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Histamine receptor subtypes: a century of rational drug design

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1. ABSTRACT

Histamine plays an important role as neurotransmitter and chemical mediator in multiple physiological and pathophysiological processes in central and peripheral tissues. In the last century the extensive study of its actions in the human body, resulted in the identification of four G protein-coupled receptor (GPCR) subtypes (H_1R-H_4R), mediating numerous effects. The successful application of H_1R and H_2R antagonists/inverse agonists in the treatment of allergic conditions and gastric ulcer proved that these two receptors are excellent drug targets. Ligands for H_3R are currently in advanced stages of clinical development for a broad spectrum of mainly central diseases (e.g. narcolepsy, Alzheimer's disease, epilepsy and schizophrenia). Meanwhile, preclinical research in the H_4R field, focused on inflammatory and immunological processes, led to the evaluation of the first H_4R-targeting clinical candidates. Drug development for each histamine receptor subtype will be discussed with a special focus on H_3R and H_4R ligands.

2. INTRODUCTION

Since the first synthesis of histamine (HA) (1) by Windaus and Vogt in 1907 (1) and the first determination of HA action in the human body by Dale and Laidlaw in 1910 (2, 3) a century of research has passed. The biogenic amine HA, 2-(1*H*-imidazol-4-yl)ethanamine, is found ubiquitously in the mammalian organism and it acts in various physiological and pathophysiological processes as chemical mediator and neurotransmitter in peripheral and central tissues. HA is produced by the decarboxylation reaction of the semi-essential amino acid L-histidine catalyzed by the enzyme histidine decarboxylase or aromatic decarboxylase. Mast cells and basophils are important sources of HA, which is released from granule stores in response to several triggers. Additionally, other cell types, including neurons, produce and release HA (4). Two major HA inactivation mechanisms can be distinguished: The ubiquitous imidazole methylation by histamine *N*tele-methyltransferase and the peripheral oxidation by diamine oxidase (5). Under physiological

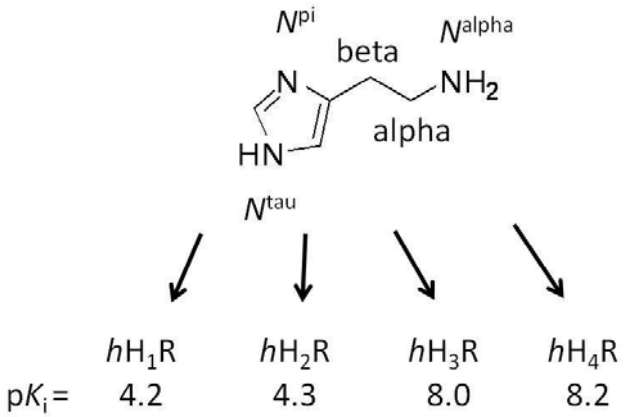

Figure 1. Histamine (1) nomenclature according to Black and Ganellin and histamine affinities to the HR subtypes.

onditions the monocationic form of HA, where the primary aliphatic amine (pK_a= 9.4) is protonated and the amidine moiety of the imidazole ring remains unchanged (pK_a = 5.8) predominates (96%) (6). For the imidazole ring two tautomeric forms (Fig.1) can be distinguished to *N*pi- and *N*tele-histamine according to Black and Ganellin (7). Additionally two conformers depending on the flexible ethylspacer exist. These conformational changes allow multiple arrangements of the acceptor/donor system and the basic moieties, which result in a variety of pharmacophoric hypotheses and possible binding modes of HA to its targets.

The pleiotropic effects of HA are mediated *via* four G protein-coupled receptor (GPCR) subtypes (H_1R–H_4R), which differ in their distribution, ligand binding, signaling pathways and functions (8). The first three subtypes have been characterized by classical pharmacological methods, using ´selective´ agonists and antagonists, whereas meanwhile all corresponding genes have been cloned in humans and in many other species. HA acts as a full agonist on the receptors with subtype-specific differences in affinity (Figure 1). Regarding human receptor subtypes (hH$_1$R–hH$_4$R), affinity of HA at the hH$_1$R and hH$_2$R is low, whereas receptor binding at the hH$_3$R and hH$_4$R is higher (9). In contrast to the ubiquitously expressed H_1R and H_2R subtypes, H_3R and H_4R have a more distinct expression pattern on neuronal and hematopoietic cells, respectively (10). Molecular aspects, distribution pattern, physiological relevance, (potential) therapeutic applications and ligands of the human histamine receptors (*h*HR) are summarized in Table 1.

The H_1R plays an important role in wakefulness, food intake and inflammatory responses, whereas the H_2R mediates gastric acid secretion. Antagonists of hH$_1$R and hH$_2$R reached blockbuster status in the therapy of allergic conditions and gastric ulcer, respectively. During the last century of HA research the knowledge of HA effects in the mammalian organism has increased. At the time of discovery some of these effects could not be linked to any known HA receptor subtype, consequently leading to the identification of the H_3R in 1983 (11) and in 2000 to the cloning of the H_4R based on its homology to the H_3R sequence (12). The H_3R controls HA-mediated neurotransmission by controlling the release of HA and other neurotransmitters. Especially antagonists/inverse agonists, designed for the treatment of a number of central disorders and for peripheral conditions such as allergic rhinitis, have entered late clinical phases (13). Preclinical evaluation of the hH$_4$R indicates its therapeutic potential as a drug target for inflammatory and immunological diseases (14) while the first H_4R ligand (UR-63325) recently entered clinical trials (15).

All *h*HRs exhibit varying degree of constitutive activity (16-19), generated by constitutively active conformations that are pre-coupled to G proteins, also found in a variety of other GPCRs (20). The extent of constitutive activity greatly differs among species, cell types, receptor isoforms and receptor coupling. This spontaneous activity in the absence of any ligand indicates the importance of the receptors in maintaining physiological balance (21). For the hH$_3$R at least 20 splice variants have been identified (16), whereas only three hH$_4$R isoforms have been described until today. In addition, species variability results in differences in potency of HR ligands, which makes the interpretation of results from *in vitro* and *in vivo* studies rather complicated. More information on species dependent isoform-specific receptor action is needed, especially for the H_4R, where the rodent homologue shows only 65–70% homology to the hH$_4$R sequence (22).

Considering the functionality of HR ligands the time of their description has to be taken into account as their classification to agonists and antagonists is still relative. With the discovery of new HR subtypes and the finding of constitutive activity (16-19) many ligands are re-classified. Following the identification of the H_4R, especially imidazole-containing compounds (methylhistamines, imetit, immepip, clobenpropit, ciproxifan, proxyfan and thioperamide), originally designed as selective H_3R ligands, were re-evaluated for their H_4R affinities and efficacies. Taking the endogenous ligand as a

Table 1. Characterization of human histamine receptor subtypes (*h*H1R – *h*H_4R)

	***h*H$_1$R**	***h*H$_2$R**	***h*H$_3$R**	***h*H$_4$R**
Postulation/Discovery	1919 (3)	1966 (40) /1972 (41)	1983 (11)	1977 (234), 1994 (136), 2000-2001 (12, 138-141, 143)
Cloning	1994 (24)	1991 (42)	1999 (63)	2000-2001 (12, 138-141, 143)
Chromosomal gene location (235)	3q25	5q35.2	20q13.33	18q11.2
Amino acids[a] (235)	487	359	445	390
Isoforms			> 20 (71, 72)	> 3 (145)
G protein coupling (8, 58, 236)	$Galpha_{q/11}$	$Galpha_s$	$Galpha_{i/o}$	$Galpha_{i/o}$
Constitutive activity	+ (17)	+ (53)	++ (16, 73, 74)	++ (19, 140)
Signal transduction (8, 58, 236)	PLC↑, Ca^{2+}↑	cAMP↑	cAMP↓, Ca^{2+}↑, MAPK↑	cAMP↓, Ca^{2+}↑, MAPK↑
Tissues (8, 237, 238)	Ubiquitous (mainly lung, CNS, blood vessels)	Ubiquitous (mainly stomach, heart, CNS)	Neurons (CNS and PNS)	Bone marrow, hematopoietic cells
Physiological relevance (8, 237, 238)	Bronchoconstriction, vasodilation, food intake, sleep-wake regulation	Gastric acid secretion	Neurotransmitter release (→ sleep-wake regulation, attention/cognition, food intake)	Immune responses (→ chemotaxis, IL-, IFN-modulation)
Pathophysiological conditions	Allergic reactions, emesis, sleep-wake disorders (8, 33, 239, 240)	Gastric ulcers (4, 8, 47, 241)	Cognitive impairement, schizophrenia, sleep-wake disorders, epilepsy, pain, etc. (10, 13)	Inflammatory diseases (allergy, asthma, pruritus, arthritis), pain, etc. (10, 14)
Reference agonists (235)	Betahistine, histaprodifen	Arpromidine, impromidine	R-alpha-Methylhistamine, imetit, immepip	4-Methylhistamine, VUF 8430
Reference antagonists/inverse agonists (235)	Diphenhydramine, pyrilamine, mepyramine	Cimetidine, tiotidine, famotidine, ranitidine	Ciproxifan, thioperamide, pitolisant	JNJ-7777120, thioperamide, ST-1012 (199)

Abbreviations: cAMP, cyclic adenosine monophosphate; CNS, central nervous system; IFN, interferone; IL, interleukin; MAPK, mitogen-activated protein kinase; PL, phospholipase; PNS, peripheral nervous system. +, ++: extent of constitutive activity [a] amino acid number given for the longest subtype

potent template, this has been a broad playground for medicinal chemists in the development of novel lead structures with higher potency and higher selectivity for the desired target (Figure 2).

3. HISTAMINE H_1 RECEPTOR

The H_1R and its involvement in allergic diseases are in the focus of research since the early 19th century and the ´antihistamines´ are still clinically successful and widely available. However, structural information about the receptor was first gained in 1991 by expression cloning of the cDNA encoding bovine H_1R protein in *Xenopus* oocytes (23). The *h*H_1R cDNA sequence (24) and the intronless genes encoding the receptor proteins in humans and other species were described soon thereafter. Receptor proteins of different species vary only slightly in length; they are highly homologous and mainly consistent in their pharmacology. The *h*H_1R gene is localized on chromosome 3 (25) and the receptor sequence consists of 487 amino acids (Table 1). Several DNA-binding motifs, including potential glucocorticoid responsive elements, were found by analysing the 5'-flanking region of the gene.

The H_1R is ubiquitously expressed and mediates its effects by $G_{q/11}$ activation *via* phospholipase C (PLC) and to a minor degree by PLA_2 and PLD. Activation of the receptor leads to an increase of inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG) associated with an increase of the intracellular Ca^{2+} concentration followed by activation of protein kinase C (PKC). Other signaling pathways, such as stimulation of the adenylyl cyclase with formation of cAMP and NF-kappaB cascade lead to a release or increase in expression of (pro)inflammatory mediators (P-selectin, ICAM-1, VCAM-1, iNOS, IL-1beta, IL-6, TNF-alpha, GM-CSF) (26, 27).

