# Structure-Activity Relationships of Histamine $H_2$ Receptor Ligands+

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**Abstract:** Recent research on histamine $H_2$ receptor agonists was focused on quantitative structure-activity relationships and receptor models explaining the activity of imidazolylpropylguanidines. Their selectivity for guinea pig *vs.* human isoforms was investigated using $H_2$ receptor-$G_{s\alpha}$ fusion proteins and attributed to amino acid differences in transmembrane domains 1 and 7. New antagonists result from approaches to improve pharmacokinetic properties and to design hybrid drugs which additionally have gastroprotective or anti *H. pylori* activity.

**Keywords:** $H_2$ receptor agonists; $H_2$ receptor antagonists; impromidine analogues; arpromidine; molecular modelling; structure-activity relationships; QSAR; site-directed mutagenesis.

+*Dedicated to Prof. Dr. Gottfried Märkl, Regensburg, on the occasion of his 75th birthday.*

## 1. INTRODUCTION

Histamine is a neurotransmitter and an autacoid and exerts its effects *via* histamine $H_1$, $H_2$, $H_3$ and $H_4$ receptors (HRs) [1-5]. The histamine $H_2$ receptor ($H_2R$) couples to the G-protein $G_s$ to mediate adenylyl cyclase activation [1] and, in some systems, also to $G_q$ related to phospholipase C stimulation [6]. The discovery and pharmacological characterisation of the $H_2R$ by Black and collaborators in 1972 [7] is closely associated with one of the greatest success stories in drug research at all, i.e. the search for $H_2R$ antagonists as drugs for the treatment of gastric and duodenal ulcer resulting in the development of cimetidine and its introduction into clinic about 30 years ago [8,9]. The application of $H_2R$ antagonists has provided evidence for an important physiological role of histamine (**1**, Fig. **1**) in the regulation of gastric acid secretion [7]. Meanwhile, $H_2Rs$ were detected in numerous other peripheral tissues and cells, for example, in leukocytes, the heart, airways, uterus and vascular smooth muscle, and in the brain (*e.g.* basal ganglia, hippocampus, amygdala, cerebral cortex, cerebellum) [1,10-13]. Subsequently, the $H_2R$ was cloned from several species including rat, guinea pig, mouse, dog and humans [14]. A large number of selective $H_2R$ agonists and antagonists have been discovered, and the pharmacological properties as well as the structure-activity relationships (SAR) of numerous ligands are summarised in previous reviews (cf., for example ref. [1,15-21]). Therefore, in the following, the relevant structural classes are only shortly described and the SAR of $H_2R$ ligands are outlined with the emphasis on some more recent results of quantitative SAR (QSAR) studies, molecular modelling and investigations of the molecular ligand/receptor interactions. In the last ten years, new series of $H_2R$ antagonists were mainly designed to improve the pharmacological and pharmacokinetic profile of antiulcer agents and not to explore SAR on a molecular level, so that the knowledge about the $H_2$ antagonistic pharmacophore and possible interaction sites has not significantly advanced. Therefore, recent experimental and theoretical investigations like *in vitro* mutagenesis studies, receptor modelling and QSAR approaches applied to the binding sites of amine and guanidine-type agonists will be the focus of this review.

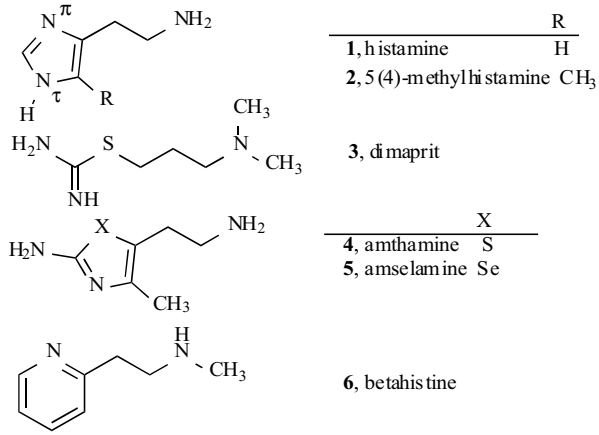


**Fig. (1).** Structures of selective $H_2$ receptor agonists **1-5** and of the unselective non-tautomeric agonist betahistine (**6**).

## 2. STRUCTURE-ACTIVITY RELATIONSHIPS OF HISTAMINE $H_2$ RECEPTOR AGONISTS

The histamine derivative 5-methylhistamine (**2**, Fig. **1**) was the first compound described to exhibit some selectivity for the $H_2R$ [7,22,23]. On one hand, extension of the side chain and replacement of the basic amino group with polar, planar groups (e.g. thiourea or cyanoguanidine) that are uncharged at physiological pH led to the discovery of the $H_2R$ antagonists. On the other hand, the first highly potent $H_2R$ agonist impromidine [24] resulted from histamine, when the chain length was extended by one methylene group and when the amine function was simultaneously replaced with a strongly basic guanidino group and combined with the 2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl moiety known from the $H_2R$ antagonist cimetidine. $H_2R$ agonists were also found among histamine-like heteroarylalkylamines and amino alkyl isothioureas. Thus, the compounds may be roughly subdivided into two structural classes: the amine- and the guanidine-type $H_2R$ agonists corresponding to histamine-like (Fig. **1**) and impromidine-like structures (Fig. **2**, Table **1**).

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1389-5575/04 $45.00+.00 

**Table 1. Structures and Histamine $H_2$ Receptor Agonism of Imidazolylpropylguanidines (General Structure See Fig. 2; Selected Data From Refs. [82, 83]; $pD_2$ ($pEC_{50}$) Values on the Isolated Guinea Pig Right Atrium Referred to Histamine, $pD_2$ = 6.0)**

