83.99 (+, 2CH), 67.11 (-, 2CH\(_2\)), 56.59 (-, 2CH\(_2\)), 56.02 (-, 2CH\(_2\)), 42.48 (+, 2CH\(_3\)), 36.85 (-, 2CH\(_2\)), 31.43 (-, 2CH\(_2\)). \text{HRMS (EI-MS) calcd for C}_{40}H_{50}N_{4}O_{4} [M+H]\(^+\) 651.3905, found 651.3910.

\(N^1,N^6\text{-Bis}(2\text{-[(2\text{-benzhydryloxy}ethyl)(methyl)amino]ethyl})\text{adipdiamide (3.9b).}\)

Compound 3.9b was prepared from compound 3.8 (542 mg, 2 mmol) and adipoyl dichloride (357 mg, 1.95 mmol) by analogy with the procedure for the preparation of compound 3.9a. Yellow oil (63 mg, 0.09 mmol, 12.4%). \(^1\text{H-NMR}\) (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.37-7.20 (m, 20H), 5.37 (s, 2H), 3.56 (t, \(J\) = 5.4 Hz, 4H), 3.37-3.27 (m, 4H), 2.71 (t, \(J\) = 5.4 Hz, 4H), 2.58 (t, \(J\) = 5.8 Hz, 4H), 2.31 (s, 6H), 1.98-1.88 (m, 4H), 1.53-1.44 (m, 4H). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 171.85 (2C \(\text{quat}\)), 141.00 (4C \(\text{quat}\)), 127.42 (+, 8Ar-CH), 126.53 (+, 4Ar-CH), 125.86 (+, 8Ar-CH), 82.95 (+, 2CH), 65.65 (-, 2CH\(_2\)), 56.42 (-, 2CH\(_2\)), 56.00 (-, 2CH\(_2\)), 42.42 (+, 2CH\(_3\)), 36.52 (-, 2CH\(_2\)), 35.97 (-, 2CH\(_2\)), 25.08 (-, 2CH\(_2\)). \text{HRMS (EI-MS) calcd for C}_{42}H_{54}N_{4}O_{4} [M+H]\(^+\) 679.4218, found 679.4221.

\(N^1,N^6\text{-Bis}(2\text{-[(2\text{-benzhydryloxy}ethyl)(methyl)amino]ethyl})\text{octanediadime (3.9c).}\)
Compound 3.9c was prepared from compound 3.8 (284 mg, 1 mmol) and octanediol dichloride (116 mg, 0.55 mmol) by analogy with the procedure for the preparation of compound 3.9a. Yellow oil (205 mg, 0.29 mmol, 58.1%). $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.34-7.20 (m, 20H), 5.37 (s, 2H), 3.56 (t, $J$ = 5.4 Hz, 4H), 3.36-3.28 (m, 4H), 2.70 (t, $J$ = 5.4 Hz, 4H), 2.57 (t, $J$ = 5.8 Hz, 4H), 2.30 (s, 6H), 1.98-1.91 (m, 4H), 1.56-1.45 (m, 4H), 1.24-1.16 (m, 4H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 173.20 (2C quat), 142.08 (4C quat), 128.47 (+, 8Ar-CH), 127.58 (+, 4Ar-CH), 126.92 (+, 8Ar-CH), 84.00 (+, 2CH), 66.76 (-, 2CH$_2$), 56.45 (-, 2CH$_2$), 56.01 (-, 2CH$_2$), 42.42 (+, 2CH$_3$), 36.50 (-, 2CH$_2$), 36.39 (-, 2CH$_2$), 28.93 (-, 2CH$_2$), 25.53 (-, 2CH$_2$). HRMS (El-MS) calcd for C$_{44}$H$_{58}$N$_4$O$_4$ [M$^+$H$^+$] 707.4531, found 707.4532.

$N^1,N^{00}$-Bis(2-[(2-(benzhydryloxy)ethyl](methyl)amino)ethyl)decanediamide (3.9d).

Compound 3.9d was prepared from compound 3.8 (284 mg, 1 mmol) and octanediol dichloride (116 mg, 0.55 mmol) by analogy with the procedure for the preparation of compound 3.9a. Yellow oil (264 mg, 0.36 mmol, 71.9%). $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.37-7.20 (m, 20H), 5.37 (s, 2H), 3.56 (t, $J$ = 5.4 Hz, 4H), 3.37-3.28 (m, 4H), 2.70 (t, $J$ = 5.4 Hz, 4H), 2.57 (t, $J$ = 5.7 Hz, 4H), 2.30 (s, 6H), 2.01-1.90 (m, 4H), 1.58-1.44 (m, 4H), 1.24-1.16 (m, 8H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 173.30 (2C quat), 142.09 (4C quat), 128.47 (+, 8Ar-CH), 127.58 (+, 4Ar-CH), 126.92 (+, 8Ar-CH), 84.00 (+, 2CH), 66.75 (-, 2CH$_2$), 56.40 (-, 2CH$_2$), 55.96 (-, 2CH$_2$), 42.39 (+, 2CH$_3$), 36.50 (-, 2CH$_2$), 36.39 (-, 2CH$_2$), 29.23 (-, 4CH$_2$), 25.69 (-, 2CH$_2$). HRMS (El-MS) calcd for C$_{46}$H$_{62}$N$_4$O$_4$ [M$^+$H$^+$] 735.4844, found 735.4846.

2,4,6-Trichloroquinazoline (3.10)

2,4,6-Trichloroquinazoline was prepared according to Smits et al.\textsuperscript{38}

tert-Butyl 4- {6- chloro- 4- ([thiophen- 2- ylmethyl)amino]quinazolin- 2- yl)piperazine- 1-carboxylate (3.11).

A mixture of compound 3.10 (2.33 g, 9.7 mmol), EtOAc (15 mL), DIPEA (2.47 g, 19.14 mmol), 2-thienylmethylamine (1.08 g, 9.55 mmol) in a 20-mL microwave tube was stirred at rt for 30 min. tert-Butyl piperazine-1-carboxylate (4.5 g, 23.9 mmol) was added, the microwave tube was sealed, and the mixture was subjected to microwave irradiation (120 °C) for 10 min.
Solid material was filtered off and washed with EtOAc. The filtrate was washed with brine, dried over Na₂SO₄ and evaporated. Purification by column chromatography (EE/PE, 1/5) yielded a white solid (3.69 g, 8.02 mmol, 83.8%); mp 184 °C. ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.42-7.35 (m, 3H), 7.20-7.14 (m, 1H), 7.01 (d, J = 2.7 Hz, 1H), 6.94-6.90 (m, 1H), 4.89 (d, J = 5.5 Hz, 2H), 3.89-3.85 (m, 4H), 3.48-3.44 (m, 4H), 1.44 (s, 9H).

6-Chloro-2-(piperazin-1-yl)-N-(thiophen-2-ylmethyl)quinazolin-4-amine (3.12).

TFA (15 mL) was added to a solution of compound 3.11 (3.69 g, 8.02 mmol) in DCM (150 mL). After stirring for 3 h, the mixture was evaporated to dryness under reduced pressure, yielding 3.12 di(hydrotrifluoroacetate) as a white solid (3.0 g, 8.06 mmol, 100%); mp 157 °C. ¹H-NMR (300 MHz, DMSO-d₆) δ [ppm]: 8.11 (d, J = 2.3 Hz, 1H), 7.53-7.48 (m, 1H), 7.38-7.34 (m, 1H), 7.27 (d, J = 8.9 Hz, 1H), 7.08 (d, J = 2.5 Hz, 1H), 6.98-6.93 (m, 1H), 4.83 (d, J = 5.6 Hz, 2H), 3.83-3.75 (d, J = 4.7 Hz, 4H), 2.82-2.78 (m, 4H).

2,2'-[Decane-1,10-diylbis(piperazine-4,1-diyl)]bis[6-chloro-N-(thiophen-2-ylmethyl)quinazolin-4-amine] (3.13a).

Compound 3.13a was prepared from compound 3.12 (360 mg, 1 mmol) and 1,10-diiododecane (182 mg, 0.5 mmol) by analogy with the procedure for the preparation of compound 3.7a. Yellow oil (30 mg, 0.03 mmol, 7%). ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.52 (d, J = 1.8 Hz, 2H), 7.42-7.38 (m, 4H), 7.24-7.20 (m, 2H), 7.06-7.04 (m, 2H), 4.93 (d, J = 5.4 Hz, 4H), 4.02-3.97 (m, 8H), 3.06-2.96 (m, 4H), 2.59 (t, J = 5.0 Hz, 8H), 2.46-2.40 (m, 4H), 1.32-1.29 (m, 12H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 158.60 (6Cquat), 141.18 (2Cquat), 133.15 (+, 2Ar-CH), 127.48 (+, 2Ar-CH), 126.69 (+, 2Ar-CH), 126.25 (+, 2Ar-CH), 125.87 (2Cquat), 125.39 (+, 2Ar-CH), 120.51 (+, 2Ar-CH), 110.98 (2Cquat), 58.71 (-, 2CH₂), 53.07 (-, 2CH₂), 45.42 (-, 4CH₂), 43.50 (-, 2CH₂), 39.74 (-, 2CH₂), 34.46 (-, 2CH₂), 29.49 (-, 2CH₂), 27.60 (-, 2CH₂), 26.24 (-, 2CH₂). HRMS (EI-MS) calcd for C₄₄H₅₄Cl₂N₁₀S₂ [M⁺] 857.3424, found 857.3417.

2,2'-[Dodecane-1,12-diylbis(piperazine-4,1-diyl)]bis[6-chloro-N-(thiophen-2-ylmethyl)quinazoline-4-amine] (3.13b).
Compound 3.13b was prepared from compound 3.12 (360 mg, 1 mmol) and 1,12-dibromododecane (164 mg, 0.5 mmol) by analogy with the procedure for the preparation of compound 3.7a. Yellow oil (22 mg, 0.02 mmol, 4%). \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.47 (s, 2H), 7.41 (d, \(J = 2.7\) Hz, 4H), 7.24-7.20 (m, 2H), 7.07-7.03 (m, 2H), 6.98-6.94 (m, 2H), 4.93 (d, \(J = 5.3\) Hz, 4H), 4.05-3.93 (m, 8H), 3.63 (t, \(J = 6.6\) Hz, 4H), 2.61-2.52 (m, 8H), 2.45-2.39 (m, 4H), 1.28-1.21 (m, 16H).

\(^1\)^13C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.54 (6C quat), 141.09 (2C quat), 133.20 (+, 2Ar-CH), 127.34 (+, 2Ar-CH), 126.73 (+, 2Ar-CH), 126.32 (+, 2Ar-CH), 125.86 (+, 2Ar-CH), 125.47 (2C quat), 123.20 (+, 2Ar-CH), 121.34 (+, 2Ar-CH), 126.32 (+, 2Ar-CH), 125.86 (+, 2Ar-CH), 125.47 (2Cquat), 120.38 (+, 2Ar-CH), 110.98 (2C quat), 62.99 (-, 2CH\(_2\)), 58.90 (-, 2CH\(_2\)), 53.27 (-, 2CH\(_2\)), 43.64 (-, 4CH\(_2\)), 43.50 (-, 2CH\(_2\)), 39.94 (-, 2CH\(_2\)), 32.80 (-, 2CH\(_2\)), 29.53 (-, 2CH\(_2\)), 27.53 (-, 2CH\(_2\)), 25.75 (-, 2CH\(_2\)). HRMS (EI-MS) calcd for C\(_{46}\)H\(_{58}\)Cl\(_2\)N\(_{10}\)S\(_2\) [M+H]\(^+\) 885.3737, found 885.3736.


To a solution of compound 3.12 (910 mg, 2.5 mmol) in acetonitrile, DIPEA (323 mg, 2.51 mmol), tert-butyl (2-bromoethyl)carbamate (614 mg, 2.75 mmol) and sodium iodide (375 mg, 2.51 mmol) were added. The reaction mixture was heated to reflux for 2 h (control by TLC), the solvent was evaporated, and the remaining mixture was poured into water. The product was extracted with DCM, the combined organic layers were dried over Na\(_2\)SO\(_4\), the solvent was removed under reduced pressure, and the residue was subjected to column chromatography (DCM/MeOH/25% aqueous ammonia, 100/2.5/1), yielding a yellow solid (680 mg, 1.35 mmol, 54.8%). m.p. 171 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.45-7.41 (m, 2H), 7.40-7.35 (m, 1H), 7.25-7.21 (m, 1H), 7.06-7.04 (m, 1H), 6.99-6.94 (m, 1H), 4.94 (d, \(J = 5.3\) Hz, 2H), 3.99-3.89 (m, 4H), 3.33-3.21 (m, 2H), 2.55-2.45 (m, 6H), 1.46 (s, 9H).


Compound 3.14b was prepared by analogy with the procedure for the preparation of 3.14a, using compound 3.12 (579 mg, 1.59 mmol) and tert-butyl(3-bromopropyl)carbamate (530 mg, 1.59 mmol). After purification of column chromatography (DCM/MeOH/25% aqueous
ammonia, 100/2.5/1), a white solid was obtained (501 mg, 0.97 mmol, 61.3%). m.p. 167 °C. 
\(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.55-7.50 (m, 1H), 7.44-7.35 (m, 2H), 7.22-7.18 (m, 1H), 7.06-7.03 (m, 1H), 6.96-6.92 (m, 1H), 4.93 (d, \(J = 5.4\) Hz, 2H), 4.00-3.89 (m, 4H), 3.26-3.15 (m, 2H), 2.56-2.40 (m, 6H), 1.74-1.63 (m, 2H), 1.43 (s, 9H). 
\(^1^3\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 158.57 (C\(_{quat}\)), 158.51 (C\(_{quat}\)), 156.16 (C\(_{quat}\)), 150.71 (C\(_{quat}\)), 141.24 (C\(_{quat}\)), 133.15 (+, Ar-CH), 127.24 (+, Ar-CH), 126.66 (+, Ar-CH), 126.30 (+, Ar-CH), 125.77 (C\(_{quat}\)), 125.38 (+, Ar-CH), 120.59 (+, Ar-CH), 110.97 (C\(_{quat}\)), 78.94 (C\(_{quat}\)), 66.83 (-, CH\(_2\)), 57.01 (-, CH\(_2\)), 53.33 (-, 2CH\(_2\)), 43.90 (-, 2CH\(_2\)), 42.75 (-, CH\(_2\)), 39.83 (-, CH\(_2\)), 28.48 (+, 3CH\(_3\)). HRMS (EI-MS) calcd for C\(_{25}\)H\(_{33}\)ClN\(_6\)O\(_2\)S \([M+H]^+\) 517.2147, found 517.2148.

\(N^1,N^4\)-Bis[2-(4-(6-chloro-2-ylmethyl)amino)quinazolin-2-yl]piperazin-1-yl)ethyl]succinamide (3.15a).

Compound 3.14a (251 mg, 0.50 mmol) was dissolved in 10 mL DCM, 10 mL of TFA was added dropwise, the mixture was stirred at rt for 3 h (control by TLC). The solvent was evaporated, the remaining oil was dissolved in anhydrous THF, succinyl dichloride (32 mg, 0.20 mmol) were added dropwise in presence of TEA (128 mg, 0.80 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for another 30 min, then allowed to warm to room temperature with stirring for 2 h (control by TLC). The solvent was evaporated, and the remaining mixture was poured into water. The aqueous layer was extracted with DCM, and the combined organic layers were dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure, and the product was purified by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/2/1). White solid (40 mg, 0.05 mmol, 20.3%), m.p. 112 °C. \(^1\)H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) [ppm]: 8.19 (d, \(J = 2.0\) Hz, 2H), 7.52-7.44 (m, 2H), 7.31-7.26 (d, \(J = 8.9\) Hz, 2H), 7.07 (d, \(J = 2.9\) Hz, 2H), 6.95-6.91 (m, 2H), 4.81 (d, \(J = 5.5\) Hz, 4H), 3.94-3.66 (m, 8H), 3.42-3.35 (m, 4H), 3.26-3.12 (m, 4H), 2.53-2.47 (m, 8H), 2.33-2.30 (m, 4H). \(^1^3\)C-NMR (75 MHz, DMSO-d\(_6\)) \(\delta\) [ppm]: 171.24 (2C\(_{quat}\)), 158.54 (2C\(_{quat}\)), 158.18 (2C\(_{quat}\)), 150.46 (2C\(_{quat}\)), 142.24 (4C\(_{quat}\)), 132.57 (+, 2Ar-CH), 126.83 (+, 2Ar-CH), 126.22 (+, 2Ar-CH), 125.87 (+, 2Ar-CH), 125.07 (+, 2Ar-CH), 122.13 (+, 2Ar-CH), 111.11 (2C\(_{quat}\)), 56.98 (-, 2CH\(_2\)), 52.64 (-, 2CH\(_2\)), 43.25 (-, 2CH\(_2\)), 38.53 (-, 4CH\(_2\)), 35.85 (-, 2CH\(_2\)), 30.80 (-, 4CH\(_2\)). HRMS (EI-MS) calcd for C\(_{42}\)H\(_{48}\)Cl\(_2\)N\(_{12}\)O\(_2\)S\(_2\) [M+H\(^+\)] 887.2914, found 887.2908.
\(N^1, N^6\)-Bis[2-(4-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl)piperazin-1-yl) ethyl]adipamide (3.15b).

Compound 3.15b was prepared by analogy with the procedure for the preparation of 3.15a, using compound 3.14a (407 mg, 0.81 mmol) and adipoyl dichloride (33 mg, 0.17 mmol). White solid (117 mg, 0.13 mmol, 32.0 %), m.p. 192 °C. \(\text{\textsuperscript{1}H-NMR} (300 \text{ MHz}, \text{CD}_{3}\text{OD}) \delta \text{ [ppm]}:
\)
8.23 (d, \(J = 2.2 \text{ Hz}, 2\text{H})\), 7.85-7.80 (m, 2\text{H}), 7.64 (d, \(J = 8.9 \text{ Hz}, 2\text{H})\), 7.34-7.30 (m, 2\text{H}), 7.15-7.12 (m, 2\text{H}), 7.00-6.96 (m, 2\text{H}), 5.04 (s, 4\text{H}), 4.40-4.20 (m, 8\text{H}), 3.67-3.45 (m, 12\text{H}), 3.36-3.32 (m, 4\text{H}), 2.35-2.25 (m, 4\text{H}), 1.70-1.63 (m, 4\text{H}).

\(\text{\textsuperscript{13}C-NMR} (75 \text{ MHz}, \text{CD}_{3}\text{OD}) \delta \text{ [ppm]}:
\)
177.32 (2\text{C quat}), 160.43 (2\text{C quat}), 153.38 (2\text{C quat}), 140.70 (4\text{C quat}), 136.88 (+, 2\text{Ar-CH}), 132.22 (2\text{C quat}), 128.11 (+, 2\text{Ar-CH}), 127.88 (+, 2\text{Ar-CH}), 126.71 (+, 2\text{Ar-CH}), 124.43 (+, 2\text{Ar-CH}), 120.82 (+, 2\text{Ar-CH}), 112.60 (2\text{C quat}), 58.00 (-, 2\text{CHZ}), 52.48 (-, 4\text{CHZ}), 43.45 (-, 4\text{CHZ}), 41.35 (-, 2\text{CHZ}), 36.42 (-, 2\text{CHZ}), 35.36 (-, 2\text{CHZ}), 28.30 (-, 2\text{CHZ}), 25.12 (-, 2\text{CHZ}). HRMS (EI-MS) calcd for C_{44}H_{52}Cl_{2}N_{12}O_{2}S_{2} [M+H] 915.3227, found 915.3226.

\(N^1, N^6\)-Bis[2-(4-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl)piperazin-1-yl) ethyl]octanediamide (3.15c).

Compound 3.15c was prepared by analogy with the procedure for the preparation of 3.15a, using compound 3.14a (231 mg, 0.46 mmol) and octanedioyl dichloride (42 mg, 0.20 mmol). White solid (110 mg, 0.12 mmol, 52.1 %), m.p. 194 °C. \(\text{\textsuperscript{1}H-NMR} (300 \text{ MHz}, \text{DMSO-d}_6) \delta \text{ [ppm]}:
\)
8.10 (d, \(J = 2.3 \text{ Hz}, 2\text{H})\), 7.51-7.46 (m, 2\text{H}), 7.37-7.33 (m, 2\text{H}), 7.26 (d, \(J = 8.9 \text{ Hz}, 2\text{H})\), 7.09-7.05 (m, 2\text{H}), 6.96-6.92 (m, 2\text{H}), 4.82 (d, \(J = 5.6 \text{ Hz}, 4\text{H})\), 3.83-3.76 (m, 8\text{H}), 3.23-3.14 (m, 4\text{H}), 2.46-2.31 (m, 12\text{H}), 2.04 (t, \(J = 7.3 \text{ Hz}, 4\text{H})\), 1.53-1.40 (m, 4\text{H}), 1.26-1.18 (m, 4\text{H}).

\(\text{\textsuperscript{13}C-NMR} (75 \text{ MHz}, \text{DMSO-d}_6) \delta \text{ [ppm]}:
\)
171.91 (2\text{C quat}), 158.52 (2\text{C quat}), 158.22 (2\text{C quat}), 150.52 (2\text{C quat}), 142.22 (2\text{C quat}), 132.56 (+, 2\text{Ar-CH}), 126.89 (+, 2\text{Ar-CH}), 126.24 (+, 2\text{Ar-CH}), 125.84 (+, 2\text{Ar-CH}), 125.10 (+, 2\text{Ar-CH}), 123.97 (2\text{C quat}), 121.91 (+, 2\text{Ar-CH}), 111.03 (2\text{C quat}), 57.14 (-, 2\text{CHZ}), 52.71 (-, 4\text{CHZ}), 43.32 (-, 4\text{CHZ}), 38.67 (-, 2\text{CHZ}), 35.91 (-, 2\text{CHZ}), 35.28 (-, 2\text{CHZ}), 28.30 (-, 2\text{CHZ}), 25.12 (-, 2\text{CHZ}). HRMS (EI-MS) calcd for C_{46}H_{52}Cl_{2}N_{12}O_{2}S_{2} [M+H] 943.3540, found 943.3533.
$N^1,N^6$-bis[2-(4-{6-chloro-4-[{(thiophen-2-ylmethyl)amino]quinazolin-2-yl}piperazin-1-yl})
ethyl]decanediamide (3.15d).