H_1Rs are distributed in a wide variety of tissues such as the central nervous system (CNS), smooth muscles, gastrointestinal tract, cardiovascular system, endothelial cells and lymphocytes (28). The effects mediated by HA *via* the H_1R can be distinguished in peripheral and central physiological and pathophysiological processes. In airways, intestine and arteries activation of the receptor leads to contraction of smooth muscles. Dilatation of arterioles and capillaries results in reduction of blood pressure. Under allergic conditions HA is released from its cellular stores (i.e. mast cells). The typical immediate responses of type I allergic reactions, such as redness, swelling and itching, are mainly caused by H_1R activation, whereas the increase in vascular permeability, responsible for swelling and itching, is caused by the stimulation of afferent neurons. In late phase reactions inflammatory events are influenced by HA, including the activation of eosinophil granulocytes. Enhancement of wakening status, vomiting and food intake are central effects of the H_1R activation (29). Considering the variety of HA-mediated effects *via* the H_1R, the great therapeutic potential of H_1R ligands becomes apparent.

3.1. Histamine H_1 receptor agonists

In spite of the outstanding clinical role of H_1-antihistamines, H_1R agonists are mainly used only as experimental pharmacological tools (30). A useful approach for developing selective H_1R agonists was the replacement of the imidazole moiety of HA. 2-(Thiazol-2-yl)ethanamine (2) (Figure 3) and pyridylethylamine are

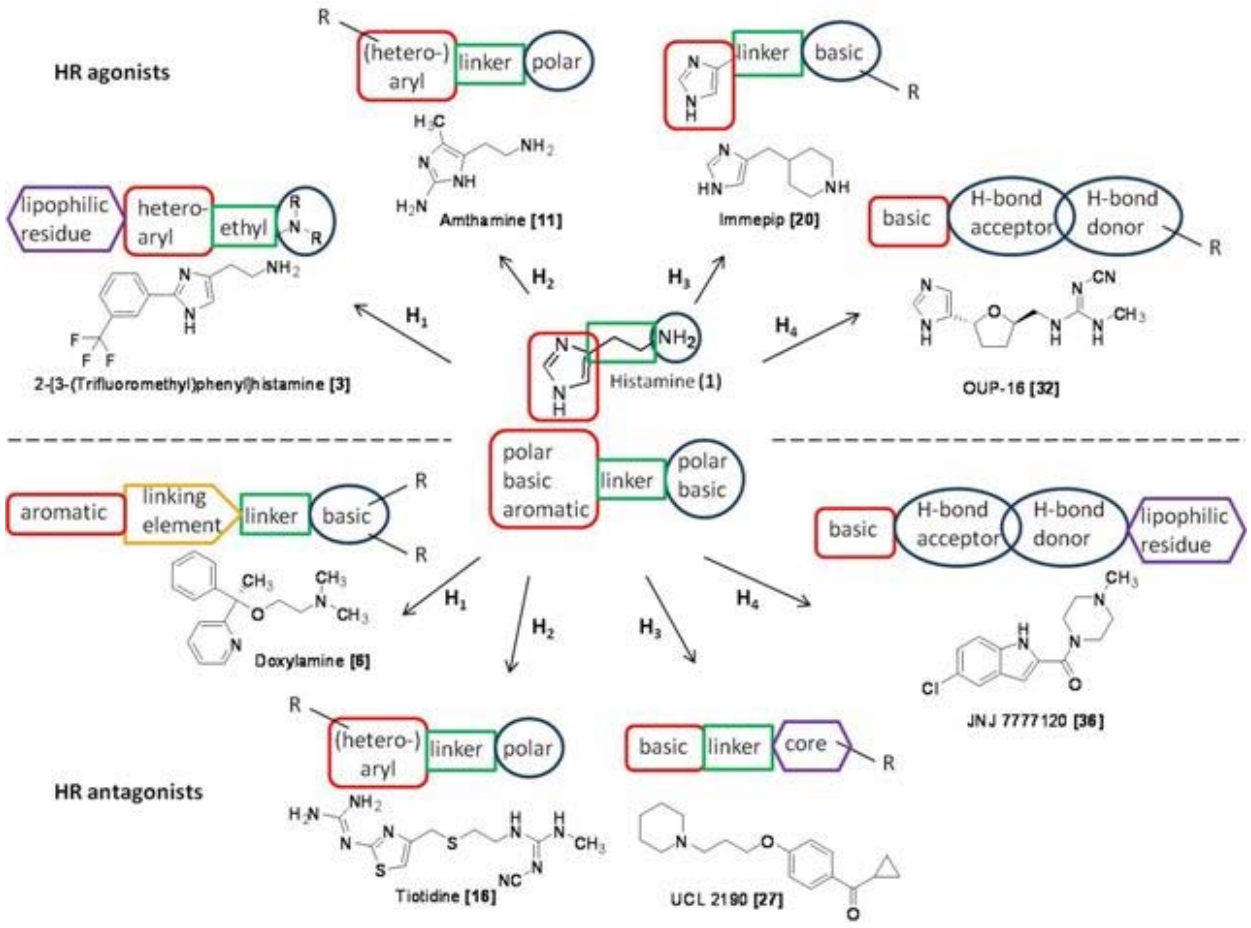

Figure 2. General structural blueprint based on the endogenous ligand histamine.

examples with some H_1R preference. A higher selectivity and affinity was achieved by HA derivatives substituted at the 2-position of the imidazole moiety such as 2-(3-(trifluoromethyl)phenyl)histamine (3) (Figure 2) and suprahistaprodifen (4) (Figure 3). Betahistine, a moderately potent H_1R agonist with additional H_3R antagonist properties, is therapeutically used in Ménière's disease (31).

3.2. Histamine H_1 receptor antagonists

In 1933 and 1937 the first compounds with HA antagonistic character were published by the group of Bovet (32). At first, H_1R antagonists (Figure 3), such as diphenhydramine (5), were used in the treatment of allergic conditions (Table 1). Due to central effects, today their indications are mainly restricted to sleep disorders (e.g. doxylamine (6) (Figure 2)) and motion sickness or they are in general used as antiemetic/antikinetic drugs (e.g. diphenhydramine (5)) (33). Many of these compounds exhibit additional pharmacological activities on other receptor systems and are used as neuroleptics with anticholinergic action. The increase in weight gain of some neuroleptics, such as olanzapine, can be directly correlated to H_1R antagonist properties (34). Due to structural similarities to anaesthetics, numerous H_1R antagonists can act as local anaestetics in high doses and are used in the treatment of urticaria and itch. After the recognition of the constitutive activity of the H_1R, most H_1R antagonists were newly characterized in pharmacological *in vitro* studies as inverse agonists inducing a reduction of basal receptor activity (27). Therefore, using the term ´H_1-antihistamines´, which also includes neutral antagonists, seems more coherent.

Differentiation of the H_1-antihistamines (Figure 3) in first and second generation drugs is based on the existence or absence of central effects. Whereas the easily brain penetrating older compounds, such as bamipine, dimetindene, doxylamine (6) and mepyramine (7), cause sedation, newer drugs such as cetirizine (8), loratadine (9) and fexofenadine offer low or even absent brain penetration. Minor structural variations resulted in second generation H_1-antihistamines, offering enhanced receptor selectivity, increased affinity, optimized kinetics, reduced or lacking central effects and additional beneficial non-H_1 mediated effects. Higher hydrophilicity or the affinity to efflux systems such as the P-glycoprotein (P-gp) or organic anion transporters (35) seem reasonable causes for poor brain penetration. Blockade of voltage-dependent human ether-a-go-go related gene potassium channels (*h*ERG),

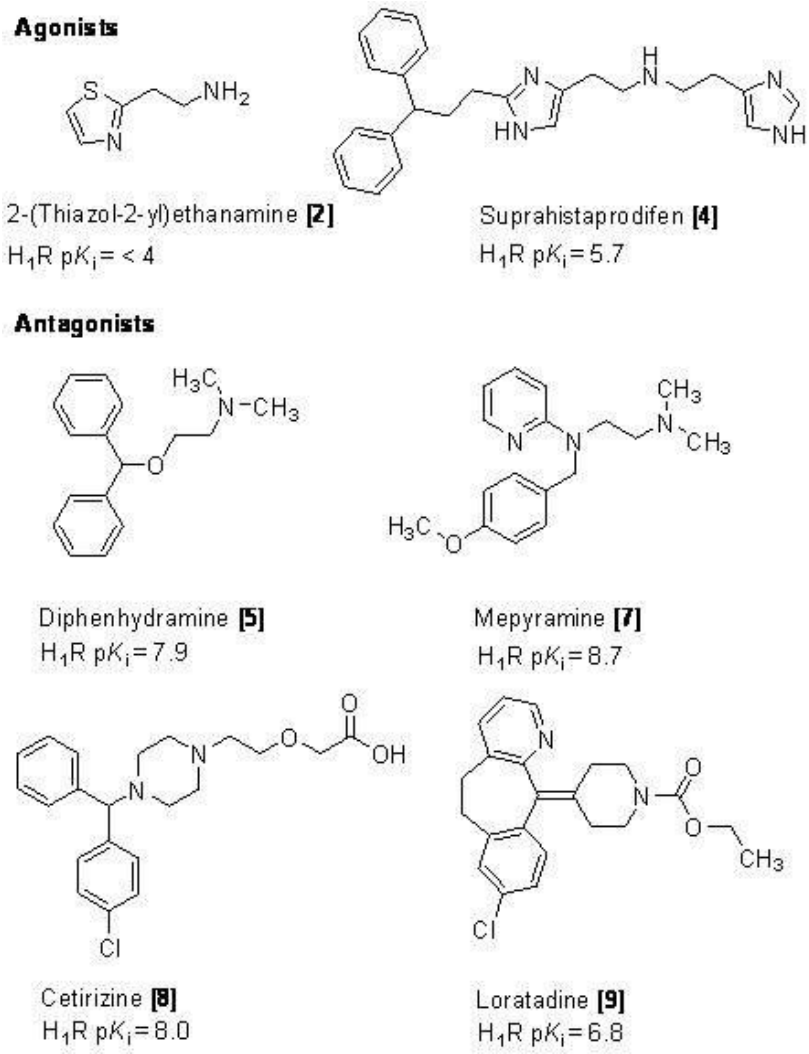

Figure 3. Representative histamine H_1 receptor ligands.

which leads to prolonged QT time and torsades de pointes arrhythmia, is a drawback for some second generation drugs (36). Due to the potential metabolic accumulation caused by inhibition of cytochrome P_{450} (CYP) enzyme CYP3A4, terfenadine and astemizol were withdrawn from the over-the-counter (OTC) market. Inhibition of mast cell degranulation (mast cell stabilization) by mizolastine, loratadine (9) and ketotifen adds another beneficial property, useful in the treatment of allergy. Anti-inflammatory 5-lipoxygenase inhibiting activity was additionally shown for mizolastine. Utilizing the brain penetration effect, first generation H_1-antihistamines are excellent tools for pharmacological investigations, including *in vivo* CNS studies with radioligands such as (^{3}H)mepyramine (37). However, species differences with regard to affinity present a major problem for many compounds.

The general pharmacophore for H_1R antagonists consists of a basic functionality, which is connected *via* different linkers to an aromatic lipophilic moiety (Figure 2). Most compounds contain a tertiary amine as basic moiety, except compounds with basic guanidine and amidine groups. Diphenyl or heteroaryl motifs are mostly used as aromatic groups. According to the linking group the majority of structures can be divided to the ethylenediamine, colamine and propylamine types (38). Due to the chirality of numerous H_1-antihistamines, stereochemical differentiation in enantiomeric/diastereomeric compounds led to pharmacologically more effective compounds such as clemastine. Mutational studies on H_1R indicated aromatic amino acids in the putative transmembrane helix 4 and 5 (Trp167, Phe433, Phe436) being responsible for binding and stereospecific interactions. Additionally, a basic amino acid (Lys200) was found to interact with the carboxylic acid structures of some second generation drugs (39).