| No. | A | X [a] | $R^1$ | Y | $R^2$ | $pD_2$ | No. | A | X [a] | $R^1$ | Y | $R^2$ | $pD_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **11a** | $-CH_2-$ | Ph | H | H | - | 5.80 | **28** | $-CH_2-$ | 2-Py | H | Ph | H | 6.35 |
| **11b** | $-(CH_2)_2-$ | Ph | H | H | - | 6.07 | **29a** | $-(CH_2)_2-$ | Ph | H | Ph | H | 7.15 |
| **11c** | $-(CH_2)_3-$ | Ph | H | H | - | 6.70 | **29b** | $-(CH_2)_2-$ | Ph | H | Ph | 4-F | 7.75 |
| **11d** | $-(CH_2)_4-$ | Ph | H | H | - | 6.00 | **29c** | $-(CH_2)_2-$ | Ph | H | Ph | 3-F | 7.35 |
| **12a** | $-(CH_2)_2-$ | 2-Py | H | H | - | 6.70 | **29d** | $-(CH_2)_2-$ | Ph | H | Ph | 3,4-diF | 7.30 |
| **12b** | $-(CH_2)_3-$ | 2-Py | H | H | - | 7.10 | **29e** | $-(CH_2)_2-$ | Ph | H | Ph | 4-Cl | 7.10 |
| **13a** | $-(CH_2)_2-$ | cHex | H | H | - | 6.80 | **29f** | $-(CH_2)_2-$ | Ph | H | Ph | 3,4-diCl | 7.45 |
| **13b** | $-(CH_2)_3$ | cHex | H | H | - | 6.40 | **29g** | $-(CH_2)_2-$ | Ph | 4-F | Ph | 4-F | 7.75 |
| **14** | $-CH_2-$ | N-(2-Py) | H | H | - | 6.90 | **29h** | $-(CH_2)_2-$ | Ph | 4-F | Ph | 3,4-diF | 7.55 |
| **15a** | $-CH_2-$ | OPh | H | H | - | 5.80 | **29i** | $-(CH_2)_2-$ | Ph | 3-F | Ph | 3-F | 7.55 |
| **15b** | $-CH(CH_3)-$ | OPh | H | H | - | 5.40 | **29j** | $-(CH_2)_2-$ | Ph | 3-F | Ph | 3,4-diF | 7.60 |
| **15c** | $-(CH_2)_2-$ | OPh | H | H | - | 6.00 | **29k** | $-(CH_2)_2-$ | Ph | 3,4-diF | Ph | 3,4-diF | 7.35 |
| **16a** | $-CH_2-$ | SPh | H | H | - | 6.70 | **29l** | $-(CH_2)_2-$ | Ph | 4-Cl | Ph | 4-Cl | 6.40 |
| **16b** | $-CH_2-$ | SPh | 2-Cl | H | - | 5.93 | **29m** | $-(CH_2)_2-$ | Ph | 4-Cl | Ph | 3,4-diCl | 6.20 |
| **16c** | $-CH_2-$ | SPh | 3-Cl | H | - | 6.48 | **29n** | $-(CH_2)_2-$ | Ph | 3-Cl | Ph | 3,4-diCl | 5.00 |
| **16d** | $-CH_2-$ | SPh | 4-F | H | - | 6.08 | **30a** | $-(CH_2)_2-$ | 2-Py | H | Ph | H | 7.39 |
| **16e** | $-CH_2-$ | SPh | 4-Cl | H | - | 6.60 | **9** [c] | $-(CH_2)_2-$ | 2-Py | H | Ph | 4-F | 8.01 |
| **16f** | $-CH_2-$ | SPh | 4-Me | H | - | 6.20 | **30b** | $-(CH_2)_2-$ | 2-Py | H | Ph | 4-Me | 7.71 |
| **17a** | $-(CH_2)_2-$ | SPh | H | H | - | 5.90 | **30c** | $-(CH_2)_2-$ | 2-Py | H | Ph | 4-Cl | 7.84 |
| **17b** | $-(CH_2)_2-$ | SPh | 2-Cl | H | - | 5.72 | **30d** | $-(CH_2)_2-$ | 2-Py | H | Ph | 3-F | 7.88 |
| **17c** | $-(CH_2)_2-$ | SPh | 3-Cl | H | - | 5.01 | **30e** | $-(CH_2)_2-$ | 2-Py | H | Ph | 3-Cl | 7.94 |
| **17d** | $-(CH_2)_2-$ | SPh | 4-F | H | - | 5.08 | **30f** | $-(CH_2)_2-$ | 2-Py | H | Ph | 2-Cl | 7.35 |
| **17e** | $-(CH_2)_2-$ | SPh | 4-Cl | H | - | 5.70 | **30g** | $-(CH_2)_2-$ | 2-Py | H | Ph | 3,4-diF | 8.12 |
| **17f** | $-(CH_2)_2-$ | SPh | 4-Me | H | - | 5.30 | **30h** | $-(CH_2)_2-$ | 2-Py | H | Ph | 3,5-diF | 8.05 |
| **18** | $-(CH_2)_2-$ | S-(2-Py) | H | H | - | 7.10 | **30i** | $-(CH_2)_2-$ | 2-Py | H | Ph | 3,4-diCl | 8.19 |
| **19** | $-(CH_2)_2O-$ | Ph | H | H | - | 6.00 | **30j** | $-(CH_2)_2-$ | 2-Py | H | Ph | 3,5-diCl | 7.37 |
| **20a** | $-(CH_2)_2S-$ | Ph | H | H | - | 7.00 | **30k** | $-(CH_2)_2-$ | 2-Py | H | Ph | 2,4-diCl | 7.72 |
| **20b** | $-(CH_2)_2S-$ | Ph | 4-Br | H | - | 6.70 | **30l** | $-(CH_2)_2-$ | 2-Py | H | Ph | 4-Br | 7.48 |
| **20c** | $-(CH_2)_2S-$ | Ph | 3-Cl | H | - | 6.50 | **30m** | $-(CH_2)_2-$ | 2-Py | H | Ph | $4-CF_3$ | 7.76 |
| **20d** | $-(CH_2)_2S-$ | Ph | 4-Cl | H | - | 6.50 | **30n** | $-(CH_2)_2-$ | 2-Py | H | Ph | 4-OMe | 7.44 |
| **20e** | $-(CH_2)_2S-$ | Ph | 3,4-diCl | H | - | 6.20 | **30o** | $-(CH_2)_2-$ | 2-Py | H | Ph | $3-NH_2$ | 7.38 |
| **20f** | $-(CH_2)_2S-$ | Ph | 3-F | H | - | 7.10 | **30p** | $-(CH_2)_2-$ | 2-Py | H | Ph | 2-F | 7.46 |
| **20g** | $-(CH_2)_2S-$ | Ph | 4-F | H | - | 6.60 | **30q** | $-(CH_2)_2-$ | 2-Py | H | Ph | $3-CF_3$ | 7.43 |
| **20h** | $-(CH_2)_2S-$ | Ph | 4-Me | H | - | 6.50 | **30r** | $-(CH_2)_2-$ | 2-Py | 3-Me, 5-Br | Ph | 4-F | 6.10 |
| **20i** | $-(CH_2)_2S-$ | Ph | 4-Et | H | - | 6.20 | **31a** | $-(CH_2)_2-$ | 3-Py | H | Ph | 4-F | 8.09 |
| **20j** | $-(CH_2)_2S-$ | Ph | $4-NO_2$ | H | - | 6.30 | **31b** | $-(CH_2)_2-$ | 4-Py | H | Ph | 4-F | 7.63 |
| **20k** | $-CH(CH_3)CH_2S-$ | Ph | H | H | - | 6.50 | **32** | $-(CH_2)_2-$ | 2-Py | H | 2-Py | H | 6.90 |
| **21a** | $-(CH_2)_2S-$ | 2-Py | H | H | - | 7.30 | **33** | $-(CH_2)_2-$ | 2-Py | H | 2-Thi | H | 7.16 |
| **21b** | $-(CH_2)_2S-$ | 3-Py | H | H | - | 6.90 | **34a** | $-(CH_2)_2-$ | 4-Im | H | Ph | 4-F | 7.62 |
| **7** [b] | $-(CH_2)_2S-$ | 4-Im | 4-Me | H | - | 7.65 | **34b** | $-(CH_2)_2-$ | 2-Im | H | Ph | 4-F | 8.22 |
| **22** | $-(CH_2)_3S-$ | Ph | H | H | - | 5.70 | **35a** | $-(CH_2)_3-$ | 2-Py | H | Ph | H | 6.61 |
| **23** | $-(CH_2)_3S-$ | 2-Py | H | H | - | 6.40 | **35b** | $-(CH_2)_3-$ | 2-Py | H | Ph | 4-F | 6.87 |
| **24** | $-(CH_2)_2-$ | $CH_3$ | - | $CH_3$ | - | 6.20 | **36** | $-(CH_2)_2-$ | Ph | 4-F | $CH_2Ph$ | H | 6.15 |
| **25a** | $-(CH_2)_2-$ | Ph | H | $CH_3$ | - | 7.50 | **37a** | $-(CH_2)_2-$ | 2-Py | H | $CH_2Ph$ | 4-F | 5.89 |
| **25b** | $-(CH_2)_2-$ | Ph | H | $C2H_5$ | - | 7.30 | **37b** | $-(CH_2)_4-$ | 2-Py | H | $CH_2Ph$ | 4-F | 6.06 |
| **26** | $-(CH_2)_2-$ | cHex | H | $CH_3$ | - | 7.50 | **38** | $-(CH_2)_2-$ | cHex | H | cHex | H | 6.50 |
| **27** | $-CH_2-$ | Ph | H | Ph | H | 6.20 | | | | | | | |