Compound 3.15d was prepared by analogy with the procedure for the preparation of 3.15a,
using compound 3.14a (231 mg, 0.46 mmol) and decanediyl dichloride (57 mg, 0.24 mmol).
White solid (140 mg, 0.14 mmol, 61.4 %), m.p. 219 °C. $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]:
7.62 (d, $J = 1.5$ Hz, 2H), 7.37 (d, $J = 8.9$ Hz, 2H), 7.26-7.19 (m, 4H), 7.07-7.03 (m, 2H),
6.98-6.93 (m, 2H), 4.90 (d, $J = 5.3$ Hz, 4H), 3.99-3.91 (m, 8H), 3.44-3.35 (m, 4H), 2.60-2.52
(m, 12H), 2.19 (t, $J = 7.6$ Hz, 4H), 1.66-1.56 (m, 4H), 1.32-1.27 (m, 8H). $^{13}$C-NMR (75 MHz,
CDCl$_3$) δ [ppm]: 173.47 (6C$_{ quar}$), 158.52 (2C$_{ quar}$), 140.69 (2C$_{ qua}$), 133.42 (+, 2Ar-CH), 126.79 (+,
2Ar-CH), 126.55 (+, 2Ar-CH), 125.43 (+, 4Ar-CH), 121.22 (+, 2Ar-CH), 110.92 (4C$_{ qua}$),
56.74 (-, 2CH$_2$), 52.79 (-, 4CH$_2$), 45.71 (-, 2CH$_2$), 43.92 (-, 2CH$_2$), 39.86 (-, 2CH$_2$), 36.70 (-,
2CH$_2$), 35.73 (-, 2CH$_2$), 29.19 (-, 2CH$_2$), 29.19 (-, 2CH$_2$), 25.71 (-, 2CH$_2$). HRMS (EI-MS)
calcd for C$_{48}$H$_{60}$Cl$_2$N$_{12}$O$_2$S$_2$ [M+H]$^+$ 971.3853, found 971.3848.

$N^1,N^6$-Bis[3-(4-{6-chloro-4-[{(thiophen-2-ylmethyl)amino]quinazolin-2-yl}piperazin-1-yl})
propyl]succinamide (3.16a).

Compound 3.16a was prepared by analogy with the procedure for the preparation of 3.15a,
using compound 3.14b (345 mg, 0.67 mmol) and succinyl dichloride (46 mg, 0.30 mmol).
White solid (180 mg, 0.20 mmol, 59.7%), m.p. 106 °C. $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]:
7.50 (d, $J = 1.9$ Hz, 2H), 7.43-7.33 (m, 4H), 7.22-7.19 (m, 2H), 7.05-7.01 (m, 2H), 6.96-6.92
(m, 2H), 4.91 (d, $J = 5.4$ Hz, 4H), 4-3.85 (m, 8H), 3.41-3.25 (m, 4H), 2.60-2.40 (m, 16H),
1.77-1.60 (m, 4H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 172.02 (2C$_{ qua}$), 158.59 (4C$_{ qua}$),
150.77 (2C$_{ qua}$), 141.23 (2C$_{ qua}$), 133.15 (+, 2Ar-CH), 127.33 (+, 2Ar-CH), 126.69 (+, 2Ar-CH),
126.26 (+, 2Ar-CH), 125.79 (2C$_{ qua}$), 125.44 (+, 2Ar-CH), 120.53 (+, 2Ar-CH), 111.01 (2C$_{ qua}$),
57.29 (-, CH$_2$), 53.31 (-, 2CH$_2$), 43.99 (-, 2CH$_2$), 39.92 (-, CH$_2$), 39.28 (-, CH$_2$), 31.95 (-, CH$_2$),
25.32 (-, 2CH$_2$). HRMS (EI-MS) calcd for C$_{44}$H$_{52}$Cl$_2$N$_{12}$O$_2$S$_2$ [M+H]$^+$ 915.3227, found 915.3227.

$N^1,N^6$-Bis[3-(4-{6-chloro-4-[{(thiophen-2-ylmethyl)amino]quinazolin-2-yl}piperazin-1-yl})
propyl]adipamide (3.16b).

Compound 3.16b was prepared by analogy with the procedure for the preparation of 3.15a,
using compound 3.14b (306 mg, 0.59 mmol) and adipoyl dichloride (46 mg, 0.25 mmol).
White solid (210 mg, 0.22 mmol, 74.1%), m.p. 91 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.50 (d, \(J = 2.0\) Hz, 2H), 7.44-7.34 (m, 4H), 7.23-7.19 (m, 2H), 7.06-7.00 (m, 2H), 6.97-6.92 (m, 2H), 4.92 (d, \(J = 5.4\) Hz, 4H), 4.00-3.85 (m, 8H), 3.43-3.34 (m, 4H), 2.60-2.39 (m, 12H), 2.20-2.10 (m, 4H), 1.73-1.61 (m, 8H). \(^1^3\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 172.63 (2C\(_{\text{quat}}\)), 158.74 (2C\(_{\text{quat}}\)), 158.62 (2C\(_{\text{quat}}\)), 150.91 (2C\(_{\text{quat}}\)), 141.25 (2C\(_{\text{quat}}\)), 133.16 (+, 2Ar-CH), 127.40 (+, 2Ar-CH), 126.69 (+, 2Ar-CH), 126.25 (+, 2Ar-CH), 125.81 (2C\(_{\text{quat}}\)), 125.43 (+, 2Ar-CH), 120.54 (+, 2Ar-CH), 111.05 (2C\(_{\text{quat}}\)), 57.59 (-, CH\(_2\)), 53.36 (-, 2CH\(_2\)), 44.06 (-, 2CH\(_2\)), 39.93 (-, CH\(_2\)), 39.45 (-, CH\(_2\)), 36.47 (-, CH\(_2\)), 25.23 (-, 4CH\(_2\)). HRMS (EI-MS) calcd for C\(_{46}\)H\(_{56}\)Cl\(_2\)N\(_{12}\)O\(_2\)S\(_2\) [M+H]\(^+\) 943.3540, found 943.3535.

\(N^1,N^6\)-Bis[3-(4-(6-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl)piperazin-1-yl)propyl]octanediamide (3.16c).

Compound 3.16c was prepared by analogy with the procedure for the preparation of 3.15a, using compound 3.14b (327 mg, 0.63 mmol) and octanediol dichloride (45 mg, 0.21 mmol). White solid (130 mg, 0.13 mmol, 41.0%), m.p. 97 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.52 (d, \(J = 2.0\) Hz, 2H), 7.47-7.41 (m, 4H), 7.22-7.18 (m, 2H), 7.04-7.00 (m, 2H), 6.96-6.92 (m, 2H), 4.92 (d, \(J = 5.4\) Hz, 4H), 4.01-3.84 (m, 8H), 3.40-3.27 (m, 4H), 2.58-2.40 (m, 12H), 2.13-2.06 (m, 4H), 1.72-1.69 (m, 4H), 1.63 – 1.54 (m, 4H), 1.27-1.23 (m, 4H). \(^1^3\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 172.95 (2C\(_{\text{quat}}\)), 158.76 (2C\(_{\text{quat}}\)), 158.65 (2C\(_{\text{quat}}\)), 150.92 (2C\(_{\text{quat}}\)), 141.27 (2C\(_{\text{quat}}\)), 133.19 (+, 2Ar-CH), 127.38 (+, 2Ar-CH), 126.68 (+, 2Ar-CH), 126.22 (+, 2Ar-CH), 125.84 (2C\(_{\text{quat}}\)), 125.40 (+, 2Ar-CH), 120.60 (+, 2Ar-CH), 111.08 (2C\(_{\text{quat}}\)), 57.76 (-, CH\(_2\)), 53.38 (-, 2CH\(_2\)), 44.05 (-, 2CH\(_2\)), 39.92 (-, CH\(_2\)), 39.55 (-, CH\(_2\)), 36.88 (-, 2CH\(_2\)), 28.97 (-, 2CH\(_2\)), 25.73 (-, 2CH\(_2\)), 25.16 (-, CH\(_2\)). HRMS (EI-MS) calcd for C\(_{48}\)H\(_{56}\)Cl\(_2\)N\(_{12}\)O\(_2\)S\(_2\) [M+H]\(^+\) 971.3853, found 971.3852.

\(N^1,N^{10}\)-Bis[3-(4-(6-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl)piperazin-1-yl)propyl]decanediamide (3.16d).

Compound 3.16d was prepared by analogy with the procedure for the preparation of 3.15a, using compound 3.14b (339 mg, 0.66 mmol) and decanediol dichloride (87 mg, 0.36 mmol). White solid (250 mg, 0.25 mmol, 76.0%), m.p. 110 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]:
7.55 (d, J = 2.0 Hz, 2H), 7.46-7.41 (m, 4H), 7.22-7.18 (m, 2H), 7.03-7.00 (m, 2H), 6.96-6.91 (m, 2H), 4.91 (d, J = 5.4 Hz, 4H), 3.97-3.85 (m, 8H), 3.43-3.27 (m, 4H), 2.59-2.41 (m, 12H), 2.13-2.03 (m, 4H), 1.75-1.62 (m, 4H), 1.63-1.50 (m, 4H), 1.30-1.15 (m, 8H). ^{13}C-NMR (75 MHz, CDCl₃ δ [ppm]: 173.08 (2C quat), 158.78 (2C quat), 158.68 (2C quat), 150.90 (2C quat), 141.31 (2Cquat), 133.18 (+, 2Ar-CH), 127.36 (+, 2Ar-CH), 126.66 (+, 2Ar-CH), 126.19 (+, 2Ar-CH), 125.85 (2Cquat), 125.36 (+, 2Ar-CH), 120.66 (+, 2Ar-CH), 111.11 (2Cquat), 57.89 (-, CH₂), 53.39 (-, 2CH₂), 44.07 (-, 2CH₂), 39.89 (-, CH₂), 39.68 (-, CH₂), 37.06 (-, 2CH₂), 29.29 (-, 2CH₂), 29.23 (-, 2CH₂), 25.93 (-, 2CH₂), 25.04 (-, CH₂). HRMS (EI-MS) calcd for C₅₀H₆₄Cl₂N₁₂O₂S₂ [M+H]⁺ 999.4166, found 999.4166.

3.3.2. Pharmacology

3.3.2.1. Competition binding experiments

Competition binding experiments were performed on membrane preparations of Sf9 insect cells expressing the hH₁R + RGS4 or the hH₄R + Gα₂ + β₁γ₂. General procedures for the generation of recombinant baculoviruses, culture of Sf9 cells and membrane preparation are described elsewhere. The respective membranes were thawed and sedimented by centrifugation at 4 °C and 13000 rpm for 10 min. Membranes were re-suspended in binding buffer (12.5 mM MgCl₂, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each tube (total volume 100 μL) contained 30 μg (hH₁R) or 100 μg (hH₄R) of membrane protein and increasing concentrations of unlabeled ligands. Radioligands: H₁R: [³H]pyrilamine, specific activity 20.0 Ci/mmol, Kᵹ = 4.5 nM, c = 5 nM, nonspecific binding determined in the presence of 10 μM of diphenhydramine; H₄R: [³H]histamine, specific activity 25 Ci/mmol, Kᵹ = 10 nM, c = 10 nM, nonspecific binding determined in the presence of 10 μM of histamine. Filtration through glass microfiber filters (for hH₄R, glass microfiber filters was pretreated with 0.3% polyethylenimine, Whatman GF/B, Maidstone, UK) using a Brandel 96 sample harvester (Brandel, Gaithersburg, MD) separated unbound from membrane associated radioligand. After three washing steps with binding buffer, filter pieces for each well were punched out and transferred into 96-well sample plates 1450-401 (Perkin Elmer, Rodgau Germany). Each well was supplemented with 200 μL of scintillation cocktail (Rotiscint Eco plus, Roth, Karlsruhe, Germany) and incubated in the dark. Radioactivity was measured with a Micro Beta² 1450 scintillation counter (Perkin
Elmer, Rodgau, Germany). Protein concentration was determined by the method of Lowry using bovine serum albumin as standard. Data analysis of the resulting competition curves was accomplished by non-linear regression analysis using the algorithms in PRISM GraphPad Software (GraphPad Prism 5.0 software, San Diego, CA). $K_i$ values were calculated according to the Cheng-Prusoff equation. Values represent the mean ± SEM of 3 independent experiments each performed in triplicate.

### 3.3.2.2. Preparation of compound stock solutions

All compounds were dissolved in 50% DMSO and 50% double distilled water (v/v) with appropriate equivalents of aqueous HCl. The final DMSO concentration was adjusted to 5% (v/v) in all assays. As demonstrated previously, DMSO concentrations up to 5% (v/v) are tolerated and have no influence on $pK_i$ and $pEC_{50}$ values. Ligand concentrations were used in the range from 0.1 nM up to 1 mM.

### 3.4. References

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Chapter 4

Benzimidazole- and Quinazoline-type histamine H₁/H₄ receptor ligands: Dual vs. subtype selective antagonism
4. Benzimidazole- and Quinazoline-type histamine $H_1/H_4$ receptor ligands: Dual vs. subtype selective antagonism

4.1. Introduction

Histamine (4.1, Figure 1) is a mediator and neurotransmitter involved in numerous physiological and pathological processes mediated via four histamine receptor subtypes, which all belong to class A of G-protein-coupled receptors. The human histamine $H_1$ receptor is expressed on various cell types including endothelial cells and smooth muscle cells and involved in allergic and inflammatory reactions, e.g., bronchial asthma, allergic rhinitis, and urticaria. The human histamine $H_4$ receptor which was identified and cloned around 2001 is mainly localized in cells of the immune system, such as neutrophils, eosinophils, basophils, dendritic cells, mast cells and monocytes. As a major player in histamine-induced immunological and inflammatory reactions, the $hH_4R$ was suggested as a potential target for the treatment of asthma, pruritus, and rheumatoid arthritis. Due to the complementary and overlapping functions of $hH_1R$ and $hH_4R$, it has been speculated that the combination of $hH_1R$ and $hH_4R$ antagonists in treating allergic diseases might be superior to monotherapy. In various experimental models of allergy, a synergistic effect of co-administered $hH_1R$ and $hH_4R$ antagonists was observed regarding inhibition of pruritus and skin inflammation from chronic dermatitis, acute hapten-induced scratching, and peanut-induced intestinal allergy. Thus, dual $hH_1R/hH_4R$ antagonists with balanced high affinities at both receptors might harbor a great potential for the treatment of allergic diseases.

In order to reach multiple molecular targets simultaneously, a prevailing way is to use cocktails of drugs. But this approach may be hampered by poor patient compliance and the risk of drug-drug interactions. In principle, dual or multiple target ligands capable of addressing multiple desired biological targets are suggested to be superior with respect to higher affinity, potency, efficacy and reduced reliance on multiple drug regimens upon
administration, and enhanced or modified physiological responses. Besides their potential clinical value, such hybrid compounds may be useful pharmacological tools for in vitro investigations. Previously, ligands showing affinities at both histamine receptor subtypes, H₃R and H₄R, were described, e.g. quinazoline, aminopyrimidine, astemizole, and loxapine derivatives (Figure 1). But due to the low homology between H₃R and H₄R (23.0%), most of the putative dual receptor ligands preferred one of the receptor subtypes and failed to bind with adequate balanced affinities to both, H₃R and H₄R.

Figure 1: Structures and affinities of selected H₃R and/or H₄R ligands. Pharmacological data were taken from a) Strasser et al. b) Igel et al. c) Smits et al. d) Wagner et al. e) Hammer et al. f) Smits et al. g) Naporra et al. h) Baumeister i) Geyer et al.
In the present work, aiming at ligands with dual actions on hH₁R and hH₄R, compounds derived from high affinity hH₁R and/or hH₄R ligands were synthesized and characterized at the four human histamine receptors. For this purpose, benzimidazole-type derivatives 4.3 and 4.4 (Figure 1) were employed. Compound 4.4, a ‘truncated’ analog of astemizole 4.7 (Figure 1), shows high affinity to the hH₁R (pKᵢ 8.77) but almost no affinity to the hH₄R (pKᵢ 4.41), compound 4.3 is a modified version of compound 4.4 with higher hH₄R affinity (pKᵢ 5.61) but reduced hH₁R affinity (pKᵢ 7.07).³⁷, ³⁸ Twenty-four derivatives with different substituents at various positions of the benzimidazole scaffold were synthesized, aiming at compounds with increased hH₄R affinity but retained hH₁R affinity (Figure 2). In addition, several compounds derived from 4.8 (Figure 1), a potent and selective hH₄R ligand,⁴², ⁴⁴ were prepared. The H₁R ligand 4.4 and the H₄R ligand 4.8 share structural features, suggesting a combination of these two moieties to increase the affinity at both receptors. In a second approach, structural modifications in 2-position of the quinazoline scaffold of compound 3.3 (Figure 1) were carried out. For both approaches, imidazole containing building blocks, e.g., histamine 4.1 and homohistamine 4.9 (Figure 1), were employed to boost the hH₄R affinity. By linking these pharmacophoric moieties to benzimidazole or quinazoline scaffold with or without a spacer, series of putative dual target ligands²⁸ were synthesized.

Figure 2 Structural variations of benzimidazoles and quinazolines synthesized as putative dual H₁ and H₄ receptor ligands.
4.2. Results and discussion

4.2.1. Chemistry For the synthesis of benzimidazole derivatives (Scheme 1 and 2), N-substituted 2-chlorobenzimidazoles 4.12a-e were allowed to react with various amines under microwave radiation in the presence of a base to obtain compounds 4.19a, 4.19b, 4.20a, 4.20b, 4.23 and intermediates 4.21a, 4.21b, 4.31a-c. In case of intermediate 4.28, the reaction was performed under acidic conditions in order to increase the nucleophilic attack in favor of the amino group compared to the hydroxy group of 4-aminophenol.45

Building blocks 4.3, 4.4, 4.14a-c were synthesized in two steps according to a procedure described by Rainer et al.46 The 2-phenylbenzimidazole scaffold 4.24 was built based on a procedure described by Xu, et al.47 The propylamine moiety was introduced to compounds 4.3, 4.4, 4.24 and 4.28 by alkylation with tert-butoxycarbonyl protected bromopropylamine. In case of compound 4.26, the benzimidazole was alkylated with 1-(chloromethyl)-4-fluorobenzene prior to the cleavage of the Boc group. Compounds 4.16a, 4.16b, 4.27, 4.30, 4.32a, 4.32b were prepared by alkylation of respective amine precursors and 4-(2-bromoethyl)-1-tritylimidazole. The corresponding intermediates were not isolated but directly subjected to de-protection, followed by column chromatography or preparative HPLC to yield the title compounds. In some cases (compounds 4.18a, 4.18b, 4.22a, 4.22b), higher yields were obtained when the imidazole moiety was left unprotected, that is, 4-(2-bromoethyl)imidazole was used instead of 4-(2-bromoethyl)-1-tritylimidazole. Urocanic acid was coupled to compounds 4.31a, 4.31c affording the corresponding amides (4.33a, 4.33b), which were subjected to hydrogenation or reduction with lithium aluminum hydride to give compounds 4.34 and 4.35a, 4.35b, respectively.
**Scheme 1** Synthesis of the benzimidazole derivatives 4.16-20, 4.22 and 4.23\(^a\)

\[
\begin{align*}
\text{Reagents and conditions: (i) } & R^2-\text{Cl, NaOH, ACN, 95 °C, 2 h; (ii) ethyl 4-aminopiperidine-1-carboxylate, 170 °C, overnight; (iii) 47% HBr in H}_{2}\text{O, 126 °C, 3 h; (iv) tert-butyl (3-bromopropyl)carbamate, NaI, DIPEA, ACN, reflux, 2 h; (v) TFA, DCM, rt, 3 h; (vi) 4-(2-bromoethyl)-1-tritylimidazole, NaI, DIPEA, ACN, reflux, 48 h; (vii) MeI, DIPEA, ACN, 0.5 h, rt; (viii) 4-(2-bromoethyl)imidazole, DIPEA, ACN, microwave, 120 °C, 10 min; (ix) histamine dihydrochloride, DIPEA, NMP, microwave, 200 °C, 10 min; (x) 2-(4-methylpiperazin-1-yl)ethan-1-amine, DIPEA, NMP, microwave, 180 °C, 2 h; (xi) piperazine, DIPEA, NMP, microwave, 180 °C, 1 h; (xii) 4-(2-bromoethyl)imidazole, DIPEA, NMP, microwave, 120 °C, 50 min; (xiii) 1-methylpiperazine, DIPEA, NMP, microwave, 180 °C, 1 h.}
\end{align*}
\]

\(^a\)Compound 4.12a is a mixture of 2,5-dichloro-1-phenethyl-1H-benz[d]imidazole and 2,6-dichloro-1-phenethyl-1H-benz[d]imidazole.
Scheme 2 Synthesis of the benzimidazole derivatives 4.26, 4.27, 4.30, 4.32, 4.33, 4.34 and 4.35

Reagents and conditions: (i) Na$_2$S$_2$O$_5$, DMF, 90 °C, 2 h, H$_2$O, 4 h, 0 °C; (ii) tert-butyl(3-bromopropyl)carbamate, K$_2$CO$_3$, acetone, 60 °C, 17 h; (iii) 1-(chloromethyl)-4-fluorobenzene, NaOH, ACN, reflux, 2 h; (iv) TFA, DCM, rt, 3 h; (v) 4-(2-bromoethyl)-1-trityl-imidazole, NaI, DIPEA, ACN, reflux, 48 h; (vi) 4-aminophenol, EtOH, HCl in iso-propanol, 48 h; (vii) diamine, DIPEA, microwave, 160-180 °C, 10 min - 3 h; (viii) urocanic acid, HOBT, TBTU, DIPEA, rt, 30 min; (ix) 5% Pd/C, H$_2$, MeOH, rt, 19 h; (x) LiAlH$_4$, THF, 0 °C to rt to 70 °C, 3h, 15% NaOH in H$_2$O, H$_2$O, 0 °C to rt, 30 min.

Quinazoline derivatives 4.40, 4.41, 4.42 and 4.44 (Scheme 3) as well as the required building blocks 4-(2-bromoethyl)-imidazole, 4-(2-bromoethyl)-1-trityl-imidazole, 4-methyl-1-piperazineethanamine, tert-butyl piperazine-1-carboxylate and 2-(1-trityl-imidazol-4-yl)ethan-1-amine were synthesized according to described procedures.
Scheme 3 Synthesis of the quinazoline derivatives 4.40, 4.41, 4.42 and 4.44*

Reagents and conditions: (i) thiophen-2-ylmethanamine, DIPEA, EtOAc, rt, 0.5 h; (ii) tert-butyl piperazine-1-carboxylate, DIPEA, EtOAc, microwave, 120 °C, 10 min; (iii) TFA, DCM, rt, 3 h; (iv) 4-(2-bromoethyl)-imidazole, DIPEA, EtOAc, microwave, 120 °C, 30 min; (v) 2-(4-methylpiperazine-1-yl)ethan-1-amine, DIPEA, NMP, microwave, 180 °C, 2 h; (vi) 1-methylpiperidin-4-amine, DIPEA, NMP, microwave, 180 °C, 50 min; (vii) 2-(1-tritylimidazol-4-yl)ethan-1-amine, DMF, microwave, 160 °C, 10 min.