Metabolism played a major role in H_1-antihistamine development. While many of the newer compounds are active metabolites of already existing drugs, such as cetirizine (8) and fexofenadine which are

metabolites of hydroxyzine and terfenadine, respectively, the antiallergic activity of loratadine (9) and ebastine is mainly attributed to their active metabolites produced by distinct presystemic metabolism.

4. HISTAMINE H_2 RECEPTOR

The first antihistaminergic compounds introduced by Bovet and Straub were not able to antagonize all HA-induced effects. In *in vitro* studies, the effect of HA on heart function, uterus and gastric acid secretion remained unaffected, leading to the assumption that HA may mediate its effects *via* more than one target. In 1966, Ash and Schild postulated two distinct subtypes (H_1R, H_2R) (40) which were finally proven by James Black and coworkers with the successful development of selective H_2R antagonists in 1972 (41). Sir James Black, Nobel laureate physican and pharmacologist, who died in March 2010, changed the face of medicine with his pioneering research in rational drug design.

The H_2R was cloned by Gantz *et al.* in canine gastric parietal cells based on known sequence similarity of various GPCRs (42). The genes encoding for rat, human, guinea-pig and mouse H_2R were identified afterwards. The chromosomal localization of the *h*H_2R was determined on chromosome 5 (43). The *h*H_2R consists of 359 amino acids. In comparison to H_1R, H_2R possesses a longer, palmitoylated C-terminus, which is assumed to be important for membrane binding. Modifications on the *N*-terminus of the receptor by glycosylation were observed as well. Signal transduction of the *h*H_2R is mainly G_s-mediated and stimulates adenylyl cyclase resulting in increased cAMP concentration. Additionally, alternative signaling pathways were identified. G_q-mediated activation of PLC leads to an increase of IP_3 and intracellular Ca^{2+}. Also cAMP-independent pathways were reported (44). Like the H_1R, the H_2R also shows constitutive activity (Table 1).

The H_2R is found in numerous tissues including the brain, stomach and the heart. Stimulation of H_2R leads to gastric acid secretion representing its most prominent effect (45). Gastrin, acetylcholine and HA are responsible for the activation of parietal cells. After binding to their respective receptors different signaling pathways lead to active gastric acid secretion by H^+/K^+-ATPase. The H_2R is also involved in a number of other physiological processes. Stimulation of the receptor could additionally lead to positive inotropic and chronotropic effects in the heart, relaxation of smooth muscles in lung and inhibition of nerve cells leading to increasing working memory in brain. Participation in the anti-nociceptive opioid system has also been reported.

4.1. Histamine H_2 receptor agonists

Usage of *h*H_2R agonists is constricted mainly to the HA effects concerning the heart (46). Some agonists were used in the treatment of heart failure, but are still obsolete. The development of H_2R agonists (Figure 4) started with resembling the endogenous ligand HA (47, 48). One of the first H_2R agonists was dimaprit (10) (49), followed by its rigid aromatic analogue amthamine (11) (Figure 2). Increasing affinity was obtained by introducing a guanidine moiety. Representative compounds such as impromidine (12) and arpromidine show positive inotropic vasodilatory effects. Due to their polarity, oral application is hampered, which was solved by the development of prodrugs with strong electron withdrawing groups on the guanidine moiety decreasing basicity. Selectivity over the other *h*HR is low (Table 2). Dimaprit and imipramine for example show activity on all known HR subtypes.

4.2. Histamine H_2 receptor antagonists

For a long time, H_2R antagonists were essential drugs for the treatment of gastric ulcer due to their inhibition of gastric acid secretion, with clear dominance over inorganic antacids. Nowadays, they are replaced by proton pump inhibitors such as omeprazole in first line therapy of acid-dependent gastric disorders.

Applying knowledge in structure-activity relationship (SAR) of other aminergic receptor systems such as the adrenergic receptors, the structure of the endogenous agonist was systematically modified and enlarged yielding antagonists (47). At the beginning of rational development of H_2R antagonists, *Nalpha*-guanylhistamine was identified as a weak partial H_2R agonist concerning gastric acid secretion. The next step in the development process was the replacement of the strong basic and physiological protonated guanidine by thiourea (non-charged, highly polar). Burimamide (13), the first selective H_2R antagonist introduced by Sir J. Black and coworkers in 1972 and showing a 100-fold increase in potency compared to *N*alpha-guanylhistamine was identified. Although the substance was used for the characterization of the H_2R, its activity as H_3R antagonist and H_4R agonist was detected later on (9). Lacking oral bioavailability, further optimization had to be done using burimamide (13) as lead structure. Introduction of an electron-withdrawing thioether in the alkyl side chain and substitution of the aromatic imidazole ring in 5-position by a methyl group led to metiamide. The 10-fold increase in affinity compared to burimamide is probably due to the influence of the methyl group on the tautomeric balance. Quite likely, the *Ntau*-H-tautomer of HA may be the biological active form at the H_2R. Based on the occurrence of agranulocytosis due to the thiourea moiety, the development strategy turned to guanidine derivatives, using electron-withdrawing nitro or cyano groups for decreasing basicity. This process led to the blockbuster drug cimetidine (14), the first H_2R antagonist on the market for the therapy of gastric ulcer (50, 51). The interaction potential of imidazole-containing compounds with CYP450 enzymes, especially as inhibitor of CYP3A4, was observed. Replacement of the 4-methylimidazole moiety of cimetidine (14), the main cause for this pharmacokinetic liability, by (hetero)aromatic ring systems resulted in the furan derivative ranitidine (15), the thiazole containing compounds famotidine and nizatidine as well as roxatidine acetate (prodrug), which contains an oxyether bound benzyl ring. Additionally, bioisosterically replacement on the guanidine structure in the eastern part of the molecule took place (diaminonitroethen, amidine substituted with

Table 2. Comparison of binding affinities (pK_i) to the respective receptor subtypes, H_4R potencies (pEC_{50}) and H_4R efficacies (alpha) of different HR ligands. (9)

					hH$_4$R		
Compounds	**hH$_1$R pK$_i$**	**hH$_2$R pK$_i$**	**hH$_3$R pK$_i$**		**pK$_i$**	**pEC$_{50}$**	**alpha**
Histamine	4.2	4.3	8.0		8.2[a]	7.7	1
H$_1$R agonists							
2-(Thiazole-2-yl)ethanamine	< 4				< 5		
Pyridylethylamine	3.8				< 5		
Histaprodifen	5.7				< 5	7.7	1
H$_1$R antagonists							
Diphenhydramine[b]	7.9	5.8[c]	< 5		< 5		
Mepyramine	8.7	5.0[c]	6.0[c]		< 5		
Ketotifen[c]	8.9	6.0	5.6		4.3[d]	4.6[d]	
Cetirizine	8.0				< 5		
Loratadine	6.8				< 5		
Fexofenadine	8.0				< 5		
Mizolastine	9.1				< 5		
Clozapine	9.4		< 5[e]		6.7	6.8	1
H$_2$R agonists							
Dimaprit		4.6	6.1[e]		6.5	5.8	0.8
Amthamine		5.2			5.3		0
Impromidine		6.3	6.8		7.6	7.6	0.5
H$_2$R antagonists							
Burimamide		5.4	7.9		7.4	7.7	0.7
Cimetidine	< 5[b]	6.2	< 5[e]		< 5		
Ranitidine	< 4[b]	7.1	< 5[b]		< 5		
Famotidine		7.8			< 5		
Tiotidine		7.8			< 5		
H$_3$R agonists							
(R)-alpha-Methylhistamine	< 5[f]	< 5[f]	8.2		6.6	6.2	1
N^{alpha}-Methylhistamine			8.4		6.5	6.1	1
Imetit			8.8		8.2	7.9	0.9
Immethridine	< 5[g]	< 5[g]	9.1		6.6	6.0	0.5
Immepip	< 5[h]	< 5[h]	9.3		7.7	7.8	0.9
Methimepip	< 5[h]	< 5[h]	9.0		5.7	5.3	0.5
H$_3$R antagonists							
Thioperamide	< 5[i]	< 5[i]	7.3		6.9	7.0	-1
Clobenpropit	5.6[i]	5.2[i]	8.6		8.1	7.7	0.8
Ciproxifan[i]	< 5	< 5	7.2		5.7		
Iodoproxyfan			9.2		7.9	7.9	0.7
Pitolisant[j]	5.9	5.3	8.3		< 4		
NNC 38-1201[k]	< 6	< 6	8.3		< 6		
JNJ 5207852[l]	< 5	< 5	9.2		< 5		
ABT-239[m]	5.8	5.2	9.4		< 5		
H$_4$R agonists							
4-Methylhistamine		5.1	< 5[n]		7.3	7.4	1
OUP-16[o]			5.7		6.9	7.1	1
VUF 6884[p]	8.6		5		7.6		
H$_4$R antagonists							
JNJ 7777120	< 5[q]	< 5[q]	5.3		7.8		0
A-940894[r]	< 5	< 5	5.4		7.1		
ST-1012[s]					6.6	7.4	-1

alpha, intrinsic activity (1 full agonism; 0 neutral antagonism; -1 full inverse agonism) [a] Ref. (22); [b] Ref. (242); [c] Ref. (243); [d] Ref. (244); [e] Ref. (139); [f] Ref. (245); [g] Ref. (246); [h] Ref. (247); [i] Ref. (248); [j] Ref. (107); [k] Ref. (110); [l] Ref. (112); [m] Ref. (249); [n] Ref. (250); [o] Ref. (168); [p] Ref. (170); [q] Ref. (159); [r] Ref. (251); [s] Ref. (199)

sulfonamide or glycolic acidamide structure), which led to the newer compounds exhibiting higher potency on *h*H_2R than cimetidine (14), improved interaction potential and enhanced risk-benefit ratio (4). As pharmacological tools, the tritium labeled [^{3}H]tiotidine (16) (Figure 2) and [^{125}I]iodoaminopotentidine are promising radioligands for obtaining further information on receptor distribution especially in brain and other tissues (37). The newly developed zolantidine (17) is even suitable for *in vivo* CNS studies (52). In contrast to the clinically used H_2R inverse agonists cimetidine (14), ranitidine (15) and famotidine, which were re-classified due to the constitutive activity of the receptor, burimamide (13) exhibits neutral antagonistic properties in many *in vivo* models (53). Representative H_2R ligands are shown in Figure 4.