[a] Ph – phenyl, Py – pyridyl-, Im – imidazolyl, cHex – cyclohexyl, Thi - thienyl. [b] Impromidine. [c] Arpromidine.

### 2.1. Amines as $H_2$ Receptor Agonists

Figure **1** shows the structures of prototypic small molecule $H_2R$ agonists. Dimaprit (**3**), amthamine (**4**) and amselamine (**5**, Fig. **1**) are similar to histamine (**1**) concerning both structural criteria and $H_2R$-agonistic activity, but are selective for the $H_2R$. In addition to the synthesis and pharmacological characterisation of histamine analogues and related small molecules, theoretical investigations were performed to identify the putative active species of the $H_2R$ agonists (i.e. the protonation site(s) at physiological pH, the conformeric and tautomeric form) and its possible interactions with the receptor protein [25-28]. Amthamine (**4**), which was designed based on quantum chemical calculations for both histamine and dimaprit, may be considered as a cyclic analogue of the isothiourea dimaprit (**3**). Whereas the sulphur atom of **3** should be a proton acceptor according to the model of receptor activation proposed by Weinstein and co-workers [27], the group of Timmerman [28] suggested an activation model in which the agonists accept a proton from a proton-donating receptor site on their double-bonded nitrogen atoms. In contrast to previously reported models [29], this new model is able to accommodate and explain the agonistic activities of all known $H_2R$ agonists, including non-tautomeric compounds like betahistine (**6**), a non-selective $H_1/H_2$ receptor agonist. The potency of **3** amounts to about 70 % of that of histamine whereas **4** and **5** are slightly more potent than **1** on the isolated spontaneously beating guinea pig right atrium. Due to its high $H_2R$ agonist activity amthamine (**4**) proved to be a valuable tool for pharmacological investigations. The seleno analogue of **4**, amselamine (**5**) [30], was found to be slightly more potent than **4** in different tissues including standard models such as the guinea pig right atrium and the gastric acid secretion.

The histamine-binding site of the $H_2R$ was identified as result of *in vitro* mutagenesis studies and modelling approaches based on bacteriorhodopsin (for schematic representation, see Fig. **3**). Investigation of $H_2R$ mutants proved an ionic interaction of the protonated amino group with Asp98 (TM3) [29]. The second and third site of the widely accepted three-point model for biogenic amine/GPCR interaction could principally be formed by the couples Asp186/Thr190 [29] or Tyr182/Asp186 in TM5 [31-33]. Based on a pure α-helical TM5, the proposed two hydrogen bonds of the imidazole ring with the $H_2R$ are only possible with Tyr182 and Asp186 [31]. This assumption is also in agreement with a pH-dependent model of $H_2R$ activation that suggests tautomerisation of the imidazole into the $N^{\pi}$-H form caused by neutralisation of histamine upon binding and accompanied by proton transfers from Tyr182 to $N^{\pi}$ and from $N^{\tau}$ to Asp186, respectively [34]. Interactions of non-tautomeric agonists with $H_2R$ are compatible with this model, too. Asn293 of the $\beta_2AR$ [35] and Phe436 of the $H_1R$ [36] have been suggested to interact with the β-OH group of epinephrine and with the imidazolylethyl moiety of histamine, respectively. The corresponding residue in TM6 of the $H_2R$, Phe254, is close to the histamine side chain only if agonists do not deeply penetrate into the GPCR core.

### 2.2. Guanidines as $H_2$ Receptor Agonists

Impromidine (**7**, Fig. **2**), the prototypical guanidine-type $H_2R$ agonist, is ~50-fold more potent than histamine in increasing heart rate in the isolated guinea pig right atrium, a standard model used for the pharmacological characterisation of $H_2R$ ligands. It is a full agonist in the atrium but, depending on the species and the tissue studied, its intrinsic activity may be lower [1,37-43].

