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Histamine H_4 receptor agonists

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ABSTRACT

Since its discovery 10 years ago the histamine H_4 receptor (H_4R) has attracted attention as a potential drug target, for instance, for the treatment of inflammatory and allergic diseases. Potent and selective ligands including agonists are required as pharmacological tools to study the role of the H_4R in vitro and in vivo. Many H_4R agonists, which were identified among already known histamine receptor ligands, show only low or insufficient H_4R selectivity. In addition, the investigation of numerous H_4R agonists in animal models is hampered by species-dependent discrepancies regarding potencies and histamine receptor selectivities of the available compounds, especially when comparing human and rodent receptors. This article gives an overview about structures, potencies, and selectivities of various compounds showing H_4R agonistic activity and summarizes the structure–activity relationships of selected compound classes.

The biogenic amine histamine (**1**) mediates its effects by four histamine receptor (HR) subtypes, designated H_1 (H_1R), H_2 (H_2R), H_3 (H_3R), and H_4 receptors (H_4R), all belonging to family A of G-protein coupled receptors.[1] The H_1R is mainly expressed on smooth muscle cells, endothelia, immune cells and the CNS. Activation of this receptor subtype is, for instance, associated with allergic reactions.[2] H_1R antagonists (the 'antihistamines') have been therapeutically used since the 1940s,[2b] and the new generation H_1R antagonists like cetirizine or loratadine are still among the top selling drugs. H_2R[3] are mainly expressed in gastric parietal cells, the heart, neurons, and immune cells and play a crucial physiological role in stimulating gastric acid secretion.[2a] Thus, in the 1970s H_2R antagonists such as cimetidine and ranitidine became first-choice drugs for the treatment of gastric and duodenal ulcer and gastroesophagal reflux disease. The third histamine receptor subtype was discovered in 1983 by Schwartz and co-workers.[4] It is mainly located on neurons, predominantly in the CNS.[5] The H_3R acts as a presynaptic auto- and heteroreceptor controlling the release of histamine and other neurotransmitters. H_3R ligands are not marketed as drugs so far. However, H_3R antagonists are considered as drug candidates, for instance, for the treatment of dementia, Alzheimer's disease, narcolepsy, attention deficit/hyperactivity disorder or allergic rhinitis, and compounds from different companies have been under clinical investigation.[6]

After cloning of the human (h) H_3R in 1999 by Lovenberg,[7] in 2000 and 2001, several research groups were able to identify and to clone the fourth histamine receptor subtype due to its rather high sequence homology with the hH_3R (about 40% overall sequence identity and about 58% sequence identity within the transmembrane domains).[8] In contrast, the homology of the hH_4R with the hH_1R and hH_2R is low (about 20% overall sequence identity).[8b] Numerous reviews on the H_4R and the potential therapeutic value of antagonists appeared over recent years.[9] However, the (patho)physiological role of the H_4R is far from being understood. The hH_4R is mainly expressed in various cells of the immune system like eosinophils, T-lymphocytes, dendritic cells, mast cells, and basophils.[8b–d,9e,10] Recently, hH_4R expression was also detected in different areas of the CNS.[11] On the cellular level, H_4R activation induces chemotaxis of mast cells and eosinophils and triggers calcium mobilization in mast cells, monocytes and eosinophils.[12] Furthermore, the H_4R modulates the release of various inflammatory mediators.[10e,12c,13] In different animal models, blocking the H_4R with selective antagonists such as JNJ7777120 (**79**) proved to be beneficial, for example, in mouse allergic airway inflammation, in a mouse peritonitis or a rat colitis model.[14] H_4R antagonists were essentially more effective in the attenuation of experimental pruritus than the classical H_1R antagonists.[15] All these results indicate the hH_4R to play an important role in different inflammatory, autoimmune, and allergic disorders. Consequently, antagonists are discussed as possible drugs for the treatment of diseases like asthma, allergic rhinitis, pruritus, pain or inflammatory bowel disease.[16] To further explore the role of the H_4R, selective ligands are required. This article gives an overview about currently known H_4R agonists.

H_1R ligands with agonistic activity at the hH_4R: Several hH_1R ligands were investigated for their affinity and activity at the hH_4R

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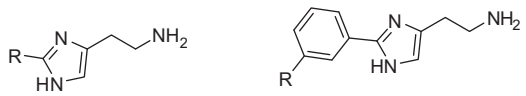
by the groups of Leurs[17] and Seifert.[18] Typical hH_1R antagonists such as mepyramine, hydroxyzine, fexofenadine, cetirizine or loratadine demonstrate negligible affinity for the hH_4R (pK_i <5). In contrast, the first described selective H_1R agonist, 2-methylhistamine (**2**),[19] behaves as an almost full agonist at the hH_4R with comparable potencies at hH_1R and hH_4R (Table 1). Many 2-phenylhistamines, originally developed as selective H_1R agonists,[20] turned out to be also moderate partial agonists at the hH_4R (pEC_{50}: 4–6). Examples are **3** and **4** with around 10-fold selectivity for the hH_1R. In contrast, histaprodifen-type H_1R agonists show no significant agonistic activity at the H_4R receptor.[18b] Taken together, as expected from the low sequence homology between the hH_1R and hH_4R,[8b] most H_1R ligands have low affinity for the hH_4R and only few compounds show partial agonistic activity.

H_2R ligands with agonistic activity at the hH_4R: Like the hH_1R, the hH_2R shows only low sequence identity with the hH_4R (22%).[8b] However, several H_2R ligands exhibit agonistic activity at the hH_4R, some even proved to be hH_4R selective (Table 2). 5(4)-Methylhistamine (**5**) was among the first described selective H_2R agonists.[3,19] However, in 2005, Lim et al.[17] revealed **5** as a potent and selective full hH_4R agonist showing >100-fold selectivity for the hH_4R over the other histamine receptor subtypes. Since this compound is well accessible, it has become the most frequently used hH_4R agonist. However, **5** has to be employed with care, as at higher concentrations, this compound is able to activate also other histamine receptor subtypes, in particular H_2Rs. Moreover, many hH_4R agonists are substantially less potent and selective in rodents.[22] In the case of **5**, the potency at the mouse H_4R (mH_4R) and rat H_4R (rH_4R) is almost 100-fold reduced (pEC_{50} mH_4R: 5.8, rH_4R: 5.6).[17] As shown for **1**, the reason could be that Phe-169 in the second extracellular loop of the hH_4R is replaced by valine in rodents.[22c] The different pharmacological behavior of agonists at the hH_4R and the m/rH_4Rs correlates with the rather low homology between the species isoforms: hH_4R and mH_4R: 68%, hH_4R and rH_4R: 69%.[22a] In contrast, the hH_2R shares a higher homology with rodent H_2Rs: hH_2R and m/rH_2R: 85%.[22a] Accordingly, **5** shows a potency of around 40% relative to histamine at both the hH_2R and the rH_2R.[17,19] Unlike agonists, H_4R antagonists obviously do not demonstrate such pronounced species differences.[22a] For example, the potent and selective H_4R antagonist JNJ 7777120 (**79**) shows almost identical affinities for the human, rat and mouse

