

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

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

an der Fakultät für Chemie und Pharmazie

der Universität Regensburg



vorgelegt von

Jianfei Wan

aus

Ürümqi (China)

2017

The experimental part of this work was carried out between January 2013 and September 2016 under the supervision of PD. Dr. Andrea Straßer and Prof. Dr. Armin Buschauer at the Institute of Pharmacy, Faculty of Natural Sciences IV - Chemistry and Pharmacy, University of Regensburg.

The thesis was submitted on:

1st, March, 2017

Date of the colloquium:

24th, March, 2017

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)

Acknowledgements

I would like to express my sincere gratitude

to my research supervisor Prof. Dr. Armin Buschauer for giving me the chance to study in Germany, and gave me such an interesting project to explore, I am grateful to his guidance and assistance for my project and the admission of GRK1910,

to my supervisor PD Dr. A. Strasser for scientific advice and valuable discussions on my project,

to Prof. Dr. Günther Bernhardt for constructive criticism on my thesis,

to Prof. Dr. Detlef Neumann (Institute of Pharmacology, Hannover medical School) and his group members for assistance for my stay in Hannover,

to Maria Beer-Krön for excellent technical assistance and Peter Richthammer for solving technical problems, Karin Reindl for helping to solve organizational matters,

to all employees of the analytical department of the University of Regensburg for the help in the interpretation of NMR, mass spectra and elemental analyses data,

to Ulla Seibel for the help in the assays,

to all members of the Buschauer- and Elz- group for their help, especially my lab mates Dr. Paul Baumeister, Dr. Christian Textor for helping me quite a lot when my PhD study started, Sabrina Biselli, Edith Bartole for providing a very enjoyable atmosphere in the lab,

to Dr. Paul Baumeister, Dr. Daniel Bücherl for all the fun times we spend together in Germany and China,

to Xueke She for her encouragement and help for solving problems in experiments, and my parents for their supports and concerns,

to China Scholarship Council and the Project „International Quality Network – Medicinal Chemistry“ for providing financial support to me, and Research Training Group GRK1910 of the Deutsche Forschungsgemeinschaft for financial support and scientific promotion.

Poster Presentations:

- 06/2014 Jianfei Wan, Hans-Joachim Wittmann, Andrea Strasser, Armin Buschauer. Synthesis and pharmacological characterization of combined histamine H₁/H₄ receptor ligands. Emil Fischer Graduate School Research Day, Erlangen, Germany.
- 09/2014 Jianfei Wan, Hans-Joachim Wittmann, Andrea Strasser, Armin Buschauer. Synthesis and pharmacological characterization of combined histamine H₁/H₄ receptor ligands. XXIIIth International Symposium on Medicinal Chemistry (EFMC-ISMC), Lisbon, Portugal.
- 09/2014 Jianfei Wan, Hans-Joachim Wittmann, Andrea Strasser, Armin Buschauer. Synthesis and pharmacological characterization of combined histamine H₁/H₄ receptor ligands. The 7th International Summer School in Medicinal Chemistry. Regensburg, Germany.
- 04/2016 Jianfei Wan, Hans-Joachim Wittmann, Andrea Strasser, Armin Buschauer. Benzimidazole type derivatives as dual histamine H₁/H₄ receptor ligands. GLISTEN meeting, Erlangen, Germany.
- 09/2016 Jianfei Wan, Hans-Joachim Wittmann, Andrea Strasser, Armin Buschauer. Benzimidazole type derivatives as dual histamine H₁/H₄ receptor ligands. The 8th International Summer School in Medicinal Chemistry. Regensburg, Germany.

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.

Contents

1. Introduction	2
1.1. G-protein coupled receptors.....	2
1.1.1. Classification	2
1.1.2. Structure	2
1.1.3. Function	3
1.1.4. Signaling	4
1.1.4.1. G-protein cycle.....	4
1.1.4.2. G-Proteins and their pathways.....	5
1.1.5. Oligomerization	5
1.1.6. Allosteric modulation	6
1.1.7. Bivalent ligands for GPCRs.....	6
1.2. Histamine and its receptors.....	7
1.2.1. Histamine	7
1.2.2. Histamine receptors	7
1.2.2.1. The histamine H ₁ R	7
1.2.2.2. The histamine H ₂ R	8
1.2.2.3. The histamine H ₃ R	9
1.2.2.4. The histamine H ₄ R	10
1.2.3. Dual ligands of histamine receptors	11
1.3. Reference.....	13
2. Scope and objectives	4
2.1. References.....	5
3. Homo-dimeric ligands as human histamine H ₁ receptor ligands or dual H ₁ /H ₄ receptor ligands.....	8
3.1. Introduction	8
3.2. Results and discussion	10
3.2.1. Chemistry	10
3.2.2. Results and discussion	12
3.2.2.1. Competition binding studies	12
3.2.2.2. Conclusion	15
3.3. Experimental section	16
3.3.1. Chemistry	16
3.3.1.1. General conditions	16
3.3.1.2. Synthesis	17
3.3.2. Pharmacology	31
3.3.2.1. Competition binding experiments.....	31
3.3.2.2. Preparation of compound stock solutions	32
3.4. References.....	32
4. Benzimidazole- and Quinazoline-type histamine H ₁ /H ₄ receptor ligands: Dual vs. subtype selective antagonism	38
4.1. Introduction	38
4.2. Results and discussion	41

4.2.1. Chemistry	41
4.2.2. Pharmacology	44
4.2.2.1. Competition binding data at the hH ₁ R and hH ₄ R.	45
4.2.2.2. Histamine receptor subtype selectivity and activity of selected compounds.....	48
4.3. Conclusion	52
4.4. Experimental section.....	53
4.4.1. Chemistry.....	53
4.4.1.1. General conditions.	53
4.4.1.2. Synthesis	53
4.4.2. Pharmacological Methods.....	80
4.4.2.1. Preparation of compound stock solutions	81
4.4.2.2. Competition binding experiments.....	81
4.4.2.3. [³⁵ S]GTPγS binding assay ^{76, 77}	82
4.5. References.....	83
5. Summary.....	90
6. Appendix	94
6.1. Abbreviations.....	94
6.2. Purity determined by HPLC.....	97
6.3. Saturation binding at the hH ₃ R with N ¹ - [3- (1H- imidazol- 4- yl)propyl]- N ² - propionylguanidine ([³ H]UR-PI294)	98
6.4. NMR spectra of selected compounds	98
6.4.1. NMR spectra (COSY, HSQC, HMBC, NOESY) of 4.13a:.....	98
6.4.2. NMR spectra (COSY, HSQC, HMBC, NOESY) of 4.13b:.....	103
6.4.3. NMR spectra (COSY, HSQC, HMBC, ROESY) of 4.16a:.....	105
6.4.4. NMR spectra (COSY, HSQC, HMBC) of 4.16b	107
6.5. References.....	108

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 genome¹ and one of the most ubiquitous and versatile receptor family². 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 β_2 adrenergic receptor (β_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 β_2 AR active state.¹⁵ Meanwhile, the structures of more than 30 different GPCRs are described in the literature.

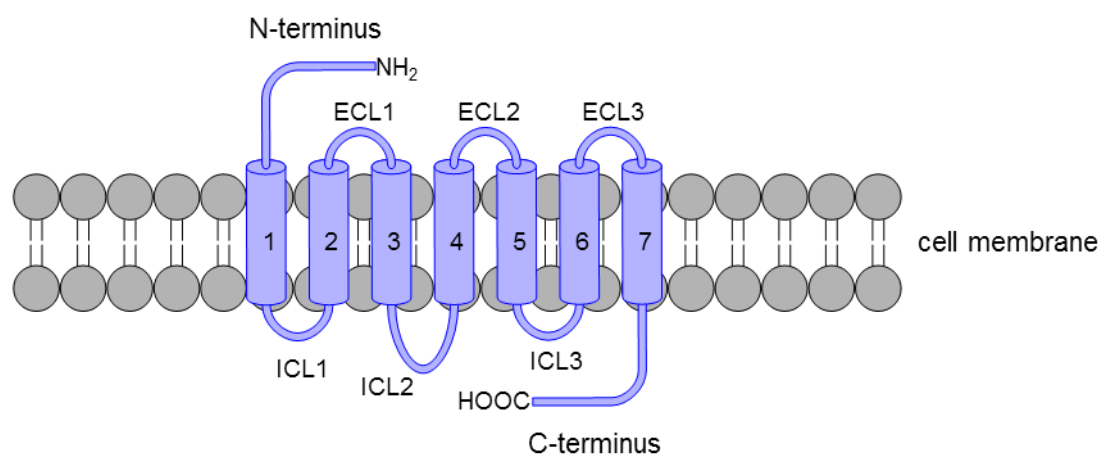


Figure. 1.1 Schematic representation of a prototypical class A GPCR (ICL = intracellular loop; ECL = extracellular loop; 1-7 = transmembrane domains)

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 %.¹⁶ 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.¹⁷

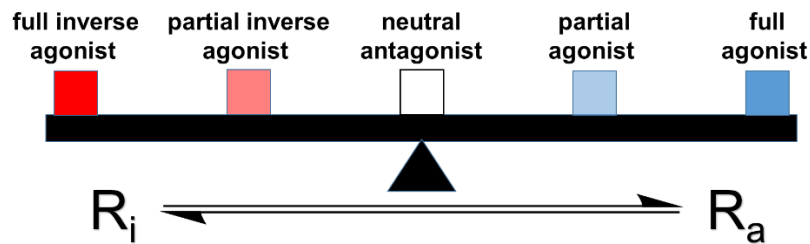


Figure. 1.2 Two state model of a GPCR. The receptor toggles between the inactive state R_i and the active state R_a . (modified according to Seifert and Wenzel-Seifert¹⁷)

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 G_α subunit with bound GDP and a $G_{\beta\gamma}$ complex) resulting in the activation of the G-protein by evoking the exchange of GDP by GTP.^{18, 19} Subsequently, the G_α subunit is detached from the $G_{\beta\gamma}$ 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 G_α subunit and re-associates with $G_{\beta\gamma}$ marking the completion of the cycle.¹¹ Regulators of G-protein signalling (RGS proteins; GTPase activating proteins, GAPs) can enhance the activity of the GTPase.²⁰⁻²²

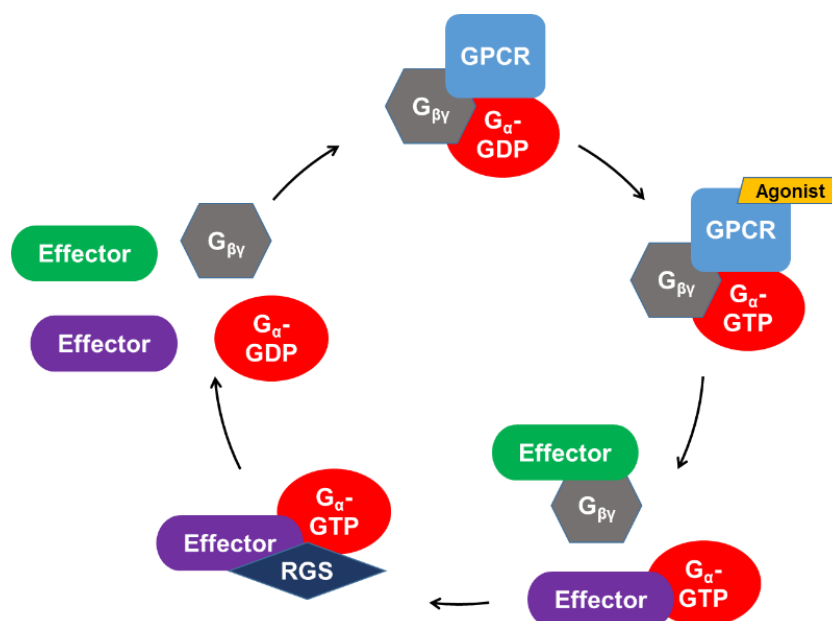


Figure. 1.3 Generalized diagram of the G-protein cycle. (modified according to Bridges et al.²³)

1.1.4.2. G-Proteins and their pathways

Till now, 16 different G_{α} -, 5 G_{β} - and 12 G_{γ} -subunits are known: The G_{α} subunits are grouped into four main families: G_{α_s} , $G_{\alpha_{i/o}}$, $G_{\alpha_{q/11}}$ and $G_{\alpha_{12/13}}$ based on the differences in structure and signaling pathway.^{24, 25} The G_{α_s} 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 $G_{\alpha_{i/o}}$ 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 $G_{\alpha_{q/11}}$ family regulates the activity of phospholipase C (PLC_{β}), leading to the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Both are second messengers, being responsible for various intracellular effects including the release of Ca^{2+} ions from intracellular stores²⁹ and the stimulation of protein kinase C (PKC) which further modulates the function of cellular proteins by phosphorylation.³⁰ The $G_{\alpha_{12/13}}$ family interferes with the cytoskeletal assembly by interaction with Rho-GEFs (Ras homology guanine nucleotide exchange factors).^{11, 24, 31} Besides the G_{α} -subunits, the $G_{\beta\gamma}$ -heterodimer is also involved in effector regulation, e.g., PLC_{β} and K^+ , Ca^{2+} 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.^{44, 45} Regarding GPCRs, an allosteric site is physically distinct from the orthosteric binding site, that is, the binding pocket of the endogenous ligand.^{23, 46-48} 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,⁴⁹ 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.⁵⁰ Allosteric modulation may offer a possibility to increase the receptor subtype selectivity of ligands due to lower conservation of allosteric compared to orthosteric sites.^{44, 51, 52}

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.⁵³⁻⁵⁵ 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**).⁵⁶⁻⁵⁸ With a spacer of appropriate length attached in the right position, bivalent ligands might achieve higher affinity and selectivity compared to their monomeric counterparts.^{42, 43, 53, 54, 59} 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.^{60, 61}

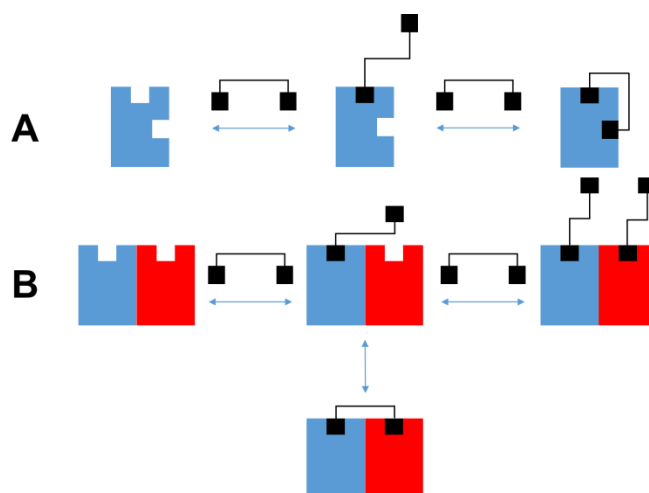


Figure 1.4 Bivalent ligand binding to (A) a GPCR with an accessory binding site or to (B) a GPCR dimer (modified according to Portoghese et al.⁵³ and Birnkammer et al.⁶¹).

1.2. Histamine and its receptors

1.2.1. Histamine

Histamine, (2-(1*H*-imidazol-4-yl)ethanamine), discovered more than a century ago,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.⁶³

1.2.2. Histamine receptors

1.2.2.1. The histamine H₁R

The histamine H₁ receptor (H₁R) is expressed mainly in mammalian brain, smooth muscle, endothelium cells and lymphocytes and is involved in pathophysiological processes such as allergic and inflammatory reactions.⁶⁴ H₁R-mediated biological effects include, for instance, vasodilatation, bronchoconstriction, increased vascular permeability, pain and itching upon insect stings.⁶⁵ The H₁R preferentially couples to the Gα_q protein, resulting in an increase in intracellular Ca²⁺.^{64, 66} Betahistine (e.g. Aequamen®) is the only marketed H₁R agonist (**Fig. 1.5**), approved for the treatment of Menière's disease. H₁R 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.^{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.^{63, 69, 70} Regardless of that, pyrilamine is still the most commonly used reference H₁R antagonist and radioligand ([³H]pyrilamine) for pharmacological studies.⁷¹ X-ray crystallography of the H₁R in complex with doxepine gave insights in ligand binding on the molecular level.⁷² Recently, Na⁺ was identified as a negative allosteric regulator bound to Asp^{2.50} of H₁R.⁷³

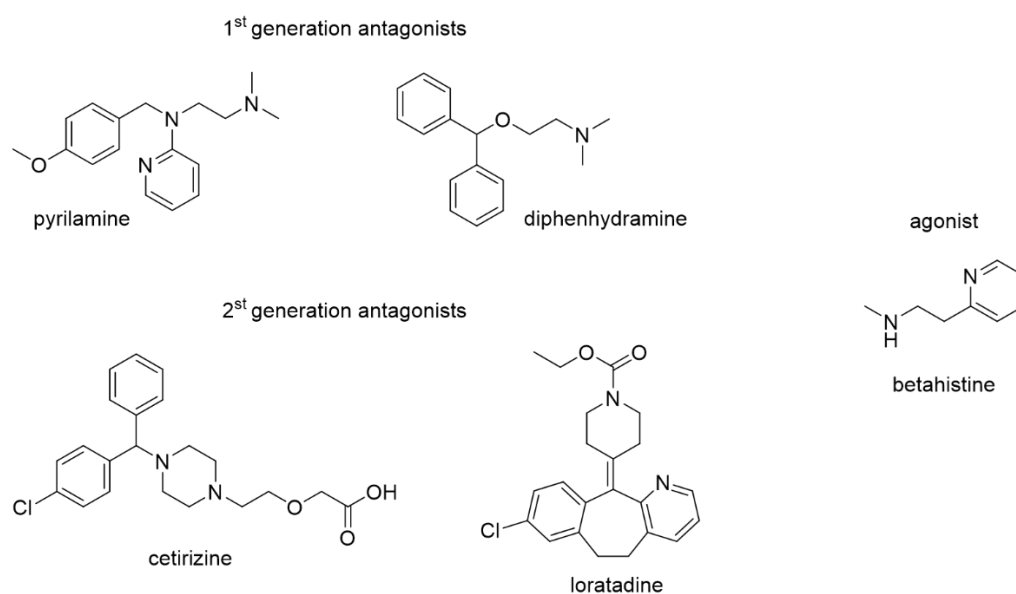


Figure 1.5 Structures of selected H₁R agonist and antagonists.

1.2.2.2. The histamine H₂R

The histamine H₂ receptor (H₂R) is expressed in a variety of tissues including brain, gastric parietal cells, heart, airways and uterus.⁶⁹ The H₂R couples to the Gα_s protein, which leads to an increase in cAMP followed by activation of PKA.⁷⁴ The H₂R was pharmacologically characterized in 1972 using the first H₂R antagonist burimamide (**Fig. 1.6**), which was able to inhibit the histamine-stimulated gastric acid secretion and the positive chronotropic effect on the heart.⁷⁵ Cimetidine (Tagamet®) was the first clinically available H₂R antagonist, followed by non-imidazoles such as ranitidine and famotidine (**Fig. 1.6**), with reduced pharmacokinetic interactions with CYP450 enzymes.⁶³ The H₂R 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 H₂R agonist demonstrated to be clinically effective in the treatment of severe catecholamine-refractory congestive heart failure.^{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

79

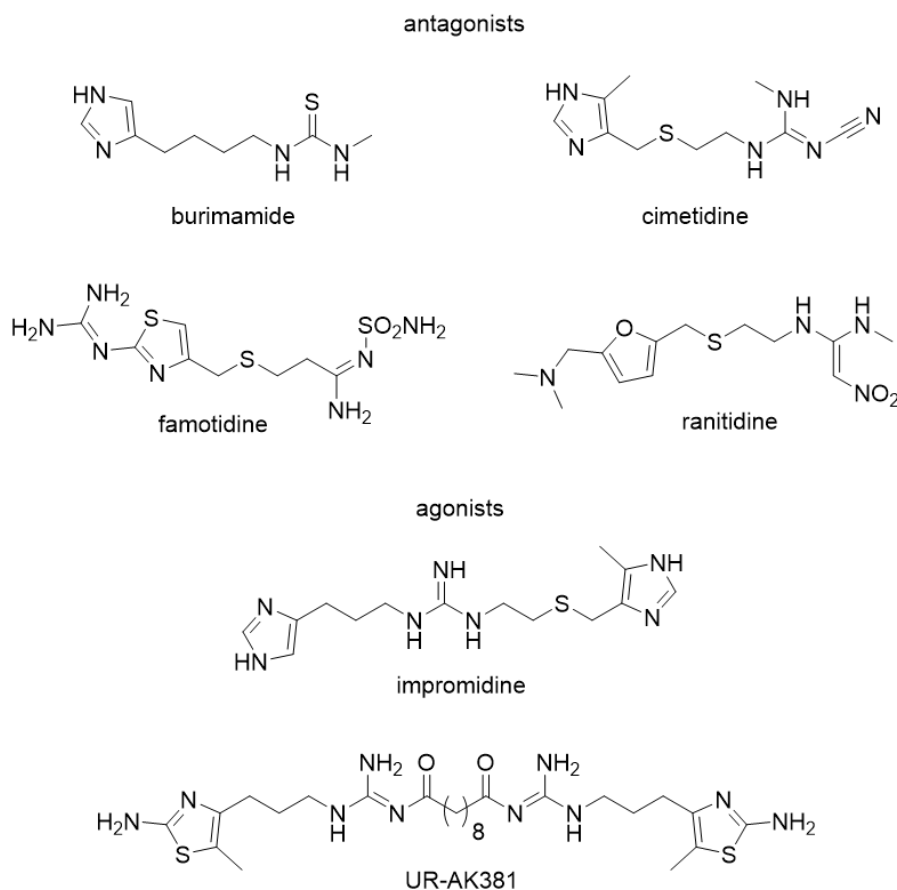


Figure 1.6 Structures of selected H₂R agonists and antagonists.

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 G $\alpha_{i/o}$ 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, clobenpropit, 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.

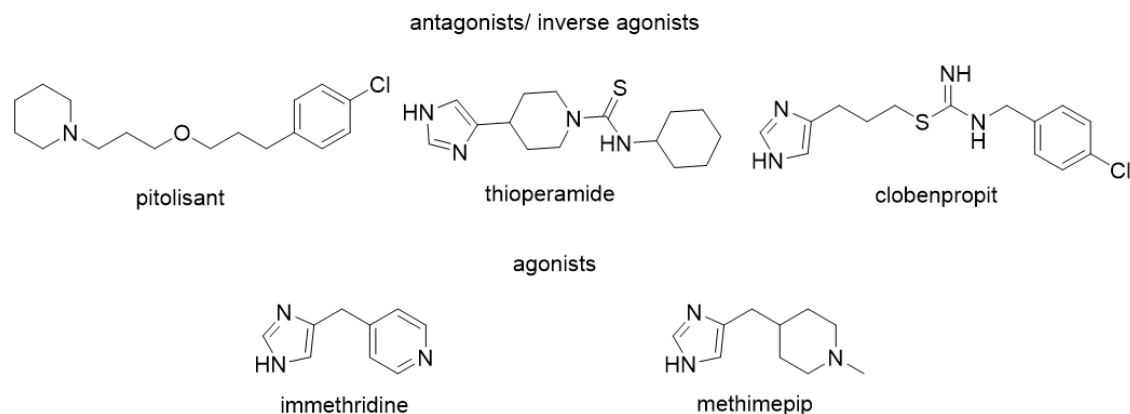


Figure 1.7 Structures of selected H₃R agonists and antagonists.

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 G $\alpha_{i/o}$ protein, leading to an inhibition of the AC and an activation of the phospholipase C- β (PLC β) via G $\beta\gamma$ 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.¹¹⁴ 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.^{115, 116} Compared with antagonists, H₄R agonists (**Fig. 1.8**) are important as pharmacological tools rather than as drugs.

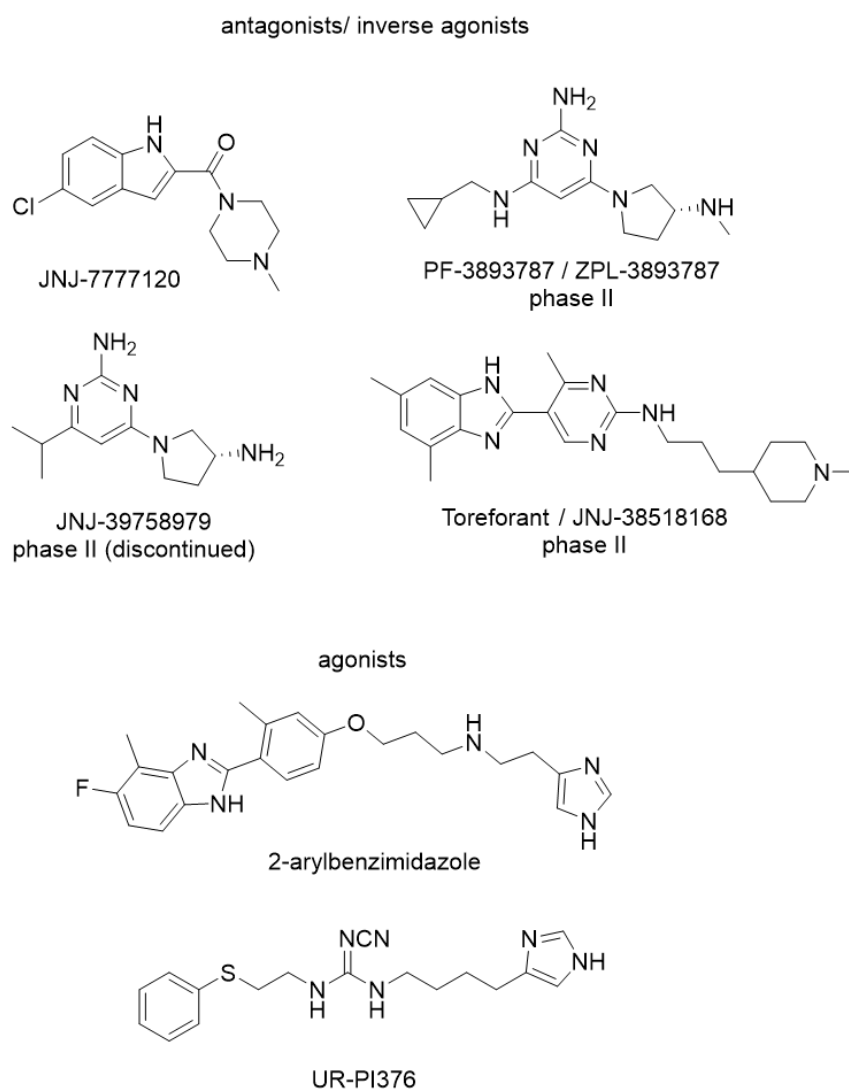


Figure 1.8 Structures of selected H₄R agonists and antagonists.

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,^{56, 117} e.g. lower the risk of drug-drug interactions compared to drug cocktails.¹¹⁸ In the histamine receptor field, a

synergistic effect of H₁R and H₄R antagonism was observed in various models of inflammation.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.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.^{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.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 effects¹²⁹.

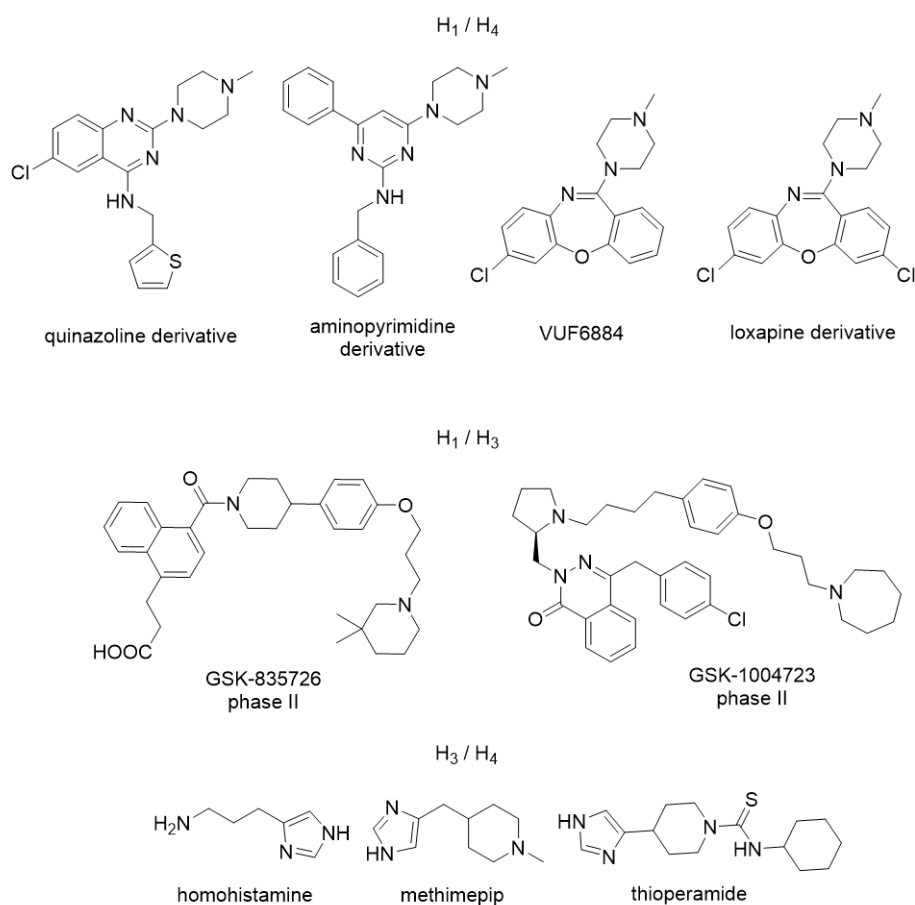


Figure 1.9 Structures of selected dual H_xR ligand structures.

1.3. Reference

1. Alexander, S. P.; Davenport, A. P.; Kelly, E.; Marrion, N.; Peters, J. A.; Benson, H. E.; Faccenda, E.; Pawson, A. J.; Sharman, J. L.; Southan, C. The Concise Guide to PHARMACOLOGY 2015/16: G protein-coupled receptors. *Br. J. Pharmacol.* **2015**, *172*, 5744-5869.
2. Pierce, K. L.; Premont, R. T.; Lefkowitz, R. J. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 639-650.
3. Lefkowitz, R. J. Historical review: a brief history and personal retrospective of seven-transmembrane receptors. *Trends. Pharmacol. Sci.* **2004**, *25*, 413-422.
4. Mombaerts, P. Genes and ligands for odorant, vomeronasal and taste receptors. *Nat. Rev. Neurosci.* **2004**, *5*, 263-278.
5. Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug. Discov.* **2006**, *5*, 993-996.
6. Rask-Andersen, M.; Masuram, S.; Schiöth, H. B. The druggable genome: evaluation of drug targets in clinical trials suggests major shifts in molecular class and indication. *Annu. Rev. Pharmacol.* **2014**, *54*, 9-26.
7. Kolakowski Jr, L. GCRDb: a G-protein-coupled receptor database. *Receptor. Channel.* **1994**, *2*, 1.
8. Schiöth, H. B.; Fredriksson, R. The GRAFS classification system of G-protein coupled receptors in comparative perspective. *Gen. Comp. Endocrinol.* **2005**, *142*, 94-101.
9. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E. Crystal structure of rhodopsin: AG protein-coupled receptor. *Science* **2000**, *289*, 739-745.
10. Kobilka, B. K. G protein coupled receptor structure and activation. *Biochim. Biophys. Acta* **2007**, *1768*, 794-807.
11. Luttrell, L. M. Reviews in molecular biology and biotechnology: transmembrane signaling by G protein-coupled receptors. *Mol. Biotechnol.* **2008**, *39*, 239-264.
12. Ji, T. H.; Grossmann, M.; Ji, I. G protein-coupled receptors I. Diversity of receptor-ligand interactions. *J. Biol. Chem.* **1998**, *273*, 17299-17302.
13. Rasmussen, S. G.; Choi, H.-J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R.; Sanishvili, R.; Fischetti, R. F. Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature* **2007**, *450*, 383-387.
14. Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K. High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258-1265.
15. Rasmussen, S. G.; Choi, H.-J.; Fung, J. J.; Pardon, E.; Casarosa, P.; Chae, P. S.; DeVree, B. T.; Rosenbaum, D. M.; Thian, F. S.; Kobilka, T. S. Structure of a nanobody-stabilized active state of the β_2 adrenoceptor. *Nature* **2011**, *469*, 175-180.
16. Kenakin, T. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J.* **2001**, *15*, 598-611.