4.2.2. Pharmacology

The title compounds were analyzed by radioligand competition binding assays on hH₁R and hH₂R, and selected compounds were additionally tested on hH₃R. For functional characterization, the compounds were investigated for agonism and antagonism in [³⁵S]GTPγS binding assays at the four human histamine receptor subtypes. All assays were performed using membrane preparations of Sf9 insect cells expressing the hH₁R + RGS4 (regulator of G-protein signaling 4), hH₂R-Gas, hH₃R + Gς₁ + Gβ₁γ₂ or the hH₄R + Gς₁ + Gβ₁γ₂.
4.2.2.1. Competition binding data at the hH₁R and hH₄R.

The affinities of the title compounds at the hH₁R and the hH₄R are summarized in Table 1. Based on the substituents at the 2-position of compounds 4.3 and 4.4 the benzimidazoles can be grouped into four types (Figure 4, red box). A piperazine moiety (“Type A”), attached directly or via a spacer decreased the affinity at both, the hH₁R and the hH₄R (e.g. compounds 4.20a, 4.20b, 4.22b, 4.23). In case of compounds bearing a piperidin-4-ylamino group (“Type B”), an aminopropyl substituent at the piperidine nitrogen (4.3) increased the hH₁R affinity but decreased hH₄R binding (4.15b). Further extension of the substituent by imidazolylethyl moiety led to a moderate increase in hH₄R affinity, whereas the pKᵢ at the hH₁R remained unchanged (4.16a and 4.16b). The structural modifications of “Type C” compounds, which are derived from 2-aryl benzimidazole 4.8, were not tolerated at the hH₁R, unless benzimidazole and phenyl ring were connected by a NH group (cf. 4.26, 4.27 and 4.30). Interestingly, compounds 4.27 and 4.30 showed a considerable increase in hH₄R affinity. Among the investigated benzimidazoles, 4.30 represented the first dual hH₁R/hH₄R ligand with nearly balanced affinities in the range of pKᵢ ≈ 8 (4.30, pKᵢ: hH₁R 8.04, hH₄R 7.74). Comparing compounds with and without an imidazole moiety (cf. 4.16b, 4.27 vs. 4.15b, 4.26), it becomes obvious that this substructure substantially contributes to H₄R binding, provided that the benzimidazole and the imidazole moieties are connected by appropriated linkers (cf. 4.16a, 4.16b vs. 4.18a, 4.18b). With this information, compounds of “Type D” were synthesized to further investigate the influence of the linker. Extending the linker length was beneficial with respect to H₄R affinity (cf. 4.19a, 4.19b, 4.32a, 4.32b, 4.35a, 4.35b). However, the decrease in hH₁R was comparable to that observed for Type C analogs. Compound 4.35b, with a distance between the imidazole and the benzimidazole ring which is 13Å, similar to that of 4.30, was identified as a dual hH₁R/hH₄R ligand with balanced affinity (Ki values in the two-digit nM range).

Replacing the 4-fluorobenzyl substituent in position 1 of the benzimidazole by 2-thienylethyl phenethyl or 4-chlorobenzyl moiety (Figure 4, purple box), however, no positive influence was observed (cf. 4.14c, 4.17c vs. 4.3, 4.17b). A 4-fluorobenzyl moiety was most favorable with respect to hH₁R binding (cf. 4.4, 4.16a, 4.18a, 4.19a, 4.20a, 4.22a compare with their counterpart 4.3, 4.16b, 4.18b, 4.19b, 4.20b, 4.22b). Chlorine atom introduced at 5- or
6-position of benzimidazole (Figure 4, blue box) proved detrimental to hH₁R and hH₄R affinity (cf. 4.14a, 4.14b, 4.17a vs. 4.3, 4.17b). Thus, introducing a piperazine moiety at 2-position, thienylethyl at 1-position and a chlorine atom at 5- or 6-position of the benzimidazole core by analogy with a report by Smits et al. on quinazoline-type ligands proved unsuccessful.

All variations of the quinazoline-type ligands (Table 2) led to a decrease in affinity at both, the hH₁R and hH₄R, except for compound 4.44, which showed a three-fold higher hH₄R affinity, compared to compound 3.3.

Table 1 hH₁R and hH₄R affinities of benzimidazole-type ligands

<table>
<thead>
<tr>
<th>cpd.</th>
<th>R</th>
<th>R²</th>
<th>(pKᵦ) hH₁R</th>
<th>(pKᵦ) hH₄R</th>
<th>cpd.</th>
<th>R</th>
<th>R²</th>
<th>(pKᵦ) hH₁R</th>
<th>(pKᵦ) hH₄R</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td></td>
<td>f</td>
<td>7.07 ± 0.04*</td>
<td>5.61 ± 0.08*</td>
<td>4.19b</td>
<td></td>
<td>f</td>
<td>6.34 ± 0.05</td>
<td>6.06 ± 0.01</td>
</tr>
<tr>
<td>4.4</td>
<td></td>
<td>f</td>
<td>8.77 ± 0.05*</td>
<td>4.41 ± 0.14*</td>
<td>4.20a</td>
<td></td>
<td>f</td>
<td>7.74 ± 0.25</td>
<td>4.68 ± 0.18</td>
</tr>
<tr>
<td>4.14a</td>
<td></td>
<td>f</td>
<td>5.87 ± 0.02</td>
<td>4.86 ± 0.07</td>
<td>4.20b</td>
<td></td>
<td>f</td>
<td>5.42 ± 0.31</td>
<td>4.72 ± 0.17</td>
</tr>
<tr>
<td>4.14b</td>
<td></td>
<td>f</td>
<td>5.52 ± 0.03</td>
<td>5.18 ± 0.17</td>
<td>4.22a</td>
<td></td>
<td>f</td>
<td>8.18 ± 0.14</td>
<td>5.10 ± 0.24</td>
</tr>
<tr>
<td>4.14c</td>
<td></td>
<td>f</td>
<td>7.11 ± 0.08</td>
<td>5.14 ± 0.16</td>
<td>4.22b</td>
<td></td>
<td>f</td>
<td>6.45 ± 0.10</td>
<td>4.93 ± 0.13</td>
</tr>
<tr>
<td>4.15b</td>
<td></td>
<td>f</td>
<td>8.22 ± 0.09</td>
<td>4.79 ± 0.30</td>
<td>4.23</td>
<td></td>
<td>f</td>
<td>6.74 ± 0.04</td>
<td>3.85 ± 0.34</td>
</tr>
<tr>
<td>4.16a</td>
<td></td>
<td>f</td>
<td>8.50 ± 0.04</td>
<td>6.61 ± 0.21</td>
<td>4.26</td>
<td></td>
<td>f</td>
<td>4.83 ± 0.08</td>
<td>4.27 ± 0.18</td>
</tr>
<tr>
<td>4.16b</td>
<td></td>
<td>f</td>
<td>6.75 ± 0.15</td>
<td>6.15 ± 0.09</td>
<td>4.27</td>
<td></td>
<td>f</td>
<td>5.51 ± 0.18</td>
<td>7.90 ± 0.04</td>
</tr>
<tr>
<td>4.17a</td>
<td></td>
<td>f</td>
<td>5.82 ± 0.03</td>
<td>4.43 ± 0.09</td>
<td>4.30</td>
<td></td>
<td>f</td>
<td>8.04 ± 0.06</td>
<td>7.74 ± 0.05</td>
</tr>
<tr>
<td>4.17b</td>
<td></td>
<td>f</td>
<td>7.46 ± 0.10</td>
<td>5.30 ± 0.02</td>
<td>4.32a</td>
<td></td>
<td>f</td>
<td>7.43 ± 0.09</td>
<td>5.51 ± 0.06</td>
</tr>
<tr>
<td>4.17c</td>
<td></td>
<td>f</td>
<td>6.64 ± 0.07</td>
<td>4.65 ± 0.29</td>
<td>4.32b</td>
<td></td>
<td>f</td>
<td>8.25 ± 0.17</td>
<td>6.01 ± 0.05</td>
</tr>
<tr>
<td>4.18a</td>
<td></td>
<td>f</td>
<td>8.06 ± 0.13</td>
<td>4.82 ± 0.10</td>
<td>4.34</td>
<td></td>
<td>f</td>
<td>7.29 ± 0.19</td>
<td>4.85 ± 0.09</td>
</tr>
<tr>
<td>4.18b</td>
<td></td>
<td>f</td>
<td>7.62 ± 0.12</td>
<td>5.05 ± 0.10</td>
<td>4.35a</td>
<td></td>
<td>f</td>
<td>7.93 ± 0.10</td>
<td>6.72 ± 0.05</td>
</tr>
<tr>
<td>4.19a</td>
<td></td>
<td>f</td>
<td>7.92 ± 0.01</td>
<td>6.05 ± 0.08</td>
<td>4.35b</td>
<td></td>
<td>f</td>
<td>7.26 ± 0.02</td>
<td>7.31 ± 0.07</td>
</tr>
</tbody>
</table>

The compounds were characterized in radioligand competition binding assays using membrane preparations of SF9 insect cells expressing the hH₁R + RGS4 or the hH₄R + Giα₂ + Gβ₁γ₂. Radioligands: H₁R: [³H]pyrilamine, Kᵦ = 4.5 nM, c = 5 nM; H₄R: [³H]histamine, Kᵦ = 10 nM, c = 10 nM. Data represent mean values ± SEM of at least three independent experiments performed in triplicate. a Reference data from Wagner et al.
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Figure 4 Summarized structure-activity relationships of the benzimidazole-type ligands at hH₁R and hH₄R

Table 2 hH₁R and hH₄R affinities of quinazoline-type ligands

<table>
<thead>
<tr>
<th>Cpd.</th>
<th>R</th>
<th>(pKᵢ) hH₁R</th>
<th>(pKᵢ) hH₄R</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td></td>
<td>6.26 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.37 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.40</td>
<td></td>
<td>5.77 ± 0.28</td>
<td>5.72 ± 0.10</td>
</tr>
<tr>
<td>4.41</td>
<td></td>
<td>4.39 ± 0.24</td>
<td>4.30 ± 0.09</td>
</tr>
<tr>
<td>4.42</td>
<td></td>
<td>5.41 ± 0.03</td>
<td>5.76 ± 0.11</td>
</tr>
<tr>
<td>4.44</td>
<td></td>
<td>5.23 ± 0.03</td>
<td>7.90 ± 0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup>(pKᵢ) hH₁R = 7.70, hH₄R = 8.12 (Smits et al.<sup>35</sup>)

The compounds were characterized in radioligand competition binding assays using membrane preparations of SF9 insect cells expressing the hH₁R + RGS4 or the hH₄R + G<sub>αi2</sub> + G<sub>βγ12</sub>. Radioligands:
H1R: [3H]pyrilamine, $K_d = 4.5 \text{ nM}, c = 5 \text{ nM};$ H3R: [3H]histamine, $K_d = 10 \text{ nM}, c = 10 \text{ nM}.$ Values represent the mean ± SEM of at least three independent experiments performed in triplicate.

### 4.2.2.2. Histamine receptor subtype selectivity and activity of selected compounds.

**Table 3** Binding and functional data of selected compounds at hH3R, determined by radioligand-competition binding and [35S]GTPγS-binding assays.

<table>
<thead>
<tr>
<th>cpd.</th>
<th>hH1R $[pK_i]$ (pEC$_{50}$ or $pK_5$)</th>
<th>hH3R $[pK_i]$ (pEC$_{50}$ or $pK_5$)</th>
<th>hH2R $[pK_i]$ (pEC$_{50}$ or $pK_5$)</th>
<th>hH4R $[pK_i]$ (pEC$_{50}$ or $pK_5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>(5.43±0.15)</td>
<td>1</td>
<td>(6.07±0.10)</td>
<td>1</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Famotidine</td>
<td>n.d.</td>
<td>n.d.</td>
<td>6.53±0.06</td>
<td>-0.33±0.06</td>
</tr>
<tr>
<td>Levocetirizine</td>
<td>7.17±0.15</td>
<td>-0.23±0.09</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

4.16a: $[8.50±0.04]$  | $[5.06±0.03]$ | $[6.79±0.05]$ | $[6.81±0.21]$ |
4.19a: $[7.92±0.01]$ | $[5.34±0.06]$ | $[7.34±0.02]$ | $[6.05±0.08]$ |
4.27: $[5.51±0.18]$ | $[5.50±0.02]$ | $[7.39±0.03]$ | $[7.90±0.04]$ |
4.30: $[8.04±0.06]$ | $[5.48±0.05]$ | $[7.26±0.01]$ | $[7.74±0.05]$ |
4.32b: $[8.25±0.17]$ | $[5.74±0.14]$ | $[5.88±0.05]$ | $[6.01±0.05]$ |
4.35a: $[7.93±0.10]$ | $[6.11±0.06]$ | $[6.89±0.03]$ | $[6.72±0.05]$ |
4.35b: $[7.26±0.02]$ | $[5.28±0.06]$ | $[6.94±0.01]$ | $[7.31±0.07]$ |
4.44: $[5.23±0.03]$ | $[6.70±0.03]$ | $[8.33±0.10]$ | $[7.90±0.02]$ |

The compounds were characterized in radioligand competition binding and [35S]GTPγS binding assays, performed on membrane preparations of SF9 insect cells expressing the hH1R + RGS4, hH3R-GGRS, hH3R + Ga2 + Gβ1γ2 or the hH2R + Ga2 + Gβ1γ2. Radioligands: H1R: [3H]pyrilamine, $K_d = 4.5 \text{ nM}, c = 5 \text{ nM};$ H3R: [3H]UR-DE 257 (N-[6-(3,4-dioxo-2-{3-[3-(piperidin-1-ylmethyl)phenoxy]propylamino}-cyclobut-1-ylamino)hexyl]-[2,3-$H_2$]-propion-amine); $K_d = 12.1 \text{ nM}, c = 20 \text{ nM};$ H2R: [3H]UR-PI 294 (N’-[3-(1H-imidazol-4-yl)propyl]-N'-propionylguanidine); $K_d = 3.3 \text{ nM}, c = 3.5 \text{ nM};$ H4R: [3H]histamine, $K_d = 10 \text{ nM}, c = 10 \text{ nM}.$ The $pK_i$ values of neutral antagonists and inverse agonists were determined in the antagonist mode in presence of histamine (10 μM for the hH1R, 1 μM for the hH2R, and 100 nM for the hH3R and the hH4R, respectively). Values represent the mean ± SEM of at least three independent experiments each performed in triplicate. $\alpha = 0$ (neutral antagonism): the measured values were in the range between ± 0.15 and not significantly different from zero.
Eight compounds exhibiting high hH,R and/or hH,R affinities (4.16a, 4.19a, 4.27, 4.30, 4.32b, 4.35a, 4.35b and 4.44) were selected for further pharmacological evaluations regarding hH,2R and hH,3R subtype affinity and functional activity at the four human histamine receptor subtypes (Table 3). Whereas hH,2R binding was low to moderate, for all imidazole containing ligands, poor selectivity for the hH,4R over the hH,3R became obvious. The selected compounds showed binding affinities in the same range at the hH,3R and the hH,4R, except for compound 19a which exhibited a preference for the hH,3R.

In the functional assays compounds 4.16a, 4.32b and 4.35a were selective hH,1R antagonists. Compound 4.19a turned out to be an hH,1R antagonist and a potent inverse agonist at the hH,3R (Table 2 and Figure 5). A dual hH,1R/hH,3R antagonist might be useful to treat allergic rhinitis59-61, e.g., a combined H,1 and H,3 receptor blockade was reported to reduce histamine-induced nasal congestion62-64.

![Image of compound 4.19a](image)

**Figure 5** Binding and function of compound 4.19a at histamine receptors. Curves shown represent mean values of at least three independent experiments performed in triplicate. A) Competition binding
curves of compound 4.19a at hHxR. Concentration-response curves of histamine and compound 4.19a at the B) hH1R, C) hH2R, D) hH3R and E) hH4R, determined in the [35S]GTPγS binding assay. All assays were performed on membrane preparations of Sf9 insect cells expressing the hH1R + RGS4, hH2R-Gsα55, hH3R + Gαi2 + Gβγ12 or the hH4R + Gαi2 + Gβγ12. Radioligands: H1R: [3H]pyrilamine, Ke = 4.5 nM, c = 5 nM; H2R: [3H]UR-DE 25757, Ke = 12.1 nM, c = 20 nM; H3R: [3H]UR-PI 29458, Ke = 3.3 nM, c = 3.5 nM; H4R: [3H]histamine, Ke = 10 nM, c = 10 nM. The pKd values of neutral antagonists and inverse agonists were determined in the antagonist mode in the presence of histamine (10 μM for the hH1R, 1 μM for the hH2R, and 100 nM for the hH3R and the hH4R, respectively). In the antagonist mode of [35S]GTPγS binding assay, the signal of histamine is referred to 100%.

The quinazoline derivative 4.44 (Table 2 and Figure 6) was a more potent dual hH3R/hH4R inverse agonist than thioperamide.
Compound \textit{4.35b} was an antagonist at the hH$_1$R, an inverse agonist at the hH$_4$R, but a weak hH$_3$R partial agonist. Thus, functional characterization revealed opposite qualities of action for compound \textit{4.35b} at the hH$_3$R and the hH$_4$R (Table 2 and Figure 7). Among the studied ligands compounds \textit{4.30} and \textit{4.35b} showed the highest (nearly) balanced affinities at both, the hH$_1$R and the hH$_4$R (Figure 8).

![Compound 4.35b](image)

\textbf{Figure 7} Binding and function of compound \textit{4.35b} at histamine receptors. Curves shown represent for mean values of at least three independent experiments performed in triplicate. A) Competition binding curves of compound \textit{4.35b} at hH$_x$R. Concentration-response curves of histamine and compound \textit{4.35b} at the B) hH$_1$R, C) hH$_2$R, D) hH$_3$R and E) hH$_4$R respectively, determined in the [\textsuperscript{35}S]GTP\textgamma{S} binding assay. For details cf. legend to \textbf{Figure 5}. 
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Figure 8 Affinity-selectivity profile of the compounds 3.3, 4.3-4.8, 4.14a-c, 4.15b, 4.16a, 4.16b, 4.17a-c, 4.18a, 4.18b, 4.19a, 4.19b, 4.20a, 4.20b, 4.22a, 4.22b, 4.23, 4.26, 4.27, 4.30, 4.32a, 4.32b, 4.34, 4.35a, 4.35b, 4.40, 4.41, 4.42, 4.44. The data were obtained from competition binding assays at hH₁R or hH₄R under comparable conditions.

4.3. Conclusion

Aiming at dual hH₁R/hH₄R antagonists, benzimidazole- and quinazoline-type compounds were synthesized and pharmacologically characterized at the four human histamine receptor subtypes. All compounds with high hH₁R and/or hH₄R affinities showed only weak affinity to the hH₂R. Unfortunately, ligands comprising imidazolylalkyl moieties did not discriminate between hH₃ and hH₄ receptors. Regardless of that, compounds 4.30 and 4.35b were identified as balanced dual hH₁R/hH₄R ligands with the highest affinities among the investigated ligands. The data of the presented benzimidazole and quinazoline derivatives are indicative of the complexity of the structure-activity relationships. It appears extremely difficult to identify a common H₁R/H₄R pharmacophore at a high level of affinity. Introducing an imidazole moiety may result in balanced H₁R/H₄R affinities. However, binding and
functional properties of such hybrid compounds become even more complex due to poor discrimination between H₄ and H₃ receptors.

4.4. Experimental section

4.4.1. Chemistry

4.4.1.1. General conditions.

See section 3.3.1.1.

4.4.1.2. Synthesis

4-(2-Bromoethyl)imidazole monohydrochloride.⁴⁸

A 250 mL round bottom flask containing 42.4 mL of 1.5 M sulfuric acid, histamine dihydrochloride (5.34 g, 28.9 mmol) and potassium bromide (11.52 g, 96.0 mmol) was fitted with a magnetic stirrer and cooled to -15 °C. A saturated solution of sodium nitrite (2.58 g, 37.2 mmol) in 3.8 mL of water was added in one portion to the magnetically stirred sulfuric acid/histamine solution at -15 °C. A color change from colorless to a deep orange-brown was observed, and a gas evolved in the reaction flask immediately upon addition. After a period of 30 minutes, the reaction mixture was warmed to room temperature. After a total of 3 hours, no more bubbling was observed and the reaction mixture had changed from a deep orange-brown to almost colorless light yellow. The reaction mixture was cooled to -15 °C and adjusted to a pH of 10 by dropwise addition of 27.6 mL of 5 M sodium hydroxide solution. This basic solution was transferred to a 100 mL separation funnel where the desired product was quickly extracted with chloroform (5 x 10 mL). The colorless chloroform layer was added directly from the separation funnel to 64 mL of 0.5 N HCl in isopropanol in a 250 mL round bottom flask. Concentration of this solution under reduced pressure gave white crystals (1.70 g, 6.72 mmol, yield 23.2%). ¹H-NMR (300 MHz, DMSO-d₆) δ ppm: 9.08 (d, J = 1.2 Hz, 1H), 7.54 (s, 1H), 3.91-3.76 (m, 2H), 3.27-3.12 (m, 2H). HRMS (EI-MS) m/z: calcd for C₅H₈BrN₂ [M+H⁺] 174.9865, found 174.9864.