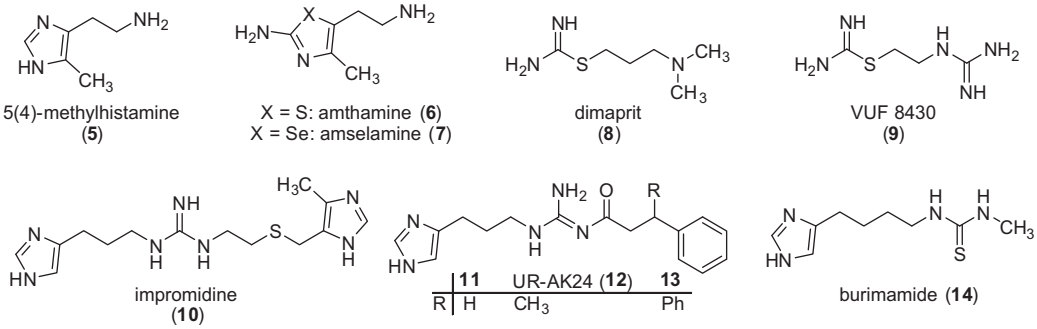
Table 1
H_1R ligands with agonistic activity at the hH_4R[a]



No.	hH_1R[b]		hH_4R[c]		
	pEC_{50}	α	pK_i	pEC_{50}	α
1	6.7	1.0	8.1	7.9	1.0
2	6.1	0.9	6.1	5.4	0.9
3	6.7	0.7	5.8	5.0	0.6
4	6.6	0.7	5.9	5.8	0.5

[a] Potencies (pEC_{50} values)[21] and intrinsic activities (α, relative to the maximal response to histamine = 1.0): determined in steady-state GTPase assays on Sf9 insect-cell membranes coexpressing the hH_1R + RGS4 or RGS19 or the hH_4R-RGS19 fusion protein, $G_{\alpha i2}$ and $G_{\beta 1\gamma 2}$; hH_4R-affinities: pK_i values[21] determined on Sf9 insect-cell membranes expressing the hH_4R-RGS19 ($+G_{\alpha i2} + G_{\beta 1\gamma 2}$), using [^{3}H]histamine as radioligand.
[b] Ref. 18a.
[c] Ref. 18b.

Table 2
H_2R ligands with agonistic activity at the hH_4R



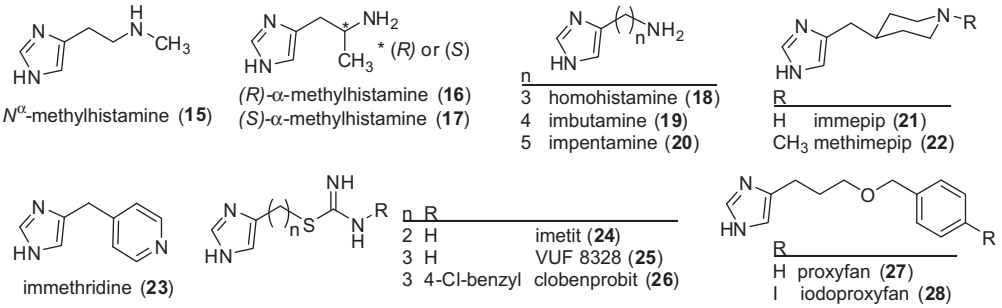
No.	hH_1R		hH_2R			hH_3R			hH_4R		
	pEC_{50}	α	pK_i	pEC_{50}	α	pK_i	pEC_{50}	α	pK_i	pEC_{50}	α
1[a]	–	–	4.3	–	–	8.0	8.3	1.0	7.8	7.7	1.0
5[a]	–	–	5.1	–	–	5.2	–	–	7.3	7.4	1.0
5[b]	4.8	0.9	–	5.5	1.0	–	–	–	–	7.2	0.9
6[a]	–	–	5.2	Agonist	–	–	–	–	5.3	–	0
7[a]	–	–	5.0	Agonist	–	–	–	–	5.6	–	0
8[a]	–	–	4.6	Agonist	–	–	–	–	6.5	5.8	0.8
9[a]	No agonistic activity		Low affinity		0.5	6.0	6.5	1.0	7.5	7.3	1.0
10[a]	–	–	6.3	Agonist	–	–	–	–	7.6	7.6	0.5
11[b]				7.2	0.8	–	8.6	0.5	–	7.8	0.7
12[b]	4.8 (pK_B)	0.4	–	7.2	0.9	–	8.6	0.2	–	7.8	0.8
13[b]	5.5 (pK_B)	0.2	–	7.1	0.7	–	7.8 (pK_B)	–	–	8.1	0.8
14[a]	–	–	5.4	–	–	–	–	–	7.4	7.7	0.7

[a] pK_i values from radioligand displacement studies: hH_2R: CHO-cells expressing the hH_2R, [^{125}I]iodoaminopotentidine; hH_3R: homogenate of SK-N-MC-cells expressing the hH_3R, [^{3}H]N^{α}-methylhistamine; hH_4R: homogenates of SK-N-MC-cells expressing the hH_4R, [^{3}H]histamine. Potencies and intrinsic activities at hH_3R and hH_4R: CRE-β-galactosidase reporter gene assays, SK-N-MC-cells expressing the hH_3R or hH_4R.[17,24]
[b] Potencies and intrinsic activities at hH_1R, hH_2R, hH_3R and hH_4R: determined in steady-state GTPase assays on Sf9 insect-cell membranes (co)expressing the receptor of interest and pertinent additional proteins (hH_1R + RGS4, hH_2R-$G_{s\alpha S}$ fusion protein, $hH_3R + G_{i\alpha 2} + G_{\beta 1\gamma 2}$ + RGS4, hH_4R-RGS19 + $G_{i\alpha 2} + G_{\beta 1\gamma 2}$).[29a,31]