17. Seifert, R.; Wenzel-Seifert, K. Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2002**, 366, 381-416.
18. Gilman, A. G. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* **1987**, 56, 615-649.
19. Offermanns, S. G-proteins as transducers in transmembrane signalling. *Prog. Biophys. Mol. Bio.* **2003**, 83, 101-130.
20. Neitzel, K. L.; Hepler, J. R. In *Cellular mechanisms that determine selective RGS protein regulation of G protein-coupled receptor signaling*, Seminars in cell & developmental biology, **2006**; Elsevier: 2006; pp 383-389.
21. Willars, G. B. In *Mammalian RGS proteins: multifunctional regulators of cellular signalling*, Seminars in cell & developmental biology, **2006**; Elsevier: 2006; pp 363-376.
22. Wieland, T.; Lutz, S.; Chidiac, P. Regulators of G protein signalling: a spotlight on emerging functions in the cardiovascular system. *Curr. Opin. Pharmacol.* **2007**, 7, 201-207.
23. Bridges, T. M.; Lindsley, C. W. G-protein-coupled receptors: from classical modes of modulation to allosteric mechanisms. *ACS Chem. Biol.* **2008**, 3, 530-541.
24. Cabrera-Vera, T. M.; Vanhauwe, J.; Thomas, T. O.; Medkova, M.; Preininger, A.; Mazzone, M. R.; Hamm, H. E. Insights into G protein structure, function, and regulation. *Endocr. Rev.* **2003**, 24, 765-781.
25. Downes, G.; Gautam, N. The G protein subunit gene families. *Genomics* **1999**, 62, 544-552.
26. Hanoune, J.; Defer, N. Regulation and role of adenylyl cyclase isoforms. *Annu. Rev. Pharmacol.* **2001**, 41, 145-174.
27. Pavan, B.; Biondi, C.; Dalpiaz, A. Adenylyl cyclases as innovative therapeutic goals. *Drug discovery today* **2009**, 14, 982-991.
28. Marinissen, M. J.; Gutkind, J. S. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends. Pharmacol. Sci.* **2001**, 22, 368-376.
29. Mikoshiba, K. IP₃ receptor/Ca²⁺ channel: from discovery to new signaling concepts. *J. Neurochem.* **2007**, 102, 1426-1446.
30. Thomsen, W.; Frazer, J.; Unett, D. Functional assays for screening GPCR targets. *Curr. Opin. Biotechnol.* **2005**, 16, 655-665.
31. Kristiansen, K. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol. Ther.* **2004**, 103, 21-80.
32. Milligan, G. G protein-coupled receptor dimerization: function and ligand pharmacology. *Mol. Pharmacol.* **2004**, 66, 1-7.
33. Milligan, G. A day in the life of a G protein-coupled receptor: the contribution to function of G protein-coupled receptor dimerization. *Br. J. Pharmacol.* **2008**, 153, S216-S229.
34. Milligan, G. The role of dimerisation in the cellular trafficking of G-protein-coupled receptors. *Curr. Opin. Pharmacol.* **2010**, 10, 23-29.

35. Palczewski, K. Oligomeric forms of G protein-coupled receptors (GPCRs). *Trends Biochem. Sci.* **2010**, 35, 595-600.
36. Gurevich, V. V.; Gurevich, E. V. How and why do GPCRs dimerize? *Trends Pharmacol. Sci.* **2008**, 29, 234-240.
37. Gomes, I.; Gupta, A.; Filipovska, J.; Szeto, H. H.; Pintar, J. E.; Devi, L. A. A role for heterodimerization of μ and δ opiate receptors in enhancing morphine analgesia. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, 101, 5135-5139.
38. Rashid, A. J.; So, C. H.; Kong, M. M.; Furtak, T.; El-Ghundi, M.; Cheng, R.; O'Dowd, B. F.; George, S. R. D₁-D₂ dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of G_{q/11} in the striatum. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, 104, 654-659.
39. Rozenfeld, R.; Devi, L. A. Receptor heterodimerization leads to a switch in signaling: β -arrestin2-mediated ERK activation by μ - δ opioid receptor heterodimers. *FASEB J.* **2007**, 21, 2455-2465.
40. White, J. F.; Grodnitzky, J.; Louis, J. M.; Trinh, L. B.; Shiloach, J.; Gutierrez, J.; Northup, J. K.; Grisshammer, R. Dimerization of the class AG protein-coupled neurotensin receptor NTS1 alters G protein interaction. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, 104, 12199-12204.
41. Uberti, M. A.; Hall, R. A.; Minneman, K. P. Subtype-specific dimerization of α 1-adrenoceptors: effects on receptor expression and pharmacological properties. *Mol. Pharmacol.* **2003**, 64, 1379-1390.
42. Messer Jr, W. S. Bivalent ligands for G protein-coupled receptors. *Curr. Pharm. Design* **2004**, 10, 2015-2020.
43. Halazy, S. G-protein coupled receptors bivalent ligands and drug design. *Expert. Opin. Ther. Pat.* **1999**, 9, 431-446.
44. Kenakin, T.; Miller, L. J. Seven Transmembrane Receptors as Shapeshifting Proteins: The Impact of Allosteric Modulation and Functional Selectivity on New Drug Discovery. *Pharmacol. Rev.* **2010**, 62, 265-304.
45. Fenton, A. W. Allostery: an illustrated definition for the 'second secret of life'. *Trends Biochem. Sci.* **2008**, 33, 420-425.
46. Conn, P. J.; Christopoulos, A.; Lindsley, C. W. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug. Discov.* **2009**, 8, 41-54.
47. Christopoulos, A. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. *Nat. Rev. Drug. Discov.* **2002**, 1, 198-210.
48. Kenakin, T. P. '7TM receptor allostery: putting numbers to shapeshifting proteins. *Trends Pharmacol. Sci.* **2009**, 30, 460-469.
49. May, L. T.; Leach, K.; Sexton, P. M.; Christopoulos, A. Allosteric modulation of G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* **2007**, 47, 1-51.
50. Gregory, K. J.; Malosh, C.; Turlington, M.; Morrison, R.; Vinson, P.; Daniels, J. S.; Jones, C.; Niswender, C. M.; Conn, P. J.; Lindsley, C. W. Identification of a high affinity MPEP-site silent allosteric modulator (SAM) for the metabotropic glutamate subtype 5 receptor (mGlu5). **2015**.
51. Christopoulos, A.; Kenakin, T. P. G protein-coupled receptor allostery and

- complexing. *Pharmacol. Rev.* **2002**, 54, 323-374.
52. Wootten, D.; Christopoulos, A.; Sexton, P. M. Emerging paradigms in GPCR allostery: implications for drug discovery. *Nat. Rev. Drug. Discov.* **2013**, 12, 630-644.
 53. Portoghese, P. S. From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. *J. Med. Chem.* **2001**, 44, 2259-2269.
 54. Portoghese, P. S. Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. *Trends. Pharmacol. Sci.* **1989**, 10, 230-235.
 55. Shonberg, J.; Scammells, P. J.; Capuano, B. Design strategies for bivalent ligands targeting GPCRs. *ChemMedChem* **2011**, 6, 963-974.
 56. Erez, M.; Takemori, A.; Portoghese, P. Narcotic antagonistic potency of bivalent ligands which contain. beta.-naltrexamine. Evidence for simultaneous occupation of proximal recognition sites. *J. Med. Chem.* **1982**, 25, 847-849.
 57. Portoghese, P.; Larson, D.; Sayre, L.; Yim, C.; Ronsisvalle, G.; Tam, S.; Takemori, A. Opioid agonist and antagonist bivalent ligands. The relationship between spacer length and selectivity at multiple opioid receptors. *J. Med. Chem.* **1986**, 29, 1855-1861.
 58. Portoghese, P.; Ronsisvalle, G.; Larson, D.; Yim, C.; Sayre, L.; Takemori, A. Opioid agonist and antagonist bivalent ligands as receptor probes. *Life Sci.* **1982**, 31, 1283-1286.
 59. Jones, R. M.; Hjorth, S. A.; Schwartz, T. W.; Portoghese, P. S. Mutational evidence for a common κ antagonist binding pocket in the wild-type κ and mutant μ [K303E] opioid receptors. *J. Med. Chem.* **1998**, 41, 4911-4914.
 60. Valant, C.; Robert Lane, J.; Sexton, P. M.; Christopoulos, A. The best of both worlds? Bitopic orthosteric/allosteric ligands of g protein-coupled receptors. *Annu. Rev. Pharmacol.* **2012**, 52, 153-178.
 61. Birnkammer, T.; Spickenreither, A.; Brunskole, I.; Lopuch, M.; Kagermeier, N.; Bernhardt, G. n.; Dove, S.; Seifert, R.; Elz, S.; Buschauer, A. The bivalent ligand approach leads to highly potent and selective acylguanidine-type histamine H₂ receptor agonists. *J. Med. Chem.* **2012**, 55, 1147-1160.
 62. Dale, H. H.; Laidlaw, P. P. The physiological action of β -iminazolylethylamine. *J. Physiol.* **1910**, 41, 318.
 63. Parsons, M. E.; Ganellin, C. R. Histamine and its receptors. *Br. J. Pharmacol.* **2006**, 147, S127-S135.
 64. Hill, S. J. Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.* **1990**, 42, 45-83.
 65. Bongers, G.; de Esch, I.; Leurs, R. Molecular pharmacology of the four histamine receptors. In *Histamine in Inflammation*, Springer: 2010; pp 11-19.
 66. Leurs, R.; Smit, M.; Timmerman, H. Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther.* **1995**, 66, 413-463.
 67. Simons, F. E. R. Advances in H₁-Antihistamines. *N. Engl. J. Med.* **2004**, 351, 2203-17.
 68. Simons, F. E. R.; Simons, K. J. Histamine and H₁-antihistamines: celebrating a century of progress. *J. Allergy Clin. Immunol.* **2011**, 128, 1139-1150. e4.
 69. Hill, S.; Ganellin, C.; Timmerman, H.; Schwartz, J.; Shankley, N.; Young, J.; Schunack, W.; Levi, R.; Haas, H. International Union of Pharmacology. XIII. Classification of

- histamine receptors. *Pharmacol. Rev.* **1997**, 49, 253-278.
70. Yanai, K.; Rogala, B.; Chugh, K.; Paraskakis, E.; Pampura, A.; Boev, R. Safety considerations in the management of allergic diseases: focus on antihistamines. *Curr. Med. Res. Opin.* **2012**, 28, 623-642.
 71. Hill, S.; Young, J.; Marrian, D. Specific binding of ³H-mepyramine to histamine H₁ receptors in intestinal smooth muscle. **1977**.
 72. Shimamura, T.; Shiroishi, M.; Weyand, S.; Tsujimoto, H.; Winter, G.; Katritch, V.; Abagyan, R.; Cherezov, V.; Liu, W.; Han, G. W. Structure of the human histamine H₁ receptor complex with doxepin. *Nature* **2011**, 475, 65-70.
 73. Hishinuma, S.; Kosaka, K.; Akatsu, C.; Uesawa, Y.; Fukui, H.; Shoji, M. Asp73-dependent and-independent regulation of the affinity of ligands for human histamine H₁ receptors by Na⁺. *Biochem. Pharmacol.* **2016**.
 74. JOHNSON, C. L.; WEINSTEIN, H.; GREEN, J. P. Studies on Histamine H₂ Receptors Coupled to Cardiac Adenylate Cyclase Blockade by H₂ and H₁ Receptor Antagonists. *Mol. Pharmacol.* **1979**, 16, 417-428.
 75. Black, J.; Duncan, W.; Durant, C. J.; Ganellin, C. R.; Parsons, E. Definition and antagonism of histamine H₂-receptors. *Nature* **1972**, 236, 385-390.
 76. Baumann, G.; Permanetter, B.; Wirtzfeld, A. Possible value of H₂-receptor agonists for treatment of catecholamine-insensitive congestive heart failure. *Pharmacol. Ther.* **1984**, 24, 165-177.
 77. Baumann, G.; Felix, S. B.; Heidecke, C. D.; Rieß, G.; Loher, U.; Ludwig, L.; Blömer, H. Apparent superiority of H₂-receptor stimulation and simultaneous β -blockade over conventional treatment with β -sympathomimetic drugs in post-acute myocardial infarction: Cardiac effects of impromidine-a new specific H₂-receptor agonist-in the surviving catecholamine-insensitive myocardium. *Agents Actions* **1984**, 15, 216-228.
 78. Aurelius, J.; Martner, A.; Brune, M.; Palmqvist, L.; Hansson, M.; Hellstrand, K.; Thoren, F. B. Remission maintenance in acute myeloid leukemia: impact of functional histamine H₂ receptors expressed by leukemic cells. *Haematologica* **2012**, 97, 1904-1908.
 79. Werner, K.; Neumann, D.; Seifert, R. Analysis of the histamine H₂-receptor in human monocytes. *Biochem. Pharmacol.* **2014**, 92, 369-379.
 80. Arrang, J.-M.; Garbarg, M.; Schwartz, J.-C. Auto-inhibition of brain histamine release mediated by a novel class (H₃) of histamine receptor. **1983**.
 81. Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erlander, M. G. Cloning and functional expression of the human histamine H₃ receptor. *Mol. Pharmacol.* **1999**, 55, 1101-1107.
 82. Schlicker, E.; Fink, K.; Detzner, M.; Göthert, M. Histamine inhibits dopamine release in the mouse striatum via presynaptic H₃ receptors. *J. Neural. Transm-Gen.* **1993**, 93, 1-10.
 83. Schlicker, E.; Betz, R.; Göthert, M. Histamine H₃ receptor-mediated inhibition of serotonin release in the rat brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, 337, 588-590.
 84. Clapham, J.; Kilpatrick, G. Histamine H₃ receptors modulate the release of [³H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible

- existence of H₃ receptor subtypes. *Br. J. Pharmacol.* **1992**, 107, 919-923.
85. Saxena, S. P.; Brandes, L. J.; Becker, A. B.; Simons, K. J.; Labella, F. S.; Gerrard, J. M. Histamine is an intracellular messenger mediating platelet aggregation. *Science* **1989**, 243, 1596-1599.
 86. Gemkow, M. J.; Davenport, A. J.; Harich, S.; Ellenbroek, B. A.; Cesura, A.; Hallett, D. The histamine H₃ receptor as a therapeutic drug target for CNS disorders. *Drug discovery today* **2009**, 14, 509-515.
 87. Bongers, G.; Bakker, R. A.; Leurs, R. Molecular aspects of the histamine H₃ receptor. *Biochem. Pharmacol.* **2007**, 73, 1195-1204.
 88. Schwartz, J. C. The histamine H₃ receptor: from discovery to clinical trials with pitolisant. *Br. J. Pharmacol.* **2011**, 163, 713-721.
 89. Inocente, C.; Arnulf, I.; Bastuji, H.; Thibault-Stoll, A.; Raoux, A.; Reimao, R.; Lin, J.-S.; Franco, P. Pitolisant, an inverse agonist of the histamine H₃ receptor: an alternative stimulant for narcolepsy-cataplexy in teenagers with refractory sleepiness. *Clin. Neuropharmacol.* **2012**, 35, 55-60.
 90. Raible, D. G.; Lenahan, T.; Fayvilevich, Y.; Kosinski, R.; Schulman, E. S. Pharmacologic characterization of a novel histamine receptor on human eosinophils. *Am. J. Respir. Crit. Care Med.* **1994**, 149, 1506-1511.
 91. Clark, R.; Gallin, J. I.; Kaplan, A. P. The selective eosinophil chemotactic activity of histamine. *J. Exp. Med.* **1975**, 142, 1462-1476.
 92. Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S.-i. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000**, 275, 36781-36786.
 93. Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L. Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.* **2001**, 59, 427-433.
 94. Nakamura, T.; Itadani, H.; Hidaka, Y.; Ohta, M.; Tanaka, K. Molecular cloning and characterization of a new human histamine receptor, hH₄R. *Biochem. Biophys. Res. Co.* **2000**, 279, 615-620.
 95. Zhu, Y.; Michalovich, D.; Wu, H.-L.; Tan, K.; Dytko, G. M.; Mannan, I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X. Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.* **2001**, 59, 434-441.
 96. Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E.; Greenfeder, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W. Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.* **2001**, 296, 1058-1066.
 97. Liu, C.; Ma, X.-J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N. Cloning and pharmacological characterization of a fourth histamine receptor (H₄) expressed in bone marrow. *Mol. Pharmacol.* **2001**, 59, 420-426.
 98. Cogé, F.; Guénin, S.-P.; Rique, H.; Boutin, J. A.; Galizzi, J.-P. Structure and expression of the human histamine H₄-receptor gene. *Biochem. Biophys. Res. Co.* **2001**, 284, 301-309.
 99. Seifert, R.; Strasser, A.; Schneider, E. H.; Neumann, D.; Dove, S.; Buschauer, A.

- Molecular and cellular analysis of human histamine receptor subtypes. *Trends Pharmacol. Sci.* **2013**, 34, 33-58.
100. Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. The role of histamine H₁ and H₄ receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug Discov.* **2008**, 7, 41-53.
101. Leurs, R.; Chazot, P. L.; Shenton, F. C.; Lim, H. D.; De Esch, I. J. Molecular and biochemical pharmacology of the histamine H₄ receptor. *Br. J. Pharmacol.* **2009**, 157, 14-23.
102. Smits, R. A.; Leurs, R.; de Esch, I. J. Major advances in the development of histamine H₄ receptor ligands. *Drug discovery today* **2009**, 14, 745-753.
103. Cowden, J. M.; Zhang, M.; Dunford, P. J.; Thurmond, R. L. The Histamine H₄ Receptor Mediates Inflammation and Pruritus in Th2-Dependent Dermal Inflammation. *J. Invest. Dermatol.* **2010**, 130, 1023-1033.
104. de Esch, I. J.; Thurmond, R. L.; Jongejan, A.; Leurs, R. The histamine H₄ receptor as a new therapeutic target for inflammation. *Trends Pharmacol. Sci.* **2005**, 26, 462-469.
105. Igel, P.; Dove, S.; Buschauer, A. Histamine H₄ receptor agonists. *Bioorg. Med. Chem. Lett.* **2010**, 20, 7191-7199.
106. Marson, C. M. Targeting the histamine H₄ receptor. *Chem. Rev.* **2011**, 111, 7121-7156.
107. Kollmeier, A.; Francke, K.; Chen, B.; Dunford, P.; Greenspan, A.; Xia, Y.; Xu, X.; Zhou, B.; Thurmond, R. The histamine H₄ receptor antagonist, JNJ 39758979, is effective in reducing histamine-induced pruritus in a randomized clinical study in healthy subjects. *J. Pharmacol. Exp. Ther.* **2014**, 350, 181.
108. Thurmond, R. L.; Chen, B.; Dunford, P. J.; Greenspan, A. J.; Karlsson, L.; La, D.; Ward, P.; Xu, X. L. Clinical and Preclinical Characterization of the Histamine H₄ Receptor Antagonist JNJ-39758979. *J. Pharmacol. Exp. Ther.* **2014**, 349, 176-184.
109. Cowden, J. M.; Yu, F.; Banie, H.; Farahani, M.; Ling, P.; Nguyen, S.; Riley, J. P.; Zhang, M.; Zhu, J.; Dunford, P. J. The histamine H₄ receptor mediates inflammation and Th17 responses in preclinical models of arthritis. *Ann. Rheum. Dis.* **2014**, 73, 600-608.
110. Jablonowski, J. A.; Grice, C. A.; Chai, W.; Dvorak, C. A.; Venable, J. D.; Kwok, A. K.; Ly, K. S.; Wei, J.; Baker, S. M.; Desai, P. J. The first potent and selective non-imidazole human histamine H₄ receptor antagonists. *J. Med. Chem.* **2003**, 46, 3957-3960.
111. Brunskole, I.; Strasser, A.; Seifert, R.; Buschauer, A. Role of the second and third extracellular loops of the histamine H₄ receptor in receptor activation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2011**, 384, 301-317.
112. Schnell, D.; Brunskole, I.; Ladova, K.; Schneider, E. H.; Igel, P.; Dove, S.; Buschauer, A.; Seifert, R. Expression and functional properties of canine, rat, and murine histamine H₄ receptors in Sf9 insect cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2011**, 383, 457-470.
113. Wifling, D.; Löffel, K.; Nordemann, U.; Strasser, A.; Bernhardt, G.; Dove, S.; Seifert, R.; Buschauer, A. Molecular determinants for the high constitutive activity of the human histamine H₄ receptor: functional studies on orthologues and mutants. *Br. J. Pharmacol.* **2015**, 172, 785-798.
114. Rosethorne, E. M.; Charlton, S. J. Agonist-biased signaling at the histamine H₄

- receptor: JNJ7777120 recruits β -arrestin without activating G proteins. *Mol. Pharmacol.* **2011**, 79, 749-757.
115. Liu, W. L. Histamine H₄ receptor antagonists for the treatment of inflammatory disorders. *Drug discovery today* **2014**, 19, 1222-1225.
 116. Ohsawa, Y.; Hirasawa, N. The role of histamine H₁ and H₄ receptors in atopic dermatitis: from basic research to clinical study. *Allergol. Int.* **2014**, 63, 533-542.
 117. Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug. Discov.* **2004**, 3, 353-359.
 118. Edwards, I. R.; Aronson, J. K. Adverse drug reactions: definitions, diagnosis, and management. *The Lancet* **2000**, 356, 1255-1259.
 119. Ohsawa, Y.; Hirasawa, N. The antagonism of histamine H₁ and H₄ receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. *Allergy* **2012**, 67, 1014-1022.
 120. Roßbach, K.; Wendorff, S.; Sander, K.; Stark, H.; Gutzmer, R.; Werfel, T.; Kietzmann, M.; Bäumer, W. Histamine H₄ receptor antagonism reduces hapten-induced scratching behaviour but not inflammation. *Exp. Dermatol.* **2009**, 18, 57-63.
 121. Wang, M.; Han, J.; Domenico, J.; Shin, Y. S.; Jia, Y.; Gelfand, E. W. Combined Blockade of the Histamine H₁ and H₄ Receptor Suppresses Peanut-Induced Intestinal Anaphylaxis by Regulating Dendritic Cell Function. *Allergy* **2016**.
 122. Smits, R. A.; Lim, H. D.; Stegink, B.; Bakker, R. A.; de Esch, I. J.; Leurs, R. Characterization of the histamine H₄ receptor binding site. Part 1. Synthesis and pharmacological evaluation of dibenzodiazepine derivatives. *J. Med. Chem.* **2006**, 49, 4512-4516.
 123. Hammer, S. G.; Gobleder, S.; Naporra, F.; Wittmann, H.-J.; Elz, S.; Heinrich, M. R.; Strasser, A. 2, 4-Diaminopyrimidines as dual ligands at the histamine H₁ and H₄ receptor—H₁/H₄-receptor selectivity. *Bioorg. Med. Chem. Lett.* **2016**, 26, 292-300.
 124. Naporra, F.; Gobleder, S.; Wittmann, H.-J.; Spindler, J.; Bodensteiner, M.; Bernhardt, G.; Hübner, H.; Gmeiner, P.; Elz, S.; Strasser, A. Dibenzo [b, f][1, 4] oxazepines and dibenzo [b, e] oxepines: Influence of the chlorine substitution pattern on the pharmacology at the H₁R, H₄R, 5-HT_{2A}R and other selected GPCRs. *Pharmacol. Res.* **2016**, 113, 610-625.
 125. Smits, R. A.; de Esch, I. J.; Zuiderveld, O. P.; Broeker, J.; Sansuk, K.; Guaita, E.; Coruzzi, G.; Adami, M.; Haaksma, E.; Leurs, R. Discovery of quinazolines as histamine H₄ receptor inverse agonists using a scaffold hopping approach. *J. Med. Chem.* **2008**, 51, 7855-7865.
 126. Procopiou, P. A.; Browning, C.; Buckley, J. M.; Clark, K. L.; Fechner, L.; Gore, P. M.; Hancock, A. P.; Hodgson, S. T.; Holmes, D. S.; Kranz, M. The discovery of phthalazinone-based human H₁ and H₃ single-ligand antagonists suitable for intranasal administration for the treatment of allergic rhinitis. *J. Med. Chem.* **2011**, 54, 2183-2195.
 127. Daley-Yates, P.; Ambery, C.; Sweeney, L.; Watson, J.; Oliver, A.; McQuade, B. The efficacy and tolerability of two novel H₁/H₃ receptor antagonists in seasonal allergic rhinitis. *Int. Arch. Allergy Appl. Immunol.* **2011**, 158, 84-98.

-
128. Neumann, D.; Beermann, S.; Burhenne, H.; Glage, S.; Hartwig, C.; Seifert, R. The dual H_{3/4}R antagonist thioperamide does not fully mimic the effects of the 'standard' H₄R antagonist JNJ 7777120 in experimental murine asthma. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2013**, 386, 983-990.
 129. Medina, V. A.; Rivera, E. S. Histamine receptors and cancer pharmacology. *Br. J. Pharmacol.* **2010**, 161, 755-767.

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.¹ A single ligand addressing multiple desired receptor targets (multitarget ligands) might be of advantage to enhance affinity, potency,² efficacy³ and safety, e.g., due to a lower risk of drug-drug interactions, compared to drug cocktails.⁴ Besides their potential clinical value, such hybrid compounds may also be useful pharmacological tools for in vitro investigations.

Among the four histamine receptor subtypes (H_1R , H_2R , H_3R , and H_4R) both, the H_1R and H_4R , were suggested to be involved in type-I allergic reactions.⁵ A synergistic effect of co-administered H_1R and H_4R antagonists was observed, e. g., regarding inhibition of pruritus and skin inflammation from chronic dermatitis⁶, acute hapten-induced scratching⁷, and peanut-induced intestinal allergy.⁸ All these results suggest that dual H_1R/H_4R antagonists may have a potential clinical value.

The aim of this work is to synthesize and characterize potential dual H_1R/H_4R antagonists. Homo- and hetero-dimeric (or bivalent) compounds will be constructed from crucial structural features of various H_1R and H_4R 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 H_1R and the H_4R on the molecular level and, possibly, to search for additional binding sites on H_1R and H_4R , respectively.

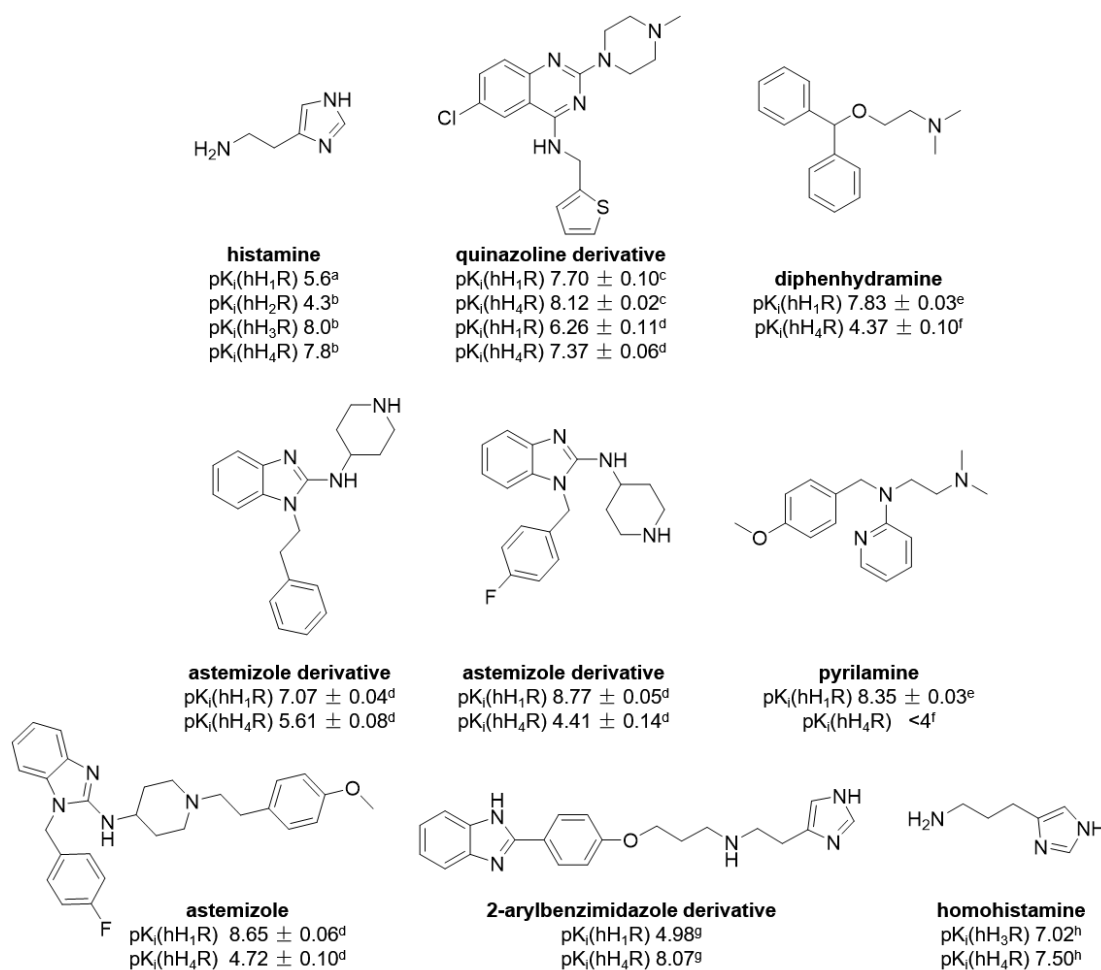


Fig. 2.1 Structures and affinities of selected H₁R and/or H₄R ligands as pharmacophores. Pharmacological data were taken from a) Strasser et al.⁹ b) Igel et al.¹⁰ c) Smits et al.¹¹ d) Wagner et al.¹² e) Wittmann et al.¹³ f) Deml et al.¹⁴ g) Baumeister¹⁵ h) Geyer et al.¹⁶

2.1. References

1. Eisen, S.; Miller, D.; Woodward, R.; Spitznagel, E.; Przybeck, T. The effect of prescribed daily dose frequency on patient medication compliance. *Arch. Intern. Med.* **1991**, 151, 1236-7.
2. Erez, M.; Takemori, A.; Portoghese, P. Narcotic antagonistic potency of bivalent ligands which contain beta-naltrexamine. Evidence for simultaneous occupation of proximal recognition sites. *J. Med. Chem.* **1982**, 25, 847-849.
3. Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug. Discov.* **2004**, 3, 353-359.
4. Edwards, I. R.; Aronson, J. K. Adverse drug reactions: definitions, diagnosis, and management. *The Lancet* **2000**, 356, 1255-1259.
5. Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. The role of histamine H₁ and H₄

- receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug Discov.* **2008**, 7, 41-53.
6. Ohsawa, Y.; Hirasawa, N. The antagonism of histamine H₁ and H₄ receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. *Allergy* **2012**, 67, 1014-1022.
 7. Roßbach, K.; Wendorff, S.; Sander, K.; Stark, H.; Gutzmer, R.; Werfel, T.; Kietzmann, M.; Bäumer, W. Histamine H₄ receptor antagonism reduces hapten-induced scratching behaviour but not inflammation. *Exp. Dermatol.* **2009**, 18, 57-63.
 8. Wang, M.; Han, J.; Domenico, J.; Shin, Y. S.; Jia, Y.; Gelfand, E. W. Combined Blockade of the Histamine H₁ and H₄ Receptor Suppresses Peanut-Induced Intestinal Anaphylaxis by Regulating Dendritic Cell Function. *Allergy* **2016**.
 9. Straßer, A.; Wittmann, H.-J.; Seifert, R. Ligand-specific contribution of the N terminus and E2-loop to pharmacological properties of the histamine H₁-receptor. *J. Pharmacol. Exp. Ther.* **2008**, 326, 783-791.
 10. Igel, P.; Dove, S.; Buschauer, A. Histamine H₄ receptor agonists. *Bioorg. Med. Chem. Lett.* **2010**, 20, 7191-7199.
 11. Smits, R. A.; de Esch, I. J.; Zuiderveld, O. P.; Broeker, J.; Sansuk, K.; Guaita, E.; Coruzzi, G.; Adami, M.; Haaksma, E.; Leurs, R. Discovery of quinazolines as histamine H₄ receptor inverse agonists using a scaffold hopping approach. *J. Med. Chem.* **2008**, 51, 7855-7865.
 12. Wagner, E.; Wittmann, H.-J.; Elz, S.; Strasser, A. Pharmacological profile of astemizole-derived compounds at the histamine H₁ and H₄ receptor—H₁/H₄ receptor selectivity. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2014**, 387, 235-250.
 13. Wittmann, H.-J.; Seifert, R.; Strasser, A. Contribution of binding enthalpy and entropy to affinity of antagonist and agonist binding at human and guinea pig histamine H₁-receptor. *Mol. Pharmacol.* **2009**, 76, 25-37.
 14. Deml, K.-F.; Beermann, S.; Neumann, D.; Strasser, A.; Seifert, R. Interactions of histamine H₁-receptor agonists and antagonists with the human histamine H₄-receptor. *Mol. Pharmacol.* **2009**, 76, 1019-1030.
 15. Baumeister, P. Molecular tools for G-protein coupled receptors: Synthesis, pharmacological characterization and [³H]-labeling of subtype-selective ligands for histamine H₄ and NPY Y₂ receptors. University of Regensburg, Regensburg, 2014.
 16. Geyer, R.; Kaske, M.; Baumeister, P.; Buschauer, A. Synthesis and functional characterization of imbutamine analogs as histamine H₃ and H₄ receptor ligands. *Arch. Pharm.* **2014**, 347, 77-88.