4-(2-Bromoethyl)-1-tritylimidazole.⁴⁹
A mixture of 4-(2-bromoethyl)imidazole hydrochloride (1.68 g, 9 mmol), triphenylmethylicloride (3.69 g, 13.4 mmol) and TEA (2.73 g, 27 mmol) in DMF (15 mL) was stirred at rt overnight. The mixture was poured into brine, extracted with DCM (90 mL), and the evaporated organic phase was subjected to column chromatography (PE/EE, 2/1). White solid (2.67g, 6.42mmol, yield 22.2%); mp 143°C.

\[
\text{^1H-NMR (300 MHz, CDCl}_3\text{)} \delta [\text{ppm}]: 7.39 (d, J = 1.3 \text{ Hz}, 1H), 7.36-7.29 (m, 9H), 7.15 (m, 6H), 6.65 (d, J = 0.5 \text{ Hz}, 1H), 3.80-3.60 (m, 2H), 3.12-2.95 (m, 2H).
\]

\[
\text{^13C-NMR (75 MHz, CDCl}_3\text{)} \delta [\text{ppm}]: 142.38 (2C_{\text{quat}}), 138.55 (+, \text{Ar-CH}), 138.37 (C_{\text{quat}}), 137.62 (C_{\text{quat}}), 129.81 (+, 6\text{Ar-CH}), 128.07 (+, 9\text{Ar-CH}), 119.39 (+, \text{Ar-CH}), 75.29 (C_{\text{quat}}), 44.07 (-, \text{CH}_2), 32.14 (-, \text{CH}_2).
\]

HRMS (EI-MS) m/z: calcd for C_{24}H_{21}BrN_{2} [M+H]^+ 417.0961, found 417.0962.

tert-Butyl [2-(4-methylpiperazin-1-yl)ethyl]carbamate.\textsuperscript{65}

To a solution of 1-methylpiperazine (500 mg, 5 mmol) and DIPEA (2 g, 15 mmol) in acetonitrile (50 mL), tert-butyl (2-bromoethyl)carbamate (1.4 g, 6 mmol) was added. The reaction mixture was heated at 50 °C overnight, the solvent was evaporated, and the remaining mixture was poured into water. The aqueous layer was extracted with DCM, and the combined organic layers were dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure. Purification of the product by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/4/1) yielded a yellow oil (140 mg, 0.20 mmol, yield 17.4%). \textsuperscript{1}H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 3.25-3.10 (m, 2H), 2.55-2.31 (m, 10H), 2.24 (s, 3H), 1.38 (s, 9H).

\[
\text{^13C-NMR (75 MHz, CDCl}_3\text{)} \delta [\text{ppm}]: 155.95 (C_{\text{quat}}), 79.12 (C_{\text{quat}}), 57.07 (-, \text{CH}_2), 55.00 (-, 2\text{CH}_2), 52.73 (-, 2\text{CH}_2), 45.94 (+, \text{CH}_3), 37.10 (-, \text{CH}_2), 28.43 (+, 3\text{CH}_3).
\]

HRMS (EI-MS) m/z: calcd for C_{12}H_{25}N_{3}O_{2} [M+H]^+ 244.2020, found 244.2021.

N-[2-(1-Triphenylmethylimidazol-4-yl)ethyl]phthalimide.\textsuperscript{52}

Ethyl phthalimide-N-carboxylate (2.4 g, 11 mmol) was added portion-wise to a stirred solution of histamine dihydrochloride (1.84 g, 10 mmol), and Na$_2$CO$_3$ (2.12 g, 20 mmol) in distilled water (50 mL) at rt. The resulting snow-white suspension was stirred vigorously at rt for 90 min. The solid was filtered off and thoroughly washed with ice-cold water (20 mL). The solid was collected and dried. The obtained white solid was dissolved in DMF (15 mL), TEA
(1.47 g, 14.6 mmol) and triphenylmethylchloride (3 g, 11 mmol) was introduced, the reaction was stirred at rt overnight. After the reaction was finished, the mixture was poured into brine and extracted with DCM (90 mL), then subjected to column chromatography (PE/EE, 1/1). White solid (2.85 g, 5.9 mmol, 81%). $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$ [ppm]: 7.83-7.76 (m, 2H), 7.73-7.65 (m, 2H), 7.35-7.21 (m, 10H), 7.10-7.01 (m, 6H), 6.58-6.48 (m, 1H), 3.97 (t, $J = 7.1$ Hz, 2H), 2.94 (t, $J = 7.0$ Hz, 2H).

2,5-Dichloro-1H-benzo[d]imidazole and 2,6-dichloro-1H-benzo[d]imidazole (4.10).$^{66}$

A mixture of urea (6 g, 100 mmol), 4-chloro-o-phenylenediamine (14.2 g, 100 mmol) and $n$-butanol (100 mL) was stirred at 120 °C for 16 h and subsequently cooled to 0 °C. The precipitate was collected and washed with $n$-butanol (5 mL) and water (200 mL). After drying, the precipitated white solid was added to 50 mL POCl$_3$ and heated at 100 °C for 18 h. The reaction mixture was cooled to rt, and the excess of POCl$_3$ was removed in vacuum. The residue was neutralized with 100 mL of saturated NaHCO$_3$ solution and extracted with EtOAc. The organic phase was washed with brine and dried over Na$_2$SO$_4$. A mixture of 2,5-dichlorobenzimidazole and 2,6-dichlorobenzimidazole was obtained as brown solid (4.62 g, 24.8 mmol, 86.1%); mp 205 °C. $^1$H-NMR (300 MHz, DMSO-d$_6$) $\delta$ [ppm]: 7.58 (d, $J = 1.9$ Hz, 1H), 7.55-7.49 (m, 1H), 7.26-7.20 (m, 1H). $^{13}$C-NMR (75 MHz, DMSO-d$_6$) $\delta$ [ppm]: 139.86 (2C$_{quat}$), 126.66 (2C$_{quat}$), 122.54 (+, 3Ar-CH).


To a solution of compound 4.10 (2.23 g, 12 mmol) in ACN (100 mL), (2-bromoethyl)benzene (4.44 g, 24 mmol) and NaOH (2.4 g, 60 mmol) was added inside, the mixture was stirred at 95 °C for 2 h (control by TLC). The solvent was evaporated yielding a yellow solid (1.12 g, 3.86 mmol, 32.2%); mp 97 °C. $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$ [ppm]: 7.61 (m, 1H), 7.32-7.16 (m, 4H), 7.13-6.99 (m, 3H), 4.43-4.30 (m, 2H), 3.07 (t, $J = 7.2$ Hz, 2H).

2-Chloro-1-phenethyl-1H-benzo[d]imidazole (4.12b).$^{67}$
Synthesized from 2-chloro-benzoimidazole (2 g, 13.16 mmol) and (2-bromoethyl)benzene (7.3 g, 37.5 mmol) by analogy with the procedure for the preparation of 4.12a. White solid (3.21 g, 12.54 mmol, 95%); mp 85 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.74-7.65 (m, 1H), 7.33-7.17 (m, 6H), 7.12-7.04 (m, 2H), 4.45-4.33 (m, 2H), 3.09 (t, \(J = 7.4\) Hz, 2H).

2-Chloro-1-(4-fluorobenzyl)-1H-benzo[d]imidazole (4.12c).\(^{68}\)
Synthesized from 2-chloro-benzoimidazole (6 g, 40 mmol) and 1-(chloromethyl)-4-fluorobenzene (17.3 g, 120 mmol) by analogy with the procedure for the preparation of 4.12a. White solid (10.17 g, 39 mmol, 97%); mp 94 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.76-7.67 (m, 1H), 7.32-7.20 (m, 3H), 7.20-7.13 (m, 2H), 7.06-6.97 (m, 2H), 5.35 (s, 2H). \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 160.85 (C\(_{quat}\)), 141.84 (C\(_{quat}\)), 140.65 (C\(_{quat}\)), 134.99 (C\(_{quat}\)), 130.85 (C\(_{quat}\)), 128.71 (+, Ar-CH), 128.60 (+, Ar-CH), 123.49 (+, Ar-CH), 122.99 (+, Ar-CH), 119.66 (+, Ar-CH), 116.18 (+, Ar-CH), 115.89 (+, Ar-CH), 109.74 (+, Ar-CH), 47.27 (-, CH\(_2\)). HRMS (EI-MS) \(m/z\): calcd for C\(_{14}\)H\(_{10}\)FN\(_2\) [MH\(^+\)] 261.0589, found 261.0589.

2-Chloro-1-(4-chlorophenethyl)-1H-benzo[d]imidazole (4.12d).
Synthesized from 2-chlorobenzimidazole (760 mg, 5 mmol) and 1-(2-bromoethyl)-4-chlorobenzene (2.18 g, 10 mmol) by analogy with the procedure for the preparation of 4.12a. Yellow-white solid (370 mg, 1.28 mmol, 25.6%); mp 69 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.79-7.61 (m, 1H), 7.31-7.15 (m, 5H), 7.06-6.91 (m, 2H), 4.37 (m, 2H), 3.06 (t, \(J = 7.2\) Hz, 2H). \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 141.68 (C\(_{quat}\)), 140.49 (C\(_{quat}\)), 135.52 (C\(_{quat}\)), 134.69 (C\(_{quat}\)), 133.06 (C\(_{quat}\)), 130.15 (+, 2Ar-CH), 128.98 (+, 2Ar-CH), 123.25 (+, Ar-CH), 122.79 (+, Ar-CH), 119.61 (+, Ar-CH), 109.74 (+, Ar-CH), 45.76 (-, CH\(_2\)), 34.95 (-, CH\(_2\)). HRMS (EI-MS) \(m/z\): calcd for C\(_{15}\)H\(_{12}\)Cl\(_2\)N\(_2\) [MH\(^+\)] 291.0450, found 291.0455.

2-Chloro-1-[2-(thiophen-2-yl)ethyl]-1H-benzo[d]imidazole (4.12e).
Synthesized from 2-chlorobenzimidazole (1 g, 6.58 mmol) and 2-(2-bromoethyl)thiophene (3.75 g, 19.74 mmol) by analogy with the procedure for the preparation of 4.12a. Yellow-white solid (730 mg, 2.79 mmol, 42.3%); mp 91 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.73-7.65
(m, 1H), 7.30-7.19 (m, 3H), 7.18-7.13 (m, 1H), 6.93-6.85 (m, 1H), 6.69-6.66 (m, 1H), 4.51-4.34 (m, 2H), 3.31 (t, J = 7.2 Hz, 2H). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 141.68 (C\(_{\text{quat}}\)), 140.56 (C\(_{\text{quat}}\)), 138.69 (C\(_{\text{quat}}\)), 134.80 (C\(_{\text{quat}}\)), 127.32 (+, Ar-CH), 126.17 (+, Ar-CH), 124.70 (+, Ar-CH), 123.25 (+, Ar-CH), 122.77 (+, Ar-CH), 119.56 (+, Ar-CH), 109.25 (+, Ar-CH), 45.99 (-, CH\(_2\)), 29.57 (-, CH\(_2\)). HRMS (EI-MS) \(m/z\): calcd for C\(_{13}\)H\(_{11}\)ClN\(_2\)S [M\(\text{H}^+\)] 263.0404, found 263.0405.

**Ethyl 4-[(5-chloro-1-phenethyl-1H-benzo[d]imidazol-2-yl)amino]piperidine-1-carboxylate (4.13a).**

Compound **4.12a** (1.11 g, 3.83 mmol) was added to ethyl 4-aminopiperidine-1-carboxylate (3.3 g, 19.15 mmol). The reaction mixture was heated to 170 °C overnight. Afterwards the mixture was poured into brine and extracted with DCM, the combined organic layers were dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure, and the remaining mixture was subjected to column chromatography (DCM/MeOH = 50/1). White solid (360 mg, 0.85 mmol, 22.2%); mp 61 °C. \(^1\text{H-NMR}\) (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.42 (d, J = 1.9 Hz, 1H), 7.29-7.26 (m, 3H), 7.03-7.00 (m, 3H), 6.96 (m, 3.5 Hz, 1H), 4.12 (q, J = 7.2 Hz, 2H), 4.09-4.06 (m, 2H), 4.05-3.85 (m, 2H), 3.78-3.69 (m, 1H), 3.03-3.00 (m, 2H), 2.93-2.83 (m, 2H), 1.86-1.77 (m, 2H), 1.26 (t, J = 7.1 Hz, 4H), 1.02-0.92 (m, 2H). \(^{13}\text{C-NMR}\) (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 155.51 (C\(_{\text{quat}}\)), 154.14 (C\(_{\text{quat}}\)), 143.39 (C\(_{\text{quat}}\)), 138.37 (C\(_{\text{quat}}\)), 132.78 (C\(_{\text{quat}}\)), 129.32 (+, 2Ar-CH), 128.94 (+, 2Ar-CH), 127.45 (+, Ar-CH), 126.81 (C\(_{\text{quat}}\)), 119.62 (+, Ar-CH), 116.51 (+, Ar-CH), 107.60 (+, Ar-CH), 61.38 (-, CH\(_2\)), 49.42 (+, CH), 44.90 (-, CH\(_2\)), 42.57 (-, 2CH\(_2\)), 35.37 (-, CH\(_2\)), 32.12 (-, 2CH\(_2\)), 14.74 (+, CH\(_3\)).

**Ethyl 4-[(6-chloro-1-phenethyl-1H-benzo[d]imidazol-2-yl)amino]piperidine-1-carboxylate (4.13b).**

Synthesized from compound **4.12a** (1.11 g, 3.83 mmol) and ethyl 4-aminopiperidine-1-carboxylate (3.3 g, 19.15 mmol) by analogy with the procedure for the preparation of **4.13a**. White solid (270 mg, 0.63 mmol, 10.3%); mp 58 °C. \(^1\text{H-NMR}\) (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.36-7.32 (m, 1H), 7.30-7.26 (m, 3H), 7.11-7.04 (m, 2H), 7.04-7.00 (m, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.07-4.04 (m, 2H), 4.03-3.89 (m, 2H), 3.77-3.69 (m, 1H), 3.03-3.01

Synthesized from 4.12b (385 mg, 1.5 mmol) and ethyl 4-aminopiperidine-1-carboxylate (1.27 g, 7.5 mmol) by analogy with the procedure for the preparation of 4.13a. Yellow-white solid (380 mg, 0.97 mmol, 65%); mp 74 °C. \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.51-7.45 (m, 1H), 7.31-7.24 (m, 3H), 7.18-6.96 (m, 5H), 4.17-4.07 (m, 4H), 4.06-3.88 (m, 2H), 3.85-3.70 (m, 1H), 3.04 (t, \(J = 5.4\) Hz, 2H), 2.96-2.81 (m, 2H), 1.91-1.75 (m, 2H), 1.26 (t, \(J = 7.1\) Hz, 3H), 1.08-0.87 (m, 2H). \(^{13}C\)-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 155.52 (C\(_{quat}\)), 153.34 (C\(_{quat}\)), 142.33 (C\(_{quat}\)), 138.64 (C\(_{quat}\)), 134.10 (C\(_{quat}\)), 129.25 (+, 2Ar-CH), 129.00 (+, 2Ar-CH), 127.33 (+, Ar-CH), 121.40 (+, Ar-CH), 119.69 (+, Ar-CH), 116.53(+, Ar-CH), 107.15 (+, Ar-CH), 61.34 (-, CH\(_2\)), 49.31 (+, CH), 44.77 (-, CH\(_2\)), 42.60 (-, 2CH\(_2\)), 35.46 (-, CH\(_2\)), 32.17 (-, 2CH\(_2\)), 14.75 (+, CH\(_3\)). HRMS (EI-MS) \(m/z\): calcd for C\(_{23}H\(_{32}\)N\(_4\)O\(_2\) [MH\(^+\)] 393.2285, found 393.2295.


Synthesized from compound 4.12e (350 mg, 1.34 mmol) and ethyl 4-aminopiperidine-1-carboxylate (919 mg, 5.34 mmol) by analogy with the procedure for the preparation of 4.13a. White solid (500 mg, 1.26 mmol, 93%); mp 97 °C. \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 7.51-7.45 (m, 1H), 7.22-7.18 (m, 1H), 7.17-7.05 (m, 3H), 6.91 (dd, \(J = 5.1, 3.4\) Hz, 1H), 6.67-6.62 (m, 1H), 4.20-4.04 (m, 6H), 3.91-3.80 (m, 1H), 3.30-3.23 (m, 2H), 3.00-2.83 (m, 2H), 2.00-1.85 (m, 2H), 1.25 (t, \(J = 7.0\) Hz, 3H), 1.17-1.04 (m, 2H). \(^{13}C\)-NMR (75 MHz, CDCl\(_3\)) \(\delta\) [ppm]: 155.55 (C\(_{quat}\)), 153.45 (C\(_{quat}\)), 142.32 (C\(_{quat}\)), 140.11 (C\(_{quat}\)), 133.95 (C\(_{quat}\)), 127.91 (+, Ar-CH), 126.77 (+, Ar-CH), 125.06 (+, Ar-CH), 121.52 (+, Ar-CH), 119.73 (+, Ar-CH), 116.53 (+, Ar-CH), 107.04 (+, Ar-CH), 61.39 (-, CH\(_2\)), 49.45 (+, CH), 44.83 (-, CH\(_2\)), 42.57 (-, 2CH\(_2\)), 35.35 (-, CH\(_2\)), 32.10 (-, 2CH\(_2\)), 14.73 (+, CH\(_3\)).
42.65 (-, 2CH₃), 32.28 (-, 2CH₂), 29.53 (-, CH₃), 14.75 (+, CH₃). **HRMS** (EI-MS) m/z: calcd for C₂₁H₂₈N₄O₂S [MH⁺] 399.1849, found 399.1849.

**Ethyl 4- ([1- (4- chlorophenethyl)- 1H- benzo[d]imidazol- 2- yl]amino)piperidine- 1- carboxylate (4.13e).**

Synthesized from compound 4.12d (350 mg, 1.21 mmol) and ethyl 4-aminopiperidine-1-carboxylate (417 mg, 2.42 mmol) by analogy according the procedure for the preparation of 4.13a. Yellow-white solid (460 mg, 1.08 mmol, 89.3%); mp 64 °C. ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.48 (d, J = 7.6 Hz, 1H), 7.27-7.22 (m, 2H), 7.17-7.06 (m, 3H), 6.97-6.91 (m, 2H), 4.24-4.01 (m, 6H), 3.91-3.76 (m, 1H), 3.01 (t, J = 6.2 Hz, 2H), 2.98-2.84 (m, 2H), 1.88-1.82 (m, 2H), 1.26 (t, J = 7.0 Hz, 3H), 1.15-0.93 (m, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 155.50 (C_quat), 153.09 (C_quat), 142.28 (C_quat), 136.89 (C_quat), 133.89 (C_quat), 133.38 (C_quat), 130.36 (+, 2Ar-CH), 129.29 (+, 2Ar-CH), 121.54 (+, Ar-CH), 119.77 (+, Ar-CH), 116.61 (+, Ar-CH), 107.14 (+, Ar-CH), 61.39 (-, CH₂), 49.56 (+, CH), 44.42 (-, CH₂), 42.66 (-, 2CH₂), 34.67 (-, CH₂), 32.35 (-, 2CH₂), 14.74 (+, CH₃). **HRMS** (EI-MS) m/z: calcd for C₂₃H₂₇ClN₄O [MH⁺] 427.1895, found 427.1893.

5-Chloro-1-phenethyl- N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4.14a).

Compound 4.13a (300 mg, 0.71 mmol) was added to 47% HBr (6 mL) and heated to 126 °C for 3 h. The solvent was removed under reduced pressure, and the remaining mixture was subjected to column chromatography (DCM/MeOH/25% aqueous ammonia, 100/10/1). Yellow oil (140 mg, 0.4 mmol, 56.1%). ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.42 (d, J = 1.8 Hz, 1H), 7.32-7.27 (m, 3H), 7.05-6.93 (m, 4H), 4.10-4.04 (m, 2H), 3.73-3.65 (m, 1H), 3.05-2.95 (m, 4H), 2.73-2.60 (m, 2H), 1.90-1.80 (m, 2H), 1.06-0.92 (m, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 154.26 (C_quat), 143.52 (C_quat), 138.29 (C_quat), 132.81 (C_quat), 129.29 (+, 2Ar-CH), 128.91 (+, 2Ar-CH), 127.44 (+, Ar-CH), 126.69 (C_quat), 119.42 (+, Ar-CH), 116.41 (+, Ar-CH), 107.50 (+, Ar-CH), 49.75 (+, CH), 45.27 (-, 2CH₂), 44.85 (-, CH₂), 35.34 (-, CH₂), 33.62 (-, 2CH₂). **HRMS** (EI-MS) m/z: calcd for C₂₀H₂₃ClN₄ [MH⁺] 355.1684, found 355.1686.

6-Chloro-1-phenethyl- N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4.14b).
Synthesized from compound 4.13b (270 mg, 0.63 mmol) by analogy with the procedure for the preparation of 4.14a. Yellow oil (140 mg, 0.4 mmol, 63.2%). ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.38-7.24 (m, 4H), 7.10-6.96 (m, 4H), 4.17-3.95 (m, 2H), 3.78-3.56 (m, 1H), 3.06-2.99 (m, 4H), 2.71-2.60 (m, 2H), 1.84-1.79 (m, 2H), 1.09-0.86 (m, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 154.01 (C₉), 141.17 (C₉), 138.30 (C₉), 134.89 (C₉), 129.32 (+, 2Ar-CH), 128.93 (+, 2Ar-CH), 127.47 (+, Ar-CH), 124.81 (C₉), 121.50 (+, Ar-CH), 117.00 (+, Ar-CH), 107.37 (+, Ar-CH), 49.82 (+, CH), 45.33 (-, 2CH₂), 44.92 (-, CH₂), 35.34 (-, CH₂), 33.73 (-, 2CH₂). HRMS (EI-MS) m/z: calcd for C₂₀H₂₅ClN₄ [MH⁺] 355.1684, found 355.1687.

N-(Piperidin-4-yl)-1-(2-(thiophen-2-yl)ethyl)-1H-benzo[d]imidazol-2-amine (4.14c).

Synthesized from compound 4.13d (500 mg, 1.26 mmol) by analogy according to the procedure for the preparation of 4.14a. Yellow oil (455 mg, 1.12 mmol, 89.2%). ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.49-7.44 (m, 1H), 7.23-7.17 (m, 1H), 7.16-7.00 (m, 3H), 6.93-6.88 (m, 1H), 6.67-6.63 (m, 1H), 4.14-4.06 (m, 2H), 3.87-3.73 (m, 1H), 3.29-3.22 (m, 2H), 3.08-2.99 (m, 2H), 2.76-2.66 (m, 2H), 2.02-1.92 (m, 2H), 1.23-1.09 (m, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 153.56 (C₉), 142.46 (C₉), 140.04 (C₉), 134.00 (C₉), 127.85 (+, Ar-CH), 126.68 (+, Ar-CH), 125.02 (+, Ar-CH), 121.39 (+, Ar-CH), 119.54 (+, Ar-CH), 116.44 (+, Ar-CH), 106.97 (+, Ar-CH), 49.65 (+, CH), 45.24 (-, 2CH₂), 44.75 (-, CH₂), 33.52 (-, 2CH₂), 29.47 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₁₈H₂₂N₄S [MH⁺] 327.1638, found 327.1640.

1-(4-Chlorophenethyl)-N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4.14d).

Synthesized from compound 4.13e (460 mg, 1.08 mmol) by analogy with the procedure for the preparation of 4.14a gave 4.14d as a yellow-white solid (455 mg, 0.97 mmol, 90%). HRMS (EI-MS) m/z: calcd for C₁₈H₂₂N₄S [MH⁺] 355.1684, found 355.1678.

N-[1-(3-Aminopropyl)piperidin-4-yl]-1-phenethyl-1H-benzo[d]imidazol-2-amine (4.15a).