H_4Rs.[14b] When considering hH_2R agonistic activity the imidazole ring of histamine can be replaced with an aminothiazole (amthamine, **6**) or an aminoselenazole (amselamine, **7**) ring. However, this modification results in a loss of agonistic activity at the hH_4R.[17] So the bioisosteric replacement of the imidazole ring in H_2R agonists with an aminothiazole or an aminoselenazole ring is an option to shift selectivity of such compounds toward the H_2R.[23] With respect to H_4R activity the imidazole ring can be replaced by an isothiourea moiety. Since the H_2R agonist dimaprit (**8**) was identified as a moderate hH_4R partial agonist, some dimaprit analogues were investigated for their H_4R activity.[24] *S*-(2-Guanidinylethyl)-isothiourea (VUF 8430, **9**), previously developed as an H_2R agonist,[25] proved to be a potent hH_4R agonist[26] with about 30-fold selectivity for the hH_4R over the hH_3R (full agonism) and negligible affinity for hH_1R (no agonism) and hH_2R (partial agonism). However, full agonistic activity of **9** at the hH_3R has to be taken into account or investigations on H_4Rs should be performed in the absence of H_3Rs to avoid misinterpretation of results. For example, H_3Rs are not expressed in most immune cells such as mast cells or eosinophils, where the H_4R is mainly located.[9a,12b,27] Imidazolylpropylguanidines such as impromidine (**10**) and arpromidine-like compounds belong to the most potent H_2R agonists.[28] In addition, these compounds are moderate H_1R antagonists and potent H_3R antagonists.[28b] At the hH_4R, **10** proved to be a potent partial agonist.[17] N^G-acylated imidazolylpropylguanidines were developed as H_2R agonists with improved pharmacokinetic properties due to strongly reduced basicity.[29] Most of these imidazoles (e.g., UR-AK51 (**11**), UR-AK24 (**12**) or UR-PG80 (**13**)) turned out be potent hH_4R agonists, even showing some selectivity for the hH_4R over the hH_2R.[29a,30] Whereas the corresponding aminothiazole bioisosteres were highly selective for the H_2R,[23c] the N^G-acylated imidazolylpropylguanidines can be considered as promising leads for the development of new H_4R agonists. Likewise, the thiourea burimamide (**14**), the first antagonist used for the pharmacological characterization of the H_2R,[3] exerts agonistic activity at the H_4R.[17] Interestingly, in contrast to the other above mentioned imidazole-type compounds, **14** does not contain a second basic group, which is obviously not required for hH_4R agonistic activity.

H_3R ligands with agonistic activity at the hH_4R: As the hH_4R shares the highest sequence homology with the hH_3R,[8c] it is not astonishing that many hH_3R ligands, in particular imidazole-containing compounds, bind to the hH_4R as well (Table 3).[17] The endogenous ligand, histamine, shows comparable affinities for the hH_3R and hH_4R ($pK_i \approx 8$).[8c,f,17,32] Most H_3R (partial) agonists also have (partial) agonistic activity at the hH_4R.[17] However, the examined compounds were 5–15,000-fold more potent at the hH_3R than at the hH_4R.[17] The reference hH_3R agonists N^{α}-methylhistamine (**15**) and (*R*)-α-methylhistamine (**16**) show 80- and 40-fold hH_3R selectivity, respectively. (*S*)-α-methylhistamine (**17**) has about 10-fold lower affinity than **16** at both subtypes. The histamine homologues homohistamine (**18**) and imbutamine (**19**) are agonists with similar hH_3R and hH_4R affinity, but hH_3R-selective potency, whereas the higher homologue impentamine (**20**) is an almost full hH_3R agonist but has no agonistic activity at the hH_4R. In this group of compounds the highest selectivity for the hH_3R resides in methimepip (**22**) and immethridine (**23**) (EC_{50} ratios of ~16,000 and ~6300, respectively). The hH_3R agonists imetit (**24**), VUF8328 (**25**), proxyfan (**27**), and iodoproxyfan (**28**) also show partial agonism, but 10–100-fold lower potency, at the hH_4R. The hH_3R inverse agonist clobenpropit (**26**) turned out to be a potent partial agonist at the hH_4R and one of a few compounds that activate the hH_4R, but not the hH_3R. This makes **26** an interesting pharmacological tool.

Table 3
H_3R ligands with agonistic activity at the hH_4R[a]



No.	hH_3R			hH_4R		
	pK_i	pEC_{50}	α	pK_i	pEC_{50}	α
1	8.0	8.3	1.0	7.8	7.7	1.0
15	8.4	9.4	1.0	6.5	6.1	1.0
16	8.2	9.5	1.0	6.6	6.2	1.0
17	7.2	8.0	1.0	5.4	4.9	1.0
18	7.3	7.4	0.9	7.5	6.7	0.8
19	8.4	9.2	1.0	8.0	7.5	0.8
20	8.3	8.4	0.9	6.6		0.0
21	9.3	10.4	1.0	7.7	7.8	0.9
22	9.0	9.5	0.8	5.7	5.3	0.5
23	9.1	9.8	0.9	6.6	6.0	0.5
24	8.8	9.9	1.0	8.2	7.9	0.9
25	8.5	9.3	1.0	8.0	7.9	0.6
26	8.6	9.4	−1.0	8.1	7.7	0.8
27	7.9	8.5	1.0	7.3	7.2	0.5
28	9.2	10.3	1.0	7.9	7.9	0.7

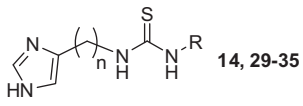
[a] Data[21] from Refs. 17,33. hH_3R affinities were determined with homogenates of SK-N-MC-cells expressing the hH_3R using [^{3}H]N^{α}-methylhistamine as radioligand, hH_4R affinities were determined with SK-N-MC-cell homogenates expressing the hH_4R and [^{3}H]histamine as radioligand, hH_3R and hH_4R potencies and intrinsic activities were determined by CRE-β-galactosidase reporter gene assays on SK-N-MC-cells expressing the hH_3R or hH_4R.[17]

In 1995, Vollinga et al. described burimamide (**14**) analogues as potent H_3R antagonists (determined at the guinea-pig ileum).[33] Like the H_2R antagonist **14**, several of these compounds proved to be potent hH_4R partial agonists (see **29**–**35**, Table 4). A four-membered carbon chain separating the imidazole ring from the thiourea moiety is essential for hH_4R agonistic activity. Small alkyl substituents at the thiourea group like methyl (**14**), ethyl (**29**), *n*-propyl (**30**) or isopropyl (**31**) are better tolerated than larger groups like benzyl (**32**) or phenylethyl (**33**).