The development of H_2R antagonists clearly demonstrates the modern concept of bioisosterical replacement in medicinal chemistry considering electronic density, hydrogen-bond acceptor/donor properties and other potential non-covalent receptor-ligand interactions (54, 55). All H_2R antagonists in the market have a general pharmacophore in common. A (hetero)aromatic ring with a basic functionality is coupled *via* a flexible linker to a highly polar moiety (Figure 2), able to build hydrogen-bonds. Considering the bioisosterical concept, all these

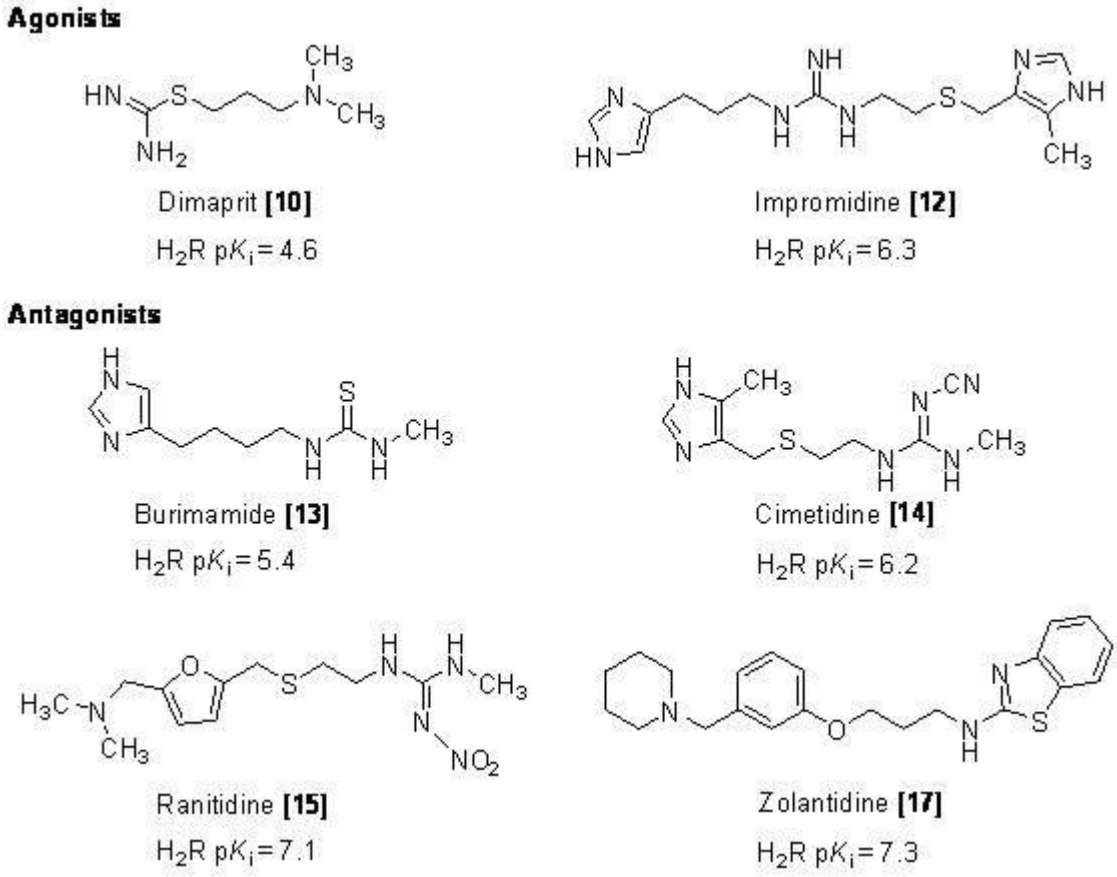

Figure 4. Representative histamine H_2 receptor ligands.

polar structural motifs have a planar electron system, high polarity and weak amphoteric properties in common and remain uncharged under physiological conditions. The basic substituent in 2-position of the ring system in the western part of the molecule preserves the basicity of the imidazole moiety (56). The receptor is sensitive to minor structural modifications. Geometry, hydrophilic properties (P, -logP), basicity/acidity (pK_a) as well as electron density (dipole moment) are important parameters to maintain affinity of the compounds towards the hH$_2$R. According to structural modifications within the scope of the pharmacophore blueprint, the obtained compounds show no significant changes in activity, but differ in receptor affinity, selectivity and pharmacokinetics.

5. HISTAMINE H_3 RECEPTOR

Investigating the role of HA as neurotransmitter, Arrang and co-workers recognized in 1983 that HA inhibits its own synthesis and release (11). This inhibitory presynaptic feedback mechanism had to be mediated by a new HR subtype because histaminergic ligands of the H_1R and H_2R showed no activity on this effect. The existence of the H_3R was proven by the development of (*R*)-alpha-methylhistamine (18) as agonist and the antagonistic ligand thioperamide (23). Besides its inhibitory activity as autoreceptor on synthesis and release of HA in histaminergic neurons, the modulation of several other neurotransmitters by H_3 heteroreceptors was observed, using these reference compounds as pharmacological tools (57). Histaminergic neurons in the CNS originate in the tuberomammillary nucleus of the posterior hypothalamus and spread to all major brain areas. The H_3R is predominantly expressed in the CNS and mainly localized in cerebral cortex, hippocampus, amygdala, nucleus accumbens, globus pallidus, striatum and hypothalamus (58). Association of the cortex and hippocampus to cognition, as well as of the hypothalamus to sleep and homeostatic regulations give a hint for possible therapeutic indications (59). Additional expression was found in the peripheral nervous system including the gastrointestinal tract, airways and the cardiovascular system, and in regions involved in nociception (60). Due to its function as heteroreceptor, the H_3R can also be found colocalized in neurons. Activation of the heteroreceptor modulates the release of several important neurotransmitters, such as dopamine, acetylcholine, norepinephrin, serotonin, gamma-aminobutyric acid, glutamate and substance P. The autoreceptor function and the cross-linking to other neurotransmitter systems play an important role in maintaining the central neurotransmitter balance, resulting in the control of multiple (patho)physiological brain functions such as vigilance, memory processes, food intake and locomotor activity (Table 1) (61, 62).

Due to the low homology of the receptor subtypes (H_3R homology to H_1R: 20%, to H_2R: 22%), H_1R and H_2R genes were unsuitable for the identification of the H_3R gene. The unknown genetic characteristics of the H_3R hampered the progress in development of H_3R ligands. Finally, in 1999 Lovenberg and co-workers succeeded in cloning the H_3R cDNA encoding for the receptor protein of 445 amino acids (63), which increased the number of focused drug discovery programs. Cloning was based on the search for novel GPCRs through homology searches of expressed sequence tag databases. Following the cloning of the H_3R from other species, including rat, mouse, guinea pig and monkey, and by using molecular biology approaches, a species-dependant H_3R pharmacology was revealed (64, 65). Significant differences in the affinity of the existing H_3R reference antagonists (thioperamide (23), clobenpropit (24), ciproxifan (25)) on the rat and human H_3R became apparent. Two amino acids in transmembrane domain 3 of the rat and human H_3R were identified by mutational studies and molecular modeling (66-68) as being responsible for the observed differences (*r*H_3R: Ala 119, Val 122; *h*H_3R: Thr 119, Ala 122).

The *h*H_3R gene is located on chromosome 20 and contains three exons interrupted by two introns (69). The gene can undergo extensive alternative splicing, resulting in 20 splice variants described in human tissues so far and additional isoforms in rodents, guinea pig and monkeys characterized at the mRNA expression level. The various H_3R isoforms differ in their species-dependent signaling properties and expression patterns (70-72). To date little is known about isoform specific H_3R action and their role in (patho)physiological processes. The H_3R is constitutively active and able to signal in an agonist independent manner (73, 74). The level of constitutive activity varies depending on the species, isoforms, receptor coupling and cell types. Probably the mechanism of constitutive activity may be attributed to a consensus region on the carboxyl terminus in the third intracellular loop of the H3R, which is highly conserved among species (65, 66). Again, the finding of constitutive activity led to a re-classification of ligands (16, 75) in agonists, neutral antagonists and inverse agonists. Also protean agonists such as proxyfan (76), behaving as agonists, neutral antagonists and inverse agonists depending on the expression level and the experimental read-out, were identified.

Stimulation of the H_3R decreases cAMP levels $G_{i/o}$-mediated *via* negative coupling to adenylyl cyclase, thereby reducing PKA activity and downstream signaling events, such as cAMP response element-binding protein (CREB)-dependent gene transcription (77). Activation of mitogen-activated protein kinase (MAPK) (78) and glycogen synthase kinase-3beta (GSK-3β) (79,80), both associated with the activation of phosphatidylinositol 3-kinase, synaptic plasticity and neuroprotection, are alternative signaling pathways. Additionally, H_3R-associated $G_{i/o}$ coupling activates PLA_2, resulting in the release of arachidonic acid and lipid mediators (81) as well as inhibition of Na^+/H^+-exchanger (82) and lowering of K^+-induced Ca^{2+} mobilization (83).

Due to the widespread distribution and the ability to affect multiple neurotransmitter systems, the *h*H_3R displays a promising target for a broad range of indications mainly concerning CNS disorders, which are discussed in detail in several recommended reviews (13,59,84-86). H_3R ligands are promising for the treatment of sleep-wake disorders, especially narcolepsy, as well as for the enhancement of cognitive and memory processes. Due to procognitive effects in animal models of learning and memory, H_3R antagonists were developed for the use in cognitive impairment and attention-deficit hyperactivity disorder. Decreases of seizure susceptibility in epilepsy models indicated an anticonvulsive effect for H_3R antagonists (87). Depression and schizophrenia (*via* serotonin and norepinephrin pathway, respectively) as well as pain and inflammatory conditions are potential therapeutic indications in the future. Additionally, the H_3R exhibits a regulatory role in food intake and antagonists are considered as potential anti-obesity drugs. Different effects of the H_3R antagonists/inverse agonists on various cancer cell lines suggested that the H_3R may act as an autoinhibitory receptor on cell growth and progression (88). Representative H_3R ligands are shown in Figure 5.

5.1. Histamine H_3 receptor agonists

While the affinity of HA is low for the *h*H_1R (pK_i value of 4.2) and the *h*H_2R (pK_i value of 4.3), the affinity for the *h*H_3R (pK_i value of 8.0) is much higher (9). Specific modifications of the endogenous ligand by mono- or di-methylation resulted in even more active and selective compounds. A drastic increase in affinity and selectivity was reached by methylation of the alpha-position of the ethylamine side chain. (*R*)-alpha-methylhistamine (18) and its 100-fold less potent (*S*)-isomer were used for receptor characterization for a long time. Additionally, tritiated radioligands are available as pharmacological tools (37). Potent stimulation of the H_3R is mainly achieved with imidazole-containing derivatives (Figure 5). Therefore, modification takes place in the eastern part of the molecule. For developing more potent agonists, the amine function was replaced by thiourea (imetit (19)) (89) or it was incorporated in ring structures (immepip (20) (Figure 2)) (90). These first generation H_3R agonists were intensively used as reference ligands until the discovery of the H_4R, when their considerable affinity for this latest HR subtype was observed (9). Afterwards, new potent and selective H_3R agonists were developed including immethridine (21) (91) and methimepip (22) (92), which are devoid of high H_4R activity (H_3R over H_4R selectivity margin of 288 and 2000, respectively) (Table 2). Being simple HA derivatives, the compounds were rapidly metabolized by histamine *N*tele-methyltransferase, which led to the development of orally available prodrugs (93, 94) such as the anti-inflammatory and antinociceptive azomethine BP2.94, which entered clinical development for different therapeutic applications (95). Additionally, several potent H_3R antagonists such as proxyfan (76), carbamate (96) and ether derivatives (97) had been described, showing agonistic properties in *in vivo* test systems. Differences in receptor structure or receptor-effector coupling according to the respective tissue used in the assay could explain these observations (8).