Chapter 3

**Homo-dimeric ligands as human histamine
H₁ receptor ligands or dual H₁/H₄ receptor
ligands**

3. Homo-dimeric ligands as human histamine H₁ receptor ligands or dual H₁/H₄ receptor ligands

3.1. Introduction

Over the past decades, our knowledge of GPCR has been changed revolutionarily with the discovery of receptor oligomerization¹⁻⁴ and allosteric modulation^{5, 6}. The existence of oligomers in class A GPCRs including opioid receptors⁷⁻⁹, adrenergic receptors¹⁰, somatostatin receptors^{11, 12}, dopamine receptors¹³⁻¹⁵, muscarinic receptors^{16, 17} and all histamine receptor subtypes¹⁸⁻²¹ has been demonstrated. It is supposed that potentially all GPCRs possess drugable allosteric sites.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.²³⁻²⁵ Homo- and hetero-dimeric ligands have emerged in the GPCR field in recent years, e.g., histamine^{26, 27}, dopamine²⁸⁻³⁰, and adenosine^{31, 32} and NPY^{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 Å²³, 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.^{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 sites³⁶.

The present study was aiming at high affinity dimeric/bivalent H₁R and/or H₄R ligands as pharmacological tools. Small series of homo-dimeric/homo-bivalent ligands were synthesized using building blocks derived from the well-known H₁R antagonists³⁷ diphenhydramine (**3.1**) and pyrilamine (**3.2**) and from a dual H₁R/H₄R antagonist, the

quinazoline derivative (**3.3**).³⁸ 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.

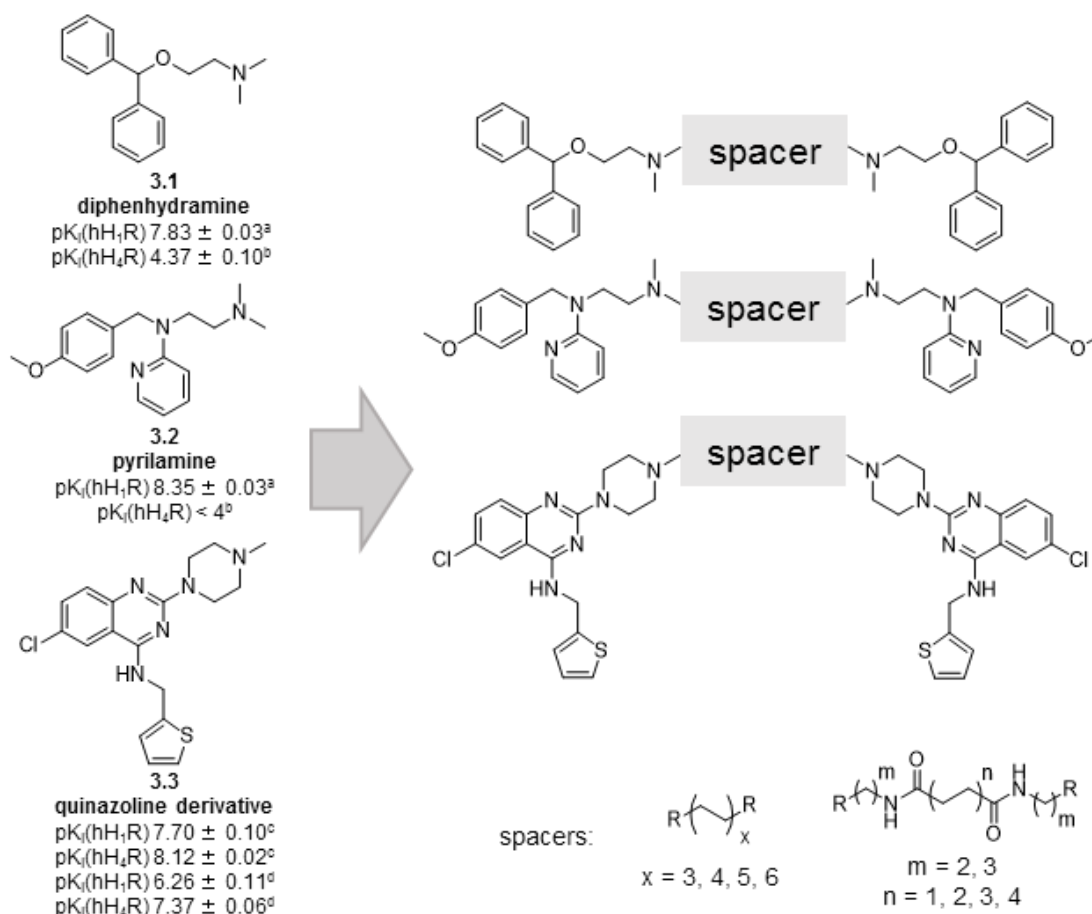
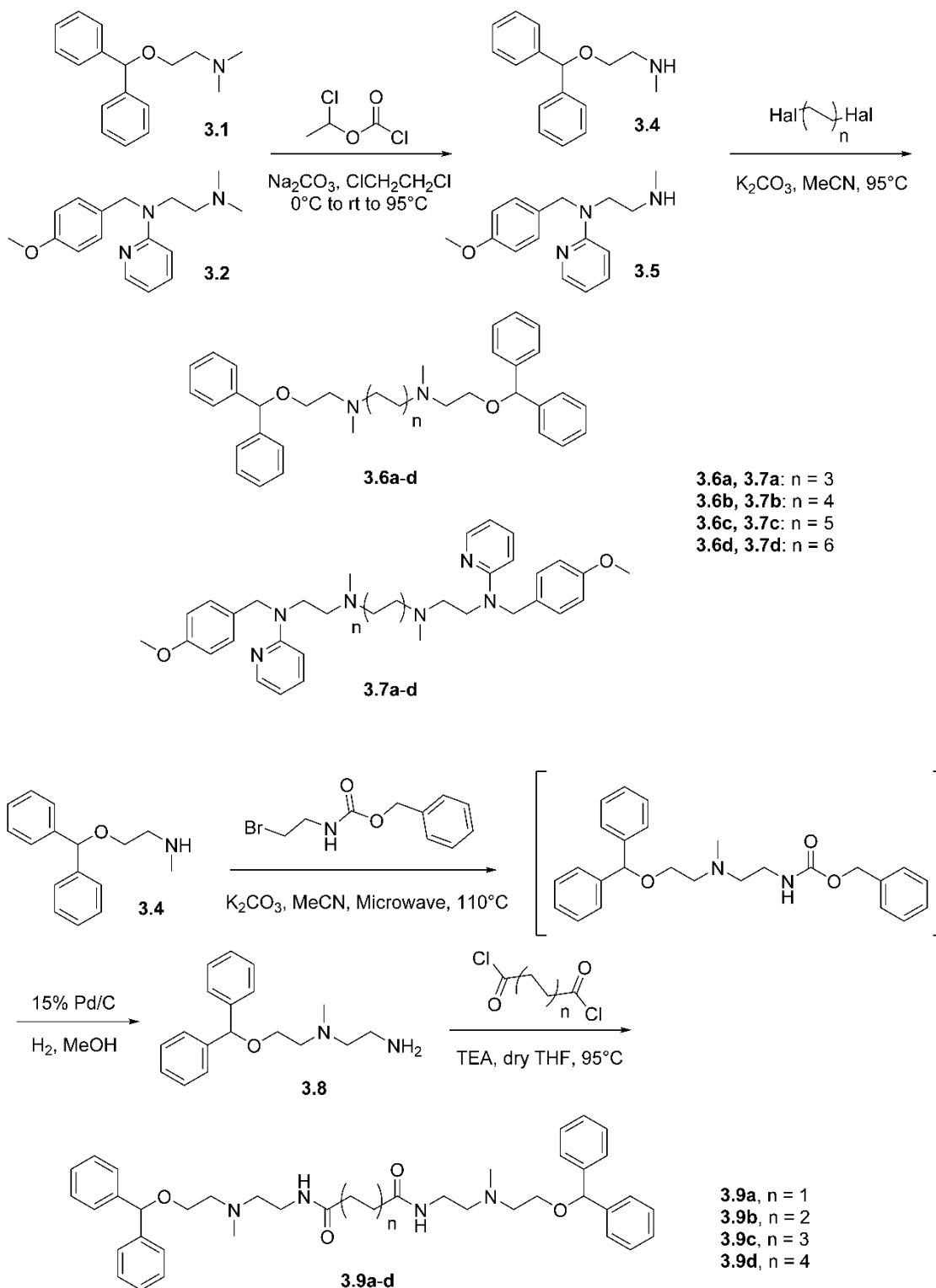


Figure 1 Selected H_1R and $\text{H}_1\text{R}/\text{H}_4\text{R}$ dual ligands, and general structures of synthesized homo-dimeric ligands. Pharmacological data were taken from a) Wittmann et al.³⁹ b) Deml et al.⁴⁰ c) Smits et al.³⁸ d) Wagner et al.⁴¹

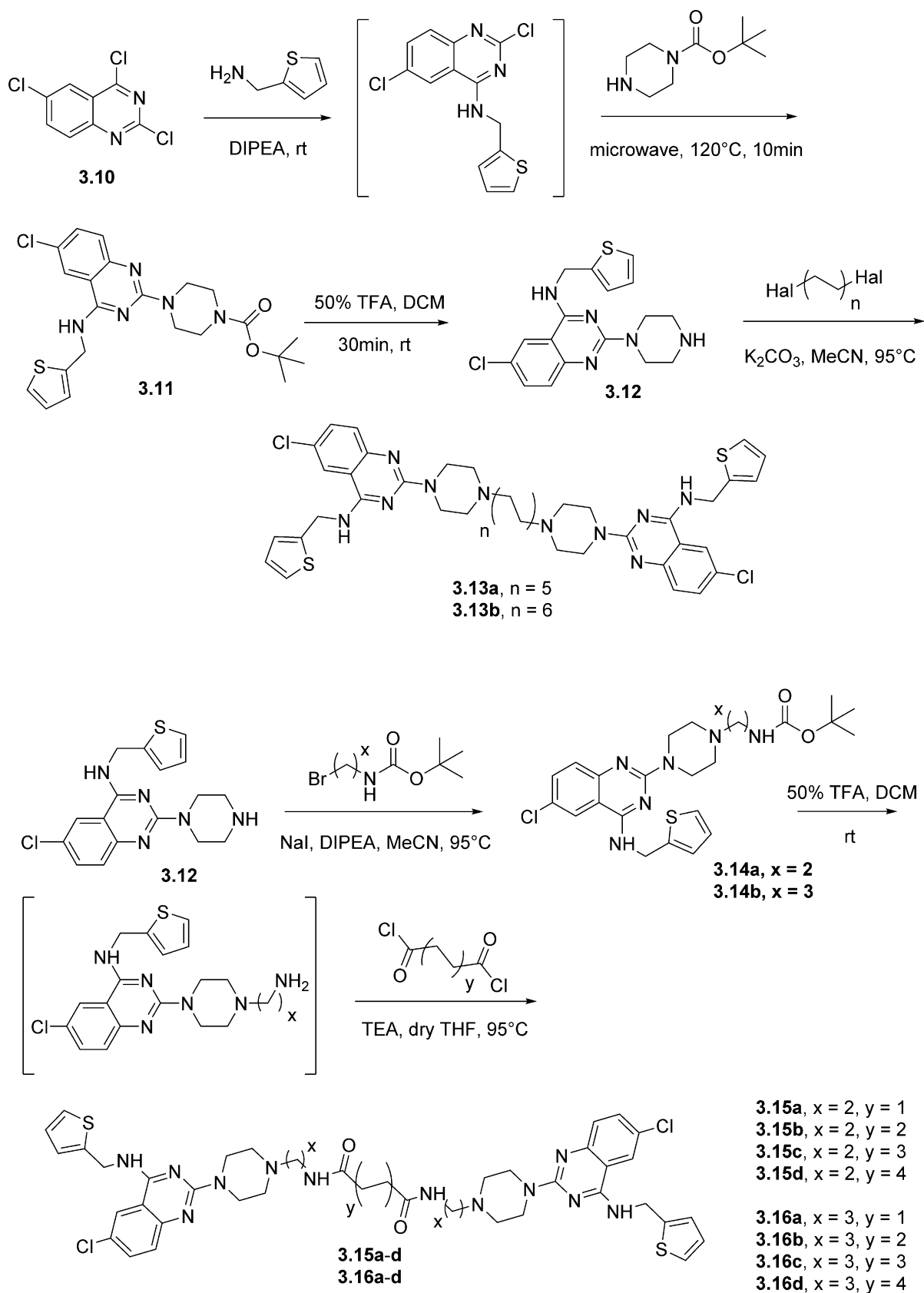
3.2. Results and discussion

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**.



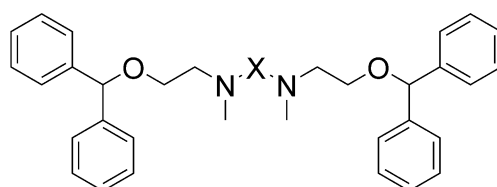
Scheme 2 Synthesis of compounds **3.13a**, **3.13b**, **3.15a-d** and **3.16a-d**.

3.2.2. Results and discussion

3.2.2.1. Competition binding studies

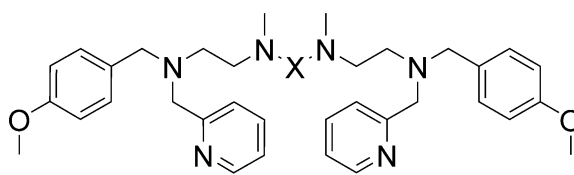
Twenty-two novel homo-dimeric ligands were synthesized and routinely analyzed by radioligand competition binding assays. All assays were performed using membrane preparations of Sf9 insect cells expressing the hH₁R + RGS4 (regulator of G-protein signaling 4)⁴² in presence of 5 nM [³H]pyrilamine, or the hH₄R + G_{αi2} + G_{β1γ2}⁴³ in presence of 10 nM [³H]histamine. The resulting affinity data are summarized in **Tables 1-3**.

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



cpd.	spacer length	spacer type (X)	(pK _i) hH ₁ R
diphenhydramine (3.1)			7.82 ± 0.03
3.6a	6	(CH ₂) ₆	6.94 ± 0.15
3.6b	8	(CH ₂) ₈	7.04 ± 0.10
3.6c	10	(CH ₂) ₁₀	6.95 ± 0.13
3.6d	12	(CH ₂) ₁₂	6.24 ± 0.19
3.9a	10	(H ₂ C) ₂ HNC(=O)(CH ₂) ₂ C(=O)NH(CH ₂) ₂	7.82 ± 0.08
3.9b	12	(H ₂ C) ₂ HNC(=O)(CH ₂) ₄ C(=O)NH(CH ₂) ₂	7.84 ± 0.12
3.9c	14	(H ₂ C) ₂ HNC(=O)(CH ₂) ₆ C(=O)NH(CH ₂) ₂	7.61 ± 0.06
3.9d	16	(H ₂ C) ₂ HNC(=O)(CH ₂) ₈ C(=O)NH(CH ₂) ₂	7.58 ± 0.08

Values represent the mean ± SEM of at least three independent experiments each performed in triplicate.

Table 2 hH₁R affinities of compound **3.7a-d**

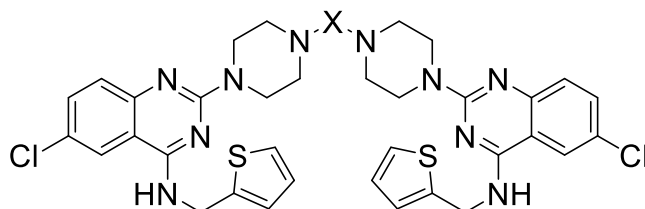
cpd.	spacer length	spacer type (X)	(pK _i) hH ₁ R
pyrilamine (3.2)			8.35 ± 0.03
3.7a	6	(CH ₂) ₆	7.94 ± 0.14
3.7b	8	(CH ₂) ₈	7.69 ± 0.08
3.7c	10	(CH ₂) ₁₀	7.91 ± 0.23
3.7d	12	(CH ₂) ₁₂	7.14 ± 0.10

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 al³⁰ 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),²³ 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**



cpd.	spacer length	spacer type (X)	(pK _i) hH ₁ R	(pK _i) hH ₄ R
3.3			6.26 ± 0.11 ^a	7.37 ± 0.06 ^a
3.13a	10	(CH ₂) ₆	5.14 ± 0.05	5.19 ± 0.19
3.13b	12	(CH ₂) ₈	<5	<5
3.15a	10	(H ₂ C) ₂ HNC(=O)(CH ₂) ₂ C(=O)NH(CH ₂) ₂	6.30 ± 0.23	<5
3.15b	12	(H ₂ C) ₂ HNC(=O)(CH ₂) ₄ C(=O)NH(CH ₂) ₂	5.77 ± 0.10	<5
3.15c	14	(H ₂ C) ₂ HNC(=O)(CH ₂) ₆ C(=O)NH(CH ₂) ₂	6.13 ± 0.13	<5
3.15d	16	(H ₂ C) ₂ HNC(=O)(CH ₂) ₈ C(=O)NH(CH ₂) ₂	6.82 ± 0.09	<5
3.16a	12	(H ₂ C) ₃ HNC(=O)(CH ₂) ₂ C(=O)NH(CH ₂) ₃	6.06 ± 0.04	5.60 ± 0.23
3.16b	14	(H ₂ C) ₃ HNC(=O)(CH ₂) ₄ C(=O)NH(CH ₂) ₃	6.48 ± 0.07	<5
3.16c	16	(H ₂ C) ₃ HNC(=O)(CH ₂) ₆ C(=O)NH(CH ₂) ₃	6.55 ± 0.07	<5
3.16d	18	(H ₂ C) ₃ HNC(=O)(CH ₂) ₈ C(=O)NH(CH ₂) ₃	6.26 ± 0.04	<5

Values represent the mean ± SEM of at least three independent experiments each performed in triplicate. ^a(pK_i) hH₁R = 7.70, hH₄R = 8.12 (Smits et al.³⁸)

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.

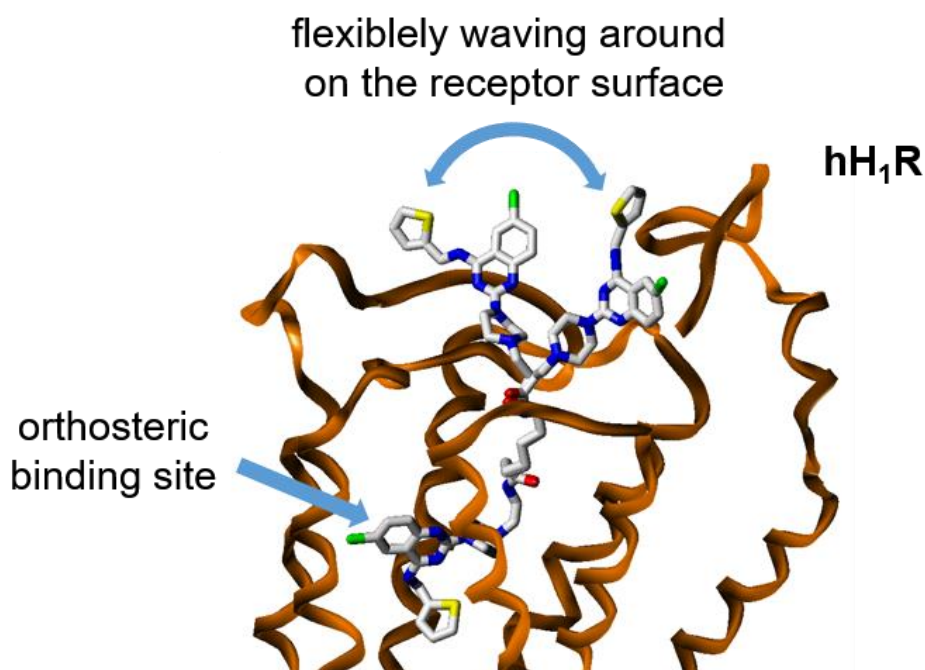


Figure 2 An overlay of two snapshots of **3.15d** bound to hH₁R. (MD simulation, performed by PD Dr. Andrea Strasser)

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 (^1H NMR and ^{13}C NMR) spectra were recorded on an Avance-300 (^1H : 300 MHz, ^{13}C : 75 MHz), Avance-400 (^1H : 400 MHz, ^{13}C : 101 MHz), or Avance-600 (^1H : 600 MHz, ^{13}C : 151 MHz) NMR spectrometer from Bruker BioSpin (Karlsruhe, Germany). Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), bs (for broad singlet), 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 ^1H 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 (t_R), the capacity (retention) factors were calculated according to $k = (t_R - t_0)/t_0$, 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.⁴⁵

Benzyl (2-bromoethyl)carbamate was synthesized according to Aissaoui et al.⁴⁵

***tert*-Butyl(3-bromopropyl)carbamate.**⁴⁶

tert-butyl(3-bromopropyl)carbamate were synthesized according to Jörg et al.⁴⁶

***tert*-Butyl piperazine-1-carboxylate.**⁴⁷

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 (C_{quat}), 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.

***N*'-(4-Methoxybenzyl)-*N*'-methyl-*N*'-(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). $^{13}\text{C-NMR}$ (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_2CO_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%). $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$ (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 (-, 2 CH_2), 57.89 (-, 2 CH_2), 56.63 (-, 2 CH_2), 42.65 (+, 2 CH_3), 27.28 (-, 2 CH_2), 26.77 (-, 2 CH_2). **HRMS** (EI-MS) calcd for $\text{C}_{38}\text{H}_{48}\text{N}_2\text{O}_2$ [MH^+] 565.3789, found 565.3790.

N^1, N^8 -Bis[2-(benzhydryloxy)ethyl]- N^1, N^8 -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%). $^1\text{H-NMR}$ (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), 2.40 (t, $J = 6.1$ Hz, 4H), 2.29 (s, 6H), 1.55-1.39 (m, 4H), 1.28-1.24 (m, 8H). $^{13}\text{C-NMR}$ (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 (-, 2 CH_2), 58.15 (-, 2 CH_2), 56.77 (-, 2 CH_2), 42.84 (+, 2 CH_3), 29.57 (-, 2 CH_2), 27.46 (-, 2 CH_2), 27.08 (-, 2 CH_2). **HRMS** (EI-MS) calcd for $\text{C}_{40}\text{H}_{52}\text{N}_2\text{O}_2$ [MH^+] 593.4102, found 593.4101.

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-diiododecane (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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 142.30 (4C_{quat}), 128.36 (+, 8Ar-CH), 127.41 (+, 4Ar-CH), 126.98 (+, 8Ar-CH), 84.01 (+, 2CH), 67.26 (-, 2CH₂), 58.19 (-, 2CH₂), 56.81 (-, 2CH₂), 42.89 (+, 2CH₃), 29.61 (-, 4CH₂), 27.51 (-, 2CH₂), 27.17 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₂H₅₆N₂O₂ [MH⁺] 621.4415, found 621.4419.

***N*¹,*N*²-Bis[2-(benzhydryloxy)ethyl]-*N*¹,*N*²-dimethyldodecane-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%). **¹H-NMR** (300 MHz, CH₃OD) δ [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). **¹³C-NMR** (75 MHz, CH₃OD) δ [ppm]: 143.88 (4C_{quat}), 129.43 (+, 8Ar-CH), 128.54 (+, 4Ar-CH), 128.15 (+, 8Ar-CH), 85.36 (+, 2CH), 67.94 (-, 2CH₂), 59.01 (-, 2CH₂), 57.68 (-, 2CH₂), 43.30 (+, 2CH₃), 30.85 (-, 2CH₂), 30.82 (-, 2CH₂), 30.78 (-, 2CH₂), 28.75 (-, 2CH₂), 27.81 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₄H₆₀N₂O₂ [MH⁺] 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, K₂CO₃ (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% NH₃, 20/1). Yellow oil (58 mg, 0.09 mmol, 14.5%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 158.66 (2C_{quat}), 158.06 (2C_{quat}), 148.02 (+, 2Ar-CH), 137.28 (+, 2Ar-CH), 130.59 (2C_{quat}), 128.16 (+, 4Ar-CH), 113.97 (+, 4Ar-CH), 111.88 (+, 2Ar-CH), 105.90 (+, 2Ar-CH), 57.75 (-, 2CH₂), 55.28 (+, 2CH₃), 54.34 (-, 2CH₂), 51.49 (-, 2CH₂), 45.92 (-, 2CH₂), 42.27 (+, 2CH₃), 27.23 (-, 4CH₂). **HRMS** (EI-MS) calcd for C₃₈H₅₂N₆O₂ [MH⁺] 625.4225, found 625.4227.

***N*¹,*N*¹-(Octane-1,8-diyl)bis[*N*²-(4-methoxybenzyl)-*N*¹-methyl-*N*²-(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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 158.62 (2C_{quat}), 158.12 (2C_{quat}), 148.04 (+, 2Ar-CH), 137.20 (+, 2Ar-CH), 130.77 (2 C_{quat}), 128.19 (+, 4Ar-CH), 113.92 (+, 4Ar-CH), 111.71 (+, 2Ar-CH), 105.78 (+, 2Ar-CH), 58.05 (-, 2CH₂), 55.26 (+, 2CH₃), 54.48 (-, 2CH₂), 51.35 (-, 2CH₂), 46.20 (-, 2CH₂), 42.52 (+, 2CH₃), 29.55 (-, 2CH₂), 27.41 (-, 2CH₂), 27.09 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₀H₅₆N₆O₂ [MH⁺] 653.4538, found 653.4535.

***N*¹,*N*¹-(Decane-1,10-diyl)bis[*N*²-(4-methoxybenzyl)-*N*¹-methyl-*N*²-(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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 158.60 (2C_{quat}), 158.17 (2C_{quat}), 148.07 (+, 2Ar-CH), 137.17 (+, 2Ar-CH), 130.89 (2C_{quat}), 128.22 (+,

4Ar-CH), 113.91 (+, 4Ar-CH), 111.64 (+, 2Ar-CH), 105.71 (+, 2Ar-CH), 58.21 (-, 2CH₂), 55.27 (+, 2CH₃), 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 C₄₂H₆₀N₆O₂ [MH⁺] 681.4851, found 681.4850.

***N',N'*- (Dodecane- 1, 12- diyl)bis[*N*²- (4- methoxybenzyl)- *N*¹- methyl- *N*²- (pyridin- 2- yl)ethane- 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%). **¹H-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). **¹³C-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 C₄₄H₆₄N₆O₂ [MH⁺] 709.5164, found 709.5167.

***N*¹-[2-(Benzhydryloxy)ethyl]-*N*¹-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%). **¹H-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*⁴-Bis(2-[[2-(benzhydryloxy)ethyl](methyl)amino]ethyl)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 Na₂SO₄. 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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 172.05 (2C_{quat}), 142.19 (4C_{quat}), 128.45 (+, 8Ar-CH), 127.52 (+, 4Ar-CH), 126.96 (+, 8Ar-CH), 83.99 (+, 2CH), 67.11 (-, 2CH₂), 56.59 (-, 2CH₂), 56.02 (-, 2CH₂), 42.48 (+, 2CH₃), 36.85 (-, 2CH₂), 31.43 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₀H₅₀N₄O₄ [MH⁺] 651.3905, found 651.3910.

***N*¹,*N*⁶-Bis(2-[[2-(benzhydryloxy)ethyl](methyl)amino]ethyl)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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 171.85 (2C_{quat}), 141.00 (4C_{quat}), 127.42 (+, 8Ar-CH), 126.53 (+, 4Ar-CH), 125.86 (+, 8Ar-CH), 82.95 (+, 2CH), 65.65 (-, 2CH₂), 56.42 (-, 2CH₂), 56.00 (-, 2CH₂), 42.42 (+, 2CH₃), 36.52 (-, 2CH₂), 35.97 (-, 2CH₂), 25.08 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₂H₅₄N₄O₄ [MH⁺] 679.4218, found 679.4221.

***N*¹,*N*⁸-Bis(2-[[2-(benzhydryloxy)ethyl](methyl)amino]ethyl)octanediamide (3.9c).**

Compound **3.9c** was prepared from compound **3.8** (284 mg, 1 mmol) and octanedioyl 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%). **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) 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 (-, 2CH₂), 56.45 (-, 2CH₂), 56.01 (-, 2CH₂), 42.42 (+, 2CH₃), 36.50 (-, 2CH₂), 36.39 (-, 2CH₂), 28.93 (-, 2CH₂), 25.53 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₄H₅₈N₄O₄ [MH⁺] 707.4531, found 707.4532.

***N',N'*⁰-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 octanedioyl 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%). **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) 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 (-, 2CH₂), 56.40 (-, 2CH₂), 55.96 (-, 2CH₂), 42.39 (+, 2CH₃), 36.53 (-, 2CH₂), 36.48 (-, 2CH₂), 29.23 (-, 4CH₂), 25.69 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₆H₆₂N₄O₄ [MH⁺] 735.4844, found 735.4846.

2,4,6-Trichloroquinazoline (3.10)

2,4,6-Trichloroquinazoline was prepared according to Smits et al.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-*d*₆) 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 (6C_{quat}), 141.18 (2C_{quat}), 133.15 (+, 2Ar-CH), 127.48 (+, 2Ar-CH), 126.69 (+, 2Ar-CH), 126.25 (+, 2Ar-CH), 125.87 (2C_{quat}), 125.39 (+, 2Ar-CH), 120.51 (+, 2Ar-CH), 110.98 (2C_{quat}), 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 [MH⁺] 857.3424, found 857.3417.

2,2'-[Dodecane-1,12-diylbis(piperazine-4,1-diyl)]bis[6-chloro-*N*-(thiophen-2-ylmethyl)quinazolin-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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [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}), 120.38 (+, 2Ar-CH), 110.98 (2C_{quat}), 62.99 (-, 2CH₂), 58.90 (-, 2CH₂), 53.27 (-, 2CH₂), 43.64 (-, 4CH₂), 43.50 (-, 2CH₂), 39.94 (-, 2CH₂), 32.80 (-, 2CH₂), 29.53 (-, 2CH₂), 27.53 (-, 2CH₂), 25.75 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₆H₅₈Cl₂N₁₀S₂ [MH⁺] 885.3737, found 885.3736.

***tert*-Butyl [2-(4-{6-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl}piperazin-1-yl)ethyl]carbamate (3.14a).**

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 Na₂SO₄, 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. **¹H-NMR** (300 MHz, CDCl₃) δ [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).

***tert*-Butyl [3-(4-{6-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl}piperazin-1-yl)propyl]carbamate (3.14b).**

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. **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [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 (-, CH₂), 57.01 (-, CH₂), 53.33 (-, 2CH₂), 43.90 (-, 2CH₂), 42.75 (-, CH₂), 39.83 (-, CH₂), 28.48 (+, 3CH₃). **HRMS** (EI-MS) calcd for C₂₅H₃₃ClN₆O₂S [MH⁺] 517.2147, found 517.2148.

***N*¹,*N*⁴-Bis[2-(4-{6-chloro-4-[(thiophen-2-ylmethyl)amino]quinazolin-2-yl})piperazin-1-yl]ethylsuccinamide (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 Na₂SO₄. 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. **¹H-NMR** (300 MHz, DMSO-d₆) δ [ppm]: 8.19 (d, *J* = 2.0 Hz, 2H), 7.52-7.44 (m, 2H), 7.37-7.31 (m, 2H), 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). **¹³C-NMR** (75 MHz, DMSO-d₆) δ [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 (-, 2CH₂), 52.64 (-, 2CH₂), 43.25 (-, 2CH₂), 38.53 (-, 4CH₂), 35.85 (-, 2CH₂), 30.80 (-, 4CH₂). **HRMS** (EI-MS) calcd for C₄₂H₄₈Cl₂N₁₂O₂S₂ [MH⁺] 887.2914, found 887.2908.