Imifuramine analogues as hH_4R agonists: The chiral disubstituted tetrahydrofuran derivative imifuramine (**38**) is a rather potent full H_3R agonist with 50-fold selectivity for the hH_3R versus the hH_4R (Table 5).[34] Hashimoto et al. synthesized imifuramine analogues and investigated these compounds at the hH_3R and hH_4R. All four stereoisomers of imifuramine are partial to full agonists at the hH_3R and hH_4R with selectivity for the hH_3R. Introduction of a non-basic cyanoguanidine moiety (**40**–**43**) instead of the primary amino group substantially reduced the activity of the compounds at the hH_3R, but improved for most isomers potency and intrinsic activity at the hH_4R. In particular, the cyanoguanidine analogue of imifuramine, the (2*R*,5*R*)-configured isomer OUP-16 (**42**),[34c] proved to be a rather potent full hH_4R agonist with 40-fold selectivity over the hH_3R, where **42** behaves as a moderate partial agonist. There is a clear stereoselectivity in favor of **42** compared to its optical antipode, the (2*S*,5*S*)-configured enantiomer **43**, with an eudismic ratio of >250 in potency at the hH_4R. The (2*R*,5*S*)-isomer **41** is also a full hH_4R agonist with about 40-fold selectivity over the hH_3R. Replacing the amino group in **38** with a benzyloxy group results in moderate to potent hH_3R and hH_4R (partial) agonists (**44**–**47**) without noteworthy selectivity for one of these receptor subtypes. As observed for burimamide (**14**) and related thioureas (**29**–**35**) the replacement of a basic amino group with a non-basic moiety (cyanoguanidine or benzyl ether) is likewise tolerated with respect to hH_4R agonistic activity. Due to the laborious preparation of the stereoisomer, compound **42** is less in use as a tool for pharmacological investigations.

Clozapine and analogues as hH_4R agonists: Already 10 years ago, when the hH_4R was cloned by several research groups, the antipsychotic drug clozapine (**48**, Table 6) was found to activate this new histamine receptor subtype.[8b,d,f,g] Based on this result, Smits et al. prepared a series of clozapine-analogues (Table 6) to investigate the structure–activity relationships at the hH_4R.[35] Replacing the NH-fragment in the diazepine-ring with a sulfur atom (**49**) or N–CH_3 (**50**) results in 10-fold lower affinity for the hH_4R. In contrast, introduction of an oxygen-atom at this position (**51**) slightly increases affinity. Exchanging the piperazine ring in **48** with a morpholine (**52**) or piperidine ring (**53**) is not tolerated. Obviously, the distal basic group in the piperazine ring is essential for hH_4R affinity. Except for the 2-chloro-derivative **54**, modification of the halogen substitution at the tricyclic ring system of **51** (introduction

Table 4
Imidazolylalkylthiourea derivatives with agonistic activity at the hH_4R



14, 29-35

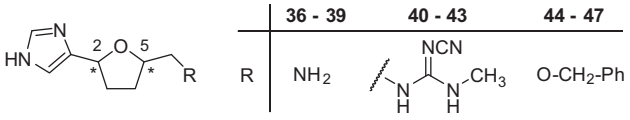
No.	*n*	R	hH_2R[a]	gpH_3R[a]	hH_4R[b]		
			pK_i	pA_2	pK_i	pEC_{50}	α
14[c]	4	CH_3	5.4	7.0	7.4	7.7	0.7
29	4	CH_2CH_3	—	7.4	7.6	7.0	0.8
30	4	$(CH_2)_2CH_3$	—	7.3	8.0	7.2	0.8
31	4	$CH(CH_3)_2$	—	7.5	8.1	7.7	0.8
32	4	CH_2Ph	—	7.1	7.3	7.1	0.3
33	4	$(CH_2)_2Ph$	—	7.0	7.2	5.9	0.3
34	5	CH_2CH_3	5.0	8.0	7.6	—	0
35	5	CH_2-(4-Cl-phenyl)	5.8	8.1	6.9	7.2	−1.0

[a] Data from Refs. 17,33, hH_2R affinities: CHO-cells expressing the hH_2R, radioligand [^{125}I]iodoaminopotentidine; gpH_3R antagonism: determined on guinea-pig jejunum preparations.[33]

[b] hH_4R: pK_i values from [^{3}H]N^{α}-methylhistamine displacement assay, pEC_{50} values and intrinsic activities (α) from CRE-β-galactosidase reporter gene assays on SK-N-MC-cells expressing the hH_4R.[17]

[c] Burimamide.

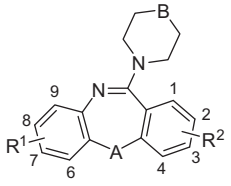
Table 5
Imifuramine analogues with agonistic activity at the hH_4R[a]



No.	Config.	hH_3R			hH_4R		
		pK_i	pEC_{50}	α	pK_i	pEC_{50}	α
1	—	7.5	8.4	1.0	—	7.7	1.0
36	2*S*,5*R*	5.8	6.1	1.0	5.2	5.1	1.0
37	2*R*,5*S*	5.7	6.1	1.0	5.6	5.3	0.9
38 (imifuramine)	2*R*,5*R*	6.6	7.4	1.0	6.1	5.7	0.7
39	2*S*,5*S*	6.7	7.0	0.9	4.9	4.5	0.6
40	2*S*,5*R*	5.1	—	<0.1	5.1	5.1	1.1
41	2*R*,5*S*	5.2	5.0	0.4	6.7	6.7	1.0
42 (OUP-16)	2*R*,5*R*	5.7	5.5	0.8	6.9	7.1	1.0
43	2*S*,5*S*	4.7	<4.0	—	4.7	4.7	1.1
44	2*S*,5*R*	5.2	5.0	0.9	4.9	4.9	0.8
45	2*R*,5*S*	6.5	6.7	1.1	6.4	6.9	0.9
46	2*R*,5*R*	6.6	7.0	1.0	6.0	6.1	0.5
47	2*S*,5*S*	5.0	5.0	0.7	4.7	4.9	0.5

[a] Data from Ref. 34c, hH_3R and hH_4R affinities pK_i values determined by displacement of [^{3}H]N^{α}-methylhistamine or [^{3}H]histamine, respectively, using homogenates of SK-N-MC-cells expressing the receptor of interest; pEC_{50} values and intrinsic activities: from CRE-β-galactosidase reporter gene assays on SK-N-MC-cells expressing the hH_3R or the hH_4R.[34c]