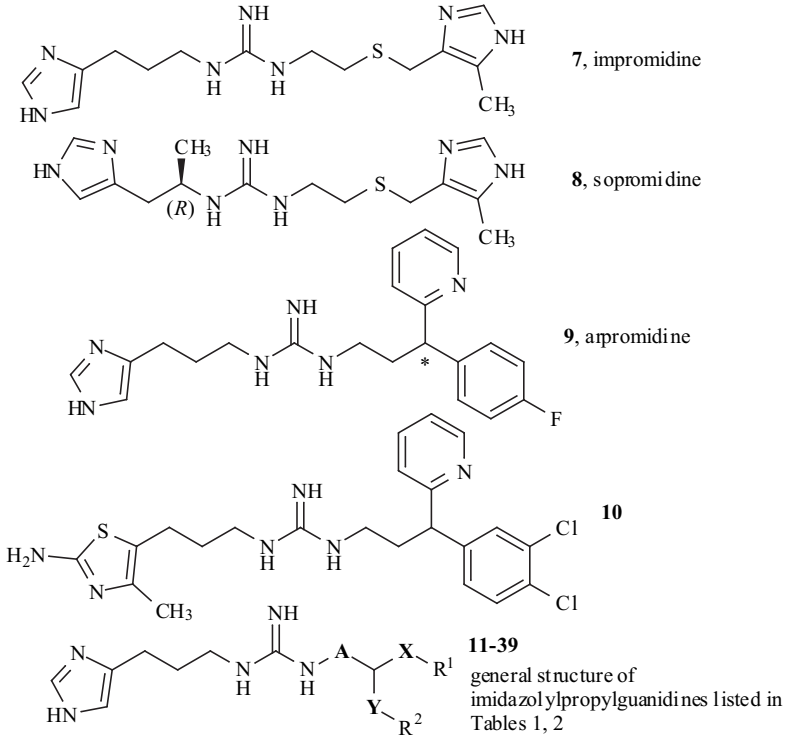


**Fig. (2).** Characteristic structures of guanidine-type histamine $H_2$ receptor agonists.

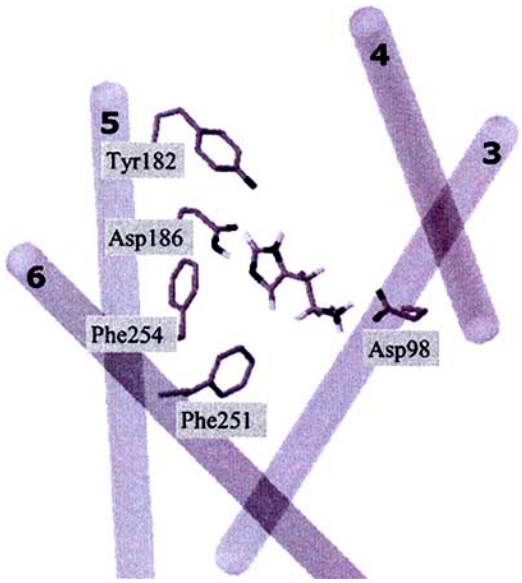


**Fig. (3).** Schematic representation of the histamine binding site of $H_2$ receptors, generated from SYBYL 6.9 (Tripos Inc.). Only TM domains 3 – 6 and amino acids proven or suggested to participate in interactions are shown.

Numerous impromidine analogues have been synthesised and analysed for agonistic activity at the $H_2R$ (for a review see [15]). Highest potency is observed for compounds with a three-membered instead of a two-membered carbon chain as in histamine connecting the imidazole ring and the basic group, although the corresponding partial structures of impromidine and histamine are considered as functionally equivalent groups that are important for the receptor activation. The lower homologue of impromidine is considerably less potent than impromidine (**7**). A unique stereochemical differentiation was found for the (*R*)-configured methyl-branched imidazolylethylguanidine, sopromidine (**8**), which proved to be an $H_2R$ agonist achieving ~7 times the potency of histamine in the guinea pig atrium, whereas the (*S*)-enantiomer has weak $H_2R$ antagonistic properties [44]. The 2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl group is assumed to contribute to $H_2R$ affinity. This partial structure shows a lower degree of stereoselectivity than the imidazolylpropyl portion [44], which is conferring efficacy and may be varied or replaced over a wide range, resulting in $H_2R$ agonists with similar or even higher potency than the parent compound. The structural analogues of impromidine described in the literature include, for example, chiral compounds with branched cimetidine-like or homohistamine partial structures [44,45] or substances characterised by other substructures from $H_2R$ antagonists like thiazoles and furans derived from tiotidine, nizatidine and ranitidine [46,47]. Completely different structures replacing the 5-methylimidazole group are present in the case of hybrid molecules combining the imidazolylpropylguanidine moiety with, for example, arylalkyl [48-56], diarylalkyl originating from $H_1R$ antagonists [57-61], dihydropyridine from calcium channel blockers [62,63] and benzoylimidazolone groups [64] from phosphodiesterase inhibitors (for reviews see [15,65,66]). Examples of $H_2R$-selective guanidine-type agonists are depicted in (Fig. **2**) and listed in Table **1** for QSAR considerations (see below).

About twenty years ago the search for new $H_2R$ agonists was considerably encouraged by clinical investigations suggesting a therapeutic potential of such substances. By using impromidine, Baumann and co-workers demonstrated that $H_2R$ stimulation may be an effective treatment in patients suffering from severe catecholamine-insensitive congestive heart failure [67]. Arpromidine (**9**) and related *N*-[3-(1*H*-imidazol-4-yl)propyl]-*N'*-[3-phenyl-3-(2-pyridyl)-propyl] guanidines were the most interesting substances of a large series of so-called 'cardiohistaminergics' developed as positive inotropic vasodilators [58,65,66,68]. The arpromidine-like $H_2R$ agonists, in particular the 3,4- and 3,5-difluorinated analogues (BU-E-75, BU-E-76, see compds. **30g**, **30h** in Table **1**) proved to be superior to impromidine in potency, hemodynamic profile and side effects when tested in the guinea pig under physiological conditions and in a pathophysiological model of severe congestive heart failure (vasopressin-induced acute heart failure [68]). Moreover, arpromidine-like substances having both $H_2R$ agonistic and $H_1R$ antagonistic properties significantly increase the survival time in rat endotoxic shock [69]. Independently from $H_2R$ agonism, such compounds were described as the first competitive non-peptide neuropeptide Y (NPY) $Y_1$ receptor antagonists [70-72]. However, those compounds exhibit only low or moderately low potency at the $Y_1$ receptor ($pK_i$ up to 6,5).

As demonstrated very recently by separation of the stereoisomers, e.g. of the 3,4-difluorinated (**30g**) and the 3,4-dichlorinated (**30i**) arpromidine analogue, the (*S*)-enantiomers are the eutomers with eudismic ratios of up to 40 [73,74]. These compounds are up to 400 times more potent than histamine in the guinea pig right atrium and are the most potent $H_2R$ agonists known so far. The absolute configuration was deduced from the X-ray structure of synthetic intermediates, phenyl(pyridyl)butanoic acids [73-77]. In addition to their agonistic activity at the $H_2R$, the impromidine- and arpromidine-like compounds are weakly to moderately active as antagonists at the $H_1R$ and have remarkable $H_3R$ antagonistic properties (in the low nanomolar range) as exemplified for some representative derivatives. Eriks *et al*. have demonstrated that the imidazole ring is not essential for $H_2$ agonism but can be replaced with an amthamine-like 2-amino-4-methylthiazole substructure (see **10**, Fig. **2**) [78] resulting in compounds which were found to be superior to the imidazole analogues concerning $H_2R$ selectivity, in particular vs. $H_3R$ [78].