***N'*,*N*⁶-Bis[2-(4-{6-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. **¹H-NMR** (300 MHz, CH₃OD) δ [ppm]: 8.23 (d, *J* = 2.2 Hz, 2H), 7.85-7.80 (m, 2H), 7.64 (d, *J* = 8.9 Hz, 2H), 7.34-7.30 (m, 2H), 7.15-7.12 (m, 2H), 7.00-6.96 (m, 2H), 5.04 (s, 4H), 4.40-4.20 (m, 8H), 3.67-3.45 (m, 12H), 3.36-3.32 (m, 4H), 2.35-2.25 (m, 4H), 1.70-1.63 (m, 4H). **¹³C-NMR** (75 MHz, CH₃OD) δ [ppm]: 177.32 (2C_{quat}), 160.43 (2C_{quat}), 153.38 (2C_{quat}), 140.70 (4C_{quat}), 136.88 (+, 2Ar-CH), 132.22 (2C_{quat}), 128.11 (+, 2Ar-CH), 127.88 (+, 2Ar-CH), 126.71 (+, 2Ar-CH), 124.43 (+, 2Ar-CH), 120.82 (+, 2Ar-CH), 112.60 (2C_{quat}), 58.00 (-, 2CH₂), 52.48 (-, 4CH₂), 43.45 (-, 4CH₂), 41.35 (-, 2CH₂), 36.42 (-, 2CH₂), 35.36 (-, 2CH₂), 26.06 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₄H₅₂Cl₂N₁₂O₂S₂ [MH⁺] 915.3227, found 915.3226.

***N'*,*N*⁶-Bis[2-(4-{6-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. **¹H-NMR** (300 MHz, DMSO-d₆) δ [ppm]: 8.10 (d, *J* = 2.3 Hz, 2H), 7.51-7.46 (m, 2H), 7.37-7.33 (m, 2H), 7.26 (d, *J* = 8.9 Hz, 2H), 7.09-7.05 (m, 2H), 6.96-6.92 (m, 2H), 4.82 (d, *J* = 5.6 Hz, 4H), 3.83-3.76 (m, 8H), 3.23-3.14 (m, 4H), 2.46-2.31 (m, 12H), 2.04 (t, *J* = 7.3 Hz, 4H), 1.53-1.40 (m, 4H), 1.26-1.18 (m, 4H). **¹³C-NMR** (75 MHz, DMSO-d₆) δ [ppm]: 171.91 (2C_{quat}), 158.52 (2C_{quat}), 158.22 (2C_{quat}), 150.52 (2C_{quat}), 142.22 (2C_{quat}), 132.56 (+, 2Ar-CH), 126.89 (+, 2Ar-CH), 126.24 (+, 2Ar-CH), 125.84 (+, 2Ar-CH), 125.10 (+, 2Ar-CH), 123.97 (2C_{quat}), 121.91 (+, 2Ar-CH), 111.03 (2C_{quat}), 57.14 (-, 2CH₂), 52.71 (-, 4CH₂), 43.32 (-, 4CH₂), 38.67 (-, 2CH₂), 35.91 (-, 2CH₂), 35.28 (-, 2CH₂), 28.30 (-, 2CH₂), 25.12 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₆H₅₆Cl₂N₁₂O₂S₂ [MH⁺] 943.3540, found 943.3533.

***N*¹,*N*⁰-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 decanedioyl dichloride (57 mg, 0.24 mmol). White solid (140 mg, 0.14 mmol, 61.4 %), m.p. 219 °C. **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 173.47 (6C_{quat}), 158.52 (2C_{quat}), 140.69 (2C_{quat}), 133.42 (+, 2Ar-CH), 126.79 (+, 2Ar-CH), 126.55 (+, 2Ar-CH), 125.43 (+, 4Ar-CH), 121.22 (+, 2Ar-CH), 110.92 (4C_{quat}), 56.74 (-, 2CH₂), 52.79 (-, 4CH₂), 45.71 (-, 2CH₂), 43.92 (-, 2CH₂), 39.86 (-, 2CH₂), 36.70 (-, 2CH₂), 35.73 (-, 2CH₂), 29.19 (-, 2CH₂), 29.19 (-, 2CH₂), 25.71 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₈H₆₀Cl₂N₁₂O₂S₂ [MH⁺] 971.3853, found 971.3848.

***N*¹,*N*⁴-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. **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 172.02 (2C_{quat}), 158.59 (4C_{quat}), 150.77 (2C_{quat}), 141.23 (2C_{quat}), 133.15 (+, 2Ar-CH), 127.33 (+, 2Ar-CH), 126.69 (+, 2Ar-CH), 126.26 (+, 2Ar-CH), 125.79 (2C_{quat}), 125.44 (+, 2Ar-CH), 120.53 (+, 2Ar-CH), 111.01 (2C_{quat}), 57.29 (-, CH₂), 53.31 (-, 2CH₂), 43.99 (-, 2CH₂), 39.92 (-, CH₂), 39.28 (-, CH₂), 31.95 (-, CH₂), 25.32 (-, 2CH₂). **HRMS** (EI-MS) calcd for C₄₄H₅₂Cl₂N₁₂O₂S₂ [MH⁺] 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. **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 172.63 (2C_{quat}), 158.74 (2C_{quat}), 158.62 (2C_{quat}), 150.91 (2C_{quat}), 141.25 (2C_{quat}), 133.16 (+, 2Ar-CH), 127.40 (+, 2Ar-CH), 126.69 (+, 2Ar-CH), 126.25 (+, 2Ar-CH), 125.81 (2C_{quat}), 125.43 (+, 2Ar-CH), 120.54 (+, 2Ar-CH), 111.05 (2C_{quat}), 57.59 (-, CH₂), 53.36 (-, 2CH₂), 44.06 (-, 2CH₂), 39.93 (-, CH₂), 39.45 (-, CH₂), 36.47 (-, CH₂), 25.23 (-, 4CH₂). **HRMS** (EI-MS) calcd for C₄₆H₅₆Cl₂N₁₂O₂S₂ [MH⁺] 943.3540, found 943.3535.

***N*¹,*N*⁸-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 octanedioyl dichloride (45 mg, 0.21 mmol). White solid (130 mg, 0.13 mmol, 41.0%), m.p. 97 °C. **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 172.95 (2C_{quat}), 158.76 (2C_{quat}), 158.65 (2C_{quat}), 150.92 (2C_{quat}), 141.27 (2C_{quat}), 133.19 (+, 2Ar-CH), 127.38 (+, 2Ar-CH), 126.68 (+, 2Ar-CH), 126.22 (+, 2Ar-CH), 125.84 (2C_{quat}), 125.40 (+, 2Ar-CH), 120.60 (+, 2Ar-CH), 111.08 (2C_{quat}), 57.76 (-, CH₂), 53.38 (-, 2CH₂), 44.05 (-, 2CH₂), 39.92 (-, CH₂), 39.55 (-, CH₂), 36.88 (-, 2CH₂), 28.97 (-, 2CH₂), 25.73 (-, 2CH₂), 25.16 (-, CH₂). **HRMS** (EI-MS) calcd for C₄₈H₆₀Cl₂N₁₂O₂S₂ [MH⁺] 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 decanedioyl dichloride (87 mg, 0.36 mmol). White solid (250 mg, 0.25 mmol, 76.0%), m.p. 110 °C. **¹H-NMR** (300 MHz, CDCl₃) δ [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). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ [ppm]: 173.08 (2C_{quat}), 158.78 (2C_{quat}), 158.68 (2C_{quat}), 150.90 (2C_{quat}), 141.31 (2C_{quat}), 133.18 (+, 2Ar-CH), 127.36 (+, 2Ar-CH), 126.66 (+, 2Ar-CH), 126.19 (+, 2Ar-CH), 125.85 (2C_{quat}), 125.36 (+, 2Ar-CH), 120.66 (+, 2Ar-CH), 111.11 (2C_{quat}), 57.89 (-, CH_2), 53.39 (-, 2CH_2), 44.07 (-, 2CH_2), 39.89 (-, CH_2), 39.68 (-, CH_2), 37.06 (-, 2CH_2), 29.29 (-, 2CH_2), 29.23 (-, 2CH_2), 25.93 (-, 2CH_2), 25.04 (-, CH_2). **HRMS** (EI-MS) calcd for $\text{C}_{50}\text{H}_{64}\text{Cl}_2\text{N}_{12}\text{O}_2\text{S}_2$ [MH^+] 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 $\text{hH}_1\text{R} + \text{RGS4}$ or the $\text{hH}_4\text{R} + \text{G}_{\alpha i2} + \beta_1\gamma_2$. General procedures for the generation of recombinant baculoviruses, culture of Sf9 cells and membrane preparation are described elsewhere.⁴⁹ 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 MgCl_2 , 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each tube (total volume 100 μL) contained 30 μg (hH_1R) or 100 μg (hH_4R) of membrane protein and increasing concentrations of unlabeled ligands. Radioligands: H_1R : [^3H]pyrilamine, specific activity 20.0 Ci/mmol, $K_d = 4.5$ nM,⁵⁰ $c = 5$ nM, nonspecific binding determined in the presence of 10 μM of diphenhydramine; H_4R : [^3H]histamine, specific activity 25 Ci/mmol, $K_d = 10$ nM,⁴³ $c = 10$ nM, nonspecific binding determined in the presence of 10 μM of histamine. Filtration through glass microfiber filters (for hH_4R , 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.⁵¹ 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.⁵² Values represent the mean \pm 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.^{50, 53} Ligand concentrations were used in the range from 0.1 nM up to 1 mM.

3.4. References

1. Kleinau, G.; Müller, A.; Biebermann, H. Oligomerization of GPCRs involved in endocrine regulation. *J. Mol. Endocrinol.* **2016**, *57*, R59-R80.
2. George, S. R.; O'Dowd, B. F.; Lee, S. P. G-protein-coupled receptor oligomerization and its potential for drug discovery. *Nat. Rev. Drug. Discov.* **2002**, *1*, 808-820.
3. Smith, N. J.; Milligan, G. Allosteric at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. *Pharmacol. Rev.* **2010**, *62*, 701-725.
4. Tadagaki, K.; Jockers, R.; Kamal, M. History and biological significance of GPCR heteromerization in the neuroendocrine system. *Neuroendocrinology* **2011**, *95*, 223-231.
5. Wootten, D.; Miller, L. J.; Koole, C.; Christopoulos, A.; Sexton, P. M. Allosteric and biased agonism at class BG protein-coupled receptors. *Chem. Rev.* **2016**.
6. Conn, P. J.; Christopoulos, A.; Lindsley, C. W. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug. Discov.* **2009**, *8*, 41-54.
7. McVey, M.; Ramsay, D.; Kellett, E.; Rees, S.; Wilson, S.; Pope, A. J.; Milligan, G. Monitoring receptor oligomerization using time-resolved fluorescence resonance energy transfer and bioluminescence resonance energy transfer the human δ -opioid receptor displays constitutive oligomerization at the cell surface, which is not

- regulated by receptor occupancy. *J. Biol. Chem.* **2001**, 276, 14092-14099.
8. Cvejic, S.; Devi, L. A. Dimerization of the δ opioid receptor: implication for a role in receptor internalization. *J. Biol. Chem.* **1997**, 272, 26959-26964.
 9. Jordan, B. A.; Devi, L. A. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* **1999**, 399, 697-700.
 10. Angers, S.; Salahpour, A.; Joly, E.; Hilairet, S.; Chelsky, D.; Dennis, M.; Bouvier, M. Detection of β 2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). *Proc. Natl. Acad. Sci. U. S. A.* **2000**, 97, 3684-3689.
 11. Grant, M.; Kumar, U. The role of G-proteins in the dimerisation of human somatostatin receptor types 2 and 5. *Regul. Peptides.* **2010**, 159, 3-8.
 12. Durán-Prado, M.; Malagón, M. M.; Gracia-Navarro, F.; Castaño, J. P. Dimerization of G protein-coupled receptors: new avenues for somatostatin receptor signalling, control and functioning. *Mol. Cell Endocrinol.* **2008**, 286, 63-68.
 13. Scarselli, M.; Novi, F.; Schallmach, E.; Lin, R.; Baragli, A.; Colzi, A.; Griffon, N.; Corsini, G. U.; Sokoloff, P.; Levenson, R. D₂/D₃ dopamine receptor heterodimers exhibit unique functional properties. *J. Biol. Chem.* **2001**, 276, 30308-30314.
 14. Lee, S. P.; So, C. H.; Rashid, A. J.; Varghese, G.; Cheng, R.; Lança, A. J.; O'Dowd, B. F.; George, S. R. Dopamine D₁ and D₂ receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *J. Biol. Chem.* **2004**, 279, 35671-35678.
 15. Łukasiewicz, S.; Polić, A.; Kędracka-Krok, S.; Wędzony, K.; Maćkowiak, M.; Dziedzicka-Wasylewska, M. Hetero-dimerization of serotonin 5-HT_{2A} and dopamine D₂ receptors. *Biochim. Biophys. Acta* **2010**, 1803, 1347-1358.
 16. Zeng, F.-Y.; Wess, J. Molecular aspects of muscarinic receptor dimerization. *Neuropsychopharmacology* **2000**, 23, S19-S31.
 17. Hern, J. A.; Baig, A. H.; Mashanov, G. I.; Birdsall, B.; Corrie, J. E.; Lazareno, S.; Molloy, J. E.; Birdsall, N. J. Formation and dissociation of M₁ muscarinic receptor dimers seen by total internal reflection fluorescence imaging of single molecules. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, 107, 2693-2698.
 18. Bakker, R. A.; Dees, G.; Carrillo, J. J.; Booth, R. G.; López-Gimenez, J. F.; Milligan, G.; Strange, P. G.; Leurs, R. Domain swapping in the human histamine H₁ receptor. *J. Pharmacol. Exp. Ther.* **2004**, 311, 131-138.
 19. Fukushima, Y.; Asano, T.; Saitoh, T.; Anai, M.; Funaki, M.; Ogihara, T.; Katagiri, H.; Matsuhashi, N.; Yazaki, Y.; Sugano, K. Oligomer formation of histamine H₂ receptors expressed in Sf9 and COS7 cells. *FEBS Lett.* **1997**, 409, 283-286.
 20. Shenton, F.; Hann, V.; Chazot, P. Evidence for native and cloned H₃ histamine receptor higher oligomers. *Inflamm. Res.* **2005**, 54, S48-S49.
 21. Van Rijn, R. M.; Chazot, P. L.; Shenton, F. C.; Sansuk, K.; Bakker, R. A.; Leurs, R. Oligomerization of recombinant and endogenously expressed human histamine H₄ receptors. *Mol. Pharmacol.* **2006**, 70, 604-615.
 22. Lane, J. R.; Sexton, P. M.; Christopoulos, A. Bridging the gap: bitopic ligands of G-protein-coupled receptors. *Trends. Pharmacol. Sci.* **2013**, 34, 59-66.
 23. Portoghese, P. S. From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. *J. Med. Chem.* **2001**, 44, 2259-2269.

24. Portoghese, P. S. Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. *Trends. Pharmacol. Sci.* **1989**, 10, 230-235.
25. Shonberg, J.; Scammells, P. J.; Capuano, B. Design strategies for bivalent ligands targeting GPCRs. *ChemMedChem* **2011**, 6, 963-974.
26. Birnkammer, T.; Spickenreither, A.; Brunskole, I.; Lopuch, M.; Kagermeier, N.; Bernhardt, G. n.; Dove, S.; Seifert, R.; Elz, S.; Buschauer, A. The bivalent ligand approach leads to highly potent and selective acylguanidine-type histamine H₂ receptor agonists. *J. Med. Chem.* **2012**, 55, 1147-1160.
27. Kagermeier, N.; Werner, K.; Keller, M.; Baumeister, P.; Bernhardt, G.; Seifert, R.; Buschauer, A. Dimeric carbamoylguanidine-type histamine H₂ receptor ligands: A new class of potent and selective agonists. *Bioorg. Med. Chem.* **2015**, 23, 3957-3969.
28. Huber, D.; Hubner, H.; Gmeiner, P. 1, 1' -Disubstituted ferrocenes as molecular hinges in mono-and bivalent dopamine receptor ligands. *J. Med. Chem.* **2009**, 52, 6860-6870.
29. Kühhorn, J.; Hübner, H.; Gmeiner, P. Bivalent dopamine D₂ receptor ligands: synthesis and binding properties. *J. Med. Chem.* **2011**, 54, 4896-4903.
30. McRobb, F. M.; Crosby, I. T.; Yuriev, E.; Lane, J. R.; Capuano, B. Homobivalent ligands of the atypical antipsychotic clozapine: design, synthesis, and pharmacological evaluation. *J. Med. Chem.* **2012**, 55, 1622-1634.
31. Soriano, A.; Ventura, R.; Molero, A.; Hoen, R.; Casadó, V.; Cortés, A.; Fanelli, F.; Albericio, F.; Lluís, C.; Franco, R. Adenosine A_{2A} receptor-antagonist/dopamine D₂ receptor-agonist bivalent ligands as pharmacological tools to detect A_{2A}-D₂ receptor heteromers. *J. Med. Chem* **2009**, 52, 5590-5602.
32. Jacobson, K. A.; Xie, R.; Young, L.; Chang, L.; Liang, B. T. A novel pharmacological approach to treating cardiac ischemia binary conjugates of A₁ and A₃ adenosine receptor agonists. *J. Biol. Chem.* **2000**, 275, 30272-30279.
33. Weiss, S.; Keller, M.; Bernhardt, G.; Buschauer, A.; König, B. Modular synthesis of non-peptidic bivalent NPY Y₁ receptor antagonists. *Bioorg. Med. Chem.* **2008**, 16, 9858-9866.
34. Keller, M.; Teng, S.; Bernhardt, G.; Buschauer, A. Bivalent Argininamide-Type Neuropeptide Y Y₁ Antagonists Do Not Support the Hypothesis of Receptor Dimerisation. *ChemMedChem* **2009**, 4, 1733-1745.
35. Valant, C.; Robert Lane, J.; Sexton, P. M.; Christopoulos, A. The best of both worlds? Bitopic orthosteric/allosteric ligands of G protein-coupled receptors. *Annu. Rev. Pharmacol.* **2012**, 52, 153-178.
36. Dupuis, D. S.; Perez, M.; Halazy, S.; Colpaert, F. C.; Pauwels, P. J. Magnitude of 5-HT_{1B} and 5-HT_{1A} receptor activation in guinea-pig and rat brain: evidence from sumatriptan dimer-mediated [³⁵S] GTPγS binding responses. *Mol. Brain. Res.* **1999**, 67, 107-123.
37. Hill, S.; Ganellin, C.; Timmerman, H.; Schwartz, J.; Shankley, N.; Young, J.; Schunack, W.; Levi, R.; Haas, H. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.* **1997**, 49, 253-278.
38. Smits, R. A.; de Esch, I. J.; Zuiderveld, O. P.; Broeker, J.; Sansuk, K.; Guaita, E.; Coruzzi, G.; Adami, M.; Haaksma, E.; Leurs, R. Discovery of quinazolines as

- histamine H₄ receptor inverse agonists using a scaffold hopping approach. *J. Med. Chem.* **2008**, 51, 7855-7865.
39. Wittmann, H.-J.; Seifert, R.; Strasser, A. Contribution of binding enthalpy and entropy to affinity of antagonist and agonist binding at human and guinea pig histamine H₁-receptor. *Mol. Pharmacol.* **2009**, 76, 25-37.
40. Deml, K.-F.; Beermann, S.; Neumann, D.; Strasser, A.; Seifert, R. Interactions of histamine H₁-receptor agonists and antagonists with the human histamine H₄-receptor. *Mol. Pharmacol.* **2009**, 76, 1019-1030.
41. Wagner, E.; Wittmann, H.-J.; Elz, S.; Strasser, A. Pharmacological profile of astemizole-derived compounds at the histamine H₁ and H₄ receptor—H₁/H₄ receptor selectivity. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2014**, 387, 235-250.
42. Seifert, R.; Wenzel-Seifert, K.; Bürckstümmer, T.; Pertz, H. H.; Schunack, W.; Dove, S.; Buschauer, A.; Elz, S. Multiple differences in agonist and antagonist pharmacology between human and guinea pig histamine H₁-receptor. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1104-1115.
43. Schneider, E. H.; Schnell, D.; Papa, D.; Seifert, R. High Constitutive Activity and a G-Protein-Independent High-Affinity State of the Human Histamine H₄-Receptor. *Biochemistry* **2009**, 48, 1424-1438.
44. Shimamura, T.; Shiroishi, M.; Weyand, S.; Tsujimoto, H.; Winter, G.; Katritch, V.; Abagyan, R.; Cherezov, V.; Liu, W.; Han, G. W. Structure of the human histamine H₁ receptor complex with doxepin. *Nature* **2011**, 475, 65-70.
45. Aissaoui, H.; Boss, C.; Koberstein, R.; Siegrist, R.; Sifferlen, T. 5, 6, 7, 8-tetrahydro-imidazo [1, 5-a] pyrazine compounds. In Google Patents: 2009.
46. Jörg, M.; May, L. T.; Mak, F. S.; Lee, K. C. K.; Miller, N. D.; Scammells, P. J.; Capuano, B. Synthesis and pharmacological evaluation of dual acting ligands targeting the adenosine A_{2A} and dopamine D₂ receptors for the potential treatment of Parkinson's disease. *J. Med. Chem.* **2014**, 58, 718-738.
47. Moussa, I. A.; Banister, S. D.; Beinart, C.; Giboureau, N.; Reynolds, A. J.; Kassiou, M. Design, synthesis, and structure–affinity relationships of regioisomeric N-benzyl alkyl ether piperazine derivatives as σ -1 receptor ligands. *J. Med. Chem.* **2010**, 53, 6228-6239.
48. Wagner, E.; Wittmann, H.-J.; Elz, S.; Strasser, A. Mepyramine–JNJ7777120-hybrid compounds show high affinity to hH₁R, but low affinity to hH₄R. *Bioorg. Med. Chem. Lett.* **2011**, 21, 6274-6280.
49. Schnell, D.; Brunskole, I.; Ladova, K.; Schneider, E. H.; Igel, P.; Dove, S.; Buschauer, A.; Seifert, R. Expression and functional properties of canine, rat, and murine histamine H₄ receptors in Sf9 insect cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2011**, 383, 457-470.
50. Straßer, A.; Striegl, B.; Wittmann, H.-J.; Seifert, R. Pharmacological profile of histaprodifens at four recombinant histamine H₁ receptor species isoforms. *J. Pharmacol. Exp. Ther.* **2008**, 324, 60-71.
51. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, 193, 265-275.
52. Yung-Chi, C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the

- concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, 22, 3099-3108.
53. Wittmann, H.-J.; Elz, S.; Seifert, R.; Straßer, A. N α-Methylated phenylhistamines exhibit affinity to the hH₄R—a pharmacological and molecular modelling study. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2011**, 384, 287-299.

Chapter 4

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

4. Benzimidazole- and Quinazoline-type histamine H₁/H₄ 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.¹⁻⁴ The human histamine H₁ receptor is expressed on various cell types including endothelial cells and smooth muscle cells⁵ and involved in allergic and inflammatory reactions, e.g., bronchial asthma, allergic rhinitis, and urticaria.⁶ The human histamine H₄ receptor which was identified and cloned around 2001,⁷⁻¹³ is mainly localized in cells of the immune system, such as neutrophils, eosinophils, basophils, dendritic cells, mast cells and monocytes.⁷⁻¹⁴ As a major player in histamine-induced immunological and inflammatory reactions, the hH₄R was suggested as a potential target for the treatment of asthma, pruritus, and rheumatoid arthritis.^{5, 15-22} Due to the complementary and overlapping functions of hH₁R and hH₄R, it has been speculated that the combination of hH₁R and hH₄R antagonists in treating allergic diseases might be superior to monotherapy.^{5, 23} In various experimental models of allergy, a synergistic effect of co-administered hH₁R and hH₄R antagonists was observed regarding inhibition of pruritus and skin inflammation from chronic dermatitis,²⁴ acute hapten-induced scratching,²⁵ and peanut-induced intestinal allergy.²⁶ Thus, dual hH₁R/hH₄R 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 compliance²⁷ and the risk of drug-drug interactions. In principle, dual or multiple target ligands²⁸ 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,^{29, 30} and enhanced or modified physiological responses.³¹⁻³⁴ 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, hH₁R and hH₄R, were described, e.g. quinazoline,³⁵ aminopyrimidine,³⁶ astemizole,^{37, 38} and loxapine derivatives^{39, 40} (**Figure 1**). But due to the low homology between hH₁R and hH₄R (23.0%),⁷ most of the putative dual receptor ligands preferred one of the receptor subtypes and failed to bind with adequate balanced affinities to both, hH₁R and hH₄R.

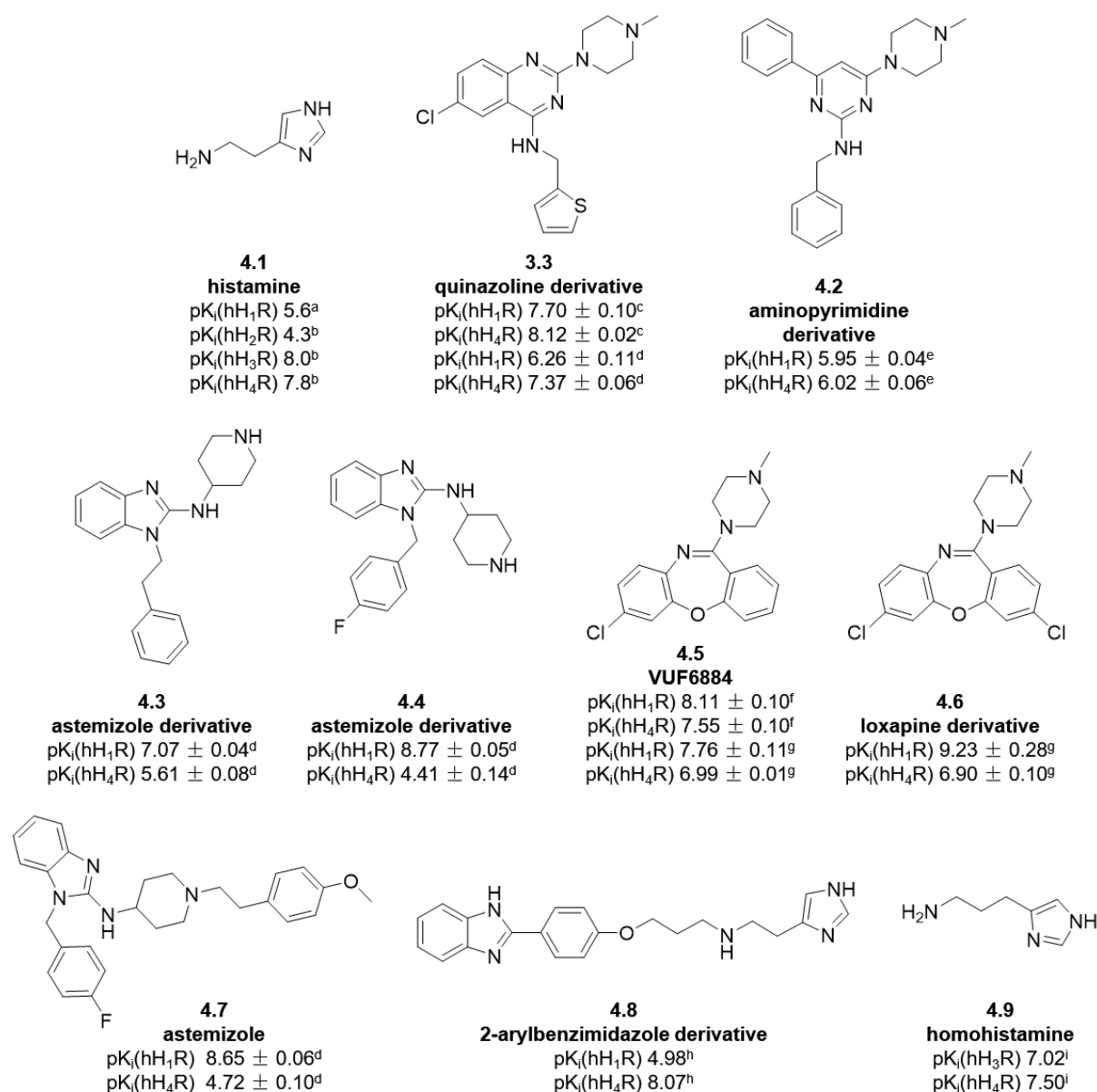


Figure 1 Structures and affinities of selected H₁R and/or H₄R ligands. Pharmacological data were taken from a) Strasser et al.⁴¹ b) Igel et al.¹⁸ c) Smits et al.³⁵ d) Wagner et al.³⁷ e) Hammer et al.³⁶ f) Smits et al.³⁹ g) Naporra et al.⁴⁰ h) Baumeister⁴² i) Geyer et al.⁴³

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 (pK_i 8.77) but almost no affinity to the hH₄R (pK_i 4.41), compound **4.3** is a modified version of compound **4.4** with higher hH₄R affinity (pK_i 5.61) but reduced hH₁R affinity (pK_i 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.

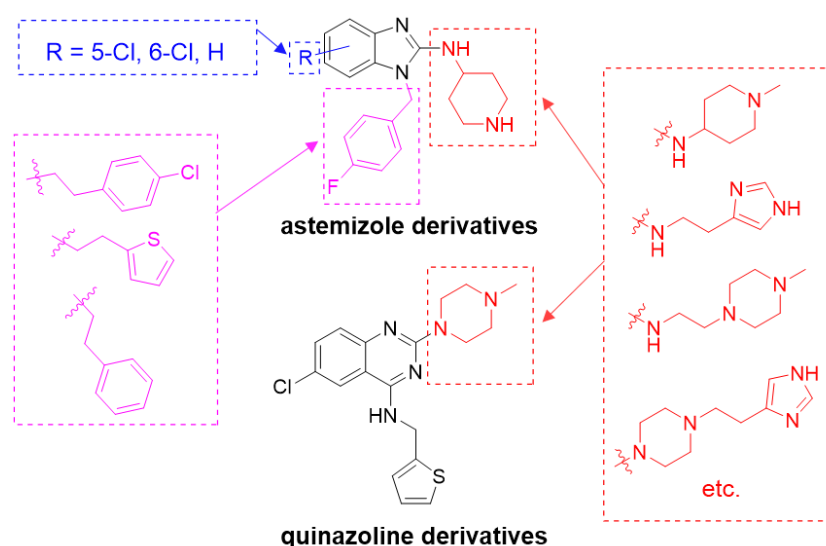
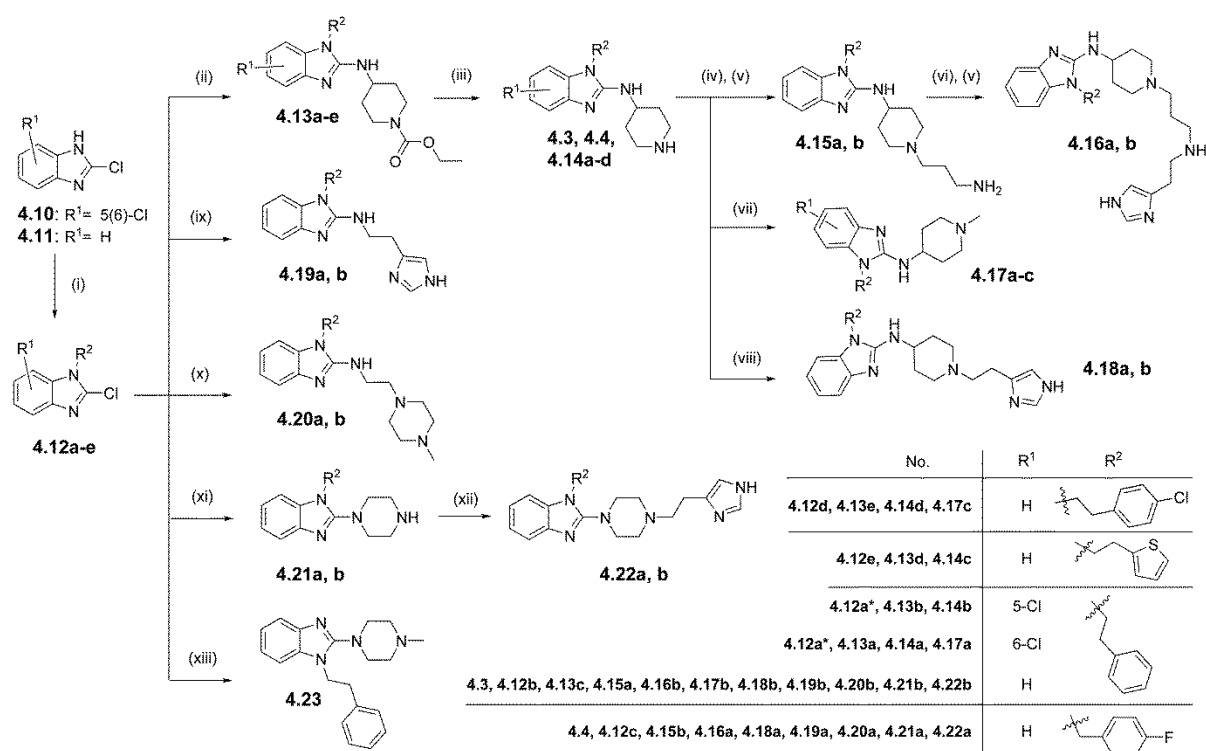


Figure 2 Structural variations of benzimidazoles and quinazolines synthesized as putative dual H₁ and H₄ receptor ligands.