Table 6
Clozapine-analogues with agonistic activity at the hH_4R



48-56

No.	A	B	R^1	R^2	hH_4R[a]		
					pK_i	pEC_{50}	α
48[b]	NH	NCH_3	8-Cl	H	6.7	6.8	1.0
49[c]	S	NCH_3	8-Cl	H	5.7	—	—
50[c]	NCH_3	NCH_3	8-Cl	H	5.9	—	—
51[c]	O	NCH_3	8-Cl	H	7.4	7.6	1.0
52[c]	NH	O	8-Cl	H	<5.0	—	—
53[c]	NH	CH_2	8-Cl	H	<5.0	—	—
54[c]	O	NCH_3	H	2-Cl	5.3	6.7	0.5
55[c]	O	NCH_3	8-Cl	4-F	7.6	7.8	1.0
56[c]	O	NCH_3	7-Cl	H	7.6	7.7	1.0

[a] Data from Ref. 35, hH_1R: pK_i values determined by displacement of [^{3}H]mepyramine, pEC_{50} values and intrinsic activities (see footnote[c]) determined by NFκB-luciferase reporter assay using COS-7 cells transiently transfected with the hH_1R; hH_2R: pK_i values: [^{125}I]iodoaminopotentidine, CHO cells expressing the hH_2R; hH_3R: pK_i values: [^{3}H]N^{α}-methylhistamine, SK-N-MC-cell homogenates expressing the hH_3R; hH_4R affinity: [^{3}H]histamine, homogenates of SK-N-MC cells expressing the hH_4R.; hH_4R agonism: CRE-β-galactosidase reporter gene assays on SK-N-MC-cells expressing the hH_4R

[b] Ref. 35, hH_1R: pK_i = 9.4.

[c] Ref. 35, selectivity data for **56**: hH_1R: pK_i = 8.1, pEC_{50} = 8.2, α = −1.0, hH_2R: pK_i = 5.1, hH_3R: pK_i = 5.0.

of fluorine and chlorine atoms at positions 2, 3, 4, 7, and 8, including twofold substitution as in **55**) did not significantly change the hH_4R affinity and agonistic activity. The 7-chloro-analogue **56** is among the most potent hH_4R agonists of this series. This compounds binds poorly to the hH_2R and hH_3R ($pK_i \approx 5$) but, as expected from **48**, shows high affinity for the hH_1R (pK_i: 8.1, inverse agonist, α: −1.0). At the hH_4R, **56** is a potent full agonist with about 10-fold higher affinity than **48**. However, since **48** binds to many different GPCRs like muscarinic, adrenergic, serotonergic, and dopaminergic receptors, a similar binding profile, that is, lack of specificity, can also be expected for the clozapine-analogue **56**. Therefore, the dibenzoxazepines have only a limited value as pharmacological tools, but the rigid structure makes these compounds interesting to study the binding site at the hH_4R and to establish a pharmacophore model.[16a,35]

Acylguanidine-type hH_4R agonists: N^G-acylated imidazolylpropylguanidines such as **11**–**13**, originally developed as H_2R agonists, are also potent hH_4R agonists.[29a] Regarding the H_2R, the imidazolylpropylguanidine moiety is considered essential for H_2R agonistic activity, whereas an acyl group of sufficient size is required to confer H_2R affinity. Inspired by the small endogenous ligand histamine (**1**), which shows considerably higher potency at the hH_4R ($pEC_{50} \approx 8$) than at the hH_2R ($pEC_{50} \approx 6$),[31] the bulky acyl substituents in acylguanidine-type hH_2R agonists[29a] were replaced with small alkanoyl residues (**58**–**61**).[31] As expected, the hH_2R agonistic potency was reduced 10-fold with decreasing the size of the acyl residue (pEC_{50}: 7.2→6.1, Table 7). In contrast, the hH_4R agonistic potency was retained or even slightly increased (cf. **58**–**61** vs **11**–**13**). Thus, by replacing the bulky residues such as 3-phenylbutanoyl (**12**) or 3,3-diphenylpropanoyl (**13**) with a small acetyl residue as in **60** (UR-PI288), the selectivity for the hH_4R compared to the hH_2R was essentially improved from 4- to 150-fold. In addition, **60** shows >1000-fold selectivity over the hH_1R and possesses only low intrinsic activity at the hH_3R. Independent of the size of the acyl-substituent, **58**–**61** are highly potent partial hH_3R agonists with low intrinsic activity. Interestingly, the weak partial H_2R agonist imidazolylpropylguanidine (SK&F 91486, **61**)[36] turned out to be a rather potent hH_3R and hH_4R partial agonist. However, compared to the acylguanidines, **61** shows a substantially higher intrinsic activity at the hH_3R. Obviously, acylation of **61** is detrimental to the intrinsic activity at the hH_3R, but not at the hH_4R.

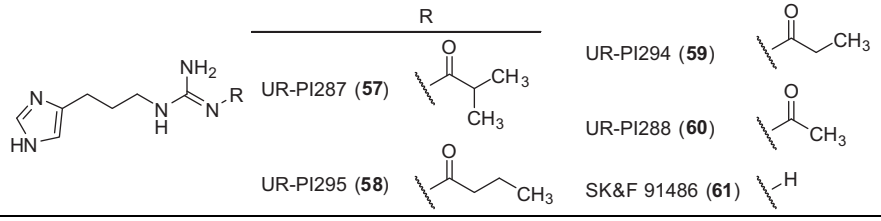
In this series the acylguanidine **59** (UR-PI294) is a compound of particular interest. Its propionyl group allows radiolabeling using commercially available succinimidyl [^{3}H]propionate.[37] The high affinity $hH_{3/4}R$ radioligand [^{3}H]UR-PI294 can be readily prepared under common laboratory conditions to yield specific activities of up to 80 Ci/mmol, which is substantially higher than that of commercially available tritiated histamine.[37]

The major drawback of these potent acylguanidine-type hH_4R agonists is the lack of selectivity compared to the hH_3R. However, these compounds including [^{3}H]-**59** are valuable pharmacological tools, in particular, for the investigation in native or recombinant systems expressing either the hH_3R or the hH_4R. For instance, due to >1000- and 100-fold selectivity over the hH_1R and hH_2R, N^G-alkanoyl imidazolylpropylguanidines can be used for pharmacological experiments on the H_4R in native systems devoid of hH_3Rs (e.g., different types of immune cells[9a,12b,27]).