To a solution of compound 4.3 (600 mg, 1.25 mmol) in acetonitrile (25 mL), tert-butyl (3-bromopropyl) carbamate (591 mg, 2.50 mmol), sodium iodide (373 mg, 2.50 mmol) and DIPEA (968 mg, 7.5 mmol) were added. The reaction mixture was heated to reflux for 2 h (control by TLC), the solvent was evaporated and the remaining mixture was poured into
water. The aqueous layer was extracted with DCM, and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure, and the resulting mixture was re-dissolved in 10 mL DCM, 10 mL of TFA was added dropwise, and the mixture was stirred at rt for 3 h. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/3/1) yielded a yellow oil (700 mg, 0.97 mmol, 77.6%).

\[ ^1H\text{-NMR} \text{(300 MHz, CD}_3\text{OD)} \delta \text{[ppm]}: 7.48-7.29 (m, 4H), 7.23-7.16 (m, 3H), 7.04-6.95 (m, 2H), 4.54-4.42 (m, 2H), 3.90-3.56 (m, 3H), 3.29-3.20 (m, 2H), 3.18-2.99 (m, 6H), 2.23-2.06 (m, 4H), 2.00-1.82 (m, 2H). \]

\[ ^{13}C\text{-NMR} \text{(75 MHz, CD}_3\text{OD)} \delta \text{[ppm]}: 150.04 (C_{quat}), 138.56 (C_{quat}), 131.81 (C_{quat}), 130.24 (C_{quat}), 130.18 (+, 2Ar-CH), 129.89(+, 2Ar-CH), 128.27 (+, Ar-CH), 125.38 (+, Ar-CH), 125.17 (+, Ar-CH), 112.90 (+, Ar-CH), 111.71 (+, Ar-CH), 52.84 (-, CH₃), 50.13 (+, CH₃), 45.21 (-, CH₂), 37.87 (-, 2CH₂), 34.59 (-, 2CH₂), 30.18 (-, CH₂), 23.54 (-, CH₂). \text{HRMS (EI-MS) m/z: calcd for C}_{23}H_{31}N_{5}[M+H]+ 378.2652, found 378.2657.\]

\[ N\text{-[1-(3-Aminopropyl)piperidin-4-yl]-1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-amine (4.15b).} \]

Synthesized from compound 4.4 (390 mg, 0.98 mmol) in acetonitrile (25 mL) and tert-butyl (3-bromopropyl) carbamate (445 mg, 1.88 mmol) by analogy with the procedure for the preparation of 4.15a. Purification by HPLC yielded 4.15b tri(hydrotrifluoroacetate) as a colorless sticky solid (435 mg, 0.6 mmol, 60.1%).

\[ ^1H\text{-NMR} \text{(400 MHz, DMSO-d}_6) \delta \text{[ppm]}: 7.56-7.52 (m, 1H), 7.42-7.35 (m, 3H), 7.32-7.13 (m, 4H), 5.50 (s, 2H), 4.14-4.00 (m, 1H), 3.76-3.58 (m, 2H), 3.30-3.18 (m, 2H), 3.12-2.86 (m, 4H), 2.38-2.18 (m, 2H), 2.10-1.87 (m, 4H). \]

\[ ^{13}C\text{-NMR} \text{(100 MHz, DMSO-d}_6) \delta \text{[ppm]}: 172.93 (C_{quat}), 153.47 (C_{quat}), 142.38 (C_{quat}), 134.50 (C_{quat}), 131.36 (C_{quat}), 128.30 (+, Ar-CH), 128.19 (+, Ar-CH), 121.50 (+, Ar-CH), 119.70 (+, Ar-CH), 116.38 (+, Ar-CH), 116.23 (+, Ar-CH), 115.94 (+, Ar-CH), 107.23 (+, Ar-CH), 52.97 (-, CH₂), 50.84 (-, CH₂), 48.98 (+, CH₃), 44.6 (-, CH₂), 36.15 (-, 2CH₂), 28.56 (-, CH₂), 21.69 (-, 2CH₂). \text{HRMS (EI-MS) m/z: calcd for C}_{22}H_{28}F_{10}N_{5}[M+H]^+ 382.2402, found 382.2407.\]

\[ N\text{-[1-(3-[[2-(1H-imidazol-4-yl)ethyl]amino]propyl)piperidin-4-yl]-1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-amine (4.16a).} \]
To a solution of compound 4.15b (810 mg, 1.12 mmol) in ACN (25 mL), 4-(2-bromoethyl)-1-trityl-1H-imidazole (700 mg, 1.67 mmol) and DIPEA (723 mg, 5.6 mmol) were added. The reaction mixture was heated at reflux for 48 h (control by TLC), the solvent was evaporated, and the remaining mixture was poured into water. The aqueous layer was extracted with DCM and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure. The mixture was dissolved in DCM (2 mL), TFA (2 mL) was added dropwise, and the mixture was stirred at rt for 3 h. Purification by HPLC yielded 4.16a tetra(hydrotrifluoroacetate) as a pale-yellow sticky solid (70 mg, 0.075 mmol, 6.70%).

^1H-NMR (600 MHz, DMSO-d₆) δ [ppm]: 9.01 (d, J = 1.1 Hz, 1H), 7.53-7.50 (m, 2H), 7.40-7.32 (m, 3H), 7.29-7.14 (m, 4H), 5.48 (s, 2H), 4.07-4.00 (m, 1H), 3.65-3.57 (m, 2H), 3.29 (t, J = 7.5 Hz, 2H), 3.24-3.17 (m, 2H), 3.08-3.02 (m, 6H), 2.29-2.23 (m, 2H), 2.11-2.03 (m, 2H), 2.02-1.92 (m, 2H).

^13C-NMR (151 MHz, DMSO-d₆) δ [ppm]: 162.98 (C quat), 161.36 (C quat), 159.56 (C quat), 149.79 (C quat), 134.79 (+, Ar-CH), 131.50 (C quat), 131.04 (C quat), 129.77 (+, Ar-CH), 129.71 (+, Ar-CH), 129.28 (+, Ar-CH), 124.11 (+, Ar-CH), 123.60 (+, Ar-CH), 117.35 (+, Ar-CH), 116.17 (+, Ar-CH), 115.52 (+, Ar-CH), 110.90 (+, Ar-CH), 53.20 (-, CH₂), 51.19 (-, CH₂), 49.36 (-, CH₃), 45.35 (-, CH₂), 45.16 (-, CH₂), 44.40 (-, CH₂), 28.89 (-, CH₂), 21.55 (-, 2CH₂), 20.85 (-, 2CH₂).

HRMS (EI-MS) m/z: calcd for C₂₇H₃₄FN₇ [M+H] 476.2932, found 476.2937.

N-[1-3-[[2-(1H-imidazol-4-yl)ethy]amino)propyl]piperidin-4-yl]-1-phenethyl-1H-benzo[d]imidazol-2-amine (4.16b).

Synthesized from compound 4.15a (170 mg, 0.23 mmol) by analogy with the procedure for the preparation of 4.15b. Yield 4.16b tetra(hydrotrifluoroacetate) as a yellow solid (90 mg, 0.097 mmol 42.2%).

^1H-NMR (600 MHz, DMSO-d₆) δ [ppm]: 9.03 (d, J = 1.3 Hz, 1H), 7.52 (d, J = 0.7 Hz, 1H), 7.48-7.43 (m, 2H), 7.28-7.21 (m, 4H), 7.20-7.14 (m, 3H), 4.49 (t, J = 6.9 Hz, 2H), 3.95-3.83 (m, 1H), 3.67-3.53 (m, 2H), 3.35-3.25 (m, 2H), 3.24-3.16 (m, 2H), 3.12-3.04 (m, 4H), 3.04-2.94 (m, 4H), 2.16-2.04 (m, 4H), 1.94-1.84 (m, 2H).

^13C-NMR (151 MHz, DMSO-d₆) δ [ppm]: 158.64 (C quat), 148.51 (C quat), 137.11 (C quat), 134.22 (+, Ar-CH), 130.28 (C quat), 128.92 (+, 2Ar-CH), 128.65 (C quat), 128.19 (+, 2Ar-CH), 126.57 (+, Ar-CH), 123.38 (+, Ar-CH), 123.06 (+, Ar-CH), 116.80 (+, Ar-CH), 111.54 (+, Ar-CH), 110.32 (+, Ar-CH), 52.52 (-, CH₂),
5-Chloro-N-(1-methylpiperidin-4-yl)-1-phenethyl-1H-benzo[d]imidazol-2-amine (4.17a).

4.14a (230 mg, 0.65 mmol) was dissolved in ACN (10 mL), DIPEA (168 mg, 1.3 mmol) was added, and MeI (83 mg, 0.58 mmol) in ACN (5 mL) was dropped slowly into the mixture at rt. After stirring for 30 min, the mixture was concentrated and subjected by HPLC giving 4.17a hydrotrifluoroacetate as a white solid (57 mg, 0.12 mmol, 20.4%). 1H-NMR (300 MHz, DMSO-d$_6$) δ [ppm]: 7.55-7.50 (m, 1H), 7.45-7.44 (m, 1H), 7.26 (m, 3H), 7.16 (m, 5H), 4.47-4.40 (m, 2H), 3.85-3.75 (m, 1H), 3.63-3.52 (m, 2H), 3.05-2.90 (m, 4H), 2.81 (s, 3H), 2.19-1.70 (m, 4H). 13C-NMR (75 MHz, DMSO-d$_6$) δ [ppm]: 153.59 (C$_{quat}$), 149.41 (C$_{quat}$), 137.05 (C$_{quat}$), 129.65 (C$_{quat}$), 128.93 (+, 2Ar-CH), 128.22 (+, 2Ar-CH), 127.27 (C$_{quat}$), 126.60 (+, Ar-CH), 122.66 (+, Ar-CH), 111.61 (+, Ar-CH), 111.51 (+, Ar-CH), 52.46 (-, 2CH$_2$), 48.54 (+, CH$_3$), 43.29 (-, CH$_2$), 42.43 (+, CH$_3$), 32.83 (-, CH$_2$), 28.63 (-, 2CH$_2$). HRMS (EI-MS) m/z: calcd for C$_{21}$H$_{25}$ClN$_4$ [MH$^+$] 369.1841, found 369.1842.

N-(1-Methylpiperidin-4-yl)-1-phenethyl-1H-benzo[d]imidazol-2-amine (4.17b).

Synthesized from compound 4.3 (240 mg, 0.50 mmol) by analogy with the procedure for the preparation of 4.17a. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1). White solid (171 mg, 0.41 mmol, 82.8%); mp 70 ºC. 1H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.52-7.44 (m, 1H), 7.32-7.26 (m, 3H), 7.16-7.00 (m, 5H), 4.14-4.05 (m, 2H), 3.71-3.56 (m, 1H), 3.07-3.01 (m, 2H), 2.77-2.63 (m, 2H), 2.25 (s, 3H), 2.12-2.01 (m, 2H), 1.89-1.80 (m, 2H), 1.23-1.01 (m, 2H). 13C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 153.59 (C$_{quat}$), 142.47 (C$_{quat}$), 138.52 (C$_{quat}$), 134.13 (C$_{quat}$), 129.24 (+, 2Ar-CH), 128.94 (+, 2Ar-CH), 127.38 (+, Ar-CH), 121.29 (+, Ar-CH), 119.50 (+, Ar-CH), 116.48 (+, Ar-CH), 107.04 (+, Ar-CH), 54.48 (-, 2CH$_2$), 46.26 (+, CH$_3$), 44.76 (-, CH$_2$), 35.43 (-, CH$_2$), 32.63 (-, 2CH$_2$). HRMS (EI-MS) m/z: calcd for C$_{21}$H$_{26}$N$_4$ [MH$^+$] 335.2230, found 335.2234.

1-(4-Chlorophenethyl)-N-(1-methylpiperidin-4-yl)-1H-benzo[d]imidazol-2-amine (4.17c).
Synthesized from compound 4.14d (344 mg, 0.97 mmol) by analogy with the procedure for the preparation of 4.17a. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1). Yellow-white solid (80 mg, 0.22 mmol, 22.0%); mp 58 °C. $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.47 (d, $J = 7.7$ Hz, 1H), 7.25-7.17 (m, 2H), 7.16-7.09 (m, 1H), 7.07-7.00 (m, 2H), 6.91 (d, $J = 8.3$ Hz, 2H), 4.05 (t, $J = 6.3$ Hz, 2H), 3.81-3.62 (m, 1H), 3.00 (t, $J = 6.2$ Hz, 2H), 2.75-2.63 (m, 2H), 2.25 (s, 3H), 2.15-2.03 (m, 2H), 1.92-1.80 (m, 2H), 1.27-1.20 (m, 2H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 153.33 (C$_{quat}$), 142.42 (C$_{quat}$), 136.73 (C$_{quat}$), 133.90 (C$_{quat}$), 133.31 (C$_{quat}$), 130.28 (+, 2Ar-CH), 129.25 (+, 2Ar-CH), 121.42 (+, Ar-CH), 119.58 (+, Ar-CH), 116.55 (+, Ar-CH), 107.07 (+, Ar-CH), 54.44 (-, 2CH$_2$), 49.04 (+, CH), 46.22 (+, CH$_3$), 44.27 (-, CH$_2$), 34.53 (-, CH$_2$), 32.77 (-, 2CH$_2$).

HRMS (EI-MS) $m/z$: calcd for C$_{21}$H$_{25}$ClN$_4$ [M+H$^+$] 369.1841, found 369.1840.

$N$-[1-[2-(1H-imidazol-4-yl)ethyl]piperidin-4-yl]-1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-amine (4.18a).

Compound 4.4 (324 mg, 1 mmol) was added to a mixture of ACN (10 mL), DIPEA (1.29 g, 10 mmol) and 4-(2-bromoethyl)-imidazole (720 mg, 3 mmol) in a 20-mL microwave tube. The microwave tube was sealed and subjected to irradiation (120 °C) for 10 min. The obtained suspension was poured into brine and extracted with EtOAc, the organic phase was dried over Na$_2$SO$_4$ and evaporated. Purification by HPLC yielded 4.18a tri(hydrotrifluoroacetate) as a white solid (77 mg, 0.10 mmol, 10%). $^1$H-NMR (300 MHz, CD$_3$OD) δ [ppm]: 7.60-7.55 (m, 1H), 7.30 (d, $J = 7.6$ Hz, 1H), 7.20-6.79 (m, 8H), 5.24 (s, 2H), 3.91-3.73 (m, 1H), 3.10-2.98 (m, 2H), 2.89-2.60 (m, 4H), 2.31 (t, $J = 11.0$ Hz, 2H), 2.15-2.04 (m, 2H), 1.72-1.56 (m, 2H). $^{13}$C-NMR (75 MHz, CD$_3$OD) δ [ppm]: 155.36 (C$_{quat}$), 143.01 (2C$_{quat}$), 136.02 (+, Ar-CH), 135.38 (C$_{quat}$), 133.90 (C$_{quat}$), 133.86 (C$_{quat}$), 129.72 (+, Ar-CH), 129.61 (+, Ar-CH), 122.52 (+, Ar-CH), 120.87 (+, Ar-CH), 116.65 (+, Ar-CH), 116.36 (+, Ar-CH), 116.10 (+, Ar-CH), 109.14 (+, 2Ar-CH), 64.36 (-, CH$_2$), 59.36 (-, CH$_2$), 53.60 (-, CH$_2$), 51.27 (+, CH), 45.28 (-, CH$_2$), 32.77 (-, 2CH$_2$), 25.19 (-, CH$_2$). HRMS (EI-MS) $m/z$: calcd for C$_{21}$H$_{27}$FN$_6$ [M+H$^+$] 419.2354, found 419.2355.
\[N\{1-\text{[2-(1H-imidazol-4-yl)ethyl]piperidin-4-yl}\}-1\text{-phenethyl-1H-benzo[}\text{d}]/\text{imidazol-2-amine (4.18b).}\]

Synthesized from compound 4.3 (480 mg, 1 mmol) and 4-(2-bromoethyl)-imidazole (290 mg, 1.5 mmol) by analogy with the procedure for the preparation of 4.18a. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/3/1), yielded a white solid (140 mg, 0.34 mmol, 33.8%); mp 61 °C. \(^1\text{H-NMR}\) (300 MHz, CD\(_3\)OD) δ [ppm]: 7.57 (d, \(J = 1.1\) Hz, 1H), 7.28-7.23 (m, 1H), 7.21-7.13 (m, 3H), 7.06-6.91 (m, 5H), 6.86-6.82 (m, 1H), 4.22 (t, \(J = 6.8\) Hz, 2H), 3.69-3.55 (m, 1H), 3.06-2.92 (m, 4H), 2.85-2.77 (m, 2H), 2.71-2.61 (m, 2H), 2.28-2.16 (m, 2H), 1.99-1.88 (m, 2H), 1.57-1.41 (m, 2H). \(^{13}\text{C-NMR}\) (75 MHz, CD\(_3\)OD) δ [ppm]: 155.16 (C\(_\text{quat}\)), 142.87 (C\(_\text{quat}\)), 139.71 (C\(_\text{quat}\)), 136.00 (C\(_\text{quat}\)), 135.99 (+, Ar-CH), 135.15 (C\(_\text{quat}\)), 130.18 (+, 3Ar-CH), 129.66 (+, 2Ar-CH), 127.78 (+, Ar-CH), 122.17 (+, Ar-CH), 120.64 (+, Ar-CH), 115.92 (+, Ar-CH), 108.92 (+, Ar-CH), 59.41 (-, CH\(_2\)), 53.67 (-, 2CH\(_2\)), 51.13 (+, CH), 44.33 (-, CH\(_2\)), 35.58 (-, CH\(_2\)), 32.84 (-, 2CH\(_2\)), 25.25 (-, CH\(_2\)). HRMS (EI-MS) \(m/z\): calcd for C\(_{25}\)H\(_{30}\)N\(_6\) [MH\(^+\)] 415.2605, found 415.2611.

\[N\{2-(1H-imidazol-4-yl)ethyl\}-1-(4-fluorobenzyl)-1H-benzo[\text{d}]/\text{imidazol-2-amine (4.19a).}\]

Compound 4.12c (260 mg, 1 mmol) was added to a mixture of NMP (8 mL), DIPEA (1.8 g, 14 mmol) and histamine dihydrochloride (1.1 g, 6 mmol) in a 20-mL microwave tube. The microwave tube was sealed and subjected to irradiation (200 °C) for 10 min. The obtained suspension was poured into brine and extracted with EtOAc, the organic phase was dried over Na\(_2\)SO\(_4\) and evaporated. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1) yielded a white solid (230 mg, 0.69 mmol, 68.7%); mp 194 °C. \(^1\text{H-NMR}\) (300 MHz, DMSO-d\(_6\)) δ [ppm]: 7.57 (d, \(J = 1.1\) Hz, 1H), 7.26-7.18 (m, 3H), 7.18-7.06 (m, 2H), 7.06-6.97 (m, 1H), 6.96-6.90 (m, 1H), 6.88-6.80 (m, 2H), 5.23 (s, 2H), 3.67-3.57 (m, 2H), 2.87 (t, \(J = 7.2\) Hz, 2H). \(^{13}\text{C-NMR}\) (75 MHz, DMSO-d\(_6\)) δ [ppm]: 162.88 (C\(_\text{quat}\)), 159.66 (C\(_\text{quat}\)), 154.39 (C\(_\text{quat}\)), 142.70 (C\(_\text{quat}\)), 134.53 (+, Ar-CH), 134.26 (C\(_\text{quat}\)), 133.16 (C\(_\text{quat}\)), 129.06 (+, Ar-CH), 128.95 (+, Ar-CH), 123.49 (+, Ar-CH), 120.36 (+, Ar-CH), 118.26 (+, Ar-CH), 115.34 (+, Ar-CH), 115.06 (+, Ar-CH), 114.92 (+, Ar-CH), 107.61 (+, Ar-CH), 43.58 (-, CH\(_2\)), 42.45 (-, CH\(_2\)), 26.83 (-, CH\(_2\)). HRMS (EI-MS) \(m/z\): calcd for C\(_{19}\)H\(_{16}\)FN\(_5\) [MH\(^+\)] 336.1619, found 336.1625.
Chapter 4

N-[2-(1H-imidazol-4-yl)ethyl]-1-phenethyl-1H-benzo[d]imidazol-2-amine (4.19b).

Synthesized from compound 4.12b (520 mg, 2 mmol) by analogy with the procedure for the preparation of 4.19a. White solid (370 mg, 1.12 mmol, 56%); mp 163 °C. ¹H-NMR (600 MHz, CD₃OD) δ [ppm]: 7.58 (d, J = 1.0 Hz, 1H), 7.27-7.25 (m, 1H), 7.20-7.12 (m, 3H), 7.07-7.03 (m, 2H), 7.01-6.97 (m, 2H), 6.95-6.90 (m, 1H), 6.84 (s, 1H), 4.15 (t, J = 7.1 Hz, 2H), 3.54 (t, J = 7.4 Hz, 2H), 2.93 (t, J = 7.1 Hz, 2H), 2.83 (t, J = 7.3 Hz, 2H). ¹³C-NMR (151 MHz, CD₃OD) δ [ppm]: 154.28 (C quat), 138.71 (2C quat), 135.57 (+, Ar-CH), 133.84 (C quat), 128.64 (+, 2Ar-CH), 128.16 (+, 2Ar-CH), 126.29 (+, 2Ar-CH), 120.66 (+, Ar-CH), 119.19 (+, Ar-CH), 114.51 (+, Ar-CH), 107.35 (+, Ar-CH), 43.03 (-, CH₂), 42.47 (-, CH₂), 34.18 (-, CH₂), 26.47 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₀H₂₁N₅ [MH⁺] 332.1870, found 332.1870.