Some general rules with respect to the SAR of imidazolylpropylguanidines are shown in Table **1**. In general, the "unbranched" derivatives **11a** to **23** (without Y in the general structure, see Fig. **2**) have an optimal chain length of four atoms between the guanidino group and ring X, whereas in the case of the branched compounds **24** to **38** the optimum is at three atoms. Only the phenylthioethers are an exception of this rule (cf. **16a** with **17a**, three atoms optimal too). Substituents at phenyl and pyridyl rings in "X position" are mostly unfavourable for $H_2R$ agonistic activity, independently of the presence of a second ring Y. In contrast, appropriate substitution at phenyl rings in "Y position" may significantly increase potency. These general observations are the basic clues for QSAR investigations and alignments of the structures (see below).

The pyridine ring in arpromidine may be replaced by different aromatic or heteroaromatic rings. Among these compounds, the 3-pyridyl analogue **31a** was about equipotent with arpromidine, whereas the corresponding 2-imidazolyl (**34b**) and 2-thiazolyl analogues proved to be even slightly more potent than the reference compound. In a series of *N*-(3,3-diphenylpropyl)-*N*'-[3-(imidazol-4-yl)propyl] guanidines (**29a** to **29n** in Table **1**) and other diphenylalkylguanidines [59,60], the SAR were similar to those of the corresponding phenyl(pyridyl) analogues [58,59]. Interestingly, neither the presence of two geminal aromatic rings nor an aromatic system at all is essential in this part of the molecule [59]. For example, phenyl or cyclohexyl combined with a methyl group in place of a second ring resulted in even higher $H_2R$ agonistic potency as in the case of the unsubstituted diphenyl analogue (cf. **25a** and **26** with **29a** in Table **1**, for details see ref. [59]).

Impromidine, arpromidine and related guanidines are nearly quantitatively protonated at physiological pH and are virtually inactive following oral administration. Changing the physicochemical properties by introducing electron-withdrawing substituents such as ethyl ester groups at the guanidine may result in prodrugs [79], which were found to produce strong positive inotropic effects in guinea pigs after introduodenal administration [66,80].

### 2.3. QSAR of Imidazolylpropylguanidines

The imidazolylpropylguanidines from the group of Buschauer and co-workers were extensively analysed by different QSAR methods. Previous results of a Free-Wilson analysis [81] are straightforward with respect to describing $pD_2$ of the guinea pig atrium assay in terms of fragment contributions. However, according to the method used, the calculations have not sufficiently explained more complex effects like "interactions" of chain length and branching or dependence of substituent influences on the nature and the arrangement of aromatic rings (see above). Therefore, Hansch analysis was applied to a large series of 141 compounds [82] (selected derivatives see Table **1**). The possible role of transport and distribution implies that hydrophobicity must be considered. Hydrophobic constants Σf of the variable part of the molecules were calculated by the fragment method of Leo and Hansch. A preliminary Hansch analysis of $pD_2$ as function of Σf, $(\Sigma f)^2$ and indicator variables, which simply describe topological properties obvious from the structural formula by values of 1 (present) or 0 (not present) was to check the applicability of additivity rules to the whole series. The influence of hydrophobicity is thereby separated from additive electrostatic and steric contributions of certain substructures. The following best equation with all regression coefficients being significant within the 95% level resulted:

The meaning of the indicator variables and their influence is as follows:

Br-α: Branching at the first C after the guanidino group reduces activity by 0.37 $pD_2$ units.

Br-γ: Branching at the third C after the guanidino group increases activity by 0.84 $pD_2$ units.

Bz-S: Benzylalkylthioether groups and their pyridyl analogues increase activity by 0.23 $pD_2$ units.

Oxy: Occurrence of ether oxygen reduces activity by 0.69 $pD_2$ units.

S-Pr: Thiopropyl groups reduce activity by 0.64 $pD_2$ units.

Ch-5: A chain length of five C atoms up to the first ring reduces activity by 0.60 $pD_2$ units.

Py: Pyridine rings increase activity by 0.47 $pD_2$ units.

R-Py: Any substituent at a pyridine ring reduces activity by 0.76 $pD_2$ units.

Without Σf and $[\Sigma f]^2$, $r^2$ amounts to 0.64, so that in eqn. (1) hydrophobicity accounts for 12.6 % of the $pD_2$ variance. Thus, a hydrophobic optimum at Σf = 2.65 leads to the highest $H_2R$ agonistic activity. It is not clear if this dependence reflects optimal organ bath-target distribution or hydrophobic binding or both. However, all favourable substituent effects are "hidden" in the correlation with Σf and $[\Sigma f]^2$, so that specific electrostatic and steric interactions related to hydrophobicity cannot be ruled out to be important. The particular influence of the indicator variables suggests that spatial effects like folding and branching as well as electrostatic interactions of heteroatoms like S, O, and N are the main forces for optimal binding of imidazolylpropylguanidines to $H_2R$ and that additivity principles of substructural effects apply to the whole series in an unique manner.

More complex substructural and substituent effects on receptor binding are of conformational nature. Such effects may be only investigated by 3D QSAR approaches. For this purpose, 142 $H_2R$ agonistic imidazolylpropylguanidines were submitted to comparative molecular field analysis (CoMFA) [82,83]. The alignment of the compounds was based on the assumption that the binding mode of the common imidazolylpropylguanidine structure is the same in all cases and was refined by the weighted field fit approach [83]. Using a region with a gridsize of 1.5 Å, 6750 steric and electrostatic field variables were calculated as interaction of a $C.sp3^+$ cation, positioned at each grid point, with the aligned conformations of the compounds.