Imidazolylbutylcyanoguanidines as hH_4R agonists: As described above, N^G-acylated imidazolylpropylguanidines are highly potent hH_4R agonists. However, the major drawback of compounds like **11**–**13** (Table 2) is their hH_2R and hH_3R (partial) agonism. Introduction of small alkanoyl residues (**57**–**60**) considerably increases selectivity for the hH_4R versus the hH_2R but these compounds still have agonistic activity at the hH_2R. A second basic group in addition to the imidazole ring is essential for H_2R activation but not required for H_4R agonism. Replacing the strongly basic guanidine in impromidine (**10**, Table 2) with a non-basic cyanoguanidine group turns H_2R agonism into H_2R antagonism.[28a] Moreover, a thiourea moiety as in **14** or a cyanoguanidine group as in **42** is compatible with H_4R agonism, and a cyanoguanidine moiety is suitable to reduce hH_3R activity (cf. **42** vs **38**). Therefore, exchanging the acylguanidine with a cyanoguanidine group was considered as a promising approach to improve hH_4R selectivity.[32c]

The cyanoguanidine **62**,[32c] a moderate hH_4R partial agonist (50-fold less potent than **11**), is only a weak hH_2R partial agonist and a moderate hH_3R inverse agonist (Table 8). The lower homologue of **62**, compound **63**, is almost inactive at the hH_4R, whereas the extension of the chain length, resulting in **64**, essentially improves hH_4R agonist potency (fivefold) and intrinsic activity (α: 0.9). This observation is consistent with the structure–activity relationships of **42**, since the distances between imidazole ring and cyanoguanidine group are comparable in both molecules. Further elongation of the chain (**65**) results in loss of hH_4R agonistic activity. In contrast to the N^G-acylated imidazolylpropylguanidines (e.g., **13**), a bulky diphenylpropyl group (**68**) is not tolerated, suggesting different binding modes of acylguanidines and cyanoguanidine-type

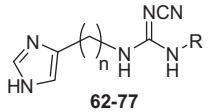
Table 7
H_xR selectivity profiles of acylguanidine-type compounds with agonistic activity at the hH_4R[a]



No.	hH_1R		hH_2R		hH_3R		hH_4R	
	pEC_{50}	α	pEC_{50}	α	pEC_{50}	α	pEC_{50}	α
1	6.7	1.0	5.9	1.0	7.6	1.0	7.9	1.0
12	–	0.4	7.2	0.9	8.6	0.2	7.8	0.8
57	5.6	0.4	7.0	0.9	8.9	0.3	8.4	0.9
58	5.7	0.3	6.9	0.8	8.8	0.4	8.6	1.0
59	5.5	0.3	6.4	0.8	8.8	0.4	8.5	0.9
60	4.9	0.2	6.1	0.8	8.4	0.3	8.3	1.0
61	–	–	5.6	0.7	8.1	0.7	8.1	0.8

[a] Data from Ref. 31. Potencies and intrinsic activities: determined in steady-state GTPase assays on Sf9 insect-cell membranes expressing the hH_1R + RGS4, the hH_2R-$G_{s\alpha S}$ fusion protein, the hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ + RGS4 or the hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$.

Table 8
Cyanoguanidine-type compounds with agonistic activity at the hH_4R[a]



62-77

No.	*n*	R	hH_1R		hH_2R		hH_3R		hH_4R	
			$pEC_{50}/(pK_B)$	α	$pEC_{50}/(pK_B)$	α	pEC_{50} /(pK_B)	α	pEC_{50} /(pK_B)	α
1			6.7	1.0	5.9	1.0	7.6	1.0	7.9	1.0
62	3	$(CH_2)_3$-Ph	–	–	4.9	0.4	(5.7)	−0.4	6.1	0.5
63	2	$(CH_2)_3$-Ph	(<5.0)	–	<5.0	–	(5.2)	−0.3	(<5.0)	–
64	4	$(CH_2)_3$-Ph	(<5.0)	–	(5.3)	0.1	(5.6)	0.0	(6.8)	0.9
65	5	$(CH_2)_3$-Ph	–	–	(5.3)	–	(6.6)	–	(7.0)	−0.3
66	4	$(CH_2)_2$-Ph	–	–	(<5.0)	–	(5.8)	−0.2	6.0	0.4
67	4	$(CH_2)_4$-Ph	–	–	(5.0)	0.1	(5.5)	−0.1	(6.3)	0.1
68	4	$(CH_2)_2$-$CHPh_2$	–	–	(<5.0)	–	–	–	(5.6)	−0.4
69	4	$(CH_2)_3$-2-Pyr	–	–	(5.5)	0.1	(5.9)	−0.4	6.2	0.6
70	4	$(CH_2)_3$-3-Pyr	–	–	(5.9)	0.2	(6.2)	−0.1	5.9	0.3
71	4	$(CH_2)_3$-4-Pyr	–	–	(5.1)	0.2	(6.1)	−0.3	5.6	0.4
72[b]	4	$(CH_2)_2$-*S*-Ph	(<5.0)	0.1	(<5.0)	0.1	(6.0)	−0.3	7.5	0.9
73	4	H	–	–	(<5.0)	0.0	6.6	0.4	6.8	0.9
74	4	CH_3	(<5.0)	–	(<5.0)	0.0	(5.7)	0.2	6.0	0.8
75	4	CH_2CH_3	(<5.0)	–	(<5.0)	0.1	(5.5)	0.1	6.2	0.8
76	4	$CH(CH_3)_2$	(<5.0)	–	(<5.0)	0.1	(5.5)	0.1	6.5	0.9
77	4	$CH_2CH(CH_3)_2$	(<5.0)	–	(<5.0)	0.1	(5.5)	−0.1	6.9	0.9

[a] Data from Ref. 32c. Potencies and efficacies determined in steady-state GTPase assays on Sf9 insect-cell membranes expressing the hH_1R + RGS4, the hH_2R-$G_{s\alpha S}$ fusion protein, the hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ and RGS4 or the hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$.
[b] UR-PI376.

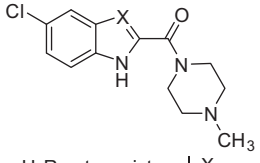
hH_4R ligands. Exchanging the phenyl ring with 2-, 3- or 4-pyridyl (**69**–**71**) reduces activity. In terms of both hH_4R agonistic potency and selectivity, replacing the benzylic methylene group by a sulfur atom (**72**, UR-PI376) turned out to be a key step. Unlike the acylguanidines **57**–**60**, compound **72** is devoid of agonistic activity at the other three histamine receptor subtypes. The selectivity of **72** for the hH_4R relative to the hH_3R is about 30-fold, and the affinities for hH_1R and hH_2R are negligible.