1-(4-Fluorobenzyl)-N-[2-(4-methylpiperazin-1-yl)ethyl]-1H-benzo[d]imidazol-2-amine (4.20a).

tert-Butyl [2-(4-methylpiperazin-1-yl)ethyl]carbamate (630 mg, 2.6 mmol) was dissolved in 20 mL DCM, 20 mL of TFA was added dropwise, the mixture was stirred at rt for 2 h. Afterward, 10 mL DCM was added and the solvent was removed under reduced pressure to yield 4-methyl-1-piperazineethanamine tri(hydrotrifluoroacetate) (1.12 g, 2.6 mmol). 4-methyl-1-piperazineethanamine tri(hydrotrifluoroacetate) (1.12 g, 2.6 mmol) and compound 4.12c (512 mg, 2 mmol) was added to a mixture of NMP (10 mL), DIPEA (3.87 g, 30 mmol) in a 20-mL microwave tube. The microwave tube was sealed and subjected to irradiation (180 °C) for 2 h. The obtained suspension was poured into brine and extracted with DCM, the organic phase was dried over Na₂SO₄ and evaporated. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1), yielded a white solid (270 mg, 0.7 mmol, 35%); mp 139 °C. ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.51 (d, J = 7.7 Hz, 1H), 7.21-6.97 (m, 8H), 5.07 (s, 2H), 3.55-3.46 (m, 2H), 2.63-2.27 (m, 10H), 2.24 (s, 3H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 154.44 (C quat), 142.29 (C quat), 134.78 (C quat), 131.60 (C quat), 131.56 (C quat), 128.45 (+, Ar-CH), 128.34 (+, Ar-CH), 121.49 (+, Ar-CH), 119.84 (+, Ar-CH), 116.59 (+, Ar-CH), 116.31 (+, Ar-CH), 116.02 (+, Ar-CH), 107.00 (+, Ar-CH), 55.86 (-, CH₂), 54.92 (-, 2CH₂), 52.45 (-, 2CH₂), 45.95 (+, CH₃), 45.06 (-, CH₂), 39.26 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₁H₂₆FN₅ [MH⁺] 368.2245, found 368.2251.
N-[2-(4-Methylpiperazin-1-yl)ethyl]-1-phenethyl-1H-benzo[d]imidazol-2-amine (4.20b).

Synthesized from compound 4.12b (512 mg, 2 mmol) by analogy with the procedure for the preparation of 4.20a. White solid (130 mg, 0.36 mmol, 18.7%); mp 89 °C. 1H-NMR (400 MHz, CDCl₃) δ [ppm]: 7.46 (d, J = 7.7 Hz, 1H), 7.32-7.20 (m, 3H), 7.16-7.07 (m, 3H), 7.03 (d, J = 4.0 Hz, 2H), 4.10 (t, J = 7.3 Hz, 2H), 3.52-3.43 (m, 2H), 3.02 (t, J = 7.3 Hz, 2H), 2.66-2.35 (m, 10H), 2.29 (s, 3H). 13C-NMR (101 MHz, CDCl₃) δ [ppm]: 154.12 (C quat), 141.92 (C quat), 137.99 (C quat), 134.08 (C quat), 128.89 (+, 4Ar-CH), 126.99 (+, Ar-CH), 121.29 (+, Ar-CH), 119.65 (+, Ar-CH), 116.21 (+, Ar-CH), 110.78 (+, Ar-CH), 56.47 (-, CH₂), 55.12 (-, 2CH₂), 52.65 (-, 2CH₂), 46.04 (+, CH₃), 44.25 (-, CH₂), 39.32 (-, CH₂), 35.24 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₂H₃⁰N₅ [M+H]+ 364.2496, found 364.2500.

1-(4-Fluorobenzyl)-2-(piperazin-1-yl)-1H-benzo[d]imidazole (4.21a).

Compound 4.12c (520 mg, 2 mmol) was added to NMP (3 mL), DIPEA (516 mg, 4 mmol) and piperazine (1.03 g, 12 mmol) in a 5-mL microwave tube. The microwave tube was sealed and subjected to irradiation (180 °C) for 1 h. The obtained suspension was poured into brine and extracted with EtOAc, the organic phase was dried over Na₂SO₄ and evaporated. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1) yielded a yellow oil (630 mg, 2 mmol, 100%). 1H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.66-7.60 (m, 1H), 7.21-6.96 (m, 7H), 5.18 (s, 2H), 3.27-3.15 (m, 4H), 3.04-2.94 (m, 4H). 13C-NMR (75 MHz, CDCl₃) δ [ppm]: 158.18 (C quat), 141.58 (C quat), 135.24 (C quat), 134.94 (C quat), 134.08 (C quat), 128.89 (+, 4Ar-CH), 126.99 (+, Ar-CH), 121.29 (+, Ar-CH), 119.65 (+, Ar-CH), 116.21 (+, Ar-CH), 110.78 (+, Ar-CH), 56.47 (-, CH₂), 55.12 (-, 2CH₂), 52.65 (-, 2CH₂), 46.04 (+, CH₃), 44.25 (-, CH₂), 39.32 (-, CH₂), 35.24 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₂H₃₀N₅ [M+H]+ 364.2496, found 364.2500.

1-Phenethyl-2-(piperazin-1-yl)-1H-benzo[d]imidazole (4.21b).

Synthesized from compound 4.3 (768 mg, 3 mmol) by analogy with the procedure for the preparation of 4.21a. Yellow oil (900 mg, 2.94 mmol, 98.0%). 1H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.70-7.60 (m, 1H), 7.21-6.96 (m, 7H), 5.18 (s, 2H), 3.27-3.15 (m, 4H), 3.04-2.94 (m, 4H). 13C-NMR (75 MHz, CDCl₃) δ [ppm]: 158.18 (C quat), 141.58 (C quat), 135.24 (C quat), 134.94 (C quat), 127.84 (+, Ar-CH), 127.73 (+, Ar-CH), 122.12 (+, Ar-CH), 121.61 (+, Ar-CH), 118.26 (+, Ar-CH), 116.13 (+, Ar-CH), 115.85 (+, Ar-CH), 109.31 (+, Ar-CH), 51.81 (-, 4CH₂), 47.02 (-, CH₂), 45.61 (-, 4CH₂).
121.48 (+, Ar-CH), 118.52 (+, Ar-CH), 109.25 (+, Ar-CH), 51.96 (-, 2CH₂), 45.69 (-, 2CH₂),
45.56 (-, CH₂), 35.13 (-, CH₂).

2-{[2-(1H-imidazol-4-yl)ethyl]piperazin-1-yl]-1-(4-fluorobenzyl)-1H-benzo[d]imidazole
(4.22a).

Compound 4.21a (310 mg, 1 mmol) was added to a mixture of NMP (10 mL), DIPEA (390
mg, 3 mmol) and 4-(2-bromoethyl)-imidazole (230 mg, 1.2 mmol) in a 20-mL microwave tube.
The microwave tube was sealed and subjected to irradiation (120 °C) for 50 min. The
obtained suspension was poured into brine and extracted with EtOAc, the organic phase was
dried over Na₂SO₄ and evaporated. Purification by column chromatography (DCM/MeOH/25%
aqueous ammonia, 100/2.5/1), yielded a white solid (180 mg, 0.44 mmol, 44.6%); mp 59 °C.

¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.62 (d, J = 7.7 Hz, 1H), 7.23-7.09 (m, 4H), 7.05-6.95 (m,
4H), 6.82 (s, 1H), 5.19 (s, 2H), 3.34-3.22 (m, 4H), 2.85-2.61 (m, 8H).

¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 163.84 (C_quad), 160.58 (C_quad), 157.88 (C_quad), 141.42 (C_quad),
135.19 (C_quad), 134.27 (+, Ar-CH), 131.85 (C_quad), 127.85 (+, Ar-CH), 127.74 (+, Ar-CH), 122.22 (+, Ar-CH),
121.77 (+, Ar-CH), 119.34 (+, Ar-CH), 118.18 (+, Ar-CH), 116.18 (+, Ar-CH), 115.89 (+, Ar-CH),
109.38 (+, Ar-CH), 57.75 (-, CH₂), 52.59 (-, 2CH₂), 50.80 (-, 2CH₂), 47.02 (-, CH₂),
23.06 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₃H₂₅FN₆ [M⁺] 405.2197, found 405.2206.

2-{[2-(1H-imidazol-4-yl)ethyl]piperazin-1-yl]-1-phenethyl-1H-benzo[d]imidazole
(4.22b).

Synthesized from compound 4.21b (306 mg, 1 mmol) by analogy with the procedure for the
preparation of 4.22a yielded a white solid (210 mg, 0.52 mmol, 52.5%); mp 130 °C. ¹H-NMR
(300 MHz, CDCl₃) δ [ppm]: 7.65-7.54 (m, 2H), 7.33-7.20 (m, 6H), 7.02-6.96 (m, 2H), 6.83 (s,
1H), 4.24 (t, J = 7.3 Hz, 2H), 3.16-3.03 (m, 6H), 2.88-2.66 (m, 4H), 2.65-2.55 (m, 4H).

¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 157.90 (C_quad), 141.47 (C_quad), 137.89 (C_quad), 134.26
(2C_quad), 133.63 (+, Ar-CH), 128.77 (+, 2Ar-CH), 128.70 (+, 2Ar-CH), 126.87 (+, Ar-CH),
121.93 (+, Ar-CH), 121.66 (+, Ar-CH), 119.58 (+, Ar-CH), 118.41 (+, Ar-CH), 109.37 (+, Ar-CH),
57.74 (-, CH₂), 52.68 (-, 2CH₂), 50.89 (-, 2CH₂), 45.54 (-, CH₂), 35.11 (-, CH₂), 22.98
2-(4-Methylpiperazin-1-yl)-1-phenethyl-1H-benzo[d]imidazole (4.23).\textsuperscript{57}

Compound 4.12b (260 mg, 1 mmol) was added to a mixture of NMP (3 mL), DIPEA (390 mg, 3 mmol) and 1-methylpiperazine (600 mg, 6 mmol) in a 5-mL microwave tube. The microwave tube was sealed and subjected to irradiation (180 °C) for 1 h. The obtained suspension was poured into brine and extracted with EtOAc, the organic phase was dried over Na\textsubscript{2}SO\textsubscript{4} and evaporated. Purification by column chromatography (DCM/MeOH, 10/1), yielded a yellow oil (310 mg, 0.96 mmol, 96.0%). \textsuperscript{1}\text{H}-NMR (300 MHz, CDCl\textsubscript{3}) δ [ppm]: 7.67-7.59 (m, 1H), 7.31-7.16 (m, 6H), 7.06-7.00 (m, 2H), 4.31-4.11 (m, 2H), 3.18-3.12 (m, 4H), 3.12-3.04 (m, 2H), 2.56-2.45 (m, 4H), 2.34 (s, 3H). \textsuperscript{13}\text{C}-NMR (75 MHz, CDCl\textsubscript{3}) δ [ppm]: 157.96 (C\textsubscript{quat}), 141.70 (C\textsubscript{quat}), 137.95 (C\textsubscript{quat}), 134.50 (C\textsubscript{quat}), 128.75 (+, 2Ar-CH), 128.71 (+, 2Ar-CH), 126.84 (+, Ar-CH), 121.77 (+, Ar-CH), 121.39 (+, Ar-CH), 118.44 (+, Ar-CH), 109.15 (+, Ar-CH), 54.81 (-, CH\textsubscript{2}), 50.59 (-, CH\textsubscript{2}), 46.22 (+, CH\textsubscript{3}), 45.66 (-, CH\textsubscript{3}), 35.12 (-, CH\textsubscript{2}). HRMS (El-MS) m/z: calcd for C\textsubscript{20}H\textsubscript{24}N\textsubscript{4} [MH\textsuperscript{+}] 321.2074, found 321.2089.

4-(1H-benzo[d]imidazol-2-yl)phenol (4.24).\textsuperscript{71}

A solution of 1,2-phenylenediamine (2.16 g, 20 mmol) and 4-hydroxybenzaldehyde (2.44 g, 20 mmol) in DMF (40 mL) was treated with Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5} (3.80 g, 20 mmol). After heating at 90 °C for 2 h, the reaction mixture was cooled to rt and subsequently diluted with ice water. The resulting suspension was stirred for 4 h, cooled to 0 °C and filtered through a glass fritted funnel. The solid was washed with cold water and dried in vacuum and used without further purification. White solid (4.17 g, 19.86 mmol, 99.3%); mp > 250 °C. \textsuperscript{1}\text{H}-NMR (300 MHz, DMSO-d\textsubscript{6}) δ [ppm]: 8.06-7.97 (m, 2H), 7.60-7.49 (m, 2H), 7.21-7.12 (m, 2H), 6.97-6.88 (m, 2H). \textsuperscript{13}\text{C}-NMR (75 MHz, DMSO-d\textsubscript{6}) δ [ppm]: 159.07 (2C\textsubscript{quat}), 151.63 (C\textsubscript{quat}), 139.17 (C\textsubscript{quat}), 128.08 (+, 2Ar-CH), 121.57 (+, 2Ar-CH), 120.68 (C\textsubscript{quat}), 115.59 (+, 2Ar-CH), 114.51 (+, 2Ar-CH). HRMS (El-MS) m/z: calcd for C\textsubscript{13}H\textsubscript{10}N\textsubscript{2}O [MH\textsuperscript{+}] 211.0866, found 211.0872.

\textit{tert}-Butyl {3-[4-(1H-benzo[d]imidazol-2-yl)phenoxy]propyl}carbamate (4.25).

To a solution of compound 4.24 (1.30 g, 6.17 mmol) in acetone, K\textsubscript{2}CO\textsubscript{3} (851 mg, 6.17 mmol) was added. The mixture was stirred for 30 min, subsequently, \textit{tert}-butyl (3-bromopropyl)carbamate (1.61 g, 6.79 mmol) was added, and the reaction mixture was
heated to reflux for 17 h. Purification by column chromatography (DCM/MeOH, 30/1). White solid (970 mg, 2.64 mmol, 42.8%); mp > 250 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ [ppm]: 8.17-8.06 (m, 2H), 7.65-7.50 (m, 2H), 7.21-7.13 (m, 2H), 7.12-7.06 (m, 2H), 4.06 (t, $J = 6.2$ Hz, 2H), 3.11 (q, $J = 6.7$ Hz, 2H), 1.87 (quint, $J = 6.5$ Hz, 2H), 1.38 (s, 9H). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) δ [ppm]: 159.86 (2C quat), 155.55 (C quat), 151.23 (2C quat), 127.87 (+, 4Ar-CH), 122.48 (C quat), 114.70 (+, 4Ar-CH), 114.51 (+, 2Ar-CH), 77.42 (C quat), 65.33 (-, CH$_2$), 36.79 (-, CH$_2$), 29.05 (-, CH$_3$), 28.14 (+, 3CH$_3$). HRMS (EI-MS) $m/z$: calcd for C$_{21}$H$_{25}$N$_3$O$_3$ [M+H]$^+$ 368.1969, found 368.1977.


To a solution of compound 4.25 (670 mg, 1.82 mmol) in ACN (50 mL), 1-(chloromethyl)-4-fluorobenzene (788 mg, 5.47 mmol) and NaOH (364 mg, 9.1 mmol) were added. The mixture was stirred at rt for 5 h, the obtained suspension was poured into brine and extracted with EtOAc. The organic phase was dried over Na$_2$SO$_4$ and evaporated, and the residue was dissolved in 10 mL of DCM, TFA (10 mL) was added dropwise, and the mixture was stirred at rt for 2 h. Purification by column chromatography (DCM/MeOH 25% aqueous ammonia, 100/5/1), yielded a pale oil (610 mg, 1.63 mmol, 89.3%). $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 7.87-7.79 (m, 1H), 7.62-7.51 (m, 2H), 7.33-7.13 (m, 3H), 7.12-6.89 (m, 6H), 5.38 (s, 2H), 4.09 (t, $J = 6.1$ Hz, 2H), 2.91 (t, $J = 6.8$ Hz, 2H), 1.94 (quint, $J = 6.5$ Hz, 2H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ [ppm]: 160.38 (C quat), 154.12 (C quat), 143.19 (C quat), 135.94 (C quat), 132.25 (C quat), 132.21 (C quat), 130.63 (+, 2Ar-CH), 127.73 (+, Ar-CH), 127.62 (+, Ar-CH), 122.88 (+, Ar-CH), 122.68 (+, Ar-CH), 122.21 (C quat), 119.84 (+, Ar-CH), 116.18 (+, Ar-CH), 115.89 (+, Ar-CH), 114.75 (+, 2Ar-CH), 110.22 (+, Ar-CH), 66.00 (-, CH$_2$), 47.75 (-, CH$_2$), 39.13 (-, CH$_3$), 32.88 (-, CH$_2$). HRMS (EI-MS) $m/z$: calcd for C$_{23}$H$_{22}$FN$_3$O [M+H]$^+$ 376.1820, found 376.1823.

N- [2- (1H- imidazol- 4- yl)ethyl]- 3- [4- [1- (4- fluorobenzyl)- 1H- benzo[d]imidazol- 2- yl]phenoxy]propan- 1- amine (4.27).

Compound 4.27 was prepared by analogy with the procedure for the preparation of compound 4.16a, using compound 4.26 (480 mg, 0.67 mmol) and
4-(2-bromoethyl)-1-trityl-1H-imidazole (416 mg, 1.0 mmol) as educts. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1) yielded yellow-white crystals (80 mg, 0.17 mmol, 26.7%); mp 120 °C. ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.81 (d, J = 7.7 Hz, 1H), 7.58-7.50 (m, 2H), 7.39 (s, 1H), 7.35-7.14 (m, 3H), 7.07-6.88 (m, 6H), 6.74 (s, 1H), 5.37 (s, 2H), 4.05 (t, J = 6.0 Hz, 2H), 2.97-2.86 (m, 2H), 2.85-2.70 (m, 4H), 1.97 (quint, J = 6.4 Hz, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 160.60 (C₉H₆), 160.38 (C₉H₆), 154.12 (C₉H₆), 142.94 (C₉H₆), 135.81 (C₉H₆), 134.66 (+, Ar-CH), 132.12 (C₉H₆), 132.08 (C₉H₆), 130.65 (+, 2Ar-CH), 127.74 (+, Ar-CH), 127.64 (+, Ar-CH), 123.03 (+, Ar-CH), 122.81 (+, Ar-CH), 122.05 (C₉H₆), 119.61 (+, 2Ar-CH), 116.20 (+, Ar-CH), 115.92 (+, Ar-CH), 114.83 (+, 2Ar-CH), 110.37 (+, Ar-CH), 66.41 (-, CH₂), 49.20 (-, CH₂), 47.73 (-, CH₂), 46.47 (-, CH₂), 29.36 (-, CH₂), 26.66.

HRMS (EI-MS) m/z: calcd for C₂₂H₂₂F₃N₅O [M+H⁺] 470.2351, found 470.2354.

4-[(1-(4-Fluorobenzyl)-1H-benzo[d]imidazol-2-yl]amino)phenol (4.28).

Compound 4.12c (1.04 g, 4.00 mmol) and 4-aminophenol (2.18 g, 20.0 mmol) were dissolved in 20 mL EtOH, and 4 mL pf HCl in iso-propanol were added dropwise while stirring. The reaction mixture was stirred and refluxed for two days, subsequently concentrated and subjected to flash column chromatography (MeOH with 10% NH₃/DCM, 5/95), yielding compound 4.28 as sticky oil (530 mg, 1.59 mmol, 40.0% yield). ¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 7.70-7.56 (m, 2H), 7.33 (d, J = 7.8 Hz, 1H), 7.27-7.20 (m, 2H), 7.19-7.11 (m, 3H), 7.05-6.91 (m, 2H), 6.80-6.68 (m, 2H), 5.47 (s, 2H). ¹³C-NMR (101 MHz, DMSO-d₆) δ [ppm]: 163.07 (C₉H₆), 160.65 (C₉H₆), 151.50 (C₉H₆), 142.69 (C₉H₆), 134.08 (C₉H₆), 133.75 (C₉H₆), 132.88 (C₉H₆), 129.31 (+, Ar-CH), 129.23 (+, Ar-CH), 121.37 (+, Ar-CH), 120.80 (+, Ar-CH), 120.70 (+, Ar-CH), 119.86 (+, Ar-CH), 116.33 (+, Ar-CH), 116.00 (+, Ar-CH), 115.79 (+, Ar-CH), 115.53 (+, Ar-CH), 115.48 (+, Ar-CH), 108.71 (+, Ar-CH), 44.47 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₀H₁₆F₃N₅O [M+H⁺] 334.1350, found 334.1356.

**tert- Butyl [3- (4- [(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl] amino) phenoxy) propyl] carbamate (4.29).**

Compound 4.29 was prepared by analogy with the procedure for the preparation of compound 4.25, using compound 4.28 (530 mg, 1.59 mmol) as educt. Purification by flash
column chromatography (MeOH with 10% NH₃ / DCM = 4/96) yielded a brown oil (290 mg, 0.59 mmol, 37%). ¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 7.50 (d, J = 7.8 Hz, 1H), 7.33-7.27 (m, 2H), 7.17-7.06 (m, 5H), 7.03-6.95 (m, 2H), 6.77-6.73 (m, 2H), 5.16 (s, 2H), 3.91-3.84 (m, 2H), 3.32-3.25 (m, 2H), 2.97-2.92 (m, 2H), 2.77 (m, 4H) 3.84 (m, 2H), 3.91 (t, J = 7.8 Hz, 2H), 3.25 (m, 2H), 1.95 (m, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ [ppm]: 163.66 (Cₜₜ), 161.21 (Cₜₜ), 156.09 (Cₜₜ), 154.91 (Cₜₜ), 151.02 (Cₜₜ), 134.05 (Cₜₜ), 131.24 (Cₜₜ), 131.20 (Cₜₜ), 128.37 (+, Ar-CH), 128.29 (+, Ar-CH), 121.92 (+, Ar-CH), 121.48 (+, Ar-CH), 120.71 (+, Ar-CH), 116.22 (+, Ar-CH), 116.01 (+, Ar-CH), 115.14 (+, 2Ar-CH), 107.83 (+, Ar-CH), 79.23 (Cₜₜ), 66.17 (-, CH₂), 45.46 (-, CH₂), 38.04 (-, CH₂), 29.55 (-, CH₂), 28.46 (+, 3CH₃). HRMS (El-MS) m/z: calcd for C₂₉H₃₃F₉N₃O₃ [MH⁺] 491.2453, found 491.2463.