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

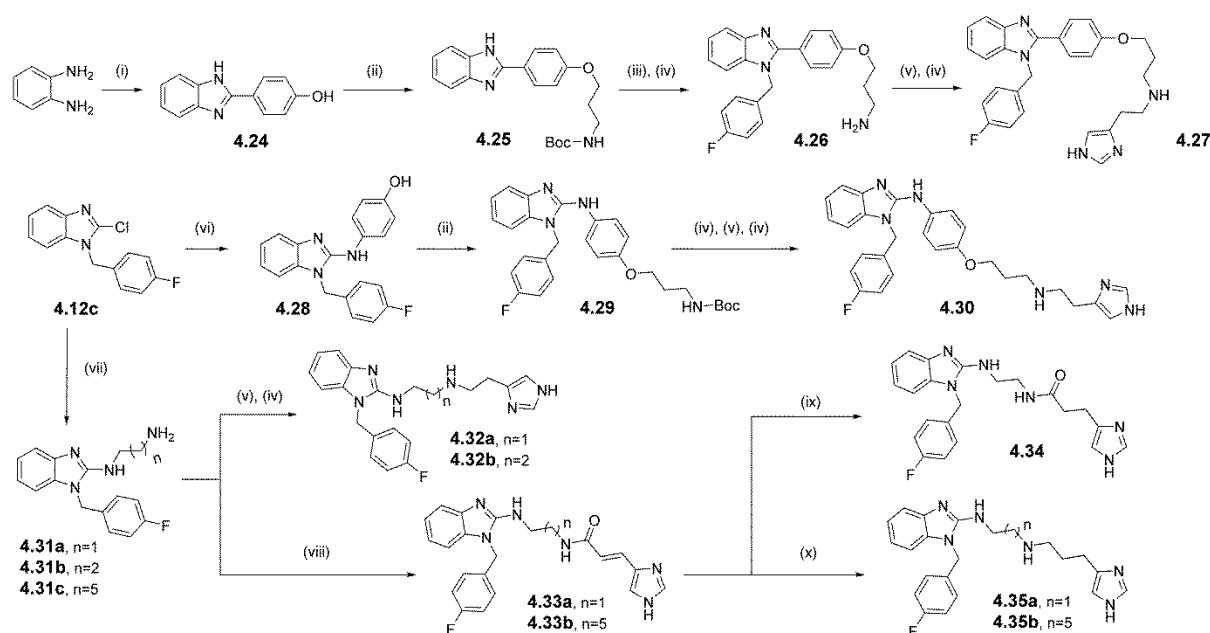
Building blocks **4.3**, **4.4**, **4.14a-c** were synthesized in two steps according to a procedure described by Rainer et al.⁴⁶ The 2-phenylbenzimidazole scaffold **4.24** was built based on a procedure described by Xu, et al.⁴⁷ The propylamine moiety was introduced to compounds **4.3**, **4.4**, **4.24** 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

^acompound **4.12a** is a mixture of 2,5-dichloro-1-phenethyl-1H-benzo[d]imidazole and 2,6-dichloro-1-phenethyl-1H-benzo[d]imidazole

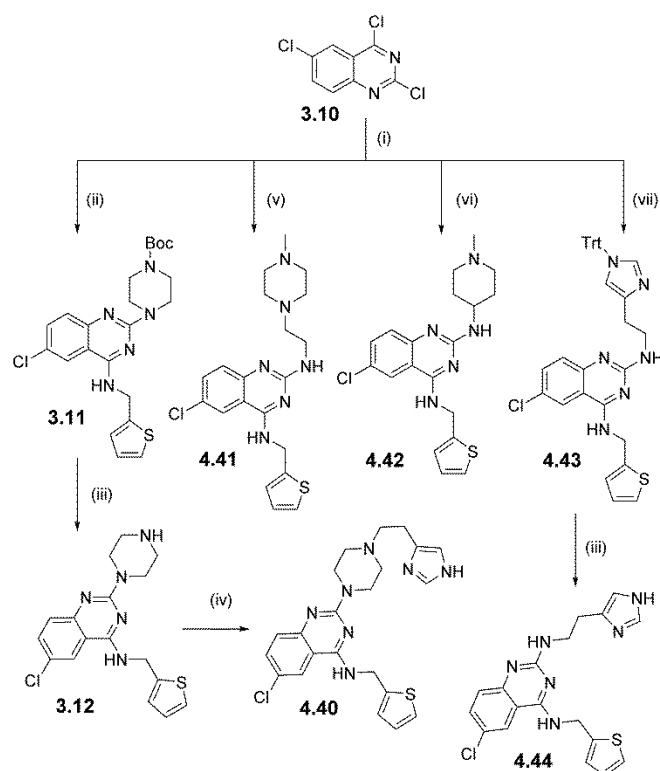
^aReagents and conditions: (i) R²-Cl, NaOH, ACN, 95 °C, 2 h; (ii) ethyl 4-aminopiperidine-1-carboxylate, 170 °C, overnight; (iii) 47% HBr in H₂O, 126 °C, 3 h; (iv) *tert*-butyl (3-bromopropyl)carbamate, NaI, DIPEA, ACN, reflux, 2 h; (v) TFA, DCM, rt, 3 h; (vi) 4-(2-bromoethyl)-1-tritylimidazole, NaI, 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.

Scheme 2 Synthesis of the benzimidazole derivatives **4.26**, **4.27**, **4.30**, **4.32**, **4.33**, **4.34** and **4.35**^a



^aReagents and conditions: (i) $\text{Na}_2\text{S}_2\text{O}_5$, DMF, 90 °C, 2 h, H_2O , 4 h, 0 °C; (ii) *tert*-butyl(3-bromopropyl)carbamate, K_2CO_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_2O , H_2O , 0 °C to rt, 30 min.

Quinazoline derivatives **4.40**, **4.41**, **4.42** and **4.44**³⁵ (**Scheme 3**) as well as the required building blocks 4-(2-bromoethyl)-imidazole,⁴⁸ 4-(2-bromoethyl)-1-trityl-imidazole,⁴⁹ 4-methyl-1-piperazineethanamine,⁵⁰ *tert*-butyl piperazine-1-carboxylate⁵¹ and 2-(1-trityl-imidazol-4-yl)ethan-1-amine,⁵² were synthesized according to described procedures.

Scheme 3 Synthesis of the quinazoline derivatives **4.40**, **4.41**, **4.42** and **4.44**^a

^aReagents 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-methylpiperazin-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 hH₄R, and selected compounds were additionally tested on hH₂R and 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),⁵³ hH₂R-G_{sas},⁵⁴ hH₃R + G_{αi2} + G_{β1γ2}⁵⁵ or the hH₄R + G_{αi2} + G_{β1γ2}⁵⁶.

4.2.2.1. Competition binding data at the hH₁R and hH₄R.

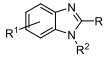
The affinities of the title compounds at the hH₁R and the hH₄R are summarized in **Table 1**. Based on the substituents at the 2-position of compounds **4.3** and **4.4** the benzimidazoles can be grouped into four types (**Figure 4**, red box). A piperazine moiety (“Type A”), attached directly or via 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 pK_i 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 pK_i ≈ 8 (**4.30**, pK_i: 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 13 Å, similar to that of **4.30**, was identified as a dual hH₁R/hH₄R ligand with balanced affinity (K_i 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.³⁵ 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



4.14a, 4.17a, R¹= 5-Cl
4.14b, R¹= 6-Cl
 Otherwise, R¹= H

cpd.	R	R ²	(pK _i) hH ₁ R	(pK _i) hH ₄ R	cpd.	R	R ²	(pK _i) hH ₁ R	(pK _i) hH ₄ R
4.3			7.07 ± 0.04 ^a	5.61 ± 0.08 ^a	4.19b			5.34 ± 0.05	6.06 ± 0.01
4.4			8.77 ± 0.05 ^a	4.41 ± 0.14 ^a	4.20a			7.74 ± 0.25	4.68 ± 0.18
4.14a			5.87 ± 0.02	4.86 ± 0.07	4.20b			5.42 ± 0.31	4.72 ± 0.17
4.14b			5.52 ± 0.03	5.18 ± 0.17	4.22a			8.18 ± 0.14	5.10 ± 0.24
4.14c			7.11 ± 0.08	5.14 ± 0.16	4.22b			6.45 ± 0.10	4.93 ± 0.13
4.15b			8.22 ± 0.09	4.79 ± 0.30	4.23			6.74 ± 0.04	3.85 ± 0.34
4.16a			8.50 ± 0.04	6.61 ± 0.21	4.26			4.83 ± 0.08	4.27 ± 0.18
4.16b			6.75 ± 0.15	6.15 ± 0.09	4.27			5.51 ± 0.18	7.90 ± 0.04
4.17a			5.82 ± 0.03	4.43 ± 0.09	4.30			8.04 ± 0.06	7.74 ± 0.05
4.17b			7.46 ± 0.10	5.30 ± 0.02	4.32a			7.43 ± 0.09	5.51 ± 0.06
4.17c			6.64 ± 0.07	4.65 ± 0.29	4.32b			8.25 ± 0.17	6.01 ± 0.05
4.18a			8.06 ± 0.13	4.82 ± 0.10	4.34			7.28 ± 0.19	4.85 ± 0.09
4.18b			7.62 ± 0.12	5.05 ± 0.10	4.35a			7.93 ± 0.10	6.72 ± 0.05
4.19a			7.92 ± 0.01	6.05 ± 0.08	4.35b			7.26 ± 0.02	7.31 ± 0.07

The compounds were characterized in radioligand competition binding assays using membrane preparations of Sf9 insect cells expressing the hH₁R + RGS4 or the hH₄R + G_{α12} + G_{β1γ2}. Radioligands: H₁R: [³H]pyrilamine, K_d = 4.5 nM, c = 5 nM; H₄R: [³H]histamine, K_d = 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.³⁷

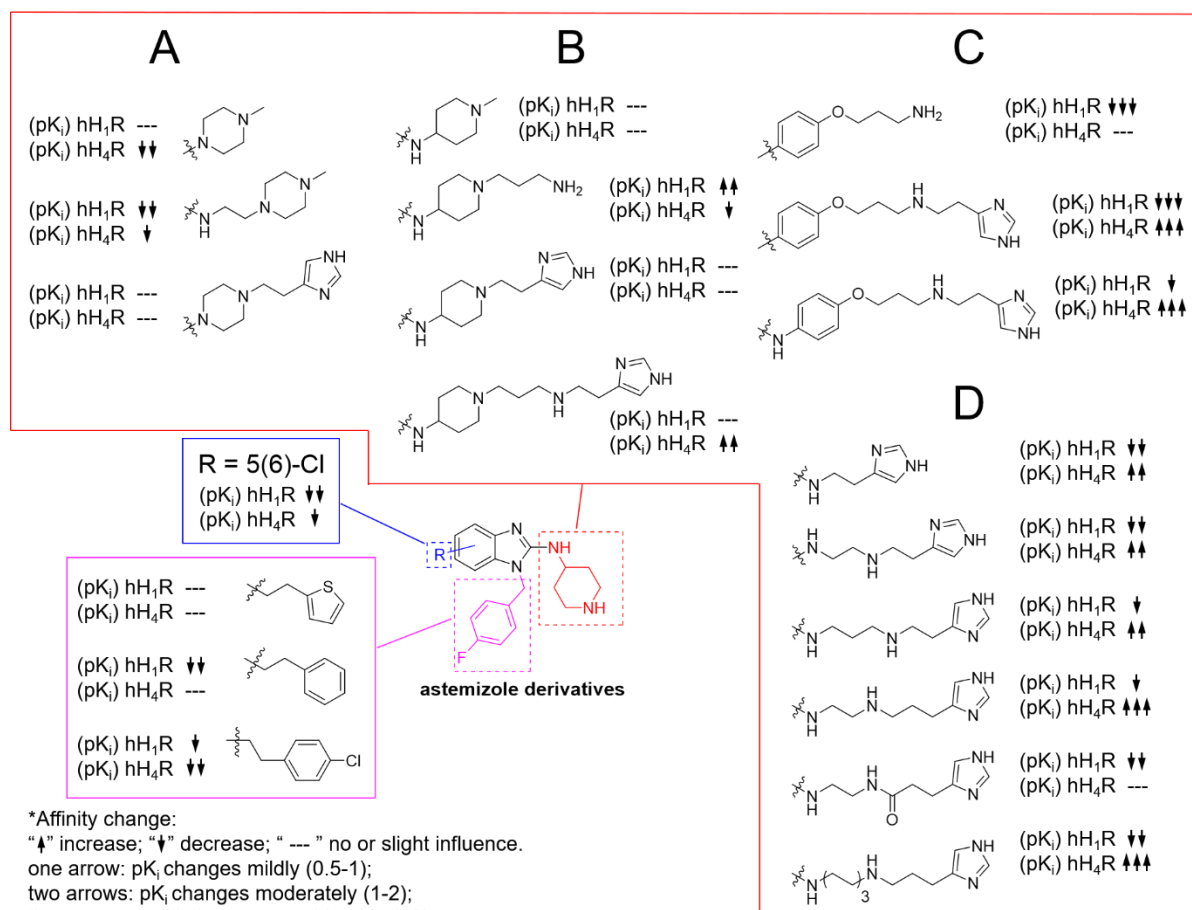
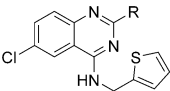
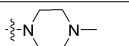
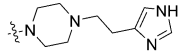
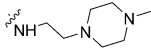
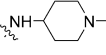
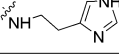


Figure 4 Summarized structure-activity relationships of the benzimidazole-type ligands at hH₁R and hH₄R

Table 2 hH₁R and hH₄R affinities of quinazoline-type ligands



Cpd.	R	(pK _i) hH ₁ R	(pK _i) hH ₄ R
3.3		6.26 ± 0.11 ^a	7.37 ± 0.06 ^a
4.40		5.77 ± 0.28	5.72 ± 0.10
4.41		4.39 ± 0.24	4.30 ± 0.09
4.42		5.41 ± 0.03	5.76 ± 0.11
4.44		5.23 ± 0.03	7.90 ± 0.02

^a (pK_i) hH₁R = 7.70, hH₄R = 8.12 (Smits et al.³⁵)

The compounds were characterized in radioligand competition binding assays using membrane preparations of Sf9 insect cells expressing the hH₁R + RGS4 or the hH₄R + G_{αi2} + G_{β1γ2}. Radioligands:

H₁R: [³H]pyrilamine, K_d = 4.5 nM, c = 5 nM; H₄R: [³H]histamine, K_d = 10 nM, c = 10 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 hH_xR, determined by radioligand-competition binding and [³⁵S]GTPγS-binding assays.

cpd.	hH ₁ R		hH ₂ R		hH ₃ R		hH ₄ R	
	[pK _i], (pEC ₅₀) or pK _b	α	[pK _i], (pEC ₅₀) or pK _b	α	[pK _i], (pEC ₅₀) or pK _b	α	[pK _i], (pEC ₅₀) or pK _b	α
Histamine	(5.43±0.15)	1	(6.07±0.10)	1	(7.97±0.04)	1	(7.73±0.06)	1
Thioperamide	n.d.	n.d.	n.d.	n.d.	7.00±0.11	-0.61±0.09	6.78±0.06	-1.03±0.08
Famotidine	n.d.	n.d.	6.53±0.06	-0.33±0.06	n.d.	n.d.	n.d.	n.d.
Levocetirizine	7.17±0.15	-0.23±0.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4.16a	[8.50±0.04]		[5.06±0.03]		[6.79±0.05]		[6.61±0.21]	
	7.09±0.10	0	<5	0	6.77±0.05	-0.41±0.08	5.82±0.06	0
4.19a	[7.92±0.01]		[5.34±0.06]		[7.34±0.02]		[6.05±0.08]	
	7.42±0.17	0	<5	0	7.38±0.01	-0.82±0.06	5.27±0.15	-0.67±0.07
4.27	[5.51±0.18]		[5.50±0.02]		[7.39±0.03]		[7.90±0.04]	
	<5	0	5.18±0.07	0	7.37±0.06	0	(7.57±0.19)	0.99±0.08
4.30	[8.04±0.06]		[5.48±0.05]		[7.26±0.01]		[7.74±0.05]	
	7.70±0.17	0	5.27±0.09	0	7.94±0.11	-0.34±0.05	(7.01±0.15)	0.84±0.08
4.32b	[8.25±0.17]		[5.74±0.14]		[5.88±0.05]		[6.01±0.05]	
	8.00±0.16	0	(6.29±0.07)	0.44±0.04	6.10±0.06	-0.66±0.05	<5	0
4.35a	[7.93±0.10]		[6.11±0.06]		[6.89±0.03]		[6.72±0.05]	
	7.05±0.09	0	<5	0	7.20±0.17	-0.63±0.05	6.83±0.08	0
4.35b	[7.26±0.02]		[5.28±0.06]		[6.94±0.01]		[7.31±0.07]	
	7.37±0.09	0	<5	0	(5.31±0.07)	0.50±0.04	6.91±0.04	-0.55±0.01
4.44	[5.23±0.03]		[6.70±0.03]		[8.33±0.10]		[7.90±0.02]	
	<5	0	6.50±0.12	0	8.88±0.05	-0.98±0.06	7.64±0.10	-1.73±0.10

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, hH₂R-G_{sαS}, hH₃R + G_{ai2} + G_{β1γ2} or the hH₄R + G_{ai2} + G_{β1γ2}. Radioligands: H₁R: [³H]pyrilamine, K_d = 4.5 nM, c = 5 nM; H₂R: [³H]UR-DE 257 (*N*-[6-(3,4-dioxo-2-{3-[3-(piperidin-1-ylmethyl)phenoxy]propylamino}-cyclobut-1-enylamino)hexyl]-[2,3-³H₂]-propion-amide)⁵⁷, K_d = 12.1 nM, c = 20 nM; H₃R: [³H]UR-PI 294 (*N*¹-[3-(1H-imidazol-4-yl)propyl]-*N*²-propionylguanidine)⁵⁸, K_d = 3.3 nM, c = 3.5 nM; H₄R: [³H]histamine, K_d = 10 nM, c = 10 nM. The pK_b 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. α = 0 (neutral antagonism): the measured values were in the range between ± 0.15 and not significantly different from zero.

Eight compounds exhibiting high hH₁R and/or hH₄R 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 hH₂R and hH₃R subtype affinity and functional activity at the four human histamine receptor subtypes (**Table 3**). Whereas hH₂R binding was low to moderate, for all imidazole containing ligands, poor selectivity for the hH₄R over the hH₃R became obvious. The selected compounds showed binding affinities in the same range at the hH₃R and the hH₄R, except for compound **19a** which exhibited a preference for the hH₃R.

In the functional assays compounds **4.16a**, **4.32b** and **4.35a** were selective hH₁R antagonists. Compound **4.19a** turned out to be an hH₁R antagonist and a potent inverse agonist at the hH₃R (**Table 2** and **Figure 5**). A dual hH₁R/hH₃R antagonist might be useful to treat allergic rhinitis⁵⁹⁻⁶¹, e.g., a combined H₁ and H₃ receptor blockade was reported to reduce histamine-induced nasal congestion⁶²⁻⁶⁴.

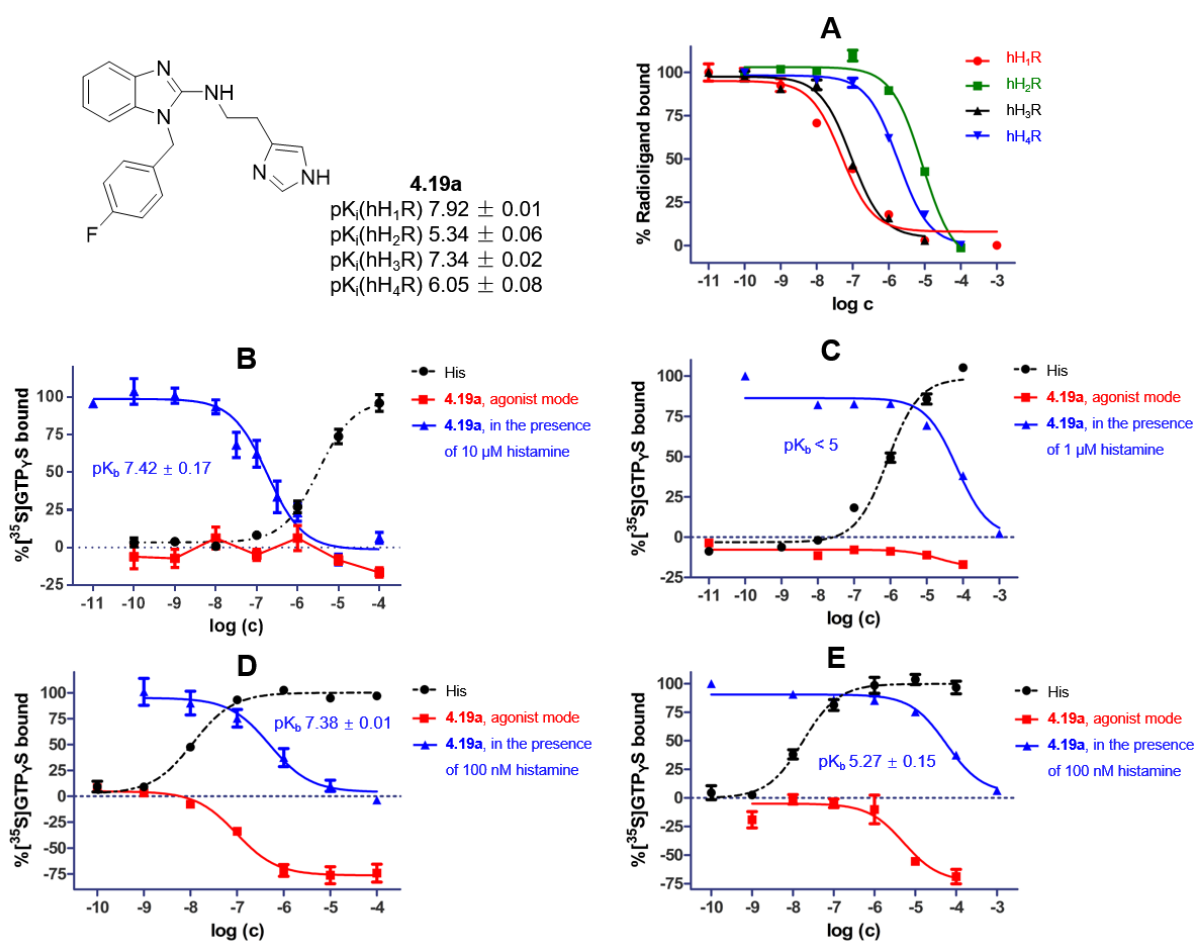


Figure 5 Binding and function of compound **4.19a** at histamine receptors. Curves shown represent mean values of at least three independent experiments performed in triplicate. **A)** Competition binding

curves of compound **4.19a** at hH_xR. 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-G_{sαs}, hH₃R + G_{αi2} + G_{β1γ2} or the hH₄R + G_{αi2} + G_{β1γ2}. Radioligands: H₁R: [³H]pyrilamine, K_d = 4.5 nM, c = 5 nM; H₂R: [³H]UR-DE 257⁵⁷, K_d = 12.1 nM, c = 20 nM; H₃R: [³H]UR-PI 294⁵⁸, K_d = 3.3 nM, c = 3.5 nM; H₄R: [³H]histamine, K_d = 10 nM, c = 10 nM. The pK_b 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.

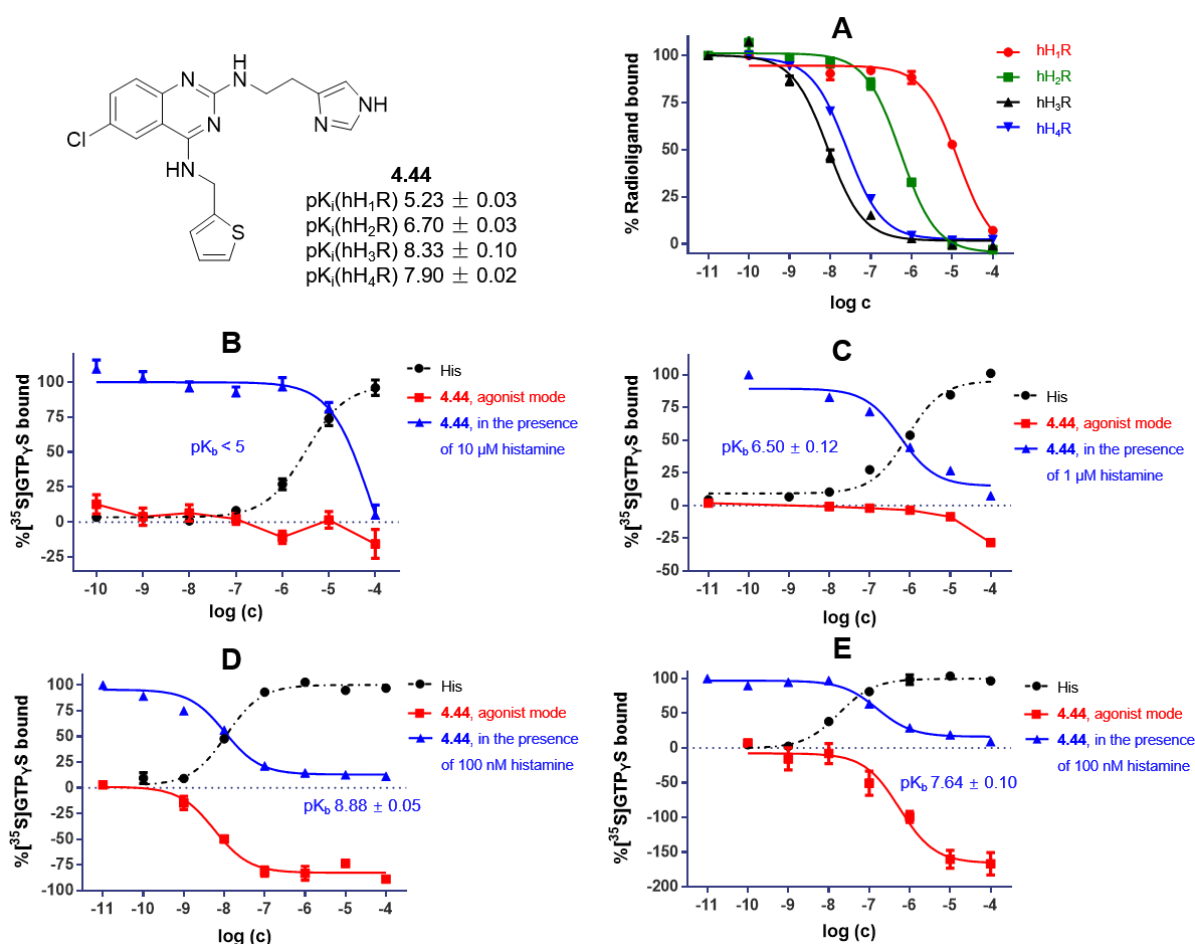


Figure 6 Binding and function of compound **4.44** at histamine receptors. Curves shown represent mean values of at least three independent experiments performed in triplicate. **A**) Competition binding curves of compound **4.44** at hH_xR. Concentration-response curves of histamine and compound **4.44** at the **B**) hH₁R, **C**) hH₂R, **D**) hH₃R and **E**) hH₄R, determined in the [³⁵S]GTPγS binding assay. For details cf. legend to **Figure 5**.

Compound **4.35b** was an antagonist at the hH₁R, an inverse agonist at the hH₄R, but a weak hH₃R partial agonist. Thus, functional characterization revealed opposite qualities of action for compound **4.35b** at the hH₃R and the hH₄R (**Table 2** and **Figure 7**). Among the studied ligands compounds **4.30** and **4.35b** showed the highest (nearly) balanced affinities at both, the hH₁R and the hH₄R (**Figure 8**).

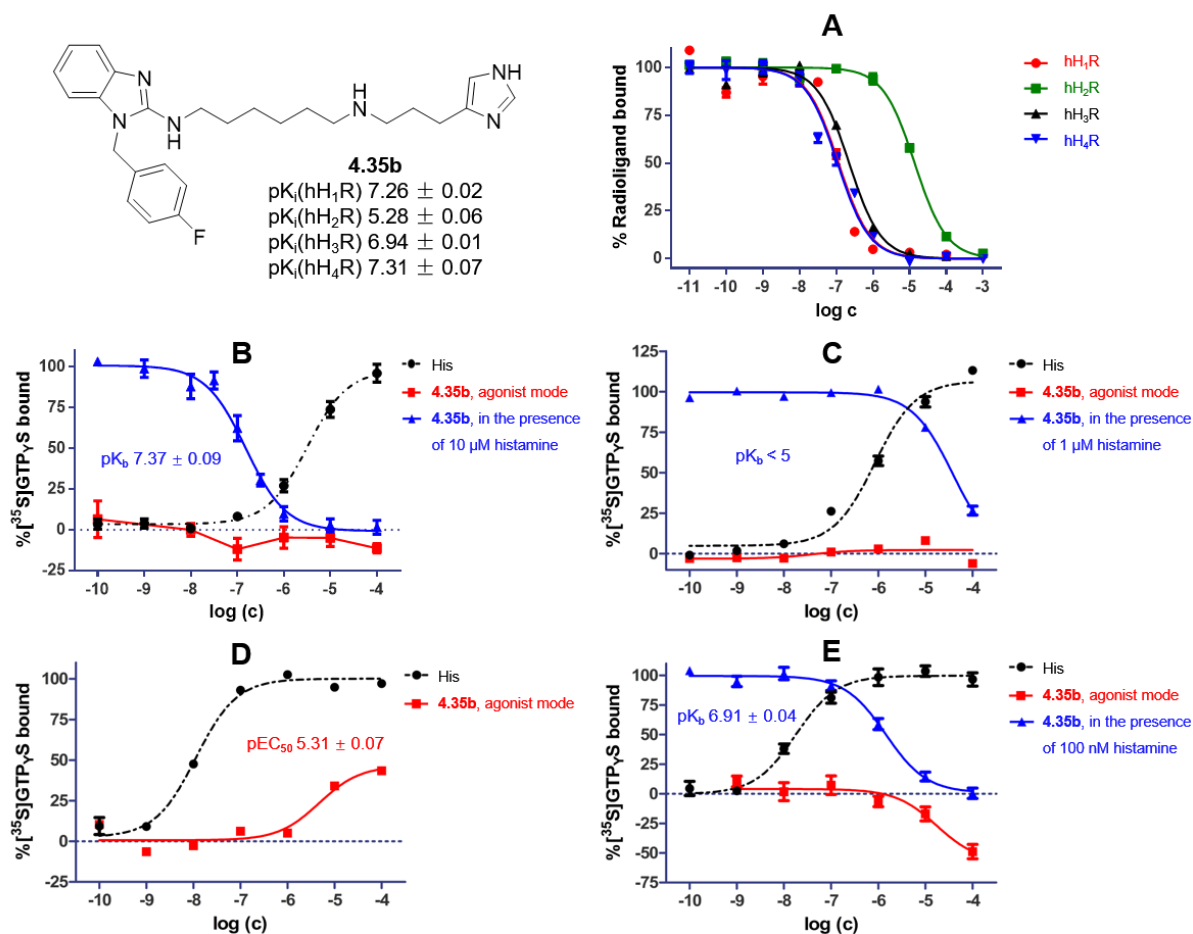


Figure 7 Binding and function of compound **4.35b** at histamine receptors. Curves shown represent for mean values of at least three independent experiments performed in triplicate. **A**) Competition binding curves of compound **4.35b** at hH_xR. Concentration-response curves of histamine and compound **4.35b** at the **B**) hH₁R, **C**) hH₂R, **D**) hH₃R and **E**) hH₄R respectively, determined in the [³⁵S]GTP_γS binding assay. For details cf. legend to **Figure 5**.