Small N^G-substituents were found to improve hH_4R selectivity over the hH_2R in the acylguanidine series (**57**–**60**),[31] The same structural modification was unsuccessful in the cyanoguanidine series.[32c] The unsubstituted parent compound **73** turned out to be a rather potent hH_4R agonist, however, selectivity was lost. In contrast to the imidazolylbutylcyanoguanidines **62**–**72**, compound **73** shows hH_3R partial agonism, suggesting a substituent 'R' of sufficient size to be required to discriminate between hH_4R and hH_3R. Indeed, methyl substitution at the cyanoguanidine group (**74**) reduces potency at both HR subtypes, but potencies at the hH_4R rise with increasing size of the alkyl substituent (**75**–**77**) whereas partial agonistic activity at the hH_3R is abolished.

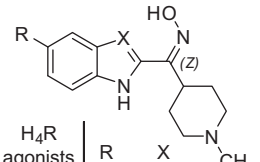
Compound **72** is the most potent and selective hH_4R agonist in this series. Compared to the acylguanidine **11**, the potencies at the hH_2R and the hH_3R were reduced by factors of >100 and about 400, respectively, whereas hH_4R agonistic potency was retained. In contrast to other selective hH_4R agonists such as 5(4)-methylhistamine (**5**), OUP-16 (**42**) or VUF8430 (**9**), UR-PI376 (**72**) does not activate other human histamine receptor subtypes (GTPase assays). However, preliminary investigations revealed that this compound—like many other hH_4R agonists—shows substantially reduced potency at the mH_4R. Therefore, **72** is in particular an interesting tool for pharmacological experiments with human H_4Rs and, for instance, not suitable for investigations on mouse H_4Rs.

Oxime analogues of JNJ7777120 as H_4R agonists: Very recently, oxime analogues (**80**–**83**) of the selective H_4R antagonists JNJ7777120 (**78**) and JNJ10191584 (VUF 6002, **79**) were reported as a new class of H_4R agonists.[38] In these compounds the piperazine ring of **78** and **79** was replaced with a piperidine ring, that is, the amide was changed to a ketone, which was converted to the oxime. Interestingly, the H_4R agonistic activity resides only in

Table 9
Oxime analogues (**80-83**) of the H_4R antagonists JNJ 7777120 (**78**) and JNJ 10191584 (**79**) as H_4R agonists[a]



H_4R antagonists	X
JNJ 7777120 (**78**)	CH
JNJ 10191584 (**79**)	N



H_4R agonists	R	X
80	H	CH
81	CH_3	CH
82	Cl	CH
83	Cl	N

No.	hH_1R	hH_2R	hH_3R	hH_4R		
	pK_i	pK_i	pK_i	pK_i	pEC_{50}	α
1	–	–	–	7.9	7.1	1.0
80	<5.0	<6.0	5.6	7.3	–	–
81	<5.0	<6.0	<5.0	7.3	7.0	0.7
82	<5.0	<6.0	<5.0	7.5	7.4	0.7
83	<6.0	<6.0	<5.0	7.8	7.1	0.6

No.	mH_4R			rH_4R			cH_4R
	pK_i	pEC_{50}	α	pK_i	pEC_{50}	α	pK_i
1	7.1	5.3	1.0	7.2	6.8	1.0	7.2
80	7.0	–	–	6.5	–	–	5.3
81	7.7	6.7	1.2	6.7	6.3	0.9	5.0
82	8.1	8.0	1.1	7.7	8.0	1.1	5.1
83	8.1	7.2	1.0	7.8	6.7	0.9	5.9

[a] Data[21] from Ref. 38. pK_i values: hH_1R, hH_3R, hH_4R, mH_4R, rH_4R: radioligand displacement assays were performed using cell pellets from SK-N-MC cells transfected with the respective histamine receptor. For the mH_3R, brain homogenates, and for the cH_4R, transiently transfected COS-7 cells were used. Functional assays were performed with cells expressing a serum-responsive element-luciferase (SRE-luciferase) reporter gene construct and G_{qi} chimera G-proteins.[38]

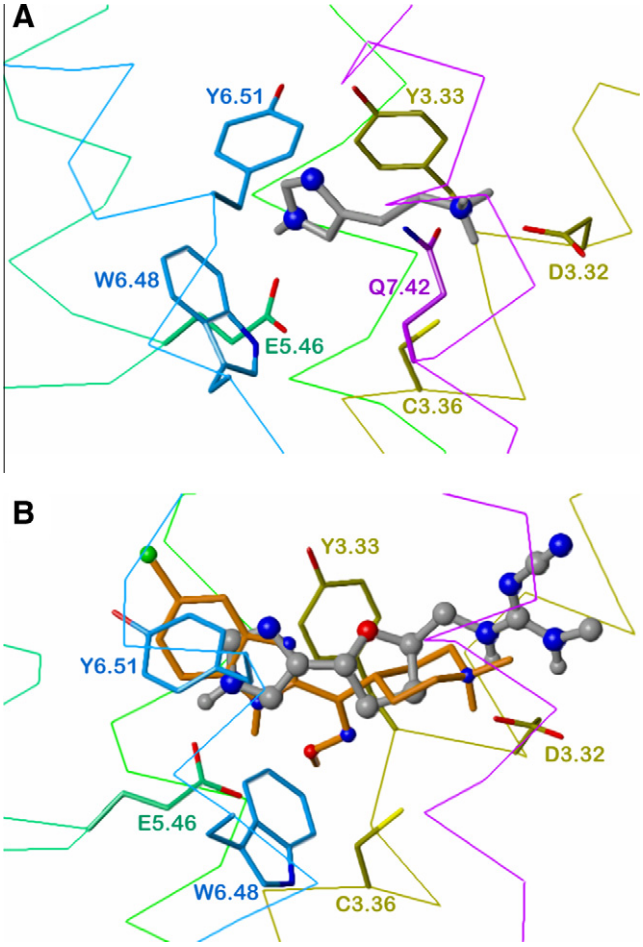
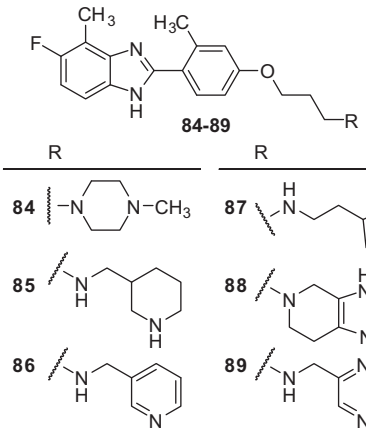