Cross validation of the best CoMFA model by the leave-one-out method led to a $q^2$ value of 0.71. Nine principal components are necessary which describe 94 % of the $pD_2$ variance in the final, non-evaluated model ($r^2$ = 0.94, s = 0.19). The field variables replace Σf, $[\Sigma f]^2$ and the indicators in eqn. (1) by going into more mechanistic and conformational detail. A considerable number of these variables seem to account only for features (bulk or charge at certain grid points), which are infrequently present in the

$$\begin{aligned} pD_2 \quad &= 1.25\ (\pm 0.41)\ \Sigma f - 0.24\ (\pm 0.07)\ [\Sigma f]^2 - 0.37\ (\pm 0.30)\ \text{Br-}\alpha \\ &+ 0.84\ (\pm 0.17)\ \text{Br-}\gamma + 0.23\ (\pm 0.20)\ \text{Bz-S} - 0.69\ (\pm 0.412)\ \text{Oxy} \\ &- 0.64\ (\pm 0.28)\ \text{S-Pr} - 0.60\ (\pm 0.40)\ \text{Ch-5} + 0.47\ (\pm 0.19)\ \text{Py} \\ &- 0.76\ (\pm 0.36)\ \text{R-Py} + 4.86\ (\pm 0.65) \qquad (1) \\ &n = 141\ r = 0.875\ r^2 = 0.765\ s = 0.384\ F = 47.4\ (p < 0.0001\ \%) \end{aligned}$$

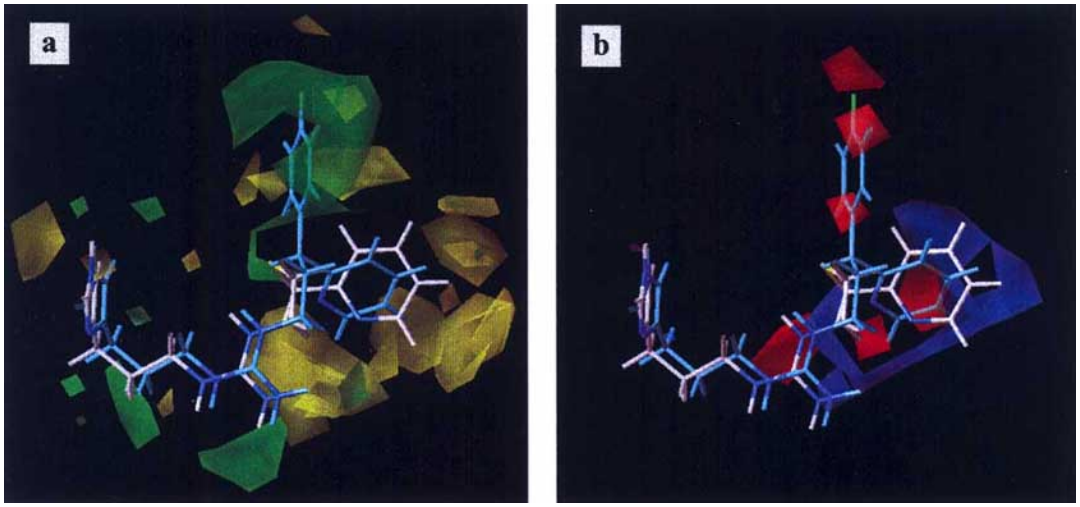


**Fig. (4).** CoMFA view (SYBYL 6.9, Tripos Inc.) of the contribution of field variables to histamine $H_2$ receptor agonism. Representative structures: **21a** (C and H atoms white) and **9** (arpromidine, C and H atoms cyan). Steric and electrostatic influences are represented by isocontour surfaces evaluated from regression coefficients of the field variables (recalculated from principal components and weighted by standard deviations). Each of the surfaces reflects a certain negative or positive effect of bulk and of interaction with a positive charge, respectively, on $pD_2$. Steric field (**a**): green - bulk favourable, yellow - bulk unfavourable. Electrostatic field (**b**): blue - positive charge favourable, red - negative charge favourable.

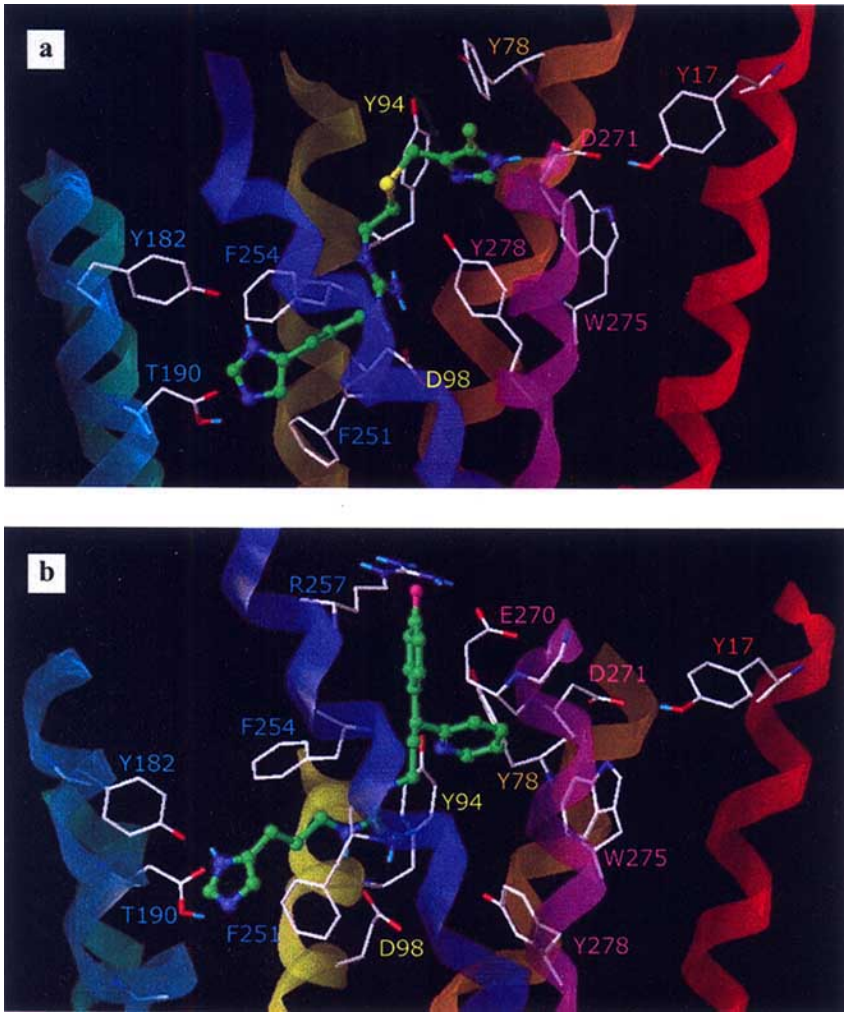


**Fig. (5).** Putative binding of (**a**) impromidine (**7**) and (**b**) arpromidine (**9**), respectively, to $gpH_2R$. The TM regions are schematically represented as ribbons in spectral colours: TM1 – red, TM2 – orange, TM3 – yellow, TM4 – green blue, TM5 – cyan, TM6 – blue, TM7 – violet. Amino acids of the binding site are denoted in the tone of their TM. The agonists are drawn in stick and ball mode with green C atoms. Models generated by SYBYL 6.9 (Tripos Inc.).

series. In part, the principal components possibly reflect positional hydrophobic effects by decomposition into steric and polar contributions, indicating that the parabolic dependence on hydrophobicity in eqn. (1) does not only describe organ bath-target distribution, but also specific hydrophobic binding. The CoMFA results with respect to potential steric and electrostatic effects of the different spatial regions of the imidazolylpropylguanidines on $H_2$ agonistic activity are summarised in (Fig. **4**), also including compounds **21a** and arpromidine (**9**) which served as base for the alignment.