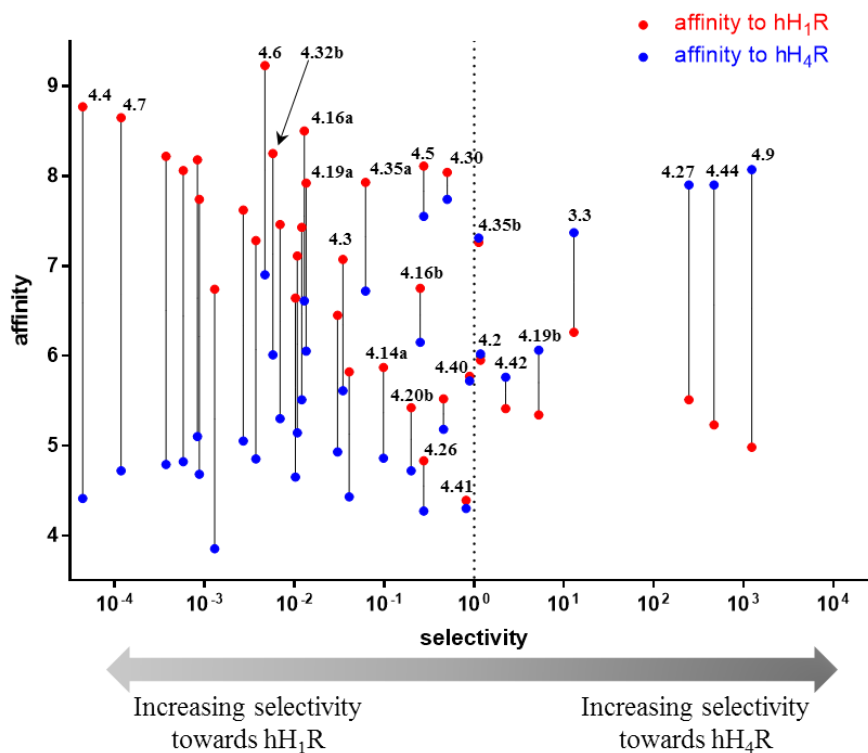


Figure 8 Affinity-selectivity profile of the compounds **3.3**, **4.3-4.8**, **4.14a-c**, **4.15b**, **4.16a**, **4.16b**, **4.17a-c**, **4.18a**, **4.18b**, **4.19a**, **4.19b**, **4.20a**, **4.20b**, **4.22a**, **4.22b**, **4.23**, **4.26**, **4.27**, **4.30**, **4.32a**, **4.32b**, **4.34**, **4.35a**, **4.35b**, **4.40**, **4.41**, **4.42**, **4.44**. The data were obtained from competition binding assays at hH_1R or hH_4R under comparable conditions.

4.3. Conclusion

Aiming at dual hH_1R/hH_4R antagonists, benzimidazole- and quinazoline-type compounds were synthesized and pharmacologically characterized at the four human histamine receptor subtypes. All compounds with high hH_1R and/or hH_4R affinities showed only weak affinity to the hH_2R . Unfortunately, ligands comprising imidazolylalkyl moieties did not discriminate between hH_3 and hH_4 receptors. Regardless of that, compounds **4.30** and **4.35b** were identified as balanced dual hH_1R/hH_4R 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 H_1R/H_4R pharmacophore at a high level of affinity. Introducing an imidazole moiety may result in balanced H_1R/H_4R 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 [MH⁺] 174.9865, found 174.9864.

4-(2-Bromoethyl)-1-tritylimidazole.⁴⁹

A mixture of 4-(2-bromoethyl)imidazole hydrochloride (1.68 g, 9 mmol), triphenylmethylchloride (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. **¹H-NMR** (300 MHz, CDCl₃) δ [ppm]: 7.39 (d, *J* = 1.3 Hz, 1H), 7.36-7.29 (m, 9H), 7.15 (m, 6H), 6.65 (d, *J* = 0.5 Hz, 1H), 3.80-3.60 (m, 2H), 3.12-2.95 (m, 2H). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 142.38 (2C_{quat}), 138.55 (+, Ar-CH), 138.37 (C_{quat}), 137.62 (C_{quat}), 129.81 (+, 6Ar-CH), 128.07 (+, 9Ar-CH), 119.39 (+, Ar-CH), 75.29 (C_{quat}), 44.07 (-, CH₂), 32.14 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₄H₂₁BrN₂ [MH⁺] 417.0961, found 417.0962.

***tert*-Butyl [2-(4-methylpiperazin-1-yl)ethyl]carbamate.**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 Na₂SO₄. 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%). **¹H-NMR** (300 MHz, CDCl₃) δ [ppm]: 3.25-3.10 (m, 2H), 2.55-2.31 (m, 10H), 2.24 (s, 3H), 1.38 (s, 9H). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 155.95 (C_{quat}), 79.12 (C_{quat}), 57.07 (-, CH₂), 55.00 (-, 2CH₂), 52.73 (-, 2CH₂), 45.94 (+, CH₃), 37.10 (-, CH₂), 28.43 (+, 3CH₃). **HRMS** (EI-MS) *m/z*: calcd for C₁₂H₂₅N₃O₂ [MH⁺] 244.2020, found 244.2021.

***N*-[2-(1-Triphenylmethylimidazol-4-yl)ethyl]phthalimide.**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 Na₂CO₃ (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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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-1*H*-benzo[*d*]imidazole and 2,6-dichloro-1*H*-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 POCl₃ and heated at 100 °C for 18 h. The reaction mixture was cooled to rt, and the excess of POCl₃ was removed in vacuum. The residue was neutralized with 100 mL of saturated NaHCO₃ solution and extracted with EtOAc. The organic phase was washed with brine and dried over Na₂SO₄. 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. **¹H-NMR** (300 MHz, DMSO-*d*₆) δ [ppm]: 7.58 (d, *J* = 1.9 Hz, 1H), 7.55-7.49 (m, 1H), 7.26-7.20 (m, 1H). **¹³C-NMR** (75 MHz, DMSO-*d*₆) δ [ppm]: 139.86 (2C_{quat}), 126.66 (2C_{quat}), 122.54 (+, 3Ar-CH).

2,5-Dichloro-1-phenethyl-1*H*-benzo[*d*]imidazole and

2,6-dichloro-1-phenethyl-1*H*-benzo[*d*]imidazole (4.12a).

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. **¹H-NMR** (300 MHz, CDCl₃) δ [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-1*H*-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. **¹H-NMR** (300 MHz, CDCl₃) δ [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)-1*H*-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. **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [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 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₁₄H₁₀FN₂ [MH⁺] 261.0589, found 261.0589.

2-Chloro-1-(4-chlorophenethyl)-1*H*-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. **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [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), 109.25 (+, Ar-CH), 45.76 (-, CH₂), 34.95 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₁₅H₁₂Cl₂N₂ [MH⁺] 291.0450, found 291.0455.

2-Chloro-1-[2-(thiophen-2-yl)ethyl]-1*H*-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. **¹H-NMR** (300 MHz, CDCl₃) δ [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) δ [ppm]: 141.68 (C_{quat}), 140.56 (C_{quat}), 138.69 (C_{quat}), 134.80 (C_{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 $\text{C}_{13}\text{H}_{11}\text{ClN}_2\text{S}$ [MH^+] 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_2SO_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) δ [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) δ [ppm]: 155.51 (C_{quat}), 154.14 (C_{quat}), 143.39 (C_{quat}), 138.37 (C_{quat}), 132.78 (C_{quat}), 129.32 (+, 2Ar-CH), 128.94 (+, 2Ar-CH), 127.45 (+, Ar-CH), 126.81 (C_{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 (-, 2 CH_2), 35.37 (-, CH_2), 32.12 (-, 2 CH_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) δ [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

(m, 2H), 2.88 (s, 2H), 1.85-1.78 (m, 2H), 1.26 (t, $J = 7.1$ Hz, 4H), 1.01-0.91 (m, 2H). **$^{13}\text{C-NMR}$** (75 MHz, CDCl_3) δ [ppm]: 155.51 (C_{quat}), 153.86 (C_{quat}), 141.01 (C_{quat}), 138.37 (C_{quat}), 134.85 (C_{quat}), 129.33 (+, 2Ar-CH), 128.96 (+, 2Ar-CH), 127.46 (+, Ar-CH), 125.01 (C_{quat}), 121.61 (+, Ar-CH), 117.08 (+, Ar-CH), 107.47 (+, Ar-CH), 61.37 (-, CH_2), 49.44 (+, CH), 44.93 (-, CH_2), 42.57 (-, 2CH_2), 35.35 (-, CH_2), 32.10 (-, 2CH_2), 14.73 (+, CH_3).

Ethyl 4- [(1- phenethyl- 1H- benzo[d]imidazol- 2- yl)amino]piperidine- 1- carboxylate (4.13c).³⁷

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. **$^1\text{H-NMR}$** (300 MHz, CDCl_3) δ [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). **$^{13}\text{C-NMR}$** (75 MHz, CDCl_3) δ [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 $\text{C}_{23}\text{H}_{28}\text{N}_4\text{O}_2$ [MH^+] 393.2285, found 393.2295.

Ethyl 4- ({1- [2- (thiophen- 2- yl)ethyl]- 1H- benzo[d]imidazol- 2- yl)amino}piperidine- 1- carboxylate (4.13d).

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. **$^1\text{H-NMR}$** (300 MHz, CDCl_3) δ [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). **$^{13}\text{C-NMR}$** (75 MHz, CDCl_3) δ [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),

42.65 (-, 2CH₂), 32.28 (-, 2CH₂), 29.53 (-, CH₂), 14.75 (+, CH₃). **HRMS** (EI-MS) *m/z*: calcd for C₂₁H₂₆N₄O₂S [MH⁺] 399.1849, found 399.1849.

Ethyl 4- {[1- (4- chlorophenethyl)- 1*H*- 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 C₂₃H₂₇ClN₄O₂ [MH⁺] 427.1895, found 427.1893.

5-Chloro-1-phenethyl-*N*-(piperidin-4-yl)-1*H*-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)-1*H*-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 (C_{quat}), 141.17 (C_{quat}), 138.30 (C_{quat}), 134.89 (C_{quat}), 129.32 (+, 2Ar-CH), 128.93 (+, 2Ar-CH), 127.47 (+, Ar-CH), 124.81 (C_{quat}), 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 C₂₀H₂₃ClN₄ [MH⁺] 355.1684, found 355.1687.

***N*-(Piperidin-4-yl)-1-(2-(thiophen-2-yl)ethyl)-1*H*-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 (C_{quat}), 142.46 (C_{quat}), 140.04 (C_{quat}), 134.00 (C_{quat}), 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)-1*H*-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-1*H*-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, 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-NMR** (300 MHz, CD₃OD) δ [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). **¹³C-NMR** (75 MHz, CD₃OD) δ [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 (-, CH₂), 50.13 (+, CH₃), 45.21 (-, CH₂), 37.87 (-, 2CH₂), 34.59 (-, 2CH₂), 30.18 (-, CH₂), 23.54 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₃H₃₁N₅ [MH⁺] 378.2652, found 378.2657.

***N*-[1-(3-Aminopropyl)piperidin-4-yl]-1-(4-fluorobenzyl)-1*H*-benzo[*d*]imidazol-2-amine (4.15b).**⁶⁹

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-NMR** (400 MHz, DMSO-*d*₆) δ [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). **¹³C-NMR** (100 MHz, DMSO-*d*₆) δ [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). **HRMS** (EI-MS) *m/z*: calcd for C₂₂H₂₈FN₅ [MH⁺] 382.2402, found 382.2407.

***N*-[1-(3-([2-(1*H*-imidazol-4-yl)ethyl]amino)propyl)piperidin-4-yl]-1-(4-fluorobenzyl)-1*H*-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-1*H*-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-*d*₆) 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-*d*₆) 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), 112.52 (+, 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 [MH⁺] 476.2932, found 476.2937.

***N*-[1-(3-([2-(1*H*-imidazol-4-yl)ethyl)amino}propyl)piperidin-4-yl]-1-phenethyl-1*H*-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-*d*₆) 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-*d*₆) 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),

50.57 (-, CH₂), 48.41 (+, CH₃), 44.74 (-, 2CH₂), 43.80 (-, CH₂), 43.16 (-, CH₂), 32.86 (-, 2CH₂), 20.95 (-, 2CH₂), 20.26 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₈H₃₇N₇ [MH⁺] 472.3183, found 472.3191.

5-Chloro-*N*-(1-methylpiperidin-4-yl)-1-phenethyl-1*H*-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), 7.45-7.38 (m, 1H), 7.29-7.12 (m, 6H), 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]: 158.39 (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 (-, 2CH₂), 48.54 (+, CH₃), 43.29 (-, CH₂), 42.43 (+, CH₃), 32.83 (-, CH₂), 28.63 (-, 2CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₁H₂₅ClN₄ [MH⁺] 369.1841, found 369.1842.

***N*-(1-Methylpiperidin-4-yl)-1-phenethyl-1*H*-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 °C. **¹H-NMR** (300 MHz, CDCl₃) 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, CDCl₃) 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 (-, 2CH₂), 46.26 (+, CH₃), 44.76 (-, CH₂), 35.43 (-, CH₂), 32.63 (-, 2CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₁H₂₆N₄ [MH⁺] 335.2230, found 335.2234.

1-(4-Chlorophenethyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-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. **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) 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 (-, 2CH₂), 49.04 (+, CH), 46.22 (+, CH₃), 44.27 (-, CH₂), 34.53 (-, CH₂), 32.77 (-, 2CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₁H₂₅ClN₄ [MH⁺] 369.1841, found 369.1840.

***N*-{1-[2-(1H-imidazol-4-yl)ethyl]piperidin-4-yl}-1-(4-fluorobenzyl)-1*H*-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 Na₂SO₄ and evaporated. Purification by HPLC yielded **4.18a** tri(hydrotrifluoroacetate) as a white solid (77 mg, 0.10 mmol, 10%). **¹H-NMR** (300 MHz, CD₃OD) 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). **¹³C-NMR** (75 MHz, CD₃OD) 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 (-, CH₂), 59.36 (-, CH₂), 53.60 (-, CH₂), 51.27 (+, CH), 45.28 (-, CH₂), 32.77 (-, 2CH₂), 25.19 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₄H₂₇FN₆ [MH⁺] 419.2354, found 419.2355.

***N*-{1-[2-(1*H*-imidazol-4-yl)ethyl]piperidin-4-yl}-1-phenethyl-1*H*-benzo[*d*]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. **¹H-NMR** (300 MHz, CD₃OD) 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). **¹³C-NMR** (75 MHz, CD₃OD) δ [ppm]: 155.16 (C_{quat}), 142.87 (C_{quat}), 139.71 (C_{quat}), 136.00 (C_{quat}), 135.99 (+, Ar-CH), 135.15 (C_{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 (-, CH₂), 53.67 (-, 2CH₂), 51.13 (+, CH), 44.33 (-, CH₂), 35.58 (-, CH₂), 32.84 (-, 2CH₂), 25.25 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₅H₃₀N₆ [MH⁺] 415.2605, found 415.2611.

***N*-[2-(1*H*-imidazol-4-yl)ethyl]-1-(4-fluorobenzyl)-1*H*-benzo[*d*]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 Na₂SO₄ 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. **¹H-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). **¹³C-NMR** (75 MHz, DMSO-*d*₆) δ [ppm]: 162.88 (C_{quat}), 159.66 (C_{quat}), 154.39 (C_{quat}), 142.70 (C_{quat}), 134.53 (+, Ar-CH), 134.26 (C_{quat}), 133.16 (C_{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 (-, CH₂), 42.45 (-, CH₂), 26.83 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₁₉H₁₈FN₅ [MH⁺] 336.1619, found 336.1625.

***N*-[2-(1*H*-imidazol-4-yl)ethyl]-1-phenethyl-1*H*-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}), 141.39 (C_{quat}), 138.11 (2C_{quat}), 134.57 (+, 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]-1*H*-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)⁵⁰ (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-1*H*-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), 107.18 (+, 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 [MH⁺] 364.2496, found 364.2500.

1-(4-Fluorobenzyl)-2-(piperazin-1-yl)-1*H*-benzo[*d*]imidazole (4.21a).⁶⁸

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}), 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).

1-Phenethyl-2-(piperazin-1-yl)-1*H*-benzo[*d*]imidazole (4.21b).⁷⁰

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]: 7.70-7.56 (m, 1H), 7.38-7.12 (m, 6H), 7.09-6.92 (m, 2H), 4.28-4.17 (m, 2H), 3.13-2.87 (m, 10H). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 158.18 (C_{quat}), 141.69 (C_{quat}), 137.98 (C_{quat}), 134.34 (C_{quat}), 128.76 (+, 2Ar-CH), 128.69 (+, 2Ar-CH), 126.83 (+, Ar-CH), 121.79 (+, Ar-CH),

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-{4-[2-(1*H*-imidazol-4-yl)ethyl]piperazin-1-yl}-1-(4-fluorobenzyl)-1*H*-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_{quat}), 160.58 (C_{quat}), 157.88 (C_{quat}), 141.42 (C_{quat}), 135.19 (C_{quat}), 134.27 (+, Ar-CH), 131.85 (C_{quat}), 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 [MH⁺] 405.2197, found 405.2206.

2-{4-[2-(1*H*-imidazol-4-yl)ethyl]piperazin-1-yl}-1-phenethyl-1*H*-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_{quat}), 141.47 (C_{quat}), 137.89 (C_{quat}), 134.26 (2C_{quat}), 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 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₄H₂₈N₆ [MH⁺] 401.2448, found 401.2449.

2-(4-Methylpiperazin-1-yl)-1-phenethyl-1H-benzo[d]imidazole (4.23).⁶⁷

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 Na₂SO₄ and evaporated. Purification by column chromatography (DCM/MeOH, 10/1), yielded a yellow oil (310 mg, 0.96 mmol, 96.0%). **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 157.96 (C_{quat}), 141.70 (C_{quat}), 137.95 (C_{quat}), 134.50 (C_{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 (-, CH₂), 50.59 (-, CH₂), 46.22 (+, CH₃), 45.66 (-, CH₂), 35.12 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₀H₂₄N₄ [MH⁺] 321.2074, found 321.2089.

4-(1H-benzo[d]imidazol-2-yl)phenol (4.24).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 Na₂S₂O₅ (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. **¹H-NMR** (300 MHz, DMSO-d₆) 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). **¹³C-NMR** (75 MHz, DMSO-d₆) δ [ppm]: 159.07 (2C_{quat}), 151.63 (C_{quat}), 139.17 (C_{quat}), 128.08 (+, 2Ar-CH), 121.57 (+, 2Ar-CH), 120.68 (C_{quat}), 115.59 (+, 2Ar-CH), 114.51 (+, 2Ar-CH). **HRMS** (EI-MS) *m/z*: calcd for C₁₃H₁₀N₂O [MH⁺] 211.0866, found 211.0872.

***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, K₂CO₃ (851 mg, 6.17 mmol) was added. The mixture was stirred for 30 min, subsequently, *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. **¹H-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). **¹³C-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 (-, CH₂), 36.79 (-, CH₂), 29.05 (-, CH₂), 28.14 (+, 3CH₃). **HRMS** (EI-MS) *m/z*: calcd for C₂₁H₂₅N₃O₃ [MH⁺] 368.1969, found 368,1977.

3-{4-[1-(4-Fluorobenzyl)-1*H*-benzo[*d*]imidazol-2-yl]phenoxy}propan-1-amine (4.26).

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 Na₂SO₄ 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%). **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) 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 (-, CH₂), 47.75 (-, CH₂), 39.13 (-, CH₂), 32.88 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₃H₂₂FN₃O [MH⁺] 376.1820, found 376.1823.

***N*-[2-(1*H*-imidazol-4-yl)ethyl]-3-{4-[1-(4-fluorobenzyl)-1*H*-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-1*H*-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 (C_{quat}), 160.38 (C_{quat}), 154.12 (C_{quat}), 142.94 (C_{quat}), 135.81 (C_{quat}), 134.66 (+, Ar-CH), 132.12 (C_{quat}), 132.08 (C_{quat}), 130.65 (+, 2Ar-CH), 127.74 (+, Ar-CH), 127.64 (+, Ar-CH), 123.03 (+, Ar-CH), 122.81 (+, Ar-CH), 122.05 (C_{quat}), 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 C₂₈H₂₈FN₅O [MH⁺] 470.2351, found 470.2354.

4-**{[1-(4-Fluorobenzyl)-1*H*-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 of 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-*d*₆) 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-*d*₆) δ [ppm]: 163.07 (C_{quat}), 160.65 (C_{quat}), 151.50 (C_{quat}), 142.69 (C_{quat}), 134.08 (C_{quat}), 133.75 (C_{quat}), 132.88 (C_{quat}), 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 C₂₀H₁₆FN₃O [MH⁺] 334.1350, found 334.1356.

tert*-Butyl [3-(4-**{[1-(4-fluorobenzyl)-1*H*-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), 1.95-1.85 (m, 2H), 1.44 (s, 9H). **¹³C-NMR** (101 MHz, CDCl₃) δ [ppm]: 163.66 (C_{quat}), 161.21 (C_{quat}), 156.09 (C_{quat}), 154.91 (C_{quat}), 151.02 (C_{quat}), 134.05 (C_{quat}), 131.24 (C_{quat}), 131.20 (C_{quat}), 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 (C_{quat}), 66.17 (-, CH₂), 45.46 (-, CH₂), 38.04 (-, CH₂), 29.55 (-, CH₂), 28.46 (+, 3CH₃). **HRMS** (EI-MS) *m/z*: calcd for C₂₈H₃₁FN₄O₃ [MH⁺] 491.2453, found 491.2463.

***N*-[4-(3-[[2-(1*H*-imidazol-4-yl)ethyl]amino}propoxy)phenyl]-1-(4-fluorobenzyl)-1*H*-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-1*H*-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 (C_{quat}), 161.00 (C_{quat}), 155.07 (C_{quat}), 152.04 (C_{quat}), 41.21 (C_{quat}), 134.86 (+, Ar-CH), 133.71 (C_{quat}), 133.39 (C_{quat}), 132.38 (C_{quat}), 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 C₂₈H₂₉FN₆O [MH⁺] 485.2460, found 485.2462.

***N'*-[1-(4-Fluorobenzyl)-1*H*-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 (C_{quat}), 154.46 (C_{quat}), 142.35 (C_{quat}), 134.67 (C_{quat}), 131.38 (C_{quat}), 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 C₁₆H₁₇FN₄ [MH⁺] 285.1510, found 285.1513.

***N'*-[1-(4-Fluorobenzyl)-1*H*-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 (C_{quat}), 160.65 (C_{quat}), 154.71 (C_{quat}), 142.57 (C_{quat}), 134.66 (C_{quat}), 131.66 (C_{quat}), 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 C₁₇H₁₉FN₄ [MH⁺] 299.1667, found 299.1670.

***N'*-[1-(4-Fluorobenzyl)-1*H*-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%). **¹H-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). **¹³C-NMR** (101 MHz, CDCl₃) δ [ppm]: 161.21 (C_{quat}), 154.30 (C_{quat}), 142.44 (C_{quat}), 134.69 (C_{quat}), 131.32 (C_{quat}), 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*¹-[2-(1*H*-imidazol-4-yl)ethyl]-*N*²-[1-(4-fluorobenzyl)-1*H*-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-1*H*-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. **¹H-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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 163.89 (C_{quat}), 160.62 (C_{quat}), 154.62 (C_{quat}), 142.06 (C_{quat}), 134.74 (+, Ar-CH), 134.54 (C_{quat}), 131.48 (C_{quat}), 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 C₂₁H₂₃FN₆ [MH⁺] 379.2041, found 379.2047.

***N*¹-[2-(1*H*-imidazol-4-yl)ethyl]-*N*²-(1-(4-fluorobenzyl)-1*H*-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 [MH⁺] 393.2197, found 393.2199.

***N*- (2- {[1- (4- Fluorobenzyl)- 1*H*- benzo[*d*]imidazol- 2- yl]amino}ethyl)- 3- (1*H*- 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-*d*₆) 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-*d*₆) 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 [MH⁺] 405.1834, found 405.1839.

***N*- [6- ({1- [4- Fluorobenzyl)- 1*H*- benzo[*d*]imidazol- 2- yl]amino}hexyl)- 3- (1*H*- 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%). **¹H-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). **¹³C-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 (-, CH₂), 42.87 (-, CH₂), 39.01 (-, CH₂), 29.75 (-, CH₂), 29.58 (-, CH₂), 26.75 (-, CH₂), 26.61 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₆H₂₉FN₆O [MH⁺] 461.2460, found 461.2465.

***N*- (2- {[1- (4- Fluorobenzyl)- 1*H*- benzo[*d*]imidazol- 2- yl]amino}ethyl)- 3- (1*H*- 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 H₂ 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. **¹H-NMR** (300 MHz, CDCl₃) 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). **¹³C-NMR** (75 MHz, CDCl₃) 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 (-, CH₂), 43.88 (-, CH₂), 40.08 (-, CH₂), 36.08 (-, CH₂), 22.65 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₂H₂₃FN₆O [MH⁺] 407.1990, found 407.1992.

***N*'-[3-(1*H*-imidazol-4-yl)propyl]-*N*'-[1-(4-fluorobenzyl)-1*H*-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 (C_{quat}), 160.57 (C_{quat}), 154.68 (C_{quat}), 142.09 (C_{quat}), 136.57 (C_{quat}), 134.56 (+, Ar-CH), 131.57 (C_{quat}), 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** (EI-MS) *m/z*: calcd for C₂₂H₂₅FN₆ [MH⁺] 393.2197, found 393.2198.

***N*'-[3-(1*H*-imidazol-4-yl)propyl]-*N*⁶-[1-(4-fluorobenzyl)-1*H*-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 (C_{quat}), 149.81 (C_{quat}), 133.67 (C_{quat}), 132.46 (C_{quat}), 130.72 (C_{quat}), 130.00 (+, Ar-CH), 129.06 (C_{quat}), 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), 25.77 (-, CH₂), 24.71 (-, CH₂), 21.01 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₆H₃₃FN₆ [MH⁺] 449.2823, found 449.2825.

2- {4- [2- (1*H*-imidazol-4-yl)ethyl]piperazin-1-yl}-6-chloro-*N*-(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 Na₂SO₄, 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. **¹H-NMR** (400 MHz, CDCl₃) δ [ppm]: 7.54 (d, *J* = 0.9 Hz, 1H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.45-7.37 (m, 2H), 7.23-7.19 (m, 1H), 7.06-7.02 (m, 1H), 6.98-6.93 (m, 1H), 6.82 (d, *J* = 0.6 Hz, 1H), 4.94 (d, *J* = 5.4 Hz, 2H), 4.03-3.96 (m, 4H), 2.85 (t, *J* = 6.7 Hz, 2H), 2.71 (t, *J* = 6.7 Hz, 2H), 2.65-2.56 (m, 4H). **¹³C-NMR** (101 MHz, CDCl₃) δ [ppm]: 158.72 (C_{quat}), 158.70 (2C_{quat}), 150.92 (C_{quat}), 141.27 (C_{quat}), 134.20 (+, Ar-CH), 133.18 (+, Ar-CH), 127.43 (+, Ar-CH), 126.71 (+, Ar-CH), 126.22 (+, Ar-CH), 125.88 (2C_{quat}), 125.40 (+, Ar-CH), 120.54 (+, 2Ar-CH), 57.92 (-, CH₂), 53.17 (-, 2CH₂), 44.06 (-, 2CH₂), 39.93 (-, 2CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₂H₂₄ClN₇S [MH⁺] 454.1575, found 454.1576.

6-Chloro-*N*²-[2-(4-methylpiperazin-1-yl)ethyl]-*N*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-Thienylmethylamine (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)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 Na₂SO₄, 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%). **¹H-NMR** (300 MHz, CDCl₃) δ [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). **¹³C-NMR** (75 MHz, CDCl₃) δ [ppm]: 159.31 (C_{quat}), 158.87 (C_{quat}), 150.50 (C_{quat}), 140.97 (2C_{quat}), 133.29 (+, Ar-CH), 126.78 (+, 2Ar-CH), 126.38 (+, Ar-CH), 125.81 (C_{quat}), 125.48 (+, Ar-CH), 120.59 (+, Ar-CH), 57.01 (-, CH₂), 55.09 (-, 2CH₂), 52.86 (-, 2CH₂), 46.03 (+, CH₃), 39.84 (-, CH₂), 38.10 (-, CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₂₀H₂₅ClN₆S [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. **¹H-NMR** (300 MHz, CD₃OD) δ [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). **¹³C-NMR** (75 MHz, CD₃OD) δ [ppm]: 160.90 (C_{quat}), 160.69 (C_{quat}), 151.49 (C_{quat}), 143.76 (C_{quat}), 134.19 (+, Ar-CH), 127.49 (+, Ar-CH), 126.92 (2C_{quat}), 126.64 (+, Ar-CH), 126.47 (+, Ar-CH), 125.58 (+, Ar-CH), 122.98 (+, Ar-CH), 76.73 (+, CH), 55.76 (-, 2CH₂), 46.30 (+, CH₃), 40.38 (-, CH₂), 33.09 (-, 2CH₂). **HRMS** (EI-MS) *m/z*: calcd for C₁₉H₂₂ClN₅S [MH⁺] 388.1357, found 388.1362.

6-Chloro-*N*⁴-(thiophen-2-ylmethyl)-*N*²-[2-(1-trityl-1*H*-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%).⁵² Compound **4.43** was synthesized from compound **3.10** (573 mg, 2.48 mmol) and

2-(1-trityl-imidazol-4-yl)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- [2- (1*H*- imidazol- 4- yl)ethyl]- 6- chloro- *N*⁴- (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-*d*₆) 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-*d*₆) 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 C₁₈H₁₇ClN₆S [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-G_{sα5}, hH₃R + G_{αi2} + β₁γ₂ or the hH₄R + G_{αi2} + β₁γ₂. General procedures for the generation of recombinant baculoviruses, culture of Sf9 cells and membrane preparation are described elsewhere.⁷² 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, K_d = 4.5 nM,⁷³ 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, K_d = 12.1 nM,⁵⁷ c = 20 nM, nonspecific binding determined in the presence of 10 μM of famotidine; H₃R: [³H]UR-PI294, specific activity 41.8 Ci/mmol, K_d = 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, K_d = 10 nM,⁵⁶ 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.⁷⁴ 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 \pm SEM of 3 independent experiments each performed in triplicate.