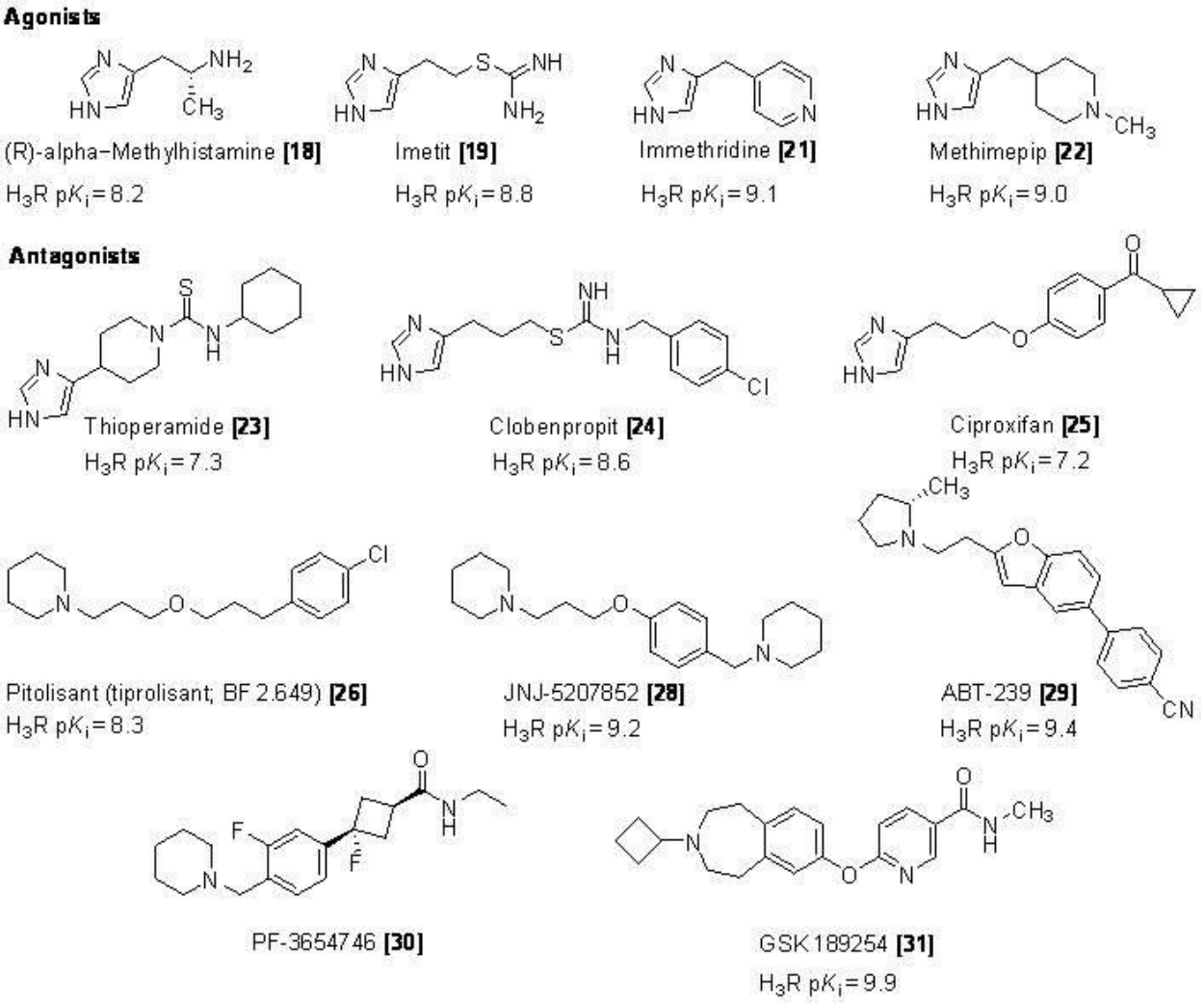

Figure 5. Representative histamine H_3 receptor ligands.

The structural diversity of H_3R agonists is limited. They all represent small HA-like compounds containing an imidazole moiety, which display the risk for drug-drug interactions. A handful of promising roles for H_3R agonists emerged from animal models of migraine, ischemic arrhythmias and obesity as well as diabetes mellitus (84, 85). The development of non-imidazole H_3R agonists seems desirable in order to generate therapeutic candidates with favourable pharmacokinetics and improved potency and selectivity.

5.2. Histamine H_3 receptor antagonists

Stepwise structural modulation of HA by enlarging the endogenous ligand led to first antagonists. Like the H_3R agonists, these early compounds closely resemble the endogenous ligand HA and contain an imidazole moiety, which was considered essential. The imidazole moiety binds to the heme iron in CYP enzymes (98). Co-administration with other CYP interacting drugs can lead to adverse side-effects through drug-drug interactions (99). The strong hydrogen bond acceptor and donor properties of the imidazole result in low bioavailability and blood-brain barrier penetration (100). The observed species differences lead to a complex pharmacological profile. The poor off-target selectivity as well as the observed H_4R affinity generate further limitations (13). The first H_3R antagonists (Figure 5) thioperamide (23) and clobenpropit (24) are additionally high potent H_4R ligands, showing only moderate affinity towards the $h$$H_3R$ (101). Based on the finding of constitutive activity, most H_3R antagonists were re-classified as inverse agonists. Proxyfan and ciprolisant (GT-2331) showed protean agonism depending on the study conditions. Iodination and labeling led to the very prominent radioligands (^{125}I)iodoproxyfan and (^{125}I)iodophenpropit used in numerous assay systems (102). However, iodoproxyfan additionally showed a potential agonistic action (103), which can be problematic for certain assays. Although the imidazole-containing H_3R antagonists showed less therapeutic potential, new structures were designed by combination of known scaffolds, such as OUP-133, leading to potent ligands with improved physicochemical and pharmacological characteristics (104).

The potential drawback of imidazole-containing compounds led to the replacement of the imidazole moiety. The new non-imidazole containing H_3R antagonists/inverse agonists provide clinical candidates with drug-like properties. Instead of the aromatic imidazole, mainly a variety of secondary and tertiary amines, partly in aliphatic heterocycles (e.g. azepine, piperazino, pyrrolidino and morpholino) were used to identify an appropriate replacement (85, 105, 106). Whereas larger substituents or oxidation of the aliphatic heterocycle reduced or abolished affinity, the piperidinyl moiety was used mainly due to the increase in potency and the decrease in pharmacokinetic interactions. In line with the imidazole-containing congener FUB 181, such a replacement led to one of the first and most prominent H_3R inverse agonist pitolisant (26) (tiprolisant; BF2.649) (107). The compound has now entered phase III clinical trials and has received orphan drug status by the European Medicines Agency for the treatment of narcolepsy. The ciproxifan analogue UCL 2190 (27) (Figure 2) contains the prominent aminopropoxyphenyl substructure, which reoccurs in many H_3R antagonist research programs. Enlargement of the alkylamine scaffold to amine-alkoxyphenyl was based on the natural ligand aplysamine-1 (108), which was one of the first non-imidazole antagonists. A second natural antagonist used as a lead structure is the non-aromatic steroid-related alkaloid conessine (109). SAR transfer from imidazole-based compounds led to guanidine derivatives discovered by the James Black Foundation.

Imidazole replacement leads to improved drug-likeness. However, antitargets (*h*ERG, CYP enzymes and P-gp) and challenging pharmacokinetic properties, such as brain residence time, plasma protein-binding and tissue distribution, have to be considered in drug discovery of non-imidazole containing compounds. Besides imidazoles, other N-heterocycles and unsatured moieties are powerful CYP inhibitors (110). Using aplysamine-1 as lead structure, modifications at its two basic moieties and the spacer length led to a variety of diamine-based ligands (111, 112). The second basic structure boosts affinity towards the H_3R probably due to strong ligand-receptor interactions (113, 114). The preclinical candidate JNJ-5207852 (28) showed efficacy in pharmacodynamic and relevant disease models (115). Brain accumulation with long brain residence times and potential induction of phospholipidosis present major drawbacks for this class of compounds (116) and are hardly desirable in the chronic treatment of CNS disorders. Positron emission tomography (PET) ligand JNJ-10181457 was developed using JNJ-5207852 as a lead compound, showing decreased CNS accumulation and a shorter half-life. Due to longer half-life, extensive tissue distribution along with toxic accumulation in the CNS and cationic channel modulation of the more potent piperidine analogs, the more hydrophilic morpholine is preferred as substituent in the eastern part of the molecule for pharmacological profiling (13). For enhancing drug-likeness, sterical restriction of the propyloxy spacer, as seen in the early benzofuran compound ABT-239 (29), was performed. Strong inhibition of *h*ERG leading to QT prolongation and arrhythmia, and the induction of phospholipidosis (due to high clogP values) halted its development (117). An advanced brain/blood ratio was reached with A-331440 (118), which shows efficacy in obesity but not in cognition unlike its predecessors (119). The higher affinity for the long human receptor form compared to the shorter one, which is present in human cortex and responsible for learning and memory functions, could provide a possible explanation. These isoform-dependent ligand interactions probably caused the variety of indications for H_3R antagonists/inverse agonists (120). Introduction of a polar imidazolidine instead of the aromatic biphenyl as linker between the two basic functions, seen in malononitrile derivatives (121, 122) offer additional pi-pi-interactions with the binding pocket (Tyr 115, Trp 371) beneficial for their binding affinities. Other examples of polar heterocyclic replacement of the aromatic phenyl core in the amine-alkoxyphenyl scaffold are shown in the literature (123-127) and in patents (128, 129). Benzo[d]azepine molecules represent potent and highly selective H_3R antagonists. GSK189254 (31) and the azepine derivative GSK 334429 showed efficacy in models of neuropathic pain (130), indicating a possible additional indication for these short-acting and poor brain penetrating ligands. Modulation of pain perception in the dorsal horn and spinal cord and cross-linkage of peripheral and central pathways of sensitization support this approach. Radiolabeling led to [^{11}C]GSK 189254, a promising PET ligand for *in vivo* CNS imaging (131).

Due to its distinct expression in the CNS and the resulting involvement in several neuronal functions, the H_3R displays a promising target for numerous CNS disorders. Clinical trials were focused on heterogeneity of potential indications. While cognitive impairment and sleep disorders are most prominent, diseases deserving medical attention such as epilepsy, attention-deficit hyperactivity disorder, neuropathic pain, obesity and allergic rhinitis are also targeted (86). Pitolisant (26) represents the most advanced clinical candidate so far with investigations related to cognition, schizophrenia, narcolepsy, epilepsy and excessive daytime sleepiness in Parkinson´s disease. Promising clinical compounds by GlaxoSmith&Kline are GSK239512 for Alzheimer's disease and schizophrenia and the dual H_1R/H_3R ligands GSK1004723/GSK835726 for the therapy of allergic rhinitis. PF-3654746 (30) developed by Pfizer shows proven efficacy in attention-deficit hyperactivity disorder, Alzheimer's disease, excessive daytime sleepiness in narcolepsy and allergic rhinitis. MK0249 (attention-deficit hyperactivity disorder, Alzheimer's disease, cognition in schizophrenia and excessive daytime sleepiness in sleep apnea), SCH497079 (obesity) and JNJ17216498 (narcolepsy) are also evaluated in phase II clinical trials (10, 59, 105). The design of multiple target compounds exhibits advantage in the treatment of complex CNS disorders, where single target approaches are often insufficient. Meanwhile, diverse intelligent H_3R hybrid approaches were developed. The attention-increasing and wake-promoting effect of H_3R antagonists were utilized for the treatment of depression in combination with serotonin reuptake inhibitors (e.g. JNJ-2853867). Enhancement of the procognitive effect of H_3R antagonists can be achieved by inhibition of the metabolizing enzyme histamine *N*tele-methyltransferase,

resulting in constantly increased levels of HA in the synaptic cleft (e.g. FUB 833). This approach is probably useful in the treatment of dementia, where acetylcholine esterase inhibitors are commonly used in the therapy of Alzheimer's disease (13). Ligands combining H_3R and H_4R binding properties are recently discussed in the literature and probably present an interesting approach for the treatment of neuropathic pain.