Regarding bulk influence (Fig. **4a**), the picture points, in summary, to a contrary effect of the upper and the lower ring as already implied in the alignment mode according to the results of the Free-Wilson [81] and Hansch analyses (see above). In the upper region, which is generally the position of a branch, bulk enhances activity in a central sphere. However, the space where bulk is favourable is limited. Steric degrees of freedom are especially near the γ branch and in *meta* and *para* position of the upper ring. In contrast, the lower ring points to a region where bulk mainly decreases activity. Therefore, prolongation of the lower chain (also in γ-unbranched compounds) and substituents $R^1$ except in *meta* position are unfavourable. Contours around the common imidazolylpropylguanidine moiety are due to the optimised field fit alignment and cannot be interpreted.

The influence of electrostatic field variables is depicted in (Fig. **4b**). Generally, negatively charged substituents $R^2$ in *meta* or *para* position of the upper ring enhance $H_2R$ agonistic activity. The large blue (positive) contour around the lower ring indicates a favourable effect of positive charges in this region. Red contours point to the activity increasing role of the nitrogen in pyridine or imidazole rings. The unfavourable effect of an oxygen atom in the chain A compared to $CH_2$ is expressed by a blue contour.

Regarding the possible mechanism of interaction, Figure **4** indicates that the QSAR of a first and a second ring, the latter being always in a branch, are markedly different. Thus, two distinct receptor sites must exist. If they would be similarly accessible, unbranched chains with three or four atoms should approach both equally well. However, many favourable substituent effects of a second ring are not present in rings of unbranched analogues. Therefore a single ring always seems to bind at the same site, which might contain an electrophilic amino acid residue preferably interacting with pyridine and imidazole nitrogen and/or a negatively charged residue near the blue contour in Fig. **4b**. The 3D distribution of field variable influences on $pD_2$ suggests that one ring or chain of branched analogues also occupies this site. To accommodate a branch with a second ring at another site, a conformational change of the receptor may take place. Having in mind the topology of transmembrane receptor domains, such change might be simply an effect of the dynamic binding of flexible, vibrating structures, involving "dipping" and "smelting" of the ligands into a region of interacting helices. Since the potency of the most active unbranched (impromidine, **7**) and branched (**34b**) compounds differs by only 0.5 log units, the binding of the second ring seems to be accompanied by conformational strain of the receptor and/or the ligand as well as by entropy loss which in part counterbalance the interaction energy.

### 2.4. Species-Selectivity of $H_2$ Receptor Agonists

For decades the guinea pig right atrium was successfully used as a pharmacological *in vitro* model for the characterisation of $H_2R$ ligands including the antagonists developed as antiulcer drugs. Nevertheless, discrepancies in potencies and efficacies of $H_2R$ agonists as well as different $H_2R$ blocking activities were found on different tissues of various species and appeared to be dependent on the lipophilicity of the compounds, e.g. in models of gastric acid secretion [38,66,84,85]. Previous investigations of guanidines on the human $H_2R$ ($hH_2R$), using neutrophils as model, and of the guinea pig $H_2R$ ($gpH_2R$), using the isolated right atrium, showed that, in contrast to $H_2R$ antagonists and amine-type $H_2R$ agonists, there were considerable differences in the potencies of guanidines between both species. Specifically, on $hH_2R$ the arpromidine-type compounds were less potent than expected and generally only partial agonists [38,58,86]. However, interpretation of the results was difficult because human neutrophils and guinea pig atrium represent very different analysis systems concerning receptor expression levels and pharmacokinetic properties [38]. With respect to potential therapeutic applications, species-specific SAR and structural differences of the receptor proteins may be useful to design $H_2R$ agonists with high affinity to the human receptor. In addition to their potential use in the treatment of cardiovascular diseases, $H_2R$ agonists could be useful, for example, as differentiation-inducing agents in acute myelogenous leukemia [87] and as anti-inflammatory drugs [38,86].

Fusion proteins of G-protein-coupled receptors (GPCRs) and G-protein α-subunits provide a defined 1:1 stoichiometry of the signaling partners and their efficient coupling with each other [88,89]. Specifically, receptor/G-protein coupling in fusion proteins can be assessed by monitoring steady-state GTPase activity. In this way, the interaction of receptors with G-proteins is measured at the most proximal level possible and independently of the availability of effector systems. Defined receptor/G-protein stoichiometry is particularly important for the analysis of agonists since potencies and efficacies of agonists are very sensitive to changes in absolute receptor and G-protein expression levels and the ratio of these proteins to each other [89]. Accordingly, the GPCR-$G_\alpha$ fusion protein technique can be applied to dissect subtle differences in the agonist pharmacology between closely related receptors [90,91].

Using the GPCR-$G_\alpha$ fusion protein technique, the groups of Seifert and Buschauer [92] have recently investigated species-differences of $H_2R$ pharmacology in detail. The coupling of $hH_2R$ and $gpH_2R$ to $G_{s\alpha S}$ could be compared under identical experimental conditions, so that an unequivocal dissection of the pharmacological differences between $hH_2R$ and $gpH_2R$ with respect to the agonistic activity of amines and guanidines was possible. This is demonstrated by the efficacies and potencies of representative $H_2R$ agonists of each structural class in the steady-state GTPase assay (Table **2**). The efficacies of histamine, dimaprit, amthamine and betahistine were similar at $hH_2R$-$G_{s\alpha S}$ and $gpH_2R$-$G_{s\alpha S}$, whereas the guanidines were all significantly less efficacious at $hH_2R$-$G_{s\alpha S}$ than at $gpH_2R$-$G_{s\alpha S}$. Elongation of the alkyl chain between the guanidino