**N-[4-(3-[[2-(1H-imidazol-4-yl)ethyl]amino]propoxy)phenyl]-1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-amine (4.30).**

Compound 4.29 (280 mg, 0.57 mmol) was dissolved in 5 mL of DCM, TFA (5 mL) was added dropwise, and the mixture was stirred at rt for 3 h. After concentration and vacuum drying overnight, 4-(2-bromoethyl)-1-trityl-1H-imidazole (200 mg, 0.48 mmol) in ACN (25 mL), DIPEA (368 mg, 2.85 mmol) and NaI (80 mg, 0.53 mmol) were added. The mixture was heated at reflux for 48 h, the solvent was evaporated, the remaining mixture was poured into brine and extracted with DCM. The combined organic layers were dried over Na₂SO₄, and the solvent was removed under reduced pressure. The residue was dissolved by 2 mL of DCM, TFA (2 mL) was added dropwise, the mixture was stirred at rt for 3 h and dried by evaporation. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/8/1) yielded a brown solid (16 mg, 0.03 mmol, 5.8%); mp 83 °C. ¹H-NMR (400 MHz, CD₃OD) δ [ppm]: 7.54 (s, 1H), 7.40-7.33 (m, 3H), 7.23-7.13 (m, 2H), 7.13-6.96 (m, 5H), 6.87-6.83 (m, 3H), 5.36 (s, 2H), 3.99 (t, J = 5.5 Hz, 2H), 2.92 (t, J = 6.9 Hz, 2H), 2.87-2.77 (m, 4H), 2.04-1.90 (m, 2H). ¹³C-NMR (101 MHz, CD₃OD) δ [ppm]: 163.43 (Cₜₜ), 161.00 (Cₜₜ), 155.07 (Cₜₜ), 152.04 (Cₜₜ), 41.21 (Cₜₜ), 134.86 (+, Ar-CH), 133.71 (Cₜₜ), 133.39 (Cₜₜ), 132.38 (Cₜₜ), 128.27 (+, Ar-CH), 128.19 (+, Ar-CH), 122.05 (+, Ar-CH), 121.37 (+, Ar-CH), 120.13 (+, Ar-CH), 115.53 (+, Ar-CH), 115.22 (+, Ar-CH), 115.00 (+, Ar-CH), 114.56 (+, Ar-CH), 108.14 (+, Ar-CH), 66.40 (-, CH₂), 48.56 (-, CH₂), 46.31 (-, CH₂), 44.38 (-, CH₂), 28.45
(-, CH₂), 26.02 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₈H₂₉FN₅O [MH⁺] 485.2460, found 485.2462.

**N’-[1-(4-Fluorobenzyl)-1H-benzo[d]imidazol-2-yl]ethane-1,2-diamine (4.31a).**

Compound **4.12c** (2.51 g, 9.65 mmol) was added to a mixture of ethane-1,2-diamine (15 mL) and DIPEA (2.49 g, 19.31 mmol) in a 20-mL microwave tube. The microwave tube was sealed and subjected to irradiation (160 °C) for 40 min. The obtained mixture was poured into brine and extracted with EtOAc, the organic layer was dried over Na₂SO₄ and evaporated. The product was used without further purification. White solid (2.64 g, 9.29 mmol, 96.3%); mp 117 °C. **¹H-NMR** (300 MHz, CDCl₃) δ [ppm]: 7.52-7.46 (m, 1H), 7.15-7.08 (m, 3H), 6.99 (m, 4H), 5.06 (s, 2H), 3.54-3.45 (m, 2H), 2.95-2.88 (m, 2H). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 160.74 (C₃₀), 154.46 (C₃₁), 142.35 (C₃₂), 134.67 (C₃₃), 131.38 (C₃₄), 128.34 (+, Ar-CH), 128.23 (+, Ar-CH), 121.53 (+, Ar-CH), 119.79 (+, Ar-CH), 116.50 (+, Ar-CH), 116.17 (+, Ar-CH), 115.88 (+, Ar-CH), 107.25 (+, Ar-CH), 45.37 (-, CH₂), 45.01 (-, CH₂), 41.00 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₁₉H₁₇FN₄ [MH⁺] 285.1510, found 285.1513.

**N’-[1-(4-Fluorobenzyl)-1H-benzo[d]imidazol-2-yl]propane-1,3-diamine (4.31b).**

Synthesized from compound **4.12c** (1.30 g, 5 mmol) and propane-1,3-diamine (15 mL) by analogy with the procedure for the preparation of **4.31a**. Sticky colorless oil (1.33 g, 4.46 mmol, 89.3%). **¹H-NMR** (300 MHz, CDCl₃) δ [ppm]: 7.50-7.45 (m, 1H), 7.15-7.05 (m, 3H), 7.04-6.93 (m, 4H), 5.03 (s, 2H), 3.61 (t, J = 6.2 Hz, 2H), 2.83 (t, J = 5.9 Hz, 2H), 1.76-1.66 (m, 2H). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 163.92 (C₃₀), 160.65 (C₃₁), 154.71 (C₃₂), 142.57 (C₃₃), 134.66 (C₃₄), 131.66 (C₃₅), 128.31 (+, Ar-CH), 128.20 (+, Ar-CH), 121.36 (+, Ar-CH), 119.49 (+, Ar-CH), 116.29 (+, Ar-CH), 115.99 (+, Ar-CH), 115.70 (+, Ar-CH), 107.06 (+, Ar-CH), 44.94 (-, CH₂), 43.25 (-, CH₂), 41.08 (-, CH₂), 31.00 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₁₉H₁₉FN₄ [MH⁺] 299.1677, found 299.1670.

**N’-[1-(4-Fluorobenzyl)-1H-benzo[d]imidazol-2-yl]hexane-1,6-diamine (4.31c).**

Synthesized from compound **4.12c** (520 mg, 2 mmol) and hexane-1,6-diamine (1.39 g, 12 mmol) by analogy with the procedure for the preparation of **4.31a**. Sticky colorless oil (440 mg,
1.29 mmol, 64.7%). **H-NMR** (400 MHz, CDCl₃) δ [ppm]: 7.50 (d, J = 7.8 Hz, 1H), 7.15-7.07 (m, 3H), 7.05-6.97 (m, 4H), 5.04 (s, 2H), 3.50-3.42 (m, 2H), 2.63 (t, J = 6.9 Hz, 2H), 1.61-1.53 (m, 2H), 1.42-1.34 (m, 2H), 1.33-1.21 (m, 4H). **C-NMR** (101 MHz, CDCl₃) δ [ppm]: 161.21 (Cquat), 154.30 (Cquat), 142.44 (Cquat), 134.69 (Cquat), 131.32 (Cquat), 128.23 (+, Ar-CH), 128.15 (+, Ar-CH), 121.51 (+, Ar-CH), 119.74 (+, Ar-CH), 116.60 (+, Ar-CH), 116.24 (+, Ar-CH), 116.03 (+, Ar-CH), 107.10 (+, Ar-CH), 45.01 (-, CH₂), 43.34 (-, CH₂), 42.05 (-, CH₂), 33.54 (-, CH₂), 29.68 (-, CH₂), 26.60 (-, CH₂), 26.52 (-, CH₂). **HRMS** (EI-MS) m/z: calcd for C₂₀H₂₅FN₄ [MH⁺] 341.2136, found 341.2143.

**N'-[2-(1H-imidazol-4-yl)ethyl]-N'-(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)ethane-1,2-diamine (4.32a).**

To a solution of compound 4.31a (330 mg, 1.16 mmol) in ACN (25 mL), 4-(2-bromoethyl)-1-trityl-1H-imidazole (488 mg, 1.17 mmol), DIPEA (454 mg, 3.52 mmol) and NaI (175 mg, 1.17 mmol) were added. The reaction mixture was heated at reflux for 40 h, the solvent was evaporated, and the remaining mixture was poured into water. The aqueous layer was extracted with DCM, and the combined organic layers were dried over Na₂SO₄ and evaporated under reduced pressure. Subsequently, the residue was dissolved in 10 mL DCM, TFA (10 mL) was added dropwise, and the mixture was stirred at rt for 24 h. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/10/1), yielded a white solid (80 mg, 0.21 mmol, 18.1%); mp 85 °C. **H-NMR** (300 MHz, CDCl₃) δ [ppm]: 7.41 (d, J = 7.6 Hz, 1H), 7.34 (s, 1H), 7.11-7.02 (m, 3H), 7.01-6.88 (m, 4H), 6.69 (s, 1H), 5.04 (s, 2H), 3.50 (t, J = 5.6 Hz, 2H), 2.84-2.75 (m, 4H), 2.65 (t, J = 6.3 Hz, 2H). **C-NMR** (75 MHz, CDCl₃) δ [ppm]: 163.89 (Cquat), 160.62 (Cquat), 154.62 (Cquat), 142.06 (Cquat), 134.74 (+, Ar-CH), 134.54 (Cquat), 131.48 (Cquat), 128.38 (+, Ar-CH), 128.27 (+, Ar-CH), 121.57 (+, Ar-CH), 119.88 (+, Ar-CH), 117.63 (+, Ar-CH), 116.02 (+, Ar-CH), 116.00 (+, Ar-CH), 115.74 (+, Ar-CH), 107.49 (+, Ar-CH), 48.69 (-, CH₂), 48.16 (-, CH₂), 44.93 (-, CH₂), 42.60 (-, CH₂), 26.68 (-, CH₂). **HRMS** (EI-MS) m/z: calcd for C₂₁H₂₅FN₆ [MH⁺] 379.2041, found 379.2047.

**N'-(2-(1H-imidazol-4-yl)ethyl)-N'-(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)propane-1,3-diamine (4.32b).**
Synthesized from compound 4.31b (600 mg, 2 mmol) by analogy with the procedure for the preparation of compound 4.32a. White solid (130 mg, 0.33 mmol, 24.9%); mp 72 °C. 1H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.46 (s, 1H), 7.39 (d, J = 7.7 Hz, 1H), 7.11-6.88 (m, 7H), 6.72 (s, 1H), 5.03 (s, 2H), 3.57 (t, J = 6.1 Hz, 2H), 2.81 (t, J = 6.3 Hz, 2H), 2.76-2.62 (m, 4H), 1.82-1.70 (m, 2H). 13C-NMR (75 MHz, CDCl₃) δ [ppm]: 160.60 (C quat), 154.80 (C quat), 142.22 (C quat), 135.46 (C quat), 134.83 (+, Ar-CH), 134.48 (C quat), 131.55 (C quat), 128.19 (+, Ar-CH), 128.08 (+, Ar-CH), 121.49 (+, Ar-CH), 119.68 (+, Ar-CH), 115.98 (+, Ar-CH), 115.81 (+, Ar-CH), 115.69 (+, Ar-CH), 107.37 (+, Ar-CH), 49.02 (-, CH₂), 47.34 (-, CH₂), 44.82 (-, CH₂), 42.39 (-, CH₂), 28.28 (-, CH₂), 26.20 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₂H₂₅FN₆ [M+H]⁺ 393.2197, found 393.2199.

N- (2- {[1- (4-Fluorobenzyl)- 1H- benzo[d]imidazol-2- yl]amino}ethyl) 3- (1H- imidazol-4- yl)acrylamide (4.33a).

To a solution of urocanic acid (690 mg, 5 mmol) in a mixture of anhydrous DMF (10 mL), HOBT (675 mg, 5 mmol), TBTU (3.21 g, 10 mmol) and DIPEA (645 mg, 5 mmol) were added. The reaction mixture was stirred at rt for 30 min, subsequently, compound 4.31a (1.42 g, 5 mmol), dissolved in DMF (5 mL), was added dropwise, and the mixture was heated to 60 °C for 1.5 h. After completion of the reaction, the mixture was poured into cold water and the pH value was adjusted to 10 by addition of 25% aqueous ammonia. Precipitated compound 4.33a was collected by filtration. White solid (1.7 g, 4.2 mmol, 84.2%); mp 141 °C. 1H-NMR (300 MHz, DMSO-d₆) δ [ppm]: 8.29-8.21 (m, 1H), 7.72 (s, 1H), 7.33-7.05 (m, 8H), 6.98-6.81 (m, 2H), 5.23 (s, 2H), 3.49-3.44 (m, 4H). 13C-NMR (75 MHz, DMSO-d₆) δ [ppm]: 165.95 (C quat), 162.88 (C quat), 159.65 (C quat), 154.39 (C quat), 142.54 (C quat), 134.26 (C quat), 133.14 (C quat), 129.09 (+, 2Ar-CH), 128.98 (+, 2Ar-CH), 120.43 (+, CH), 118.37 (+, Ar-CH), 118.19 (+, Ar-CH), 115.37 (+, 2Ar-CH), 115.09 (+, 2Ar-CH), 114.95 (+, CH), 107.69 (+, Ar-CH), 43.66 (-, CH₂), 42.09 (-, CH₂), 38.42 (-, CH₂). HRMS (EI-MS) m/z: calcd for C₂₂H₂₁FN₆O [M+H]⁺ 405.1834, found 405.1839.

N- [6- {(1- [4- Fluorobenzyl]- 1H- benzo[d]imidazol-2- yl]amino}hexyl)- 3- (1H- imidazol-4- yl)acrylamide (4.33b).
Synthesized from compound 4.31c (400 mg, 1.18 mmol) and urocanic acid (163 mg, 1.18 mmol) by analogy with the procedure for the preparation of 4.33a. Yellow oil (300 mg, 0.63 mmol, 53.6%). $^1$H-NMR (400 MHz, DMSO-d$_6$) δ [ppm]: 8.00-7.93 (m, 1H), 7.70 (s, 1H), 7.31-7.24 (m, 1H), 7.24-7.11 (m, 5H), 7.06 (d, J = 7.4 Hz, 1H), 6.95-6.89 (m, 1H), 6.87-6.77 (m, 2H), 5.24 (s, 2H), 3.40-3.36 (m, 2H), 3.17-3.10 (m, 2H), 1.66-1.54 (m, 2H), 1.48-1.38 (m, 2H), 1.36-1.26 (m, 4H). $^{13}$C-NMR (101 MHz, DMSO-d$_6$) δ [ppm]: 163.04 (C$_{quat}$), 160.62 (C$_{quat}$), 155.14 (C$_{quat}$), 143.29 (C$_{quat}$), 134.87 (C$_{quat}$), 133.83 (C$_{quat}$), 133.80 (C$_{quat}$), 129.43 (+, 2Ar-CH), 129.35 (+, 2Ar-CH), 120.89 (+, CH), 119.03 (+, Ar-CH), 118.74 (+, 2Ar-CH), 115.89 (+, 2Ar-CH), 115.67 (+, CH), 115.43 (+, Ar-CH), 108.11 (+, Ar-CH), 44.09 (-, CH$_2$), 42.87 (-, CH$_2$), 39.01 (-, CH$_2$), 29.75 (-, CH$_2$), 26.75 (-, CH$_2$), 26.61 (-, CH$_2$). HRMS (EI-MS) m/z: calcd for C$_{26}$H$_{29}$FN$_6$O [M+H$^+$] 461.2460, found 461.2465.

$N$-{2-[[1-(4-Fluorobenzyl)-1H-benzo[d]imidazol-2-yl]amino]ethyl}-3-[(1H-imidazol-4-yl)propanamide (4.34).

Compound 4.33a (1010 mg, 2.48 mmol) was hydrogenated over 500 mg of 5% Pd/C-catalyst in 30 mL of MeOH in an autoclave filled with H$_2$ at 10 bar for 19 h. The catalyst was filtered off, the filtrate was concentrated, and the residue was purified by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/5/1). White solid (1.17 g, 2.88 mmol, 82.3%); mp 101 °C. $^1$H-NMR (300 MHz, CDCl$_3$) δ [ppm]: 8.15-8.00 (m, 1H), 7.35-7.28 (m, 2H), 7.08-6.92 (m, 6H), 6.59 (s, 1H), 5.00 (s, 2H), 3.54-3.27 (m, 4H), 2.75 (t, J = 6.4 Hz, 2H), 2.38 (t, J = 6.5 Hz, 2H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ 174.39 (C$_{quat}$), 163.81 (C$_{quat}$), 160.54 (C$_{quat}$), 154.73 (C$_{quat}$), 141.81 (C$_{quat}$), 134.59 (C$_{quat}$), 134.48 (+, Ar-CH), 131.52 (C$_{quat}$), 128.36 (+, Ar-CH), 128.25 (+, Ar-CH), 121.60 (+, Ar-CH), 119.90 (+, Ar-CH), 117.20 (+, Ar-CH), 115.85 (+, Ar-CH), 115.57 (+, 2Ar-CH), 107.68 (+, Ar-CH), 44.81 (-, CH$_2$), 43.88 (-, CH$_2$), 40.08 (-, CH$_2$), 36.08 (-, CH$_2$), 22.65 (-, CH$_2$). HRMS (EI-MS) m/z: calcd for C$_{22}$H$_{29}$FN$_6$O [MH$^+$] 407.1990, found 407.1992.

$N^1$-[3-(1H-imidazol-4-yl)propyl]-$N^2$-[1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl]ethane-1,2-diamine (4.35a).
To LiAlH₄ (57 mg, 1.5 mmol) in a 25 mL argon flushed flask, anhydrous THF (5 mL) was dropped slowly under ice cooling. Subsequently, a solution of compound 4.33a (200 mg, 0.5 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at rt for 21 h and afterwards at 70 °C for 3 h. To quench the reaction, 57 µL water, 57 µL aqueous solution of 15% NaOH and 171 µL of water were dropped to the reaction mixture under ice cooling, and the mixture was stirred at rt for 30 min. A white precipitate was filtered off, and the filtrate was concentrated and subjected to column chromatography (DCM/MeOH/25% aqueous ammonia, 100/7/1). White solid (90 mg, 0.23 mmol, 46%); mp 91 °C. ¹H-NMR (300 MHz, CDCl₃) δ [ppm]: 7.43-7.35 (m, 2H), 7.10-6.93 (m, 5H), 6.92-6.83 (m, 2H), 6.65 (s, 1H), 5.06 (s, 2H), 3.52-3.45 (m, 2H), 2.77 (t, J = 5.2 Hz, 2H), 2.57-2.45 (m, 4H), 1.66 (quint, J = 6.8 Hz, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ [ppm]: 163.84 (Cquat), 160.57 (Cquat), 154.68 (Cquat), 142.09 (Cquat), 136.57 (Cquat), 134.56 (+, Ar-CH), 131.57 (Cquat), 128.34 (+, Ar-CH), 128.23 (+, Ar-CH), 121.52 (+, Ar-CH), 119.82 (+, Ar-CH), 117.19 (+, Ar-CH), 115.94 (+, Ar-CH), 115.90 (+, Ar-CH), 115.66 (+, Ar-CH), 107.49 (+, Ar-CH), 65.89 (-, CH₂), 48.26 (-, CH₂), 44.89 (-, CH₂), 42.27 (-, CH₂), 29.41 (-, CH₂), 24.32 (-, CH₂). HRMS (El-MS) m/z: calcd for C₂₂H₂₅FN₆ [M+H⁺] 393.2197, found 393.2198.

N¹-[3-(1H-imidazol-4-yl)propyl]-N⁶-[1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl]hexane-1,6-diamine (4.35b).

Synthesized from compound 4.33b (270 mg, 0.57 mmol) by analogy with the procedure for the preparation of 35a. However, purification was performed by HPLC. Yield 4.35b tri(hydrotrifluoroacetate) as a colorless sticky solid (10 mg, 0.013 mmol, 2.2%). ¹H-NMR (600 MHz, CD₃OD) δ [ppm]: 8.82 (s, 1H), 7.47-7.43 (m, 1H), 7.38-7.24 (m, 6H), 7.14-7.06 (m, 2H), 5.41 (s, 2H), 3.51 (t, J = 7.2 Hz, 2H), 3.10-2.96 (m, 4H), 2.84 (t, J = 7.6 Hz, 2H), 2.12-2.02 (m, 2H), 1.79-1.66 (m, 4H), 1.48-1.39 (m, 4H). ¹³C-NMR (151 MHz, CD₃OD) δ [ppm]: 161.77 (Cquat), 149.81 (Cquat), 133.67 (Cquat), 132.46 (Cquat), 130.72 (Cquat), 130.00 (+, Ar-CH), 129.06 (Cquat), 128.48 (+, Ar-CH), 128.42 (+, Ar-CH), 123.99 (+, Ar-CH), 123.71 (+, Ar-CH), 115.76 (+, Ar-CH), 115.60 (+, Ar-CH), 115.45 (+, Ar-CH), 111.37 (+, Ar-CH), 110.04 (+, Ar-CH), 49.03 (-, CH₂), 48.60 (-, CH₂), 46.46 (-, CH₂), 44.75 (-, CH₂), 43.27 (-, CH₂), 28.26 (-, CH₂), 25.80 (-, CH₂).
CH\textsubscript{2}), 25.77 (-, CH\textsubscript{2}), 24.71 (-, CH\textsubscript{2}), 21.01 (-, CH\textsubscript{2}). \textbf{HRMS} (EI-MS) \textit{m/z}: calcd for C\textsubscript{26}H\textsubscript{33}FN\textsubscript{6} [MH\textsuperscript{+}] 449.2823, found 449.2825.

\textbf{2- [4- (1H- imidazol- 4- yl)ethyl]piperazin- 1- yl]- 6- chloro- N\textsuperscript{2} (thiophen- 2- ylmethyl) quinazolin- 4- amine} (4.40).

A mixture of compound 3.12 (360 mg, 1 mmol), ACN (10 mL), DIPEA (387 mg, 3 mmol) and 4-(2-bromoethyl)-imidazole (225 mg, 1.2 mmol) in a 20-mL microwave tube was subjected to microwave irradiation (120 °C) for 30 min. The obtained suspension was poured into brine and extracted with EtOAc, the organic layer was dried over Na\textsubscript{2}SO\textsubscript{4}, and the solvent was removed. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/3.3/1) yielded a white solid (140 mg, 0.31 mmol, 31.4%); mp 157 °C. \textbf{\textsuperscript{1}H-NMR} (400 MHz, CDCl\textsubscript{3}) \textit{δ} [ppm]: 7.54 (d, \textit{J} = 0.9 Hz, 1H), 7.51 (d, \textit{J} = 2.0 Hz, 1H), 7.45-7.37 (m, 2H), 7.23-7.19 (m, 1H), 7.06-7.02 (m, 1H), 6.82 (d, \textit{J} = 0.6 Hz, 1H), 4.94 (d, \textit{J} = 5.4 Hz, 2H), 4.03-3.96 (m, 4H), 2.85 (t, \textit{J} = 6.7 Hz, 2H), 2.71 (t, \textit{J} = 6.7 Hz, 2H), 2.65-2.56 (m, 4H). \textbf{\textsuperscript{13}C-NMR} (101 MHz, CDCl\textsubscript{3}) \textit{δ} [ppm]: 158.72 (C\textsubscript{quat}), 158.70 (2C\textsubscript{quat}), 150.92 (C\textsubscript{quat}), 141.27 (C\textsubscript{quat}), 134.20 (+, Ar-CH), 133.18 (+, Ar-CH), 127.43 (+, Ar-CH), 126.71 (+, Ar-CH), 126.22 (+, Ar-CH), 125.88 (2C\textsubscript{quat}), 125.40 (+, Ar-CH), 120.54 (+, 2Ar-CH), 57.92 (-, CH\textsubscript{2}), 53.17 (-, 2CH\textsubscript{2}), 44.06 (-, 2CH\textsubscript{2}), 39.93 (-, 2CH\textsubscript{2}). \textbf{HRMS} (EI-MS) \textit{m/z}: calcd for C\textsubscript{22}H\textsubscript{24}ClN\textsubscript{7}S [MH\textsuperscript{+}] 454.1575, found 454.1576.

\textbf{6-Chloro- N\textsuperscript{2} [2-(4-methylpiperazin-1-yl)ethyl]-N\textsuperscript{4}- (thiophen-2-ylmethyl)quinazoline-2,4- diamine} (4.41).