Since the identification and the cloning of the H_3R, many H_3R ligands were developed by the academia and industry, which led to extensive knowledge of antagonist/inverse agonist pharmacophores and ligand-receptor interactions (13, 59, 105). Due to the potential drawbacks of the imidazole moiety the early antagonist pharmacophore blueprint was modified and extended (106). Among the diversity of structural classes, almost all compounds follow a general *h*H_3R antagonist blueprint, which contains a basic moiety, mostly a tertiary amine, as replacement for the former imidazole, linked by a (partly rigidified) alkyl spacer to a central core (Figure 2). Initially, using aromatic systems polar groups or combinations of both are also accepted by the receptor as central core. The western part of the pharmacophore is mainly responsible for receptor targeting due to ionic interactions (amine structure with Asp114 on transmembrane domain 3) and pi-pi-interactions (central core and Trp371 and Tyr115). The central core might be substituted by a variety of structural elements providing different physicochemical properties. Polar, basic, lipophilic and recently reported acidic elements (or moieties showing combination of these attributes) are possible substituents (132). A second basic moiety boosts affinity due to additional beneficial interactions with Glu206 on transmembrane domain 5 (113, 114) in the receptor's binding pocket but also shows the risk of phospholipidosis induction (120). Coupling of lipophilic groups, often in combination with a second basic moiety such as fluorophores (133), tricyclic compounds (134) and fluor-containing heterocycles (135), leads to a number of affine H_3R antagonists/inverse agonists indicating the robustness of the receptor's binding pocket to the eastern part of the molecule, which is involved in potency modulation.

6. HISTAMINE H_4 RECEPTOR

In 1994, Raible and co-workers identified HA-induced pertussis toxin-sensitive cytosolic Ca^{2+} increase in eosinophils, which could be inhibited by the H_3R antagonist thioperamide and also induced by the agonist (*R*)-alpha-methylhistamine in lower potency (136). Investigations concerning the HA uptake and release of hematopoietic progenitor cells demonstrated a H_3R-independent mechanism, which could not be influenced by H_3R agonists (137). Based on these observations another HR subtype was suggested. First, the long-awaited cloning of the H_3R gene enabled the proof of responsibility of the observed effects by this new HR. Using the H_3R DNA sequence several research groups in 2000-2001 independently identified an unexplored GPCR sequence in the human genome as new H_4R based on its overall homology to the H_3R gene (37%; homology to H_1R and H_2R only approx. 19%) (12, 138-142). Secondary protein and gene structure are similar in H_4R and H_3R (143). The *h*H_4R gene, encoding for a 390 amino acid protein, is localized on chromosome 18 and contains three exons, interrupted by two introns (144). While such intron-exon distribution may result in a number of H_3R isoforms, so far only three H_4R isoforms have been reported (Table 1). The existing shorter non-signaling, non-7-transmembrane isoforms act as negative regulatory elements on the function of the full-length *h*H_4R isoform, probably due to heterodimer formation (145). Like the other members of the HR family, the H_4R protein possesses all consensus motifs identified for class A rhodopsin-like GPCRs. Besides the conserved aspartate residue ($Asp94^{3.32}$) in the transmembranedomain 3, a glutamate in transmembrane domain 5 ($Glu182^{5.46}$), which is present in H_3R and H_4R, was found to play an essential role in HA binding. The H_4R has two potential glycosylation sites and one palmitoylation site. Two cysteine residues, conserved among the GPCRs, are also present in H_4R. They potentially form disulphide bridges, connecting transmembrane domain 3 and the second extracellular loop, which is probably essential for HA binding (144). In addition to the identification of the *h*H_4R, the cDNA of mouse, rat, guinea pig, pig, dog and monkey H_4R were cloned, which show only moderate sequence homology of 67%-72% to the *h*H_4R (except for monkey H_4R: 92% homology) (Figure 6) (22, 146-148). These observations result in substantial differences in binding behavior of the H_4R ligands on the H_4R species variants (Table 3), making the preclinical development of H_4R ligands more complicated due to the lack of reliable animal models. However, the H_4R displays similar tissue distribution in different species, suggesting involvement of the H_4R in common physiological processes among these species.

In human tissues *h*H_4R expression was detected in bone marrow, peripheral blood, spleen, thymus, small intestine, colon, heart and lung at mRNA levels (138-142). These mRNA studies present discrepancies in the expression pattern in distinct cells, which underline the importance of anti-H_4 receptor antibodies in the immunohistochemical detection of the receptor. The majority of H_4R expressing cells indeed belong to the hematopoietic system (neutrophils, mast cells, eosinophils, basophils, dendritic cells, monocytes and T cells). Additionally, the H_4R was found to be expressed in endocrine cells in the gastrointestinal tract (149), on dermal fibroblasts (150) and nerves of the human nasal mucosa (151). Besides the existence of H_4R in the enteric nervous system in rodents, expression in the CNS was also confirmed (152).

Similarly to the H_3R, the H_4R is coupled to pertussis-toxin-sensitive $Galpha_{i/o}$-proteins (Table 1) (12, 139, 142). Activation of the receptor inhibits forskolin-induced cAMP formulation and modulates PKA and its downstream signals such as transcription of genes regulated by cAMP-responsive elements. Signaling *via* MAPK phosphorylation (140) and activation of PLC associated with Ca^{2+} mobilization were also detected (153). Increased intracellular Ca^{2+} led to actin polymerization, change in cell

Table 3: Binding affinities of ligands on H_4R of different species

Ligand	Human[a]	Pig[b]	Dog[c]	Rat[a]	Mouse[a]	Guinea pig[a]						
Histamine	5.9	± 0.4	26.3	± 12.4	29	± 8.5	70	± 7	43	± 9	11.4	± 1.3
Imetit	1.3	± 0.1	79.9	± 45.2	55	± 12	6.9	± 4.1	6.8	± 3.5	12.9	± 1.0
(*R*)-alpha-Methylhistamine	144	± 8	249	± 84	93	± 9.2	700	± 274	397	± 70	203	± 30
N^{alpha}-Methylhistamine	50	± 0.8			553	± 120	316	± 146	92	± 3		
Clobenpropit	4.9	± 1.1	401	± 86		64	± 2	15	± 10	1.5	± 0.1	
Thioperamide	52	± 28	406	± 139	80	± 17	28	± 9	23	± 9	34	± 7
Burimamide	124	± 19			960	± 188	725	± 121	351	± 168		
4(5)-Methylhistamine	48	± 8		112	± 6.3							
Clozapine	625	± 181	>10000		2197	± 867	2890	± 1640	78	± 16		
JNJ 7777120	4	± 1.0		50	± 11	2.6	± 0.3[d]	4.6	± 0.3[d]			

*K*i values (nM) determined by [^{3}H]histamine receptor binding assay. [a] Ref. (22); [b] Ref. (146); [c] Ref. (148); [d] Ref. (159)

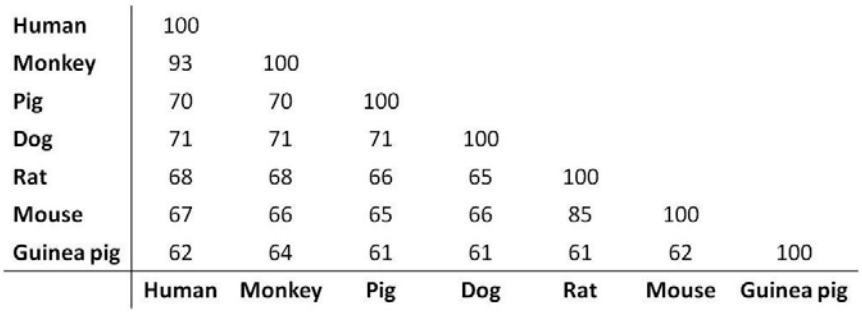

Figure 6. Homology of protein sequence (%) of the H_4R in several species. (233)

shape and is potentially linked to the migration of mast cells, eosinophils and monocyte-derived dendritic cells into sites of inflammation (153-157). Further details are reported in a number of articles of this issue.

The expression of the H_4R mainly in cells of the human immune system and the distinct effects on chemotaxis of several cell types highlight its physiological relevance in inflammatory and immunological responses. *Via* H_4R, HA mediates the expression of adhesion molecules on eosinophils. The constitutively expressed H_4R on mast cells provides autocrine as well as paracrine regulation of HA-induced processes (158, 159). Differentiation of monocytes as well is mediated *via* the H_4R. Down-regulation of chemokine CCL2 synthesis and release results in reduced monocyte recruitment (12, 140, 142, 160). Additionally, dendritic cells and T cells are involved in the control of cytokine and chemokine production (161). Chemotaxis due to H_4R activation was observed (142, 155, 162). Furthermore, the T helper cell (T_h1/T_h2) differentiation is influenced by this receptor. The immunomodulatory effects of HA mediated by the H_4R and the potential therapeutic possibilities of H_4R ligands have been recently presented in several excellent reviews (10, 14, 163). The H_4R is a promising target for the therapy of asthma, allergy and skin inflammation, such as chronic pruritus. Other therapeutic useful effects of H_4R ligands were indicated by animal models of various inflammatory conditions, such as arthritic disorders, autoimmune diseases and pain. The existing interplay of HR in inflammatory processes suggests a potential synergistic benefit of combined H_1R and H_4R antagonism (164). So far, the physiological role of the H_4R was mainly restricted to its pro-inflammatory actions. Following the controversial findings in a murine bronchial asthma model (165), the need for further basic research to finally understand the function of the H_4R becomes evident.