4.4.2.3. [³⁵S]GTP γ S binding assay^{76, 77}

The Sf9 cell membranes used in [³⁵S]GTP γ 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 MgCl₂, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each assay tube contained Sf9 membranes expressing the respective H_xR subtype (10-20 μ G-Protein/tube), 1 μ M GDP, 0.05% (w/v) bovine serum albumin, 0.2 nM [³⁵S]GTP γ 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 μ L). In case of H₁R assays the binding buffer additionally contained 150 mM of NaCl and 50 μ g/mL of saponin, H₄R assays were performed with buffer containing 100 mM of NaCl. For the determination of K_b values (antagonist mode of the [³⁵S]GTP γ S binding assay) histamine was added to the reaction mixtures (final concentrations: hH_{1/2}R: 1 μ M, hH_{3/4}R: 100 nM). Incubation was performed at 25 °C and shaking at 250 rpm for 90 min. Bound [³⁵S]GTP γ S was separated from free [³⁵S]GTP γ 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 [³⁵S]GTP γ S added was bound to the filters. Nonspecific binding was determined in the presence of 10 μ M unlabeled GTP γ S. IC₅₀ values were converted to K_b values using the Cheng-Prusoff equation.⁷⁵ EC₅₀ and K_b values from the functional GTP γ 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

1. Jacoby, E.; Bouhelal, R.; Gerspacher, M.; Seuwen, K. The 7 TM G-protein-coupled receptor target family. *ChemMedChem* **2006**, 1, 760-782.
2. Hill, S.; Ganellin, C.; Timmerman, H.; Schwartz, J.; Shankley, N.; Young, J.; Schunack, W.; Levi, R.; Haas, H. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.* **1997**, 49, 253-278.
3. Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug. Discov.* **2006**, 5, 993-996.
4. Jassal, B.; Jupe, S.; Caudy, M.; Birney, E.; Stein, L.; Hermjakob, H.; D'Eustachio, P. The systematic annotation of the three main GPCR families in Reactome. *Database* **2010**, 2010, baq018.
5. Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. The role of histamine H₁ and H₄ receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug. Discov.* **2008**, 7, 41-53.
6. Leurs, R.; Smit, M.; Timmerman, H. Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther.* **1995**, 66, 413-463.
7. Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S.-i. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000**, 275, 36781-36786.
8. Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L. Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.* **2001**, 59, 427-433.
9. Nakamura, T.; Itadani, H.; Hidaka, Y.; Ohta, M.; Tanaka, K. Molecular cloning and characterization of a new human histamine receptor, hH₄R. *Biochem. Bioph. Res. Co.* **2000**, 279, 615-620.
10. Zhu, Y.; Michalovich, D.; Wu, H.-L.; Tan, K.; Dytko, G. M.; Mannan, I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X. Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.* **2001**, 59, 434-441.
11. Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E.; Greenfeder, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W. Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.* **2001**, 296, 1058-1066.
12. Liu, C.; Ma, X.-J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N. Cloning and pharmacological characterization of a fourth histamine receptor (H₄) expressed in bone marrow. *Mol. Pharmacol.* **2001**, 59, 420-426.
13. Cogé, F.; Guénin, S.-P.; Rique, H.; Boutin, J. A.; Galizzi, J.-P. Structure and expression of the human histamine H₄-receptor gene. *Biochem. Bioph. Res. Co.* **2001**, 284, 301-309.
14. Zampeli, E.; Tiligada, E. The role of histamine H₄ receptor in immune and inflammatory disorders. *Br. J. Pharmacol.* **2009**, 157, 24-33.
15. Smits, R. A.; Leurs, R.; de Esch, I. J. Major advances in the development of histamine

- H₄ receptor ligands. *Drug discovery today* **2009**, 14, 745-753.
16. Cowden, J. M.; Zhang, M.; Dunford, P. J.; Thurmond, R. L. The Histamine H₄ Receptor Mediates Inflammation and Pruritus in Th2-Dependent Dermal Inflammation. *J. Invest. Dermatol.* **2010**, 130, 1023-1033.
 17. de Esch, I. J.; Thurmond, R. L.; Jongejan, A.; Leurs, R. The histamine H₄ receptor as a new therapeutic target for inflammation. *Trends. Pharmacol. Sci.* **2005**, 26, 462-469.
 18. Igel, P.; Dove, S.; Buschauer, A. Histamine H₄ receptor agonists. *Bioorg. Med. Chem. Lett.* **2010**, 20, 7191-7199.
 19. Marson, C. M. Targeting the histamine H₄ receptor. *Chem. Rev.* **2011**, 111, 7121-7156.
 20. Kollmeier, A.; Francke, K.; Chen, B.; Dunford, P.; Greenspan, A.; Xia, Y.; Xu, X.; Zhou, B.; Thurmond, R. The histamine H₄ receptor antagonist, JNJ 39758979, is effective in reducing histamine-induced pruritus in a randomized clinical study in healthy subjects. *J. Pharmacol. Exp. Ther.* **2014**, 350, 181.
 21. Thurmond, R. L.; Chen, B.; Dunford, P. J.; Greenspan, A. J.; Karlsson, L.; La, D.; Ward, P.; Xu, X. L. Clinical and Preclinical Characterization of the Histamine H₄ Receptor Antagonist JNJ-39758979. *J. Pharmacol. Exp. Ther.* **2014**, 349, 176-184.
 22. Cowden, J. M.; Yu, F.; Banie, H.; Farahani, M.; Ling, P.; Nguyen, S.; Riley, J. P.; Zhang, M.; Zhu, J.; Dunford, P. J. The histamine H₄ receptor mediates inflammation and Th17 responses in preclinical models of arthritis. *Ann. Rheum. Dis.* **2014**, 73, 600-608.
 23. Daugherty, B. L. Histamine H₄ antagonism: a therapy for chronic allergy? *Br. J. Pharmacol.* **2004**, 142, 5-7.
 24. Ohsawa, Y.; Hirasawa, N. The antagonism of histamine H₁ and H₄ receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. *Allergy* **2012**, 67, 1014-1022.
 25. Roßbach, K.; Wendorff, S.; Sander, K.; Stark, H.; Gutzmer, R.; Werfel, T.; Kietzmann, M.; Bäumer, W. Histamine H₄ receptor antagonism reduces hapten-induced scratching behaviour but not inflammation. *Exp. Dermatol.* **2009**, 18, 57-63.
 26. Wang, M.; Han, J.; Domenico, J.; Shin, Y. S.; Jia, Y.; Gelfand, E. W. Combined Blockade of the Histamine H₁ and H₄ Receptor Suppresses Peanut - Induced Intestinal Anaphylaxis by Regulating Dendritic Cell Function. *Allergy* **2016**.
 27. Eisen, S.; Miller, D.; Woodward, R.; Spitznagel, E.; Przybeck, T. The effect of prescribed daily dose frequency on patient medication compliance. *Arch. Intern. Med.* **1991**, 151, 1236-7.
 28. Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, 48, 6523-6543.
 29. Chen, X.; Decker, M. Multi-target compounds acting in the central nervous system designed from natural products. *Curr. Med. Chem.* **2013**, 20, 1673-1685.
 30. Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* **2008**, 51, 347-372.
 31. Shonberg, J.; Scammells, P. J.; Capuano, B. Design strategies for bivalent ligands targeting GPCRs. *ChemMedChem* **2011**, 6, 963-974.
 32. Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug.*

- Discov.* **2004**, 3, 353-359.
33. Erez, M.; Takemori, A.; Portoghese, P. Narcotic antagonistic potency of bivalent ligands which contain. beta.-naltrexamine. Evidence for simultaneous occupation of proximal recognition sites. *J. Med. Chem.* **1982**, 25, 847-849.
 34. Russo, O.; Berthouze, M.; Giner, M.; Soulier, J.-L.; Rivail, L.; Sicsic, S.; Lezoualc'h, F.; Jockers, R.; Berque-Bestel, I. Synthesis of specific bivalent probes that functionally interact with 5-HT₄ receptor dimers. *J. Med. Chem.* **2007**, 50, 4482-4492.
 35. Smits, R. A.; de Esch, I. J.; Zuiderveld, O. P.; Broeker, J.; Sansuk, K.; Guaita, E.; Coruzzi, G.; Adami, M.; Haaksma, E.; Leurs, R. Discovery of quinazolines as histamine H₄ receptor inverse agonists using a scaffold hopping approach. *J. Med. Chem.* **2008**, 51, 7855-7865.
 36. Hammer, S. G.; Gobleder, S.; Naporra, F.; Wittmann, H.-J.; Elz, S.; Heinrich, M. R.; Strasser, A. 2, 4-Diaminopyrimidines as dual ligands at the histamine H₁ and H₄ receptor—H₁/H₄-receptor selectivity. *Bioorg. Med. Chem. Lett.* **2016**, 26, 292-300.
 37. Wagner, E.; Wittmann, H.-J.; Elz, S.; Strasser, A. Pharmacological profile of astemizole-derived compounds at the histamine H₁ and H₄ receptor—H₁/H₄ receptor selectivity. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2014**, 387, 235-250.
 38. Janssens, F.; Torremans, J.; Janssen, M.; Stokbroekx, R. A.; Luyckx, M.; Janssen, P. A. New antihistaminic N-heterocyclic 4-piperidinamines. 2. Synthesis and antihistaminic activity of 1- [(4- fluorophenyl) methyl]- N- (4- piperidinyl)- 1H-benzimidazol- 2- amines. *J. Med. Chem.* **1985**, 28, 1934-1943.
 39. Smits, R. A.; Lim, H. D.; Stegink, B.; Bakker, R. A.; de Esch, I. J.; Leurs, R. Characterization of the histamine H₄ receptor binding site. Part 1. Synthesis and pharmacological evaluation of dibenzodiazepine derivatives. *J. Med. Chem.* **2006**, 49, 4512-4516.
 40. Naporra, F.; Gobleder, S.; Wittmann, H.-J.; Spindler, J.; Bodensteiner, M.; Bernhardt, G.; Hübner, H.; Gmeiner, P.; Elz, S.; Strasser, A. Dibenzo [b, f][1, 4] oxazepines and dibenzo [b, e] oxepines: Influence of the chlorine substitution pattern on the pharmacology at the H₁R, H₄R, 5-HT_{2A}R and other selected GPCRs. *Pharmacol. Res.* **2016**, 113, 610-625.
 41. Straßer, A.; Wittmann, H.-J.; Seifert, R. Ligand-specific contribution of the N terminus and E2-loop to pharmacological properties of the histamine H₁-receptor. *J. Pharmacol. Exp. Ther.* **2008**, 326, 783-791.
 42. Baumeister, P. Molecular tools for G-protein coupled receptors: Synthesis, pharmacological characterization and [³H]-labeling of subtype-selective ligands for histamine H₄ and NPY Y₂ receptors. University of Regensburg, Regensburg, 2014.
 43. Geyer, R.; Kaske, M.; Baumeister, P.; Buschauer, A. Synthesis and functional characterization of imbutamine analogs as histamine H₃ and H₄ receptor ligands. *Arch. Pharm.* **2014**, 347, 77-88.
 44. Savall, B. M.; Edwards, J. P.; Venable, J. D.; Buzard, D. J.; Thurmond, R.; Hack, M.; McGovern, P. Agonist/antagonist modulation in a series of 2-aryl benzimidazole H₄ receptor ligands. *Bioorg. Med. Chem. Lett.* **2010**, 20, 3367-3371.
 45. Rzasas, R. M.; Hu, E.; Rumfelt, S.; Chen, N.; Andrews, K. L.; Chmait, S.; Falsey, J. R.; Zhong, W.; Jones, A. D.; Porter, A. Discovery of selective biaryl ethers as PDE10A

- inhibitors: improvement in potency and mitigation of Pgp-mediated efflux. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7371-7375.
46. Martin, R. E.; Green, L. G.; Guba, W.; Kratochwil, N.; Christ, A. Discovery of the first nonpeptidic, small-molecule, highly selective somatostatin receptor subtype 5 antagonists: a chemogenomics approach. *J. Med. Chem.* **2007**, *50*, 6291-6294.
 47. Xu, H.; Yu, D.-H.; Liu, L.-L.; Yan, P.-F.; Jia, L.-W.; Li, G.-M.; Yue, Z.-Y. Small molecular glasses based on multiposition encapsulated phenyl benzimidazole iridium (III) complexes: toward efficient solution-processable host-free electrophosphorescent diodes. *J. Phys. Chem. B* **2009**, *114*, 141-150.
 48. Ting, R.; Lermer, L.; Perrin, D. M. Triggering DNAzymes with light: a photoactive C8 thioether-linked adenosine. *J. Am. Chem. Soc.* **2004**, *126*, 12720-12721.
 49. Altman, J.; Wilchek, M. 4 (5)-vinylimidazole by dehydrobromination of 1-triphenylmethyl-4-(2-bromoethyl) imidazole. *J. Heterocycl. Chem.* **1988**, *25*, 915-916.
 50. Kumar, P.; Mane, U. R.; Gupta, R. C.; Nadkarni, S. S.; Mohanan, A.; Tandon, R.; Munshi, S. 2-propene-1-ones as hsp 70 inducers. In Google Patents: 2005.
 51. Moussa, I. A.; Banister, S. D.; Beinart, C.; Giboureau, N.; Reynolds, A. J.; Kassiou, M. Design, synthesis, and structure– affinity relationships of regioisomeric N-benzyl alkyl ether piperazine derivatives as σ -1 receptor ligands. *J. Med. Chem.* **2010**, *53*, 6228-6239.
 52. Lee, Y.; Park, G. Y.; Lucas, H. R.; Vajda, P. L.; Kamaraj, K.; Vance, M. A.; Milligan, A. E.; Woertink, J. S.; Siegler, M. A.; Narducci Sarjeant, A. A. Copper (I)/O₂ Chemistry with Imidazole Containing Tripodal Tetradentate Ligands Leading to μ -1, 2-Peroxo–Dicopper (II) Species. *Inorg. Chem.* **2009**, *48*, 11297-11309.
 53. Seifert, R.; Wenzel-Seifert, K.; Bürckstümmer, T.; Pertz, H. H.; Schunack, W.; Dove, S.; Buschauer, A.; Elz, S. Multiple differences in agonist and antagonist pharmacology between human and guinea pig histamine H₁-receptor. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 1104-1115.
 54. Wenzel-Seifert, K.; Kelley, M. T.; Buschauer, A.; Seifert, R. Similar apparent constitutive activity of human histamine H₂-receptor fused to long and short splice variants of G α . *J. Pharmacol. Exp. Ther.* **2001**, *299*, 1013-1020.
 55. Schnell, D.; Burleigh, K.; Trick, J.; Seifert, R. No evidence for functional selectivity of proxyfan at the human histamine H₃ receptor coupled to defined G β /G γ protein heterotrimers. *J. Pharmacol. Exp. Ther.* **2010**, *332*, 996-1005.
 56. Schneider, E. H.; Schnell, D.; Papa, D.; Seifert, R. High Constitutive Activity and a G-Protein-Independent High-Affinity State of the Human Histamine H₄-Receptor. *Biochemistry* **2009**, *48*, 1424-1438.
 57. Baumeister, P.; Erdmann, D.; Biselli, S.; Kagermeier, N.; Elz, S.; Bernhardt, G.; Buschauer, A. [³H] UR-DE257: Development of a Tritium-Labeled Squaramide-Type Selective Histamine H₂ Receptor Antagonist. *ChemMedChem* **2015**, *10*, 83-93.
 58. Igel, P.; Schnell, D.; Bernhardt, G.; Seifert, R.; Buschauer, A. Tritium-Labeled N¹-[3-(1H-imidazol-4-yl) propyl]-N²-propionylguanidine ([³H] UR-PI294), a High-Affinity Histamine H₃ and H₄ Receptor Radioligand. *ChemMedChem* **2009**, *4*, 225-231.
 59. Slack, R.; Russell, L.; Hall, D.; Luttmann, M.; Ford, A.; Saunders, K.; Hodgson, S.; Connor, H.; Browning, C.; Clark, K. Pharmacological characterization of GSK1004723,

- a novel, long-acting antagonist at histamine H₁ and H₃ receptors. *Br. J. Pharmacol.* **2011**, 164, 1627-1641.
60. Procopiou, P. A.; Browning, C.; Buckley, J. M.; Clark, K. L.; Fechner, L.; Gore, P. M.; Hancock, A. P.; Hodgson, S. T.; Holmes, D. S.; Kranz, M. The discovery of phthalazinone-based human H₁ and H₃ single-ligand antagonists suitable for intranasal administration for the treatment of allergic rhinitis. *J. Med. Chem.* **2011**, 54, 2183-2195.
61. Daley-Yates, P.; Ambery, C.; Sweeney, L.; Watson, J.; Oliver, A.; McQuade, B. The efficacy and tolerability of two novel H₁/H₃ receptor antagonists in seasonal allergic rhinitis. *Int. Arch. Allergy Appl. Immunol.* **2011**, 158, 84-98.
62. Taylor-Clark, T.; Foreman, J. Histamine-mediated mechanisms in the human nasal airway. *Curr. Opin. Pharmacol.* **2005**, 5, 214-220.
63. McLeod, R. L.; Mingo, G. G.; Herczku, C.; DeGennaro-Culver, F.; Kreutner, W.; Egan, R. W.; Hey, J. A. Combined histamine H₁ and H₃ receptor blockade produces nasal decongestion in an experimental model of nasal congestion. *Am. J. Rhinol.* **1999**, 13, 391-399.
64. Procopiou, P. A.; Ancliff, R. A.; Gore, P. M.; Hancock, A. P.; Hodgson, S. T.; Holmes, D. S.; Keeling, S. P.; Looker, B. E.; Parr, N. A.; Rowedder, J. E.; Slack, R. J. The Discovery of Quinoline Based Single-ligand Human H₁ and H₃ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2016**.
65. Díaz, J. L.; Christmann, U.; Fernández, A.; Torrens, A.; Port, A.; Pascual, R.; Álvarez, I. s.; Burgueño, J.; Monroy, X.; Montero, A. Synthesis and Structure–Activity Relationship Study of a New Series of Selective σ 1 Receptor Ligands for the Treatment of Pain: 4-Aminotriazoles. *J. Med. Chem.* **2015**, 58, 2441-2451.
66. Ogino, Y.; Ohtake, N.; Nagae, Y.; Matsuda, K.; Moriya, M.; Suga, T.; Ishikawa, M.; Kanesaka, M.; Mitobe, Y.; Ito, J. Design, syntheses, and structure–activity relationships of novel NPY Y₅ receptor antagonists: 2-{3-Oxospiro [isobenzofuran-1 (³H), 4'-piperidin]-1'-yl} benzimidazole derivatives. *Bioorg. Med. Chem. Lett.* **2008**, 18, 5010-5014.
67. Iemura, R.; Kawashima, T.; Fukuda, T.; Ito, K.; Tsukamoto, G. Synthesis of 2-(4-substituted-1-piperazinyl) benzimidazoles as H₁-antihistaminic agents. *J. Med. Chem.* **1986**, 29, 1178-1183.
68. Musonda, C. C.; Whitlock, G. A.; Witty, M. J.; Brun, R.; Kaiser, M. Chloroquine–astemizole hybrids with potent in vitro and in vivo antiplasmodial activity. *Bioorg. Med. Chem. Lett.* **2009**, 19, 481-484.
69. Janssens, F.; Stokbroekx, R.; Torremans, J.; Luyckx, M. N-Heterocyclyl-4-piperidinamines. In Google Patents: 1980.
70. Parihar, H. S.; Suryanarayanan, A.; Ma, C.; Joshi, P.; Venkataraman, P.; Schulte, M. K.; Kirschbaum, K. S. 5-HT₃R Binding of lerisetron: an interdisciplinary approach to drug–Receptor interactions. *Bioorg. Med. Chem. Lett.* **2001**, 11, 2133-2136.
71. Takata, Y.; Tao, M.; Yokota, K. Synthesis of 2-phenylbenzimidazole derivatives. III. Condensation of o-phenylenediamine with aromatic carboxylic acids in the presence of p-toluenesulfonic acid. *Bull. Fac. Eng., Univ. Hokkaido* **1979**, 95, 81-86.
72. Schnell, D.; Brunskole, I.; Ladova, K.; Schneider, E. H.; Igel, P.; Dove, S.; Buschauer,

- A.; Seifert, R. Expression and functional properties of canine, rat, and murine histamine H₄ receptors in Sf9 insect cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2011**, 383, 457-470.
73. Straßer, A.; Striegl, B.; Wittmann, H.-J.; Seifert, R. Pharmacological profile of histaprodifens at four recombinant histamine H₁ receptor species isoforms. *J. Pharmacol. Exp. Ther.* **2008**, 324, 60-71.
74. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, 193, 265-275.
75. Yung-Chi, C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, 22, 3099-3108.
76. HILF, G.; GIERSCHIK, P.; JAKOBS, K. H. Muscarinic acetylcholine receptor-stimulated binding of guanosine 5'-O-(3-thiotriphosphate) to guanine-nucleotide-binding proteins in cardiac membranes. *Eur. J. Biochem.* **1989**, 186, 725-731.
77. Asano, T.; Pedersen, S. E.; Scott, C. W.; Ross, E. Reconstitution of catecholamine-stimulated binding of guanosine 5'-O-(3-thiotriphosphate) to the stimulatory GTP-binding protein of adenylate cyclase. *Biochemistry* **1984**, 23, 5460-5467.

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 K_i 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

α	intrinsic activity or selectivity factor
A	agonist
abs	absolute
AC	adenylyl cyclase
aq	aqueous
atm	atmosphere
Boc	<i>tert</i> -butoxycarbonyl
Boc ₂ O	di- <i>tert</i> -butyl dicarbonate
BSA	bovine serum albumin
[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
δ	chemical shift
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
dd	doublet of doublets
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d ₆	per-deuterated dimethylsulfoxide
EC ₅₀	agonist concentration which induces 50 % of the maximum response
EDC	<i>N</i> -(3-dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide hydrochloride
eq	equivalents
EtOAc	ethylacetate
Et ₂ O	diethylether
EtOH	ethanol

FCS	fetal bovine serum
G	G-Protein
GDP	guanosine diphosphate
GTP	guanosine triphosphate
GPCR	G-protein coupled receptor
h	hour(s) or human
HCl	hydrochloric acid
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum correlation
HOBt	1-Hydroxybenzotriazole hydrate
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	hertz
IC ₅₀	radioligand binding assay: ligand concentration inhibiting the binding of a radioligand by 50 %
IP ₃	inositol-1,4,5-trisphosphate
<i>J</i>	coupling constant
k	capacity factor
K _b	dissociation constant (functional assay)
KBr	potassium bromide
K ₂ CO ₃	potassium carbonate
K _d	dissociation constant (saturation binding)
KHSO ₄	potassium bisulfate
K _i	dissociation constant (competition binding)
L	liter
LiAlH ₄	Lithiumaluminiumhydrid
m	multiplet
M	molar (mol/L)
MeCN	acetonitrile
MeOH	methanol
Mel	methyl iodide
mol	mole (s)
min	minute(s)
μ	micro
mp.	melting point
HR	histamine receptor
H _x R	histamine H _x receptor (x = 1,2,3,4)
MS	mass spectrometry
n	nano or amount of substance
NaHCO ₃	sodium bicarbonate
NaI	sodium iodide
Na ₂ SO ₄	sodium sulfate
NEt ₃	triethylamine
NHS	N-hydroxysuccinimide

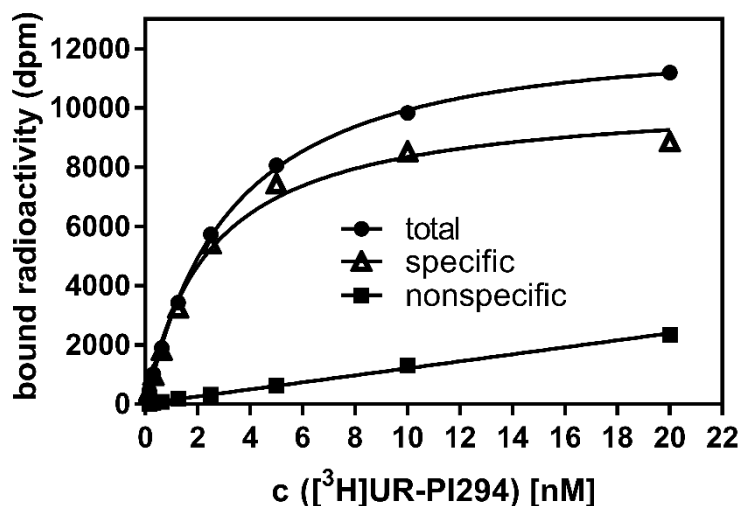
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
PE	petroleum ether
pEC ₅₀	negative decadic logarithm of the molar concentration of the agonist causing 50 % of the maximal response
Ph	phenyl
Ph ₃ P	triphenylphosphine
PIP ₂	Phosphatidylinositol-4,5-bisphosphate
PKC	protein kinase C
pK _b	negative 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	singlet or second(s)
sat.	saturated
SEM	standard error of the mean
t	triplet
t ₀	dead time
TBDPS	<i>tert</i> -butyldiphenysilyl
TBTU	2-(1 <i>H</i> -Benzotriazole-1-yl)-1,1,3,3-tetramethylammonium tetrafluoroborate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	transmembrane
TM	transmembrane
TMS	trimethylsilyl
t _R	retention time
UV	ultraviolet

6.2. Purity determined by HPLC

cpd.	t _R (min)	k	Purity (%)	Method	cpd.	t _R (min)	k	Purity (%)	Method
3.6a	9.12	2.18	97.34	b	4.16a	15.06	4.25	96.10	d
3.6b	12.54	3.37	97.26	b	4.16b	15.12	4.27	96.85	d
3.6c	14.43	4.03	98.53	b	4.17a	13.51	3.71	98.85	c
3.6d	15.35	4.35	99.94	b	4.17b	11.57	3.03	100.00	c
3.7a	11.15	2.89	96.80	a	4.17c	13.47	3.69	97.15	c
3.7b	5.43	0.89	100.00	b	4.18a	9.71	2.38	97.84	c
3.7c	16.24	4.66	98.60	a	4.18b	15.47	4.39	98.29	d
3.7d	19.16	5.68	95.33	a	4.19a	9.76	2.40	100.00	c
3.9a	18.53	5.46	97.02	c	4.19b	9.89	2.45	100.00	c
3.9b	18.81	5.55	97.57	c	4.20a	8.73	2.04	98.46	c
3.9c	19.87	5.92	96.66	c	4.20b	9.59	2.34	96.71	c
3.9d	21.29	6.42	95.18	c	4.22a	15.08	4.25	97.68	d
3.13a	12.35	3.30	100.00	a	4.22b	14.45	4.03	99.99	d
3.13b	13.81	3.81	96.35	a	4.23	9.64	2.36	98.92	c
3.15a	12.91	3.50	99.14	c	4.26	16.83	4.86	97.51	d
3.15b	13.54	3.72	97.07	c	4.27	16.01	4.57	95.13	d
3.15c	14.15	3.93	97.93	c	4.30	16.86	4.87	98.78	d
3.15d	14.78	4.15	97.60	c	4.32a	15.05	4.24	95.53	d
3.16a	13.33	3.64	97.58	c	4.32b	14.98	4.22	98.23	d
3.16b	13.63	3.75	95.85	c	4.34	15.75	4.49	99.04	d
3.16c	14.05	3.90	100.00	c	4.35a	15.09	4.26	98.11	d
3.16d	14.81	4.16	99.99	c	4.35b	16.36	4.70	95.15	d
4.14a	13.4	3.67	99.24	c	4.40	8.55	1.98	97.70	c
4.14b	13.61	3.74	99.87	c	4.41	9.12	2.18	96.19	c
4.14c	10.73	2.74	99.89	c	4.42	10.92	2.80	97.41	c
4.15b	8.59	2.00	99.93	c	4.44	11.9	3.15	99.87	c

t₀ = 2.87 min; 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 *N*¹-[3-(1*H*-imidazol-4-yl)propyl]-*N*²-propionylguanidine ([³H]UR-PI294)



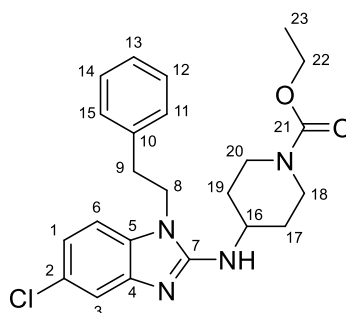
Representative *N*¹-[3-(1*H*-imidazol-4-yl)propyl]-*N*²-propionylguanidine ([³H]UR-PI294) saturation binding experiment on Sf9 insect cell membranes expressing hH₃R + G_{αi2} + G_{β1γ2} (performed in triplicate). Membranes were incubated with increasing concentrations of *N*¹-[3-(1*H*-imidazol-4-yl)propyl]-*N*²-propionylguanidine ([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 *N*¹-[3-(1*H*-imidazol-4-yl)propyl]-*N*²-propionylguanidine ([³H]UR-PI294) at a given concentration. ($K_d = 3.27 \pm 0.38$ nM from three independent experiments performed in triplicate, ref¹: 1.1 nM)

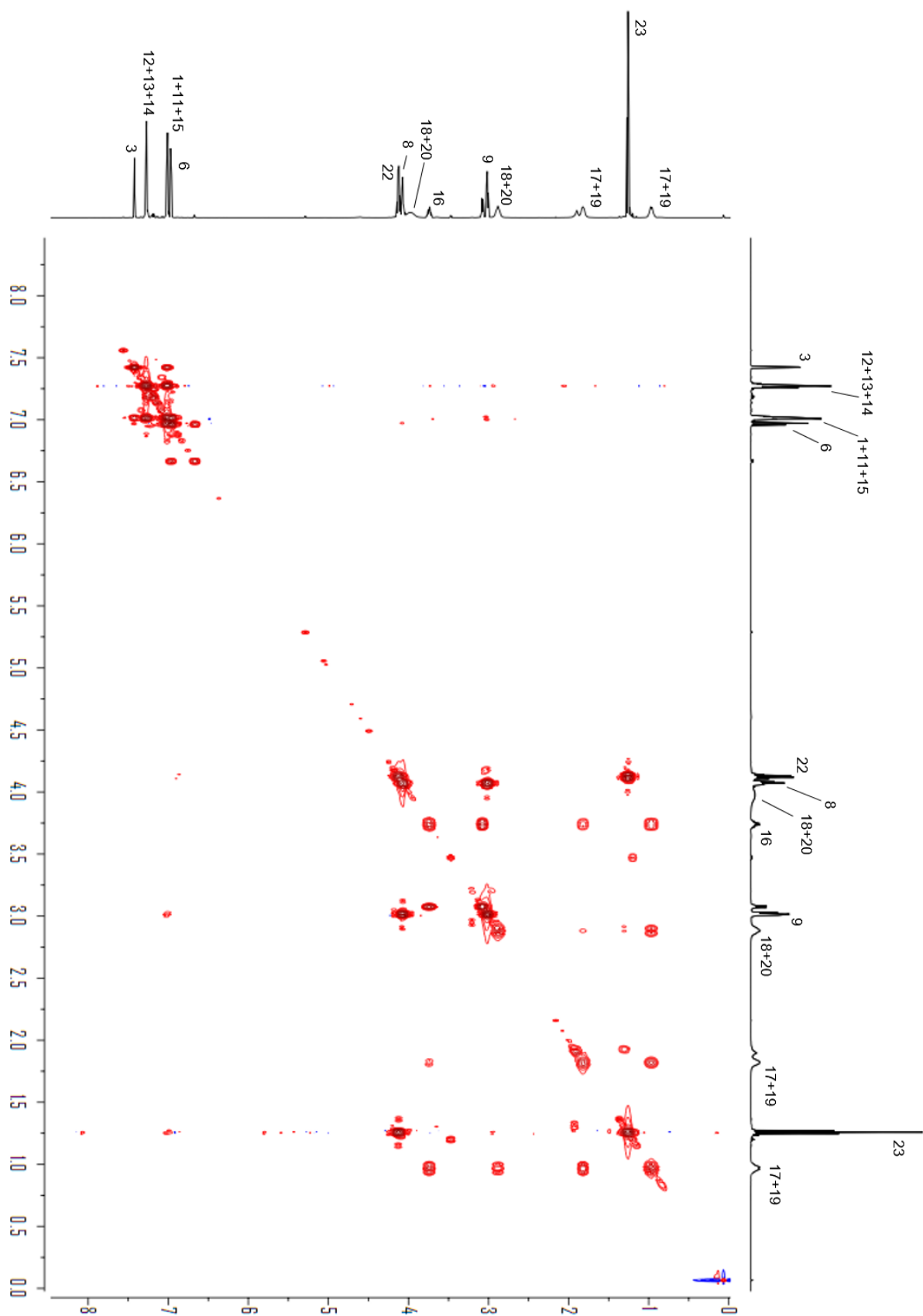
6.4. NMR spectra of selected compounds

To verify the position of Cl at benzimidazole scaffold, 2D-NMR spectra of compounds

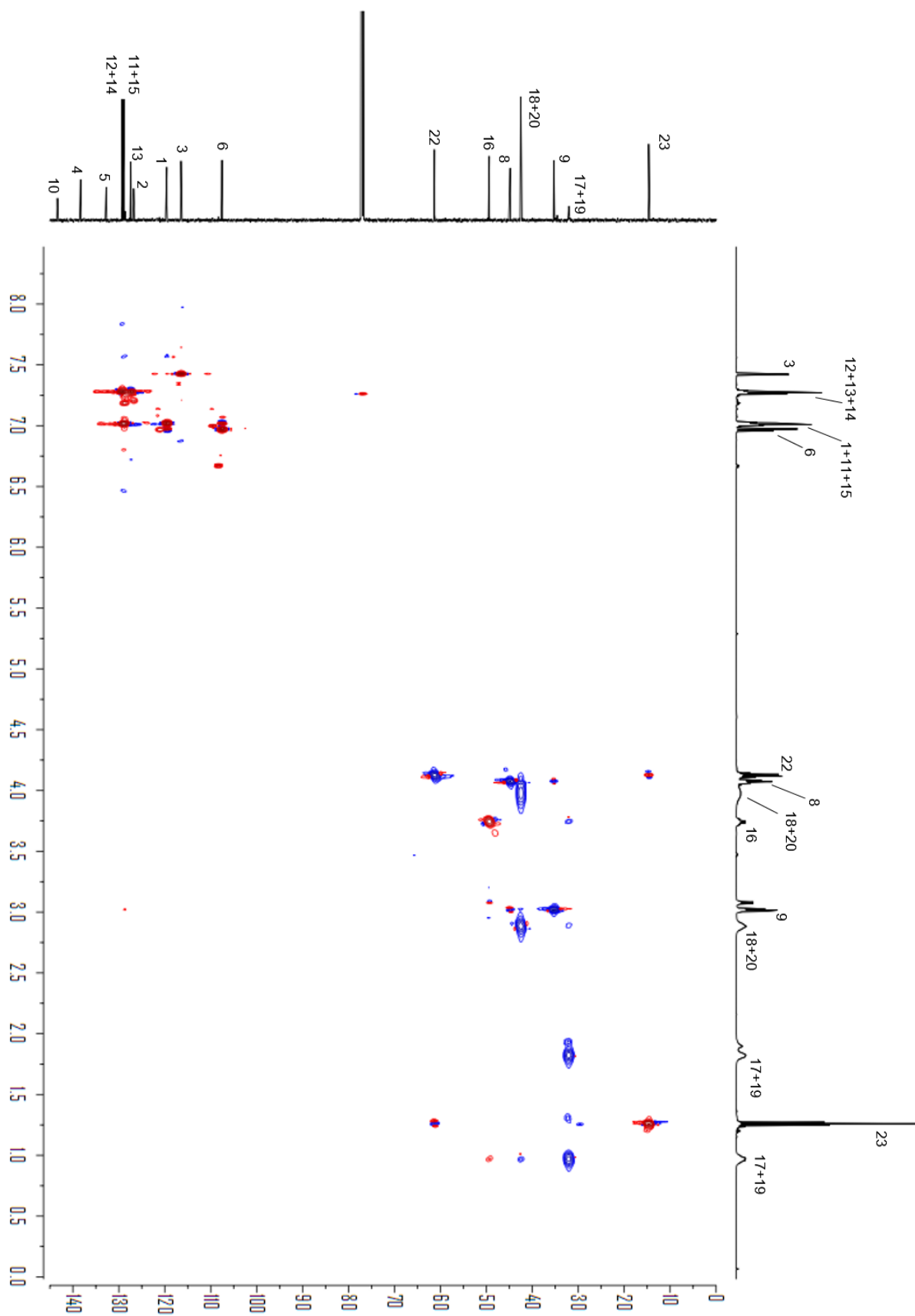
4.13a and 4.13b were recorded.