Compound 3.10 (69 mg, 0.3 mmol) was added to a mixture of EtOAc (5 mL) and DIPEA (77 mg, 0.6 mmol) in a 5-mL microwave tube. 2-Thienymethylamine (34 mg, 0.3 mmol) was added, and the mixture was stirred at rt for 30 min. The solvent was removed by evaporation, and the residue dissolved in DMF. Subsequently, 4-methyl-1-piperazineethanamine tri(hydrotrifluoroacetate)\textsuperscript{50} (448 mg, 0.9 mmol) was added, the microwave tube was sealed and subjected to irradiation (160 °C) for 10 min. The obtained suspension was poured into brine and extracted with EtOAc, the organic layer was dried over Na\textsubscript{2}SO\textsubscript{4}, the solvent was removed and the residue subjected to HPLC, yielded 4.41 tri(hydrotrifluoroacetate) as a white
solid (31 mg, 0.04 mmol, 13.6%). $^1$$H$-NMR (300 MHz, CDCl$_3$) $\delta$ [ppm]: 7.51 (d, $J = 2.0$ Hz, 1H), 7.45-7.31 (m, 2H), 7.25-7.17 (m, 1H), 7.05-7.01 (m, 1H), 6.97-6.93 (m, 1H), 4.93 (d, $J = 5.0$ Hz, 2H), 3.66-3.50 (m, 2H), 2.69-2.34 (m, 10H), 2.27 (s, 3H).

$^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$ [ppm]: 159.31 (C$\text{quat}$), 158.87 (C$\text{quat}$), 150.50 (C$\text{quat}$), 140.97 (2C$\text{quat}$), 133.29 (+, Ar-CH), 126.78 (+, 2Ar-CH), 126.38 (+, Ar-CH), 125.81 (C$\text{quat}$), 125.48 (+, Ar-CH), 120.59 (+, Ar-CH), 57.01 (-, CH$_2$), 55.09 (-, 2CH$_2$), 46.03 (-, CH$_3$), 39.84 (-, CH$_2$), 38.10 (-, CH$_2$). HRMS (EI-MS) $m/z$: calcd for C$_{20}$H$_{25}$ClN$_6$S [MH$^+$] 417.1623, found 417.1624.

6-Chloro-N$^2$-(1-methylpiperidin-4-yl)-N$^4$-(thiophen-2-ylmethyl)quinazoline-2,4-diamine (4.42).

Synthesized from compound 3.10 (230 mg, 1 mmol) and 1-methylpiperidin-4-amine (460 mg, 4 mmol) by analogy with the procedure for the preparation of 4.41. Purification by column chromatography (DCM/MeOH/25% aqueous ammonia, 100/4/1) yielded a white solid (320 mg, 0.83 mmol, 82.7%); mp 93 °C. $^1$$H$-NMR (300 MHz, CD$_3$OD) $\delta$ [ppm]: 7.92-7.88 (m, 1H), 7.48-7.40 (m, 1H), 7.34-7.18 (m, 2H), 7.05-7.00 (m, 1H), 6.94-6.88 (m, 1H), 4.90 (s, 2H), 4.03-3.85 (m, 1H), 2.88-2.76 (m, 2H), 2.27 (s, 3H), 2.23-2.07 (m, 2H), 2.05-1.90 (m, 2H), 1.65-1.48 (m, 2H). $^{13}$C-NMR (75 MHz, CD$_3$OD) $\delta$ [ppm]: 160.90 (C$\text{quat}$), 160.69 (C$\text{quat}$), 151.49 (C$\text{quat}$), 143.76 (C$\text{quat}$), 134.19 (+, Ar-CH), 127.49 (+, Ar-CH), 126.92 (2C$\text{quat}$), 126.64 (+, Ar-CH), 126.47 (+, Ar-CH), 125.58 (+, Ar-CH), 122.98 (+, Ar-CH), 76.73 (+, CH), 55.76 (-, 2CH$_2$), 46.30 (+, CH$_3$), 40.38 (-, CH$_2$), 33.09 (-, 2CH$_2$). HRMS (EI-MS) $m/z$: calcd for C$_{19}$H$_{22}$ClN$_5$S [MH$^+$] 388.1357, found 388.1362.

6-Chloro-N$^4$-(thiophen-2-ylmethyl)-N$^2$-[2-(1-trityl-1H-imidazol-4-yl)ethyl]quinazoline-2,4-diamine (4.43).

To a solution of N-[2-(1-Triphenylmethylimidazol-4-yl)ethyl]phthalimide (1.90 g, 3.93 mmol) in EtOH (50 mL), hydrazine hydrate (1.18 g, 23.6 mmol) was added and stirred at room temperature for 2 h. After reaction (control by TLC), a white precipitate formed was removed by filtration. The filtrate was evaporated to dryness yielded 2-(1-trityl-imidazol-4-yl)ethan-1-amine as a yellow oil (1.37 g, 3.88 mmol, 98.8%). Compound 4.43 was synthesized from compound 3.10 (573 mg, 2.48 mmol) and
2-(1-trityl-imidazol-4-y)ethan-1-amine (865 mg, 2.45 mmol) by analogy with the procedure for the preparation of 4.41. Purification by column chromatography (MeOH/DCM, 1/20) yielded a brown oil (170 mg, 0.27 mmol, 10.9%). **HRMS** (EI-MS) m/z: calcd for C₃₇H₃₁ClN₆S [MH⁺] 627.2092, found 627.2096.

\[N^2- \text{[2-}(1H\text{-imidazol-4-yl})\text{ethyl]-6- chloro- } N^4\text{- (thiophen-2-ylmethyl)quinazoline-2,4-diamine (4.44).}\]

Compound 4.43 (130 mg, 0.21 mmol) was dissolved in 10 mL DCM, TFA (10 mL) was added dropwise, the mixture was stirred overnight at rt (control by TLC). The solvent was evaporated, and the remaining oil was subjected to HPLC, yielding 4.44 tri(hydrotrifluoroacetate) as a white solid (60 mg, 0.08 mmol, 39.4%). **¹H-NMR** (300 MHz, DMSO-d₆) δ [ppm]: 8.99 (s, 1H), 8.40 (d, J = 2.1 Hz, 1H), 7.87-7.81 (m, 1H), 7.54-7.36 (m, 3H), 7.15 (d, J = 2.8 Hz, 1H), 7.00-6.96 (m, 1H), 4.98 (d, J = 5.4 Hz, 2H), 3.88-3.78 (m, 2H), 2.99 (t, J = 6.3 Hz, 2H). **¹³C-NMR** (75 MHz, DMSO-d₆) δ [ppm]: 158.71 (C quat), 153.00 (C quat), 139.60 (C quat), 138.21 (C quat), 135.21 (+, Ar-CH), 133.79 (+, Ar-CH), 130.42 (C quat), 128.03 (C quat), 126.87 (+, Ar-CH), 126.67 (+, Ar-CH), 125.79 (+, Ar-CH), 123.44 (+, Ar-CH), 118.67 (+, Ar-CH), 116.42 (+, Ar-CH), 110.56 (C quat), 39.63 (-, CH₂), 39.44 (-, CH₂), 24.10 (-, CH₂). **HRMS** (EI-MS) m/z: calcd for C₁₉H₁₇ClN₆S [MH⁺] 385.0997, found 385.1001.

**4.4.2. Pharmacological Methods**

Histamine dihydrochloride was from Alfa Aesar (Karlsruhe, Germany). Guanosine diphosphate (GDP) was from Sigma-Aldrich Chemie (Munich, Germany), unlabeled GTPγS was from Roche (Mannheim, Germany) and thioperamide was from R&D Systems (Wiesbaden, Germany) [³H]pyrilamine and [³H]histamine were from Hartmann Analytic (Braunschweig, Germany). [³⁵S]GTPγS was from PerkinElmer Life Sciences (Boston, MA) or from Hartmann Analytic (Braunschweig, Germany). GF/C filters were from Whatman (Maidstone, UK). [³H]UR-PI294⁵⁸ and [³H]UR-DE257⁵⁷ were synthesized as described previously.
4.4.2.1. Preparation of compound stock solutions

See section 3.3.2.2.

4.4.2.2. Competition binding experiments

Competition binding experiments were performed on membrane preparations of Sf9 insect cells expressing the hH1R + RGS4, hH2R-GsαS, hH3R + Gi2 + β1γ2 or the hH4R + Gi2 + β1γ2. General procedures for the generation of recombinant baculoviruses, culture of Sf9 cells and membrane preparation are described elsewhere. The respective membranes were thawed and sedimented by centrifugation at 4 °C and 13000 rpm for 10 min. Membranes were re-suspended in binding buffer (12.5 mM MgCl₂, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4).

Each tube (total volume 100 μL) contained 30 μg (hH1R), 40 μg (hH2R), 60 μg (hH3R) or 100 μg (hH4R) of membrane protein and increasing concentrations of unlabeled ligands. Radioligands: H₁R: [³H]pyrilamine, specific activity 20.0 Ci/mmol, Kᵟ = 4.5 nM, c = 5 nM, nonspecific binding determined in the presence of 10 μM of diphenhydramine; H₂R: [³H]UR-DE257 (radioligand was diluted with unlabeled ligand due to economic reasons), specific activity 33.0 Ci/mmol, Kᵟ = 12.1 nM, c = 20 nM, nonspecific binding determined in the presence of 10 μM of famotidine; H₃R: [³H]UR-PI294, specific activity 41.8 Ci/mmol, Kᵟ = 3.3 nM, c = 3.5 nM, nonspecific binding determined in the presence of 10 μM of thioperamide; H₄R: [³H]histamine, specific activity 25 Ci/mmol, Kᵟ = 10 nM, c = 10 nM, nonspecific binding determined in the presence of 10 μM of histamine. Filtration through glass microfiber filters (for hH₄R, glass microfiber filters was pretreated with 0.3% polyethylenimine, Whatman GF/B, Maidstone, UK) using a Brandel 96 sample harvester (Brandel, Gaithersburg, MD) separated unbound from membrane associated radioligand. After three washing steps with binding buffer, filter pieces for each well were punched out and transferred into 96-well sample plates 1450-401 (Perkin Elmer, Rodgau Germany). Each well was supplemented with 200 μL of scintillation cocktail (Rotiscint Eco plus, Roth, Karlsruhe, Germany) and incubated in the dark. Radioactivity was measured with a Micro Beta² 1450 scintillation counter (Perkin Elmer, Rodgau, Germany). Protein concentration was determined by the method of Lowry using bovine serum albumin as standard. Data analysis of the resulting competition curves was accomplished by non-linear regression analysis using the algorithms in PRISM GraphPad.
Software (GraphPad Prism 5.0 software, San Diego, CA). \(K_i\) values were calculated according to the Cheng-Prusoff equation.\(^75\) Values represent the mean ± SEM of 3 independent experiments each performed in triplicate.

### 4.4.2.3. \([^{35}S]GTP\gamma S\) binding assay\(^76,77\)

The Sf9 cell membranes used in \([^{35}S]GTP\gamma S\) binding assay and their preparations were the same as described above. The respective membranes were thawed and sedimented by 10 min centrifugation at 4 °C and 13000 rpm. Membranes were re-suspended 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 H\(_x\)R subtype (10-20 μg-Protein/tube), 1 μM GDP, 0.05% (w/v) bovine serum albumin, 0.2 nM \([^{35}S]GTP\gamma S\) and the investigated ligands (dissolved in Millipore water or in a mixture (v/v) of 50% Millipore water and 50% DMSO) at various concentrations in binding buffer (total volume 100 μL). In case of H\(_1\)R assays the binding buffer additionally contained 150 mM of NaCl and 50 μg/mL of saponin, H\(_4\)R assays were performed with buffer containing 100 mM of NaCl. For the determination of \(K_b\) values (antagonist mode of the \([^{35}S]GTP\gamma S\) binding assay) histamine was added to the reaction mixtures (final concentrations: \(hH_{1/2}R: 1 \mu M, hH_{3/4}R: 100 nM\)). Incubation was performed at 25 °C and shaking at 250 rpm for 90 min. Bound \([^{35}S]GTP\gamma S\) was separated from free \([^{35}S]GTP\gamma S\) by filtration through GF/C filters, followed by three washing steps with 2 mL of binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined by liquid scintillation counting after an equilibration phase of at least 12 h. The experimental conditions chosen ensured that not more than 10% of the total amount of \([^{35}S]GTP\gamma S\) added was bound to the filters. Nonspecific binding was determined in the presence of 10 μM unlabeled GTP\(\gamma S\). IC\(_{50}\) values were converted to \(K_b\) values using the Cheng-Prusoff equation.\(^75\) EC\(_{50}\) and \(K_b\) values from the functional GTP\(\gamma S\) assays were analyzed by nonlinear regression and best fit to sigmoidal concentration-response curves (GraphPad Prism 5.0 software, San Diego, CA).
4.5. References


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Chapter 5

Summary
5. Summary

G-protein coupled receptors are the most important class of biological targets for drug development. Among them, the histamine receptors may be considered as representative examples for aminergic GPCRs. The latest member of the histamine receptor family, the H₄R, was reported to be involved in immunological processes and inflammatory diseases. However, the (patho)physiological role of the H₄R is far from being fully understood. Thus, potent and selective pharmacological tools in various forms targeting H₄R are required. Based on complementary and overlapping functions of hH₁R and hH₄R, it is presumed that combined H₁R/H₄R antagonism might be superior to monotherapy in the treatment of allergic diseases.

In the first part of this thesis, twenty-two novel homo-dimeric ligands based on the prominent H₁R antagonists diphenhydramine (3.1), pyrilamine (3.2) and dual H₁R/H₄R antagonist quinazoline derivative (3.3), were synthesized to probe putative accessory binding sites on hH₁R and hH₄R. Furthermore, their binding affinities were determined at the hH₁R and/or hH₄R. Between the two types of spacers which were employed in this study, connecting chains comprising amide groups exhibited similar affinity compared to their monomeric counterparts. By contrast, alkyl chains without amides showed decreased affinity at the hH₁R, suggesting that the lipophilicity of the compounds plays an important role in binding affinity, e.g., high lipophilicity may result a poor solubility and high non-specific binding of the compound in assays. Since the variation of the spacer length had no significant influence on binding affinities, it may be suggested that one pharmacophore of the homo-dimeric ligand was not binding to the hH₁R, thus it did not contribute to receptor-ligand binding. At the hH₄R, all bivalent quinazoline-type ligands showed no obvious activity. This may be interpreted that the quinazoline-type homo-dimeric ligands are not tolerated at the hH₄R. In a word, the data of the present study were not sufficient to prove the existence of accessory binding sites on hH₁R and hH₄R.

The second part of this thesis was focused on developing dual hH₁R/hH₄R antagonists. Thirty benzimidazole- and quinazoline-type compounds were synthesized and
pharmacologically characterized at the four human histamine receptor subtypes. The incorporation of an imidazole moiety, separated from the benzimidazole moiety by an appropriate linker, largely improved the binding affinities at the hH₄R and resulted in a balanced dual hH₁R/hH₄R antagonist (compound 4.35b) with Kᵢ values in the two-digit nM range. However, ligands comprising imidazolylalkyl moieties did not discriminate between hH₃R and hH₄R.

In summary, although the dimeric approach may be an interesting and useful strategy in developing bitopic and dual target ligands targeting H₁R and/or H₄R, there are numerous problems associated with this approach. Due to the low homology between ligand binding sites of H₁R and H₄R, it appears extremely difficult to identify a common H₁R/H₄R pharmacophore at a high level of affinity. Moreover, as the H₄R has a relatively high homology with the H₃R, a poor discrimination between both receptor subtypes may result in more complex binding and functional properties of hybrid compounds targeting the H₄R.
Chapter 6

Appendix
6. Appendix

6.1. Abbreviations

\( \alpha \) intrinsic activity or selectivity factor
A agonist
abs absolute
AC adenylyl cyclase
aq aqueous
atm atmosphere
Boc \( \text{tert-} \)butoxycarbonyl
Boc\(_2\)O di-\( \text{tert-} \)butyl dicarbonate
BSA bovine serum albumin
\([\text{Ca}^{2+}]_i\) intracellular calcium ion concentration
calcd. calculated
cAMP cyclic 3', 5'-adenosine monophosphate
CH\(_2\)Cl\(_2\) dichloromethane
CHCl\(_3\) chloroform
CH\(_3\)CN acetonitrile
Ci curie
CNS central nervous system
COSY correlated spectroscopy
cpm counts per minute
d day(s) or doublet
DAG diacylglycerol
\( \delta \) chemical shift
DCC \( N,N'\)-dicyclohexylcarbodiimide
DCM dichloromethane
dd doublet of doublets
DIPEA diisopropylethylamine
DMAP 4-dimethylaminopyridine
DMF dimethylformamide
DMSO dimethylsulfoxide
DMSO-d\(_6\) per-deuterated dimethylsulfoxide

\( \text{EC}_{50} \) agonist concentration which induces 50 % of the maximum response
EDC \( N\)-(3-dimethylaminopropyl)-\( N'\)-ethylcarbodiimide hydrochloride
eq equivalents
EtOAc ethylacetate
Et\(_2\)O diethylether
EtOH ethanol
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<th>Abbreviation</th>
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<td>FCS</td>
<td>fetal bovine serum</td>
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<tr>
<td>G</td>
<td>G-Protein</td>
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<td>guanosine diphosphate</td>
</tr>
<tr>
<td>GTP</td>
<td>guanosine triphosphate</td>
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<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
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<tr>
<td>h</td>
<td>hour(s) or human</td>
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<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
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<tr>
<td>HMBC</td>
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<tr>
<td>HSQC</td>
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<td>HOBr</td>
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<td>μ</td>
<td>micro</td>
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<td>mp.</td>
<td>melting point</td>
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<td>HR</td>
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<td>HₓR</td>
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<td>MS</td>
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<tr>
<td>n</td>
<td>nano or amount of substance</td>
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<td>NaHCO₃</td>
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<td>NHS</td>
<td>N-hydroxsuccinimide</td>
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NMR  nuclear magnetic resonance
PBS  phosphate buffered saline
PE  petroleum ether
pEC$_{50}$  negative decadic logarithm of the molar concentration of
         the agonist causing 50% of the maximal response
Ph  phenyl
Ph$_3$P  triphenylphosphine
PIP$_2$  Phosphatidylinositol-4,5-bisphosphate
PKC  protein kinase C
pK$_b$  negative decadic logarithm of the dissociation constant
         (functional assay)
pK$_i$  negative decadic logarithm of the dissociation constant
         (competition binding assay)
ppm  parts per million
Py  pyridyl or pyrylium
q  quartet
ref  reference
R$_f$  retardation factor
RGS  regulator of G-protein signaling
RP  reversed phase
rpm  revolutions per minute
rt  room temperature
s  singulet or second(s)
sat.  saturated
SEM  standard error of the mean
t  triplet
t$_0$  dead time
TBDPS  tert-butyldiphenysily
TBTU  2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium
tetrafluoroborate
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TLC  thin layer chromatography
      TM transmembrane
TM  transmembrane
TMS  trimethylsilyl
t$_r$  retention time
UV  ultraviolett
6.2. Purity determined by HPLC

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<td>95.15</td>
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<td>c</td>
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<td>9.12</td>
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<td>11.9</td>
<td>3.15</td>
<td>99.87</td>
<td>c</td>
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</tbody>
</table>

\[ t_0 = 2.87 \text{ min}; \text{ gradient mode: a) MeCN/0.1\% TFA (aq.): 0 min: 30/70, 30 min: 90/10, 31 min: 95/5, 40 min: 95/5, 41 min: 20/80, 50 min: 20/80; b) MeCN/0.1\% TFA (aq.): 0 min: 50/50, 30 min: 85/15, 32 min: 95/5, 40 min: 95/5, 41 min: 5/95, 50 min: 5/95; c) MeCN/0.1\% TFA (aq.): 0 min: 20/80, 30 min: 90/10, 31 min: 95/5, 40 min: 95/5, 41 min: 20/80, 50 min: 20/80; d) MeCN/0.1\% TFA (aq.): 0 min: 5/95, 30 min: 85/15, 32 min: 95/5, 40 min: 95/5, 41 min: 5/95, 50 min: 5/95. \]
6.3. Saturation binding at the hH3R with N1- [3- (1H-imidazol-4-yl)propyl]-N2-propionylguanidine ([3H]UR-PI294)

Representative N1-[3-(1H-imidazol-4-yl)propyl]-N2-propionylguanidine ([3H]UR-PI294) saturation binding experiment on Sf9 insect cell membranes expressing hH3R + Gαi2 + Gβ1γ2 (performed in triplicate). Membranes were incubated with increasing concentrations of N1-[3-(1H-imidazol-4-yl)propyl]-N2-propionylguanidine ([3H]UR-PI294). Nonspecific binding was determined in the presence of 10 μM thioperamide. Specific binding is the difference between the total and nonspecific binding of N1-[3-(1H-imidazol-4-yl)propyl]-N2-propionylguanidine ([3H]UR-PI294) at a given concentration. (Kd = 3.27 ± 0.38 nM from three independent experiments performed in triplicate, ref1: 1.1 nM)

6.4. NMR spectra of selected compounds

To verify the position of Cl at benzimidazole scaffold, 2D-NMR spectra of compounds 4.13a and 4.13b were recorded.

6.4.1. NMR spectra (COSY, HSQC, HMBC, NOESY) of 4.13a:
NMR spectra (COSY) of compound 4.13a.
NMR spectra (HSQC) of compound 4.13a.
NMR spectra (HMBC) of compound 4.13a.
NMR spectra (NOESY) of compound 4.13a.
6.4.2. NMR spectra (COSY, HSQC, HMBC, NOESY) of 4.13b:

NMR spectra (COSY) of compound 4.13b.

NMR spectra (HSQC) of compound 4.13b.
NMR spectra (HMBC) of compound 4.13b.

NMR spectra (NOESY) of compound 4.13b.
To verify the alkylation reaction taking place at 4-position instead of imidazole ring of histamine, 2D-NMR spectra of compound 4.16a and 4.16b were recorded.

6.4.3. NMR spectra (COSY, HSQC, HMBC, ROESY) of 4.16a:

NMR spectra (COSY) of compound 4.16a.

NMR spectra (HSQC) of compound 4.16a.
NMR spectra (HMBC) of compound 4.16a.

NMR spectra (ROESY) of compound 4.16a.
6.4.4. NMR spectra (COSY, HSQC, HMBC) of 4.16b

NMR spectra (COSY) of compound 4.16b.

NMR spectra (HSQC) of compound 4.16b.
NMR spectra (HMBC) of compound 4.16b.

6.5. References

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Regensburg,