Figure 1. Putative hH_4R binding site of the agonists **1** (histamine), **42** (OUP-16), and **83**, based on a model of the active hH_4R state (for details of generation, cf.[32c,39]). The backbone (Cα trace, lines) and C atoms of side chains are drawn in spectral colors: TM3, yellow, TM4, green, TM5, green-blue, TM6, blue, TM7, violet. Nitrogens, blue, oxygens, red, sulfur, yellow, chlorine, green. Amino acids are labeled by the generic numbering scheme proposed by Ballesteros and Weinstein.[40] (A) Key interactions of histamine (C and essential H atoms, gray) with the hH_4R. The binding mode was derived from Lim et al.[22b] The protonated amino group forms a salt bridge with $Asp^{3.32}$ and an H-bond with $Gln^{7.42}$. The imidazolylethyl side chain ring is surrounded by $Tyr^{3.33}$ and $Tyr^{6.51}$, and the imidazole NH interacts with $Glu^{5.46}$ via a charge-assisted H-bond. (B) Key interactions of OUP-16 (ball and stick model, C and essential H atoms, gray) and the oxime **83** (thin stick model, heteroatoms, balls, C and essential H atoms, orange) with the hH_4R. Both agonists may reproduce the binding mode of histamine (interactions of the heterocycles with $Tyr^{3.33}$ and $Tyr^{6.51}$ and of an NH function with $Glu^{5.46}$). The salt bridge of the amino group of **83** with $Asp^{3.32}$ is replaced by two charge-assisted H-bonds of the cyanoguanidine moiety in the case of OUP-16. All agonists affect the putative toggle switch $Trp^{6.48}$.

the *Z*-configured oximes. Compounds **80**–**83** are hH_4R partial agonists in a luciferase assay (Table 9), and **81** (JNJ 28610244) acts as a full hH_4R agonist in a cAMP-controlled β-galactosidase assay.[38] The potencies of the compounds also vary depending on the type of assay. All four oximes **80**–**83** are selective for the hH_4R and show only low (if any) affinities for the other histamine receptor subtypes. In contrast to other H_4R agonists such as 5(4)-methylhistamine (**5**) or UR-PI376 (**72**), the oxime-analogues are almost full agonists of comparable potency at both rodent and human H_4Rs.[38] In addition, these compounds possess 10- to 450-fold selectivity over the mH_3R. The H_4R agonism of **81** (JNJ 28610244) was confirmed in vivo. The compound induced scratching in wild-type mice but not in H_4R knock-out mice.[38] Evidently, these oxime-type agonists are valuable pharmacological tools for investigating the H_4R in rodent animal models or on rodent cells. The

Table 10
2-Arylbenzimidazoles as H_4R agonists[a]



No.	hH_4R		
	pK_i	pEC_{50}	α
84	7.5	7.3	0.7
85	7.6	8.2	1.0
86	8.3	8.5	0.9
87	9.7	9.3	1.0
88	8.5	8.5	0.6
89	<5.0		

[a] Data[21] cf.[42] H_4R affinities: displacement of [^{3}H]histamine from recombinant hH_4R, hH_4R agonism: CRE-β-galactosidase reporter gene assays on SK-N-MC-cells expressing the hH_4R.

compounds also bind with moderate to high affinity at the monkey and guinea-pig H_4R whereas affinities at the dog H_4R (cH_4R) are only poor.[38]

Figure 1, derived from docking of agonists on a model of the active state, suggests overlapping binding modes of the oxime **83**, the cyanoguanidine OUP-16 (**42**) and histamine (**1**) at the hH_4R. The three agonists form the same key interactions with the receptor. A similar binding mode was also assumed for clozapine.[22b] Because of conformational constraints and stereochemistry, OUP-16 and its isomers are of particular interest for further docking studies.

2-Arylbenzimidazoles as H_4R agonists: 2-arylbenzimidazoles have been developed as H_4R antagonists by Johnson & Johnson.[41] Since **84** shows partial agonistic activity at the hH_4R (Table 10),[42] the terminal basic group was modified with the aim to obtain antagonists. However, several derivatives (**85**–**88**) revealed to be full agonists with even higher potency than **84** at the hH_4R. Compound **87**, which is characterized by a histamine substructure, has sub-nanomolar hH_4R affinity and is among the most potent hH_4R agonists described so far. In addition, **87** shows a >600-fold selectivity over the hH_2R (pK_i: 6.9), *a* >1700-fold selectivity over the hH_3R (pK_i: 6.4) and negligible activity at the hH_1R (pK_i <5). Like many other H_4R agonists, **87** is considerably (>50-fold) less potent at the mH_4R (pEC_{50}: 7.4, α: 0.8) than at the hH_4R. The conformationally constrained analogue **88** is about 10-fold less potent than **87** at the hH_4R. Interestingly, minor structural modifications of the terminal amine function can substantially change both affinity and quality of action at the H_4R. For example, replacing the pyridine ring in **86** with a pyrazine ring (**89**) results in a >1500-fold reduced H_4R affinity.

Conclusion: Numerous H_4R agonists have been identified in different structural classes. In many cases H_4R affinity, selectivity and quality of action are very sensitive toward minor structural changes (cf. cyanoguanidines **62**–**77**, oximes **78**–**81**, 2-arylbenzimidazoles **85**–**90**). Up to now, a common hH_4R-agonistic pharmacophore can be based only on a two-point model representing key

interactions with $Asp^{3.32}$ and $Glu^{5.46}$. Therefore, new leads are difficult to predict.

The selectivity-profile of available H_4R agonists is a key issue from different perspectives. Many 'selective' H_4R agonists like 5(4)-methylhistamine (**5**) also activate—predominantly at higher concentrations—other histamine receptor subtypes. In this respect UR-PI376 (**72**) is a promising H_4R agonist for investigating the hH_4R since it shows no agonistic activity at other human histamine receptor subtypes. However, the investigation of the (patho)physiological role of the histamine H_4-receptor (H_4R) in animal models is hampered by species-dependent discrepancies regarding potencies, receptor selectivities and even by opposite qualities of action of the available pharmacological tools. This especially holds for H_4R agonists, when studied on human and rodent receptors. Thus, optimized specific agonists are required to explore ligand receptor interactions in more detail, to gain deeper insight into the molecular determinants of receptor subtype and species selectivity and to provide pharmacological tools for in vitro and in vivo studies on H_4Rs. Recently discovered oxime-type compounds might supersede currently preferred H_4R agonists. It remains to be explored whether there is also a value of H_4R agonists beyond their application as tools in preclinical investigations.

Acknowledgments

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