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

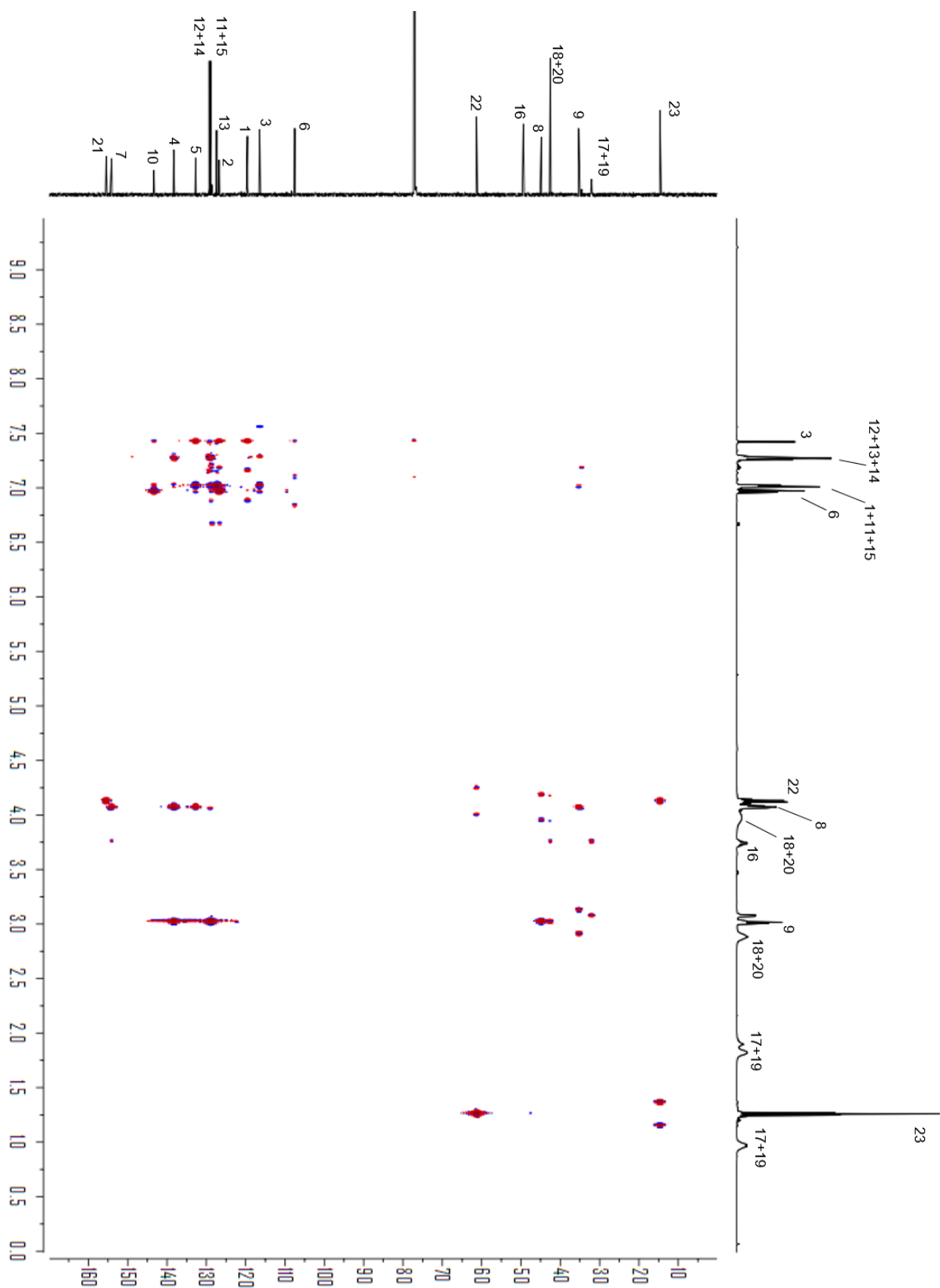


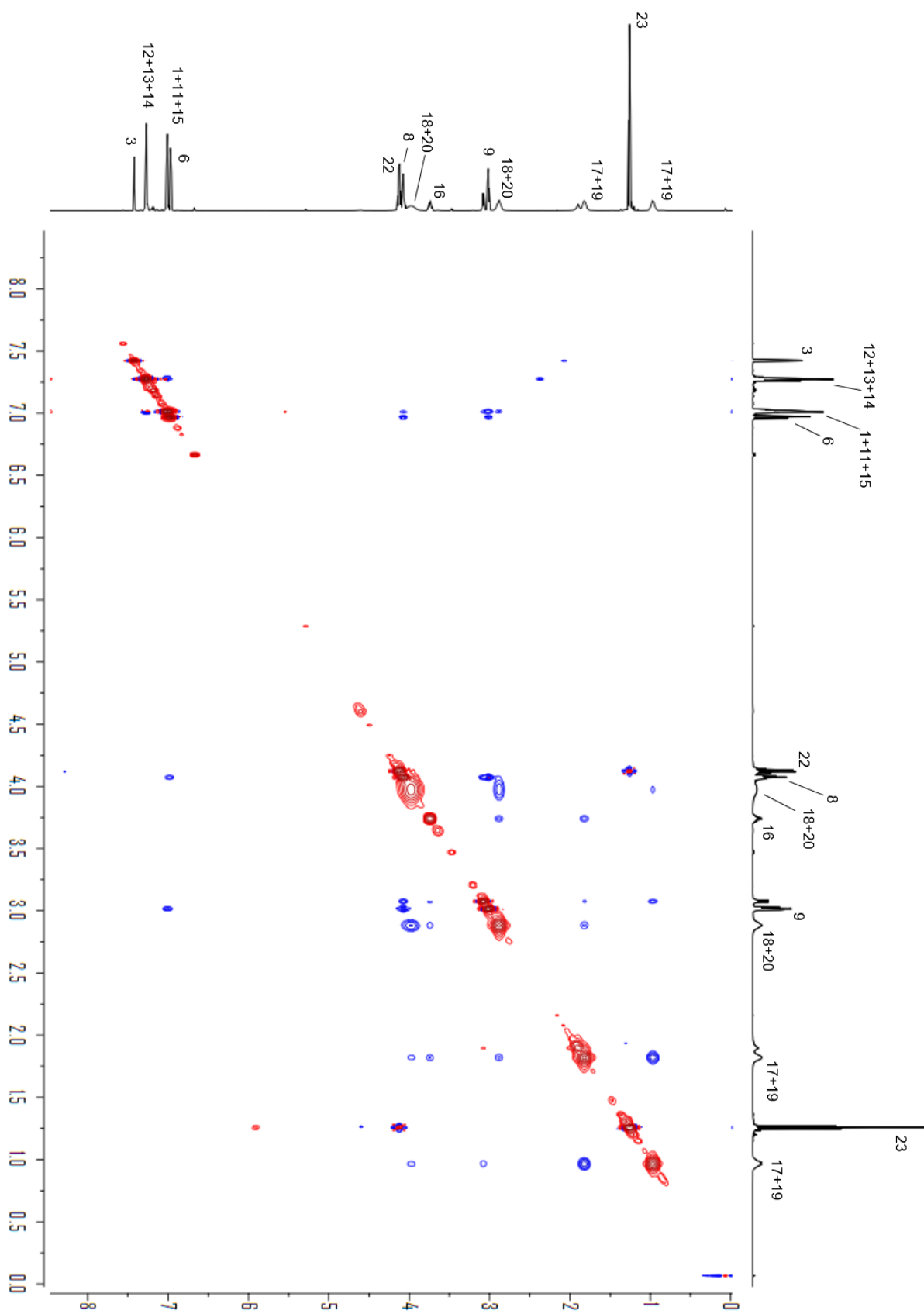


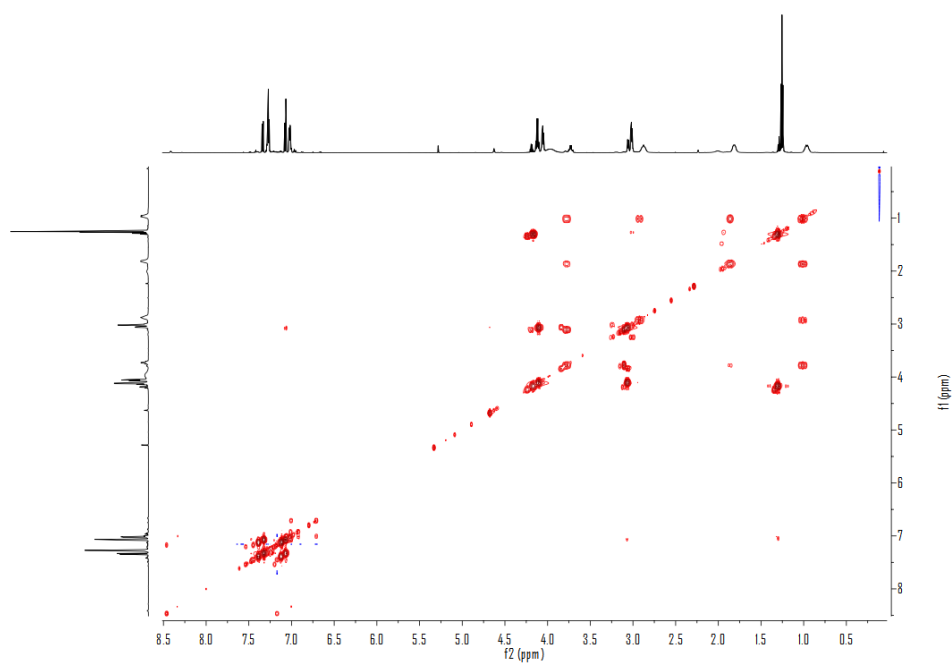
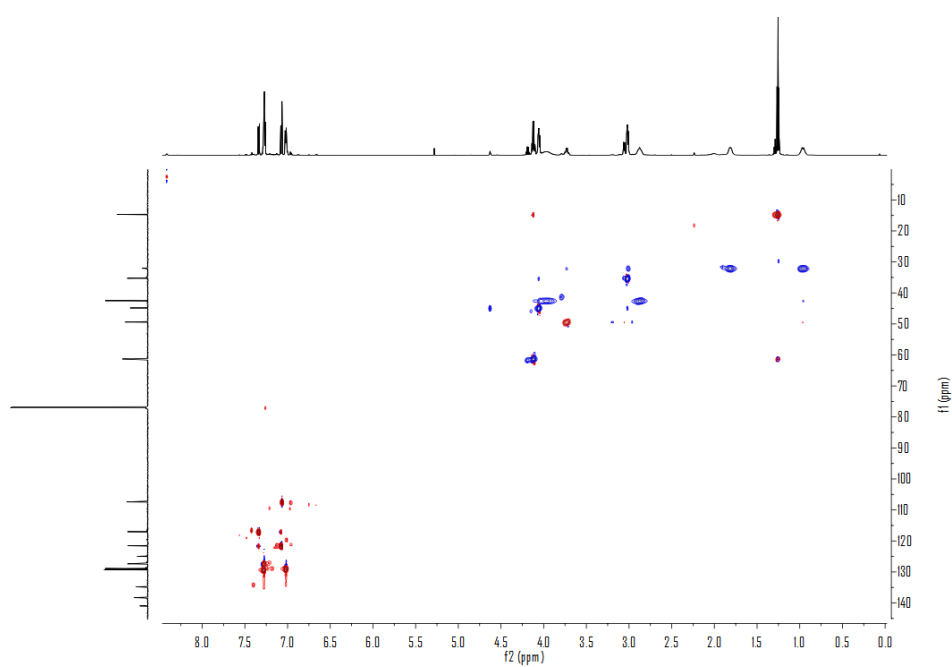
NMR spectra (COSY) of compound 4.13a.

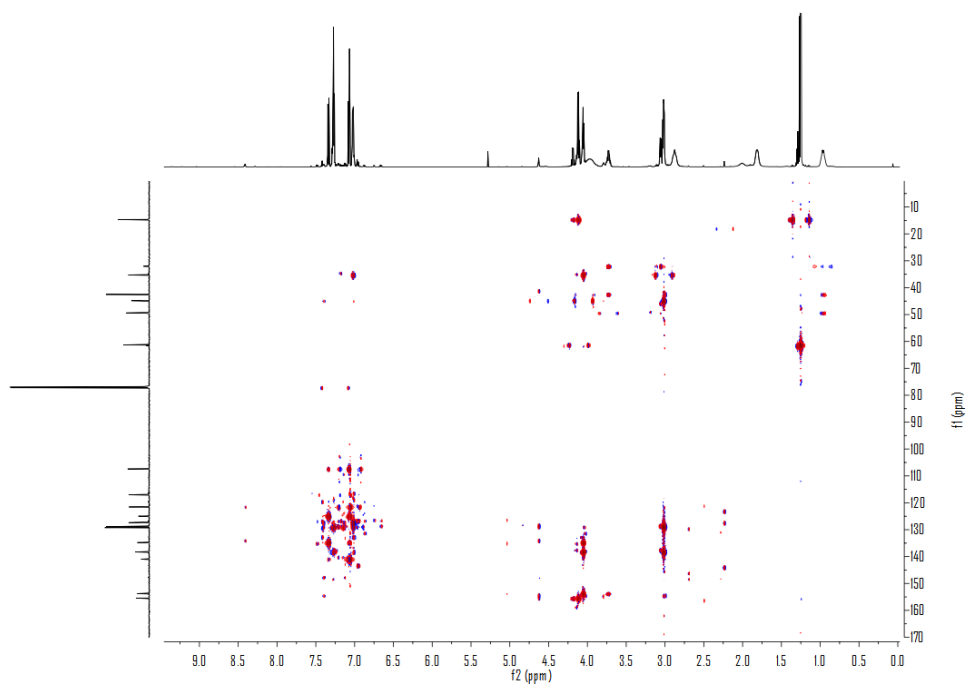


NMR spectra (HSQC) of compound 4.13a.

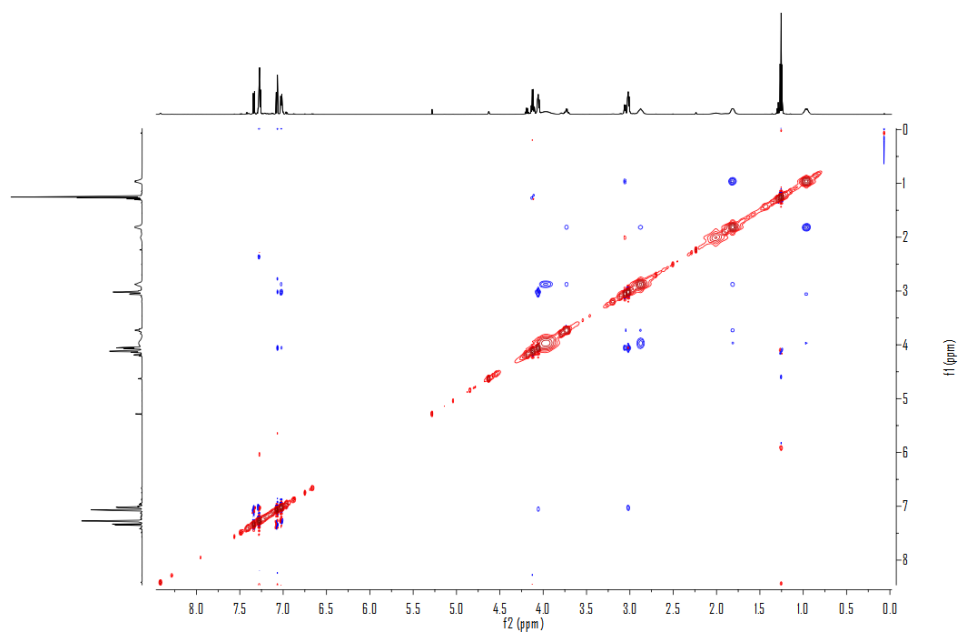
NMR spectra (HMBC) of compound **4.13a**.

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**.



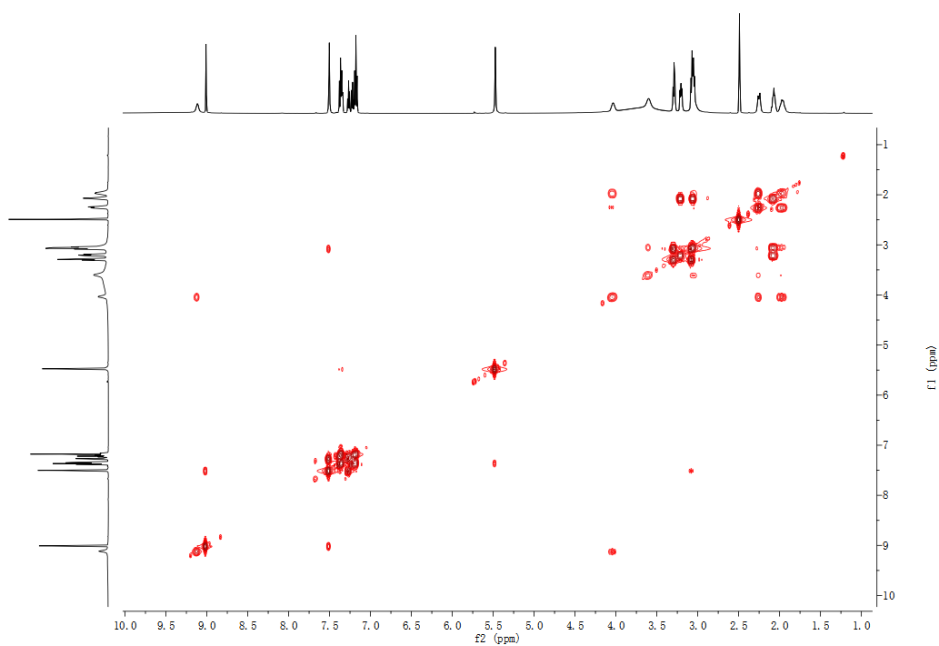
NMR spectra (HMBC) of compound **4.13b**.



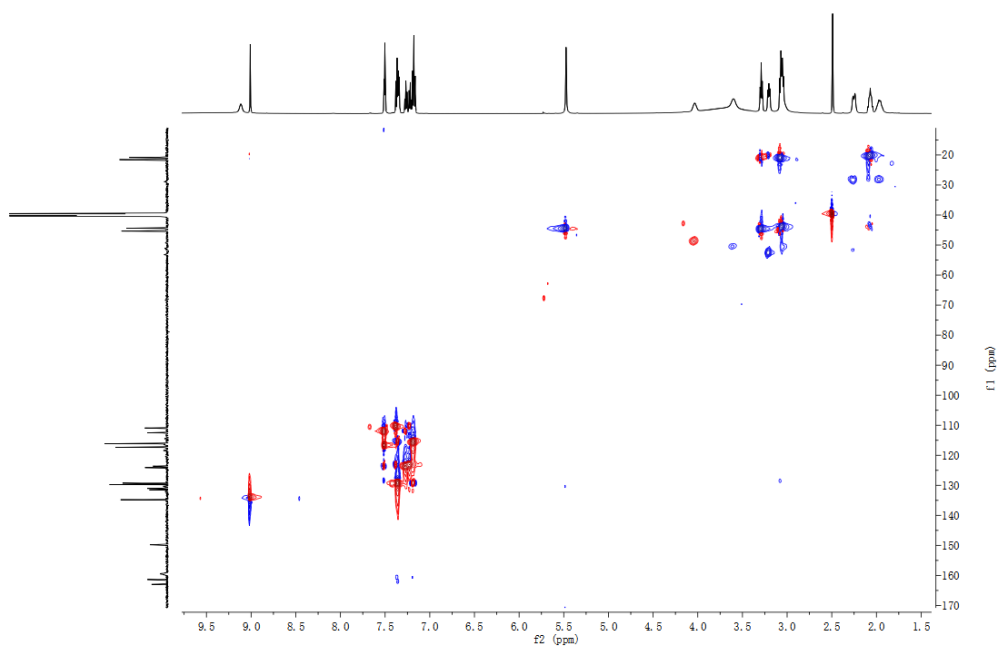
NMR spectra (NOESY) of compound **4.13b**.

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.

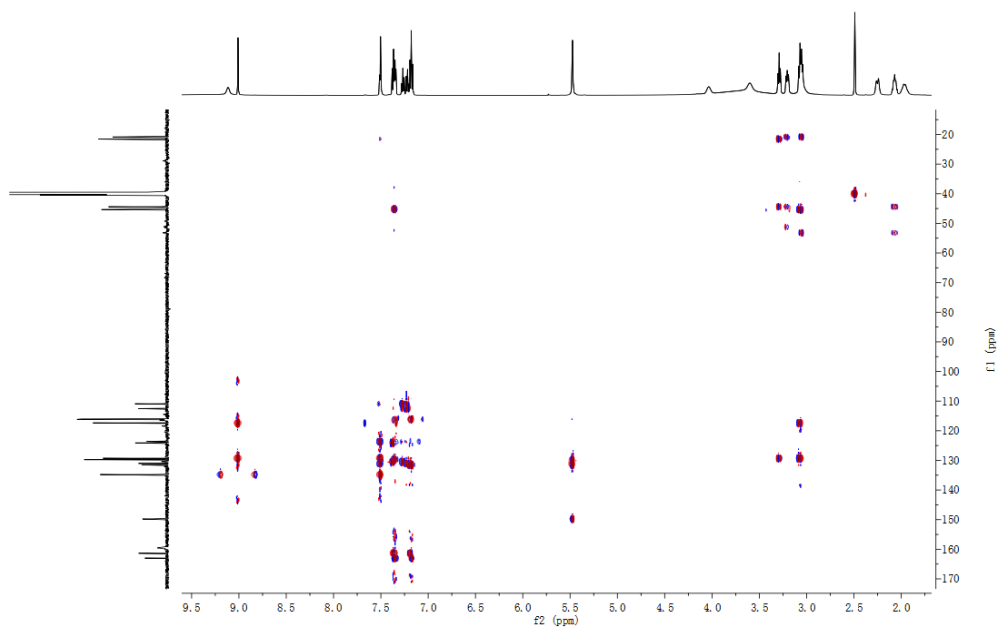
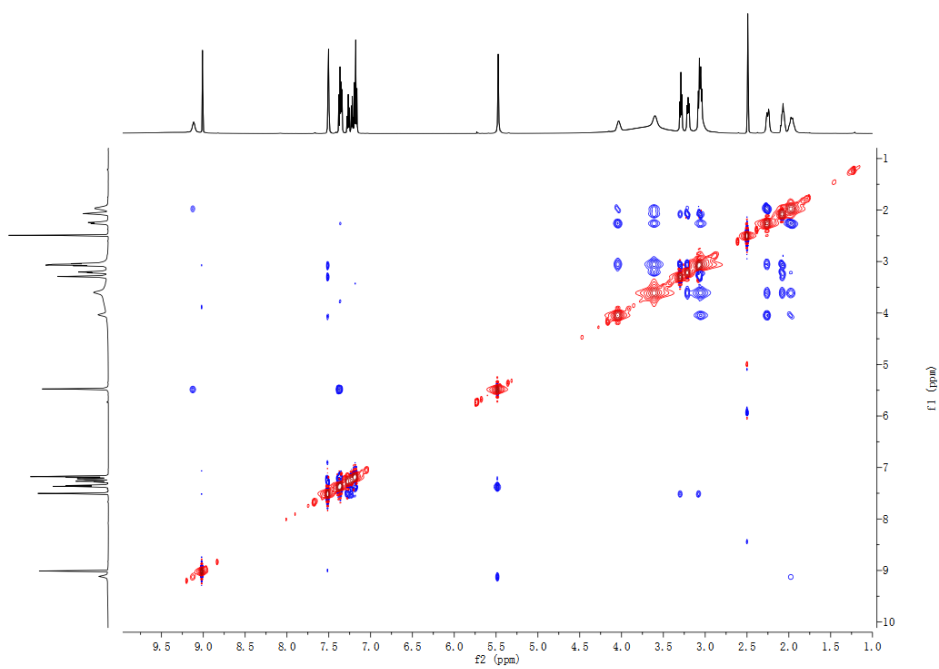
6.4.3. NMR spectra (COSY, HSQC, HMBC, ROESY) of **4.16a**:



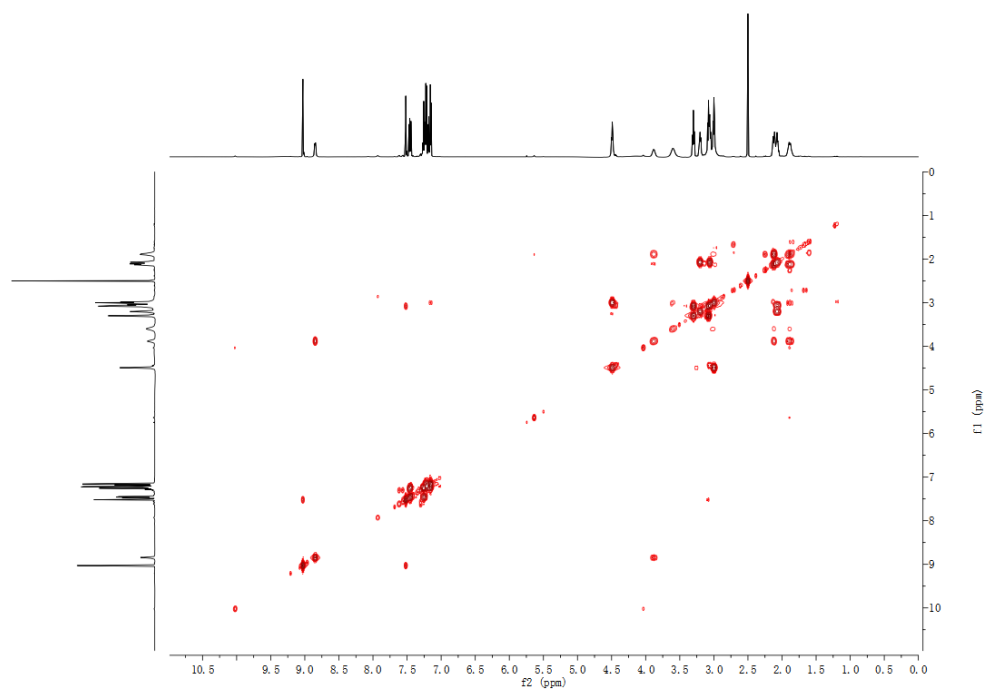
NMR spectra (COSY) of compound **4.16a**.



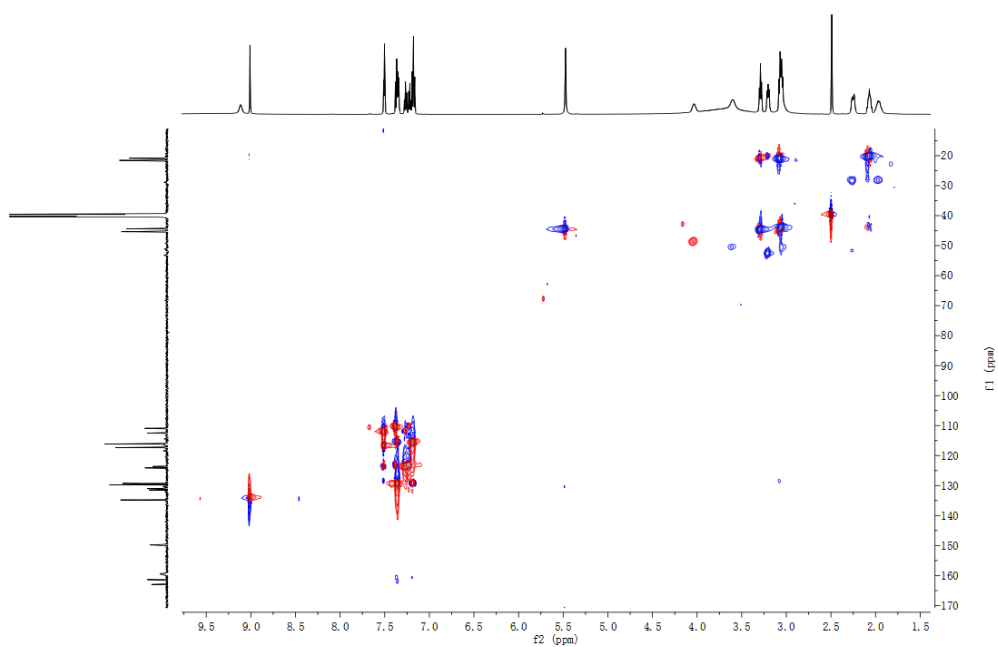
NMR spectra (HSQC) of compound **4.16a**.

NMR spectra (HMBC) of compound **4.16a**.NMR spectra (ROESY) of compound **4.16a**.

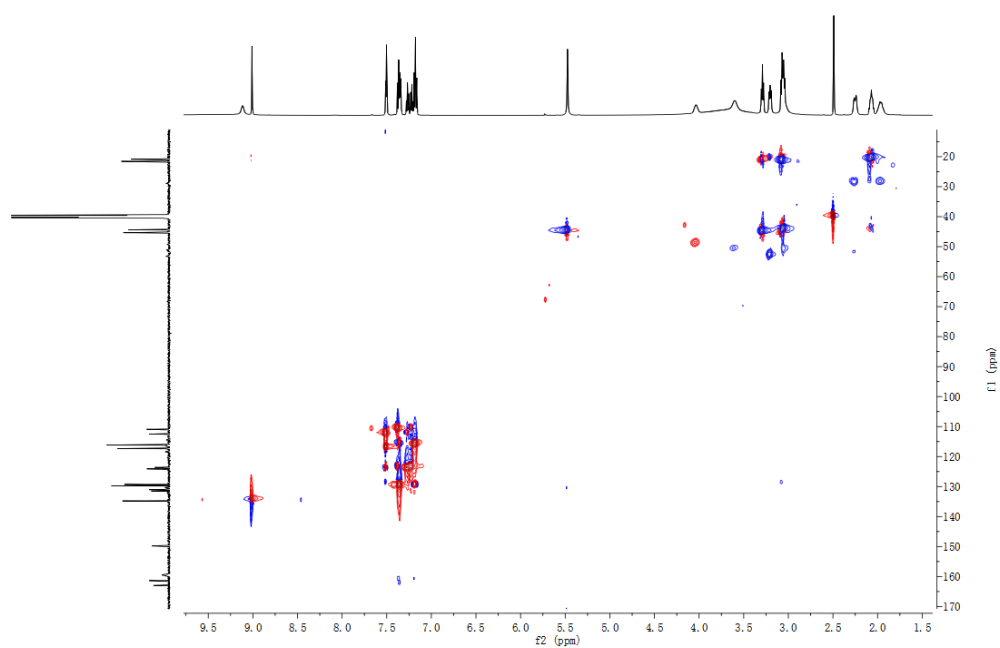
6.4.4. NMR spectra (COSY, HSQC, HMBC) of 4.16b



NMR spectra (COSY) of compound 4.16b.



NMR spectra (HSQC) of compound 4.16b.



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

6.5. References

1. Igel, P.; Schnell, D.; Bernhardt, G.; Seifert, R.; Buschauer, A. Tritium-Labeled *N*¹-[3-(1*H*-imidazol-4-yl) propyl]-*N*²-propionylguanidine ([³H] UR-PI294), a High-Affinity Histamine H₃ and H₄ Receptor Radioligand. *ChemMedChem* **2009**, *4*, 225-231.

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,