6.1. Histamine H_4 receptor agonists

Regarding the results from *in vitro* and *in vivo* studies H_4R antagonists demonstrate a greater therapeutic potential, whereas agonists (Figure 7) are useful pharmacological tools for the identification of H_4R functionality (144). As mentioned above, many of the imidazole-containing first generation H_3R ligands also possess significant H_4R affinity (85), as it became apparent after the discovery of the H_4R. Imetit (19), imbutamine, immepip (20) and clobenpropit (24) show agonist properties, whereas thioperamide (23) turned out to be a potent inverse agonist. These non-selective dual H_3R/H_4R ligands were used for the early pharmacological and (patho)physiological characterization of the H_4R (154, 155, 166, 167). As first H_4R agonists, a series of methylcyanoguanidine derivatives of 2,5-disubstituted tetrahydrofuranylimidazoles was reported. The full agonist OUP-16 (32) (EC_{50} value of 78 nM) shows moderate affinity (K_i value of 130 nM) and a 15-fold selectivity over the H_3R (Table 2) (168). Evaluation of a small library of known histaminergic ligands discovered during H_1R-H_3R research, led to the identification of the potent H_4R full agonist 4 (5)-methylhistamine (33) (K_i value of 50 nM; EC_{50} value of 40 nM, 100-fold selectivity) (9), originally developed in the H_2R field (Table 2). Binding affinities of selected HR ligands on the respective receptor subtypes are summarized in Table 2. The imidazole moiety probably needed for H_3R agonism seems not to be necessary for H_4R agonists, as demonstrated by the non-selective H_4R ligand clozapine. The antipsychotic drug clozapine offers antagonistic properties for several GPCRs (169), while it acts as full agonist on the H_4R. Based on its dibenzodiazepine scaffold, minor structural modifications led to increased affinity and selectivity in its close analog VUF 6884 (34) (K_i value of 25 nM; EC_{50} value of 20 nM). The rigidified structure of this dual H_1R antagonist/H_4R full

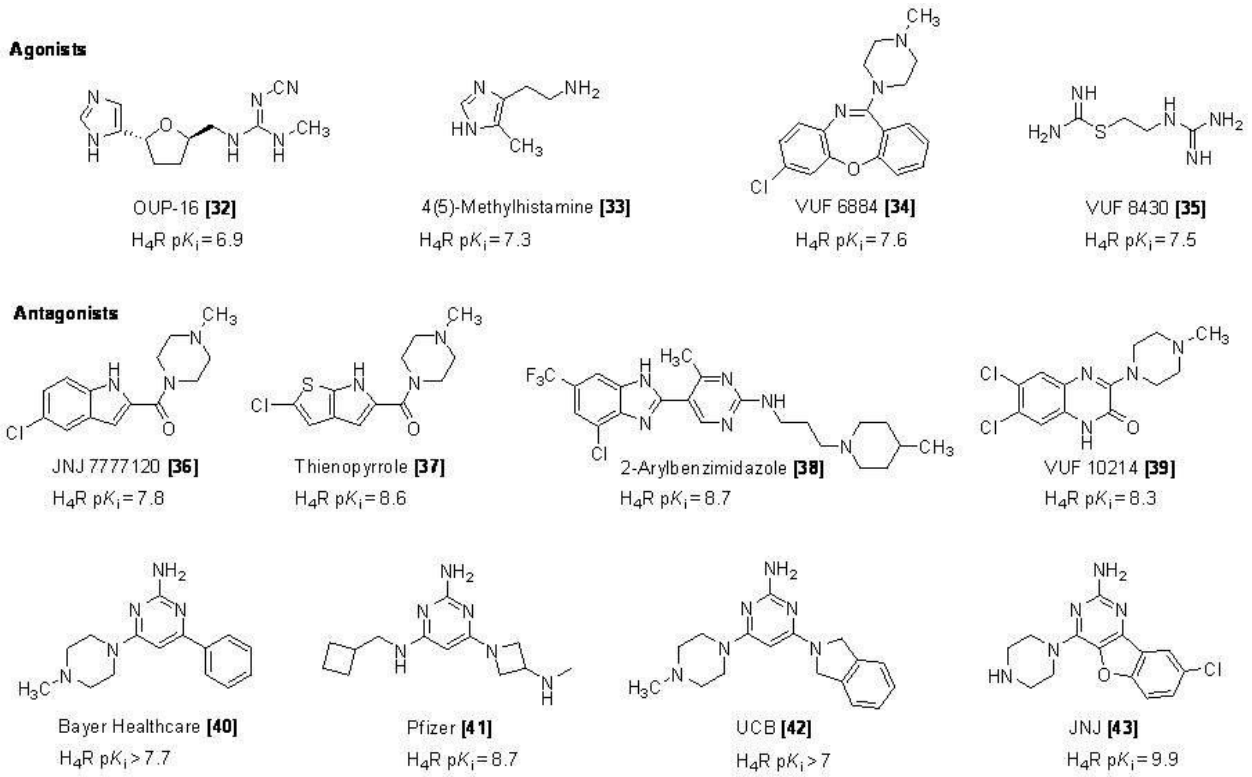

Figure 7. Representative histamine H_4 receptor ligands.

agonist was used for the investigation of the H_4R ligand binding site and construction of a pharmacophore model used for the design of new H_4R ligands (170, 171). A similar approach was carried out in the optimization of the non-imidazole compound dimaprit (H_2R/H_4R agonist and H_3R antagonist) (9). Improved potency and selectivity over the H_2R/H_3R was found in VUF 8430 (35) (K_i value of 32 nM), where the alkylthiourea structure of dimaprit was substituted by a guanidyl moiety (172, 173). VUF 8430 (35) and dimaprit, both originated by H_2R ligand development programs, show indistinguishable functionalities as full agonists on the H_4R (EC_{50} value of 50 nM). Originally developed as 5-HT_3 ligands (174) in the 1980s, a class of 2-quinoxalines were discovered as high affine H_4R ligands (175) (partial agonist pEC_{50} value of 8.99, unpublished data) (176). This scaffold was additionally identified in a fragment-based drug design approach (171). Recently, synthesis and pharmacological characterization of oxime-containing H_4R agonists were reported (177). Structural variations of the potent H_4R antagonists JNJ 7777120 (36) and JNJ 10191584 surprisingly led to H_4R agonism. A series of indole and benzimidazole analogs, where piperazine was substituted by piperidine and the carbonyl converted into an oxime moiety, resulted in promising research tools with high affinity (comparable in different species), agonistic potency and selectivity over other HRs (e.g. JNJ 28610244). There is still a lack of highly potent and selective H_4R agonists and the use of the standard references, such as 4 (5)-methylhistamine (33), remains problematic in several assay sytems.

6.2. Histamine H_4 receptor antagonists

The first H_4R antagonists were identified along with the cloning of the receptor due to the evaluation of H_4R activity of known imidazole-containing H_3R ligands (9). Thioperamide (23) acts as inverse agonist and shows two or threefold lower affinity for the H_4R than for the H_3R. Avoiding drawbacks arose during the development of the first H_3R ligands; the discovery of H_4R compounds was mainly supported by computational chemistry. The development of lead structures was no longer simply based on modifications of the endogenous ligand HA and high-throughput screening of compound libraries took advantage of structural knowledge of GPCRs and molecular-biology approaches.

Therefore, it was not suprising that the first non-imidazole H_4R antagonist JNJ 7777120 (36) (Figure 7) was identified by an in-house high-throughput screening at Johnson & Johnson (J&J) (178). The indole carboxamide JNJ 7777120 (36) binds to the hH$_4$R with a K_i value of 4nM and high selectivity. Actual discussions about its functional activity as partial agonist with low intrinsic activity, which seemed to be assay system- and species-dependent have taken place. Nevertheless, JNJ 7777120 (36) is so far used as reference antagonist to investigate the H_4R and has shown effectiveness in a variety of animal models. Due to a poor metabolic stability in human and rat liver microsomes and a resulting short *in vivo* half-life in rats ($t_{1/2}$ of 3h) (179) its use in studies of chronic inflammatory conditions is restricted. Bioisosteric replacements of indole, carbonyl and piperazine groups

were performed to investigate the possible chemical variations accepted by the H_4R. Using a scaffold-hopping approach, benzimidazole and thienopyrrole derivatives (37), which showed comparable affinities but still poor metabolic stability *in vivo* or even loss of oral bioavailability, respectively, were identified (179, 180). Pfizer reported a series of comparable benzimidazole-containing compounds obtained by bioisosteric replacement of the *N*-methylpiperazine with octahydropyrrolo (3,4-c)pyrrole and introduction of an amidine instead of the carbonyl function (181).

Again, high-throughput screening led to another promising new class of H_4R ligands (182-185). Additionally, the 2-arylbenzimidazole scaffold was found in pharmacophores from the area of H_3R research. Optimization of the lead structure by variations in the alkyloxy or alkyl amine spacer, the (hetero)aromate and the amino moiety resulted in high affine compounds such as derivative (38) (up to a K_i value of 2 nM) (Figure 7). The functionality of the 2-arylbenzimidazole series remains quite complex. As reported recently, modifications in the terminal amino group could result in partial agonism and full antagonism of the compounds (186). Additional series, in which the benzimidazole group was replaced by a substituted imidazole moiety, were reported (187, 188).

A class of methylpiperazine substituted 2-quinoxalinones was introduced (175). Optimization could be reached by substitution of the quinoxaline heterocycle. A pharmacophore model based on clozapine and JNJ 7777120 (36) used by Smits and co-workers resulted in similar compounds, such as the benzyl substituted quinoxaline VUF 10148 (K_i value of 40 nM) or compound (39) (Figure 7) (171). Based on SAR gained by the quinoxaline series, a successful scaffold hopping approach identified quinazoline derivatives as potent H_4R ligands (189). Structural optimization led to sulfonamide substituted analogs displaying high affinity for the H_4R and acting as inverse agonists (190). The compounds tolerate a large variety of aromatic, aliphatic and fused ring systems as substituents on the sulfonamide moiety by maintaining their binding affinity.

Since the first patent for 2-aminopyrimidines (Figure 7) as H_4R ligands by Bayer Healthcare AG (e.g. compound (40)) several other groups identified this scaffold and utilized it for developing their own compound series. The optimization of the 2-aminopyrimidine scaffold was based on the substituents in 4- and 6-position, namely the charged amino moiety and the aryl residue (191, 192). Regarding the patents in general, tolerance was found for a variety of substituents on 5- and 6-position of the scaffold, whereas the 2-amino group was kept constant. Although, most potent H_4R ligands contain *N*-methylpiperazine, its replacement is possible by considering the restriction of size (e.g. azetidine, aminopyrrolidine or diazepane rings). Replacement of the aryl group by aliphatic residues led to a potent H_4R ligand series by J&J (K_i value of 1nM) (193). Hereafter, further structurally diverse aminopyrimidine derivatives were reported, indicating great tolerance towards either aromatic or aliphatic lipophilic residues. Additionally, in compounds reported by Pfizer such as (41), aryl replacement was successfully shown with different aliphatic amino-alkyl moieties (K_i value of 2.7 nM) (194). Researchers at Palau Pharma introduced a series of diaminopyrimidines. In contrast to the majority of reported compounds, the two aromatic moieties (phenyl and pyrimidine) were linked by an amino group. Variations in aromatic and basic substituents enlarge the stuctural diversity (195). UR-63325, indicated for allergic respiratory diseases, represents the first clinical candidate in the H_4R field (15). Some further 2-aminopyrimidine derivatives were reported by UCB Celltech showing different (partially) saturated (hetero)cycles (e.g. piperidine, 2-methylpyrrolidine, cyclohexane) instead of the aryl group leading to moderate affine compounds (42) (K_i value < 100 nM) (196). Following lead optimization after high throughput screening, Abbott identified another aminopyrimidine compound (197, 198) showing high affinity, good oral bioavailability and *in vivo* efficacy in different animal models. Due to decreased potency on the rat H_4R (rH_4R), further optimization was done, which did not lead to enhanced rH_4R affinity. Based on a virtual screening approach 2,4-diaminopyrimidines were identified as potent H_4R ligands in our research group by Sander *et al.* (e.g. ST-1012) (199, 200). SAR revealed that slight structural variations in the aryl substitution pattern result in differences in functional properties of the ligands, probably due to different binding modes of the ligands found in a molecular dynamics simulation (201).

Conformational constrained derivatives display another playground in the 2-aminopyrimidine field. Argenta introduced benzofuro- and benzothieno-pyrimidines, where the 2-amino group was substituted or even eliminated (202, 203). Investigations were mainly focused on *N*-methylpiperazine replacement (204-207). Additionally, benzofuro- and benzothieno-pyrimidines were reported by J&J (e.g. compound (43)) (208, 209). Abbott also prepared a series of conformational constrained aminopyrimidines, combining high H_4R potency across the species and improved pharmacokinetic properties and efficacy in *in vivo* animal models for pain and inflammation (e.g. A-943931; K_i value of 4.7 nM) (210-212). Introduction of an octa-hydrobenzofuranoquinazoline system led to A-987306, showing high affinity (K_i value of 5.8 nM) and efficacy in blocking pain responses (ED_{50} value of 42µmol/kg in rat carrageenan-induced thermal hyperalgesia model) (213). Using a large-scale structure-based virtual screening based on a homology model of the hH_4R Gedeon Richter identified several potent H_4R ligands, which probably can be used as lead structures for the development of structural new H_4R ligands (214).

A rough H_4R antagonist blueprint can be deduced from different reference structures, consisting of a basic moiety, mostly an *N*-alkylated tertiary aliphatic cyclic amine, a (conjugated) hydrogen-bond acceptor/donor system and a lipophilic residue (Figure 2). Representative H_4R antagonists are shown in Figure 7. In several new structural classes even minor structural variations resulted in modulated functional activity of the H_4R ligands. Computer-aided investigations such as molecular dynamics

simulations, regarding the binding mode of the compounds and resulting changes in receptor conformation could help to find explanations. Additionally, further SAR investigations concerning the structural modifications with keeping their functional activities in mind are needed.

7. COMPUTATIONAL METHODS

In general, two major approaches for the development of new lead structures of GPCRs can be distinguished: A) the classical ligand-based design strategies according to endogenous or surrogate ligands and B) the receptor-based methods considering structural features of the target. Rational drug design starts from SAR of natural ligands and known reference compounds, leading to the development of pharmacophore models (215). The generation of valid pharmacophore models benefits from the availability of potent, structurally diverse, conformational restricted receptor ligands. These models are used in virtual screenings for identifying novel scaffolds (scaffold hopping) and application of approved scaffolds for new targets (repurposing) (216). Similarities in small-molecule binding sites of GPCRs led to privileged structural motifs, which are present in ligands for diverse GPCRs. Cross-target analysis of GPCR ligands recognize these common structural features, which can be used for the design and synthesis of combinatorial libraries finding new scaffolds. Due to promiscuous binding behavior, selectivity can first be addressed during the lead optimization process. Molecular similarity search in compound libraries is based on the assumption that similar molecules offer similar ligand properties leading to consistent binding behaviour. Different molecular and pharmacophoric descriptors, such as physicochemical properties relevant for receptor-ligand interactions, topological and distance information or the presence of functional fragments, can be used for defining molecular similarity (217). Lead optimization strategies, such as quantitative SAR models, regard physicochemical and topological (steric and electronic field environment of ligands) ligand properties in correlation with their biological activity (218). Besides pharmacodynamic adjustment the optimization of pharmacokinetic properties is also important for drug-likeness of a compound. Here, *in silico* models predicting absorption, distribution, metabolism and excretion (ADME) processes based on the ligand structure (e.g. Lipinski's rule of Five) are developed (217).

Structural biology, homology modelling and mutagenesis studies provided insights into GPCR structure and receptor-ligand interactions. Due to conformational heterogeneity of the membrane bound proteins, crystallization of GPCRs is still challenging. Advantageously, the families of GPCRs contain highly conserved structural motifs, which allow the generalization of the findings concerning receptor conformation and activation according to agonist binding observed in the crystallized structures of rhodopsin, $beta_1$ and $beta_2$ adrenergic receptors and adenosine 2A receptor (217, 219). Most small molecules bind in the transmembrane domain, mainly in a region flanked by helices 3, 5, 6 and 7. The crystallized receptors show a hydrogen-bond network between transmembrane domain 3 and 6 (E (D)RY motif) including a cluster of ordered water molecules from binding pocket to the cytoplasmic ends of the helices involved in G-protein binding. This network contains several most highly conserved residues of family A rhodopsin-like GPCRs (220). The inactive state of the receptor is stabilized by an ionic lock (221). Activation of the receptor disrupts the intramolecular interactions, leading to rearrangement of the conserved hydrogen-bond network, resulting in conformational change of the receptor observed in several biophysical and functional studies. The outward movement of the cytoplasmic end of transmembrane domain 6 opens the ionic lock and allows G-protein activation. The active receptor state is stabilized by the formation of several new intramolecular bridges (220). Recently, investigations by Schneider *et al.* concerning the H_4R indicated the ionic lock as being not essential for receptor stabilization in the inactive state for all class A GPCRs (222). After crystallization of $beta_2$ adrenergic receptor, a difference in distance of cytoplasmic ends of transmembrane domain 3 and 6 was observed, evidently greater in $beta_2$ adrenergic receptor than in rhodopsin receptor. This larger space associated with the more open conformation is probably responsible for constitutive activity found in many of the small-molecule binding GPCRs (223).

Computer-aided drug development in the HA research field becomes mainly apparent in H_3R and H_4R ligand development. Many pharmacophore models were reported in literature (113, 114, 170, 189, 224-227), also a few modeling approaches for the H_1R (39) and the H_2R (228) exist. Initial models for the investigation of the HA binding mode mainly in H_4R led to controversial results. Involvement of $Asp94^{3.32}$ (transmembrane domain 3) and $Glu182^{5.46}$ (transmembrane domain 5) was proven in different models. Whereas Shin *et al.* (229) concluded an ionic interaction of the deprotonated $Asp94^{3.32}$ with the cationic amine of the ethylamine side chain and $Glu182^{5.46}$ with the imidazole NH, Kiss *et al.* (230) offered an alternative binding mode for HA. Additional interactions with $Asn147^{4.57}$ (transmembrane domain 4) and $Ser320^{6.52}$ (transmembrane domain 6), which play a role in receptor activation and Thr323, respectively, were identified in these studies. $Glu182^{5.46}$ (present in H_3R and H_4R) is replaced by aspartic acid in H_1R. Lower interaction strength of HA and the aspartic acid residue could explain the different binding affinities to the receptor subtypes, which is much lower for H_1R than for H_3R and H_4R (230). Jongejan *et al.* identified in an H_4R agonist binding exercise a hydrogen-bond network containing of protonated $Glu182^{5.46}$, $Ser320^{6.52}$ and $Trp316^{6.48}$, which stabilize the trans-rotamer state of the tryptophan residue, indicated for GPCR activation (231). In a molecular dynamics simulation of Jójárt *et al.* an interaction of $Glu182^{5.46}$ and the amine *N*alpha was observed again, whereas no stable interaction of the imidazole NH and $Asp94^{3.32}$ was found. An additional hydrogen-bond interaction of imidazole *N*pi with $Asn147^{4.57}$ important for H_4R activation was detected (232). The models described so far have good description parameters for the binding, but the X-ray structures of the agonist- and antagonist-bound receptors are eagerly awaited.

8. PERSPECTIVE

The histamine receptor subtypes offered a great research field to medicinal chemists of numerous generations. H_1-Antihistamines are still well established drugs. As sedatives and antikinetic agents, first generation H_1R inverse agonists are used due to their brain penetrating properties. For the treatment of allergy, compounds of the second generation are indicated lacking some efficacy in chronical allergic conditions. Here, the use of dual acting ligands targeting H_1R and H_4R were discussed. H_2R antagonists lost their long standing predominance in the therapy of gastric ulcer due to the development of proton pump inhibitors. A small number of new H_1R and even a smaller number of H_2R ligands can be expected in the future. Ligands of the H_3R, especially inverse agonists, exhibit an advanced drug development status and first compounds are in a late phase of clinical trials. Numerous lead structures provide several pharmacodynamic and pharmacokinetic properties, which allow the design of a desired affinity and efficacy profile. However, some challenges still exist in the development processes of drug-like H_3R ligands for the market. Affinities for antitargets, such as CYP450 enzymes or P-gp as well as *h*ERG, responsible for severe side effects, may display problems. Due to high lipophilicity needed for CNS penetration and high plasma protein binding, many H_3R ligands show low blood/brain ratios, long half-lifes and extensive distribution in distinct tissues. Resulting brain accumulation and long duration of action of the compounds and/or active metabolites are linked to receptor tolerance, undesired wake-promoting effects and the induction of phospholipidosis. As a recent finding in clinical trials, insomnia arises as a pharmacodynamic problem. The early assessment for potential liabilities is recommended for success in drug development. So far, all of the drug-like H_3R ligands offer antagonistic properties even for the presynaptic H_3 autoreceptor. The development of clinically useful agonistic compounds as well as isoform-specific or downstream signaling-specific ligands could extend the scope for potential indications in the H_3R field. Although numerous H_3R compounds are currently in the pipeline, further variations and back-up candidates can be expected. The first H_3R inverse agonists are soon awaited for their market approval.

The growing interest in its function in inflammatory conditions and related disorders highlights the position of the H_4R as a promising drug target. Preclinical data support the importance of H_4R antagonists, being more efficient than the classical H_1-antihistamines in the treatment of allergy, inflammatory disorders and neuropathic pain. Meanwhile the first clinical candidate, UR-63325 for respiratory diseases (asthma, allergic rhinitis), has entered clinical phases. Indications for ligands of the H_4R as latest HR family member remain speculative and its (patho)physiological role and reliable animal models with high predictive value have to be further investigated. Based on preclinical studies, antagonists are probably indicated in (chronic) inflammatory conditions, such as inflammatory skin diseases (e.g. pruritus, atopic dermatitis) and respiratory diseases (e.g. asthma, allergic rhinitis). Beside the local application, systemic effects concerning autoimmune or arthritic diseases as well as the treatment of pain conditions are conceivable indications. Whereas H_3R ligands are mainly indicated in central disorders, H_4R are primarily connected to peripheral effects. Ligands combining H_3R and H_4R properties are currently being discussed as a potential treatment approach in neuropathic pain. So far, highly potent and selective H_4R ligands exist. Numerous different lead structures successfully face the problems of selectivity, species differences and pharmacokinetics (e.g. elongation of half-life). Management of adverse reactions arising from H_4R-mediated modulation of hematopoieses and immune response might be challenging for the development of clinical useful H_4R ligands. The therapeutic utility of H_4R ligands has to be proven in further preclinical and clinical investigations. Clear therapeutic indications with proof of concept in patients are still eagerly awaited.

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Abbreviations: ADME: absorption, distribution, metabolism and excretion; CNS: central nervous system; cAMP: cyclic adenosine monophosphate; CYP450: cytochrome P450; DAG: 1,2-diacylglycerol; GSK-3beta: glycogen synthase kinase-3beta; GM-CSF: granulocyte macrophage colony-stimulating factor; GPCR: G protein-coupled receptor; HA: histamine; *h*HR: human histamine receptor; *h*ERG: human ether-a-go-go related gene potassium channel; ICAM-1: inter-cellular adhesion molecule 1; IL-1beta: interleukin 1beta; iNOS: inducible nitric oxide synthase; IP_3: inositol-1,4,5-triphosphate; MAPK: mitogen-activated protein kinase; NF-kappaB: nuclear factor kappaB; P-gp: P-glycoprotein; PL: phospholipase; PK: protein kinase; SAR: structure-activity relationship; TNF-alpha: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein.

Key Words: Histamine, Receptor, Drug Design, Medicinal Chemistry, Drug Development, Review

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