**Table 2. Agonist Efficacies and Potencies at $hH_2R$-$G_{s\alpha s}$, $gpH_2R$-$G_{s\alpha s}$, and at the $hH_2R$-A271D-$G_{s\alpha s}$ Mutant Expressed in Sf9 Cells, Results from GTPase Assay on Cell Membrane Preparations (Data From Ref. [92])**

| No.[a] | | | | | | $hH_2R$-$G_{s\alpha S}$ | | $gpH_2R$-$G_{s\alpha S}$ | | $hH_2R$-A271D-$G_{s\alpha S}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Efficacy | $pD_2$ | efficacy | $pD_2$ | Efficacy | $pD_2$ |
| | **Amines[b]** | | | | | | | | | | |
| **1** | histamine | | | | | 1.00 | 5.90 | 1.00 | 5.92 | 1.00 | 6.46 |
| **3** | dimaprit | | | | | 0.85 | 5.71 | 0.93 | 5.92 | - | - |
| **4** | amthamine | | | | | 0.90 | 6.35 | 1.04 | 6.36 | - | - |
| **6** | betahistine | | | | | 0.73 | 4.47 | 0.73 | 4.29 | - | - |
| | **Imidazolylpropylguanidines[c]** | | | | | | | | | | |
| | **A** | **X** | **$R^1$** | **Y** | **$R^2$** | | | | | | |
| **7** | $-(CH_2)_2S-$ | 1*H*-Imidazol-4-yl | 4-Me | H | - | 0.84 | 6.70 | 1.00 | 7.41 | 0.85 | 7.57 |
| **30a** | $-(CH_2)_2-$ | 2-Pyridyl | H | Ph | H | 0.86 | 6.38 | 1.02 | 7.14 | 0.95 | 7.10 |
| **9** | $-(CH_2)_2-$ | 2-Pyridyl | H | Ph | 4-F | 0.79 | 6.72 | 1.02 | 7.12 | 0.91 | 7.22 |
| **30c** | $-(CH_2)_2-$ | 2-Pyridyl | H | Ph | 4-Cl | 0.77 | 6.82 | 1.07 | 7.40 | - | - |
| **30g** | $-(CH_2)_2-$ | 2-Pyridyl | H | Ph | 3,4-diF | 0.87 | 6.48 | 1.04 | 7.31 | 0.80 | 7.40 |
| **30l** | $-(CH_2)_2-$ | 2-Pyridyl | H | Ph | 4-Br | 0.70 | 6.85 | 1.00 | 7.53 | 0.83 | 7.52 |
| **35a** | $-(CH_2)_3-$ | 2-Pyridyl | H | Ph | H | 0.56 | 6.55 | 0.93 | 6.72 | 0.76 | 6.55 |
| **39a** | $-(CH_2)_2-$ | 2-Thiazolyl | H | Ph | 4-F | 0.78 | 6.51 | 0.99 | 7.09 | - | - |
| **39b** | $-(CH_2)_2-$ | 2-Thiazolyl | H | Ph | 3,4,5-triCl | 0.51 | 6.09 | 0.87 | 6.68 | 0.62 | 6.96 |

[a]Numbers of amines as in Fig. 1, numbers of imidazolylpropylguanidines as in Table 1 (except **39a**, **39b**). [b]For structures of amines, see Fig. 1. [c]For general structure of imidazolylpropylguanidines see Fig. **2**.

group and the phenyl ring (**30a** *vs*. **35a**) and introduction of a Br (**30l**) or of multiple Cl atoms into the phenyl ring (**39b**) strongly decreased agonist efficacy at $hH_2R$-$G_{s\alpha S}$ but not at $gpH_2R$-$G_{s\alpha S}$. These results indicate that the $hH_2R$-$G_{s\alpha S}$ and $gpH_2R$-$G_{s\alpha S}$ conformations stabilised by one of the small amines similarly promote GDP/GTP exchange. In contrast, the guanidines stabilise a $hH_2R$-$G_{s\alpha S}$ conformation considerably less efficient for GDP/GTP exchange than the corresponding $gpH_2R$-$G_{s\alpha S}$ conformation.

The potencies of amines differed by not more than 0.21 log units between $hH_2R$-$G_{s\alpha S}$ and $gpH_2R$-$G_{s\alpha S}$ (Table **2**). All guanidines except **35a** were significantly less potent at $hH_2R$-$G_{s\alpha S}$ than at $gpH_2R$-$G_{s\alpha S}$. Their $pD_2$ differences between $hH_2R$-$G_{s\alpha S}$ and $gpH_2R$-$G_{s\alpha S}$ are rather similar (ca. 0.4 – 0.8), indicating a nearly constant contribution of the guanidinoalkylaryl moiety to the different ligand interactions with $hH_2R$ and $gpH_2R$. Agonist potency was decreased by almost three-fold at $gpH_2R$-$G_{s\alpha S}$ by elongation of the alkyl chain between the guanidino group and the phenyl ring (**30a** *vs*. **35a**), but slightly increased at $hH_2R$-$G_{s\alpha S}$. Taken together, guanidines stabilise an active conformation in $gpH_2R$ not only more efficiently but also with higher affinity than in $hH_2R$, and the structure-activity relationships for guanidines at $hH_2R$ and $gpH_2R$ are slightly different.

Considerations from a sequence alignment of $hH_2R$ and $gpH_2R$ and from $gpH_2R$ models (see below) suggested that an exchange of Ala271 ($hH_2R$) against Asp271 ($gpH_2R$) in TM7 should be the main difference of the agonist-binding site between both receptor species. Results on a $hH_2R$-A271D-$G_{s\alpha S}$ fusion protein mutant [92] confirmed this hypothesis (Table **2**). The $pD_2$ values of guanidines at $hH_2R$-A271D-$G_{s\alpha S}$ and $gpH_2R$-$G_{s\alpha S}$ are nearly identical. Thus, the Ala-271→Asp-271 mutation increased the potency of $hH_2R$ for guanidines to the level of $gpH_2R$. These findings indicate that ion-dipole or H-bond interactions with Asp271 may play a role. Such interactions cannot occur with Ala271 in $hH_2R$, explaining why at $hH_2R$ guanidines exhibit substantially lower potencies than at $gpH_2R$. Regarding the properties of some specific agonists, it becomes obvious that elongation of the alkyl chain between the guanidino group and the phenyl ring (**30a** *vs*. **35a**) decreased agonist potency at $hH_2R$-A271D-$G_{s\alpha S}$ by 0.55 $pD_2$ units (Table **2**). This decrease in potency is similar to that observed at $gpH_2R$-$G_{s\alpha S}$. Conversely, at $hH_2R$-$G_{s\alpha S}$ the longer alkyl chain slightly increased agonist potency. These data suggest that the amino acid at position 271 of $H_2R$s affects the size and flexibility of the guanidine-binding pocket. With Ala271, the binding pocket is wider and more flexible and accommodates the longer (**35a**) as well as the shorter guanidine (**30a**). In contrast, with Asp271, the fit of the longer guanidine must probably be enforced by conformational strain.

Among all guanidines studied, the amino acid substitution at position 271 had the greatest and most

consistent impact on the potency of impromidine (**7**). With Asp271 the potencies ($pD_2$ values) of impromidine (**7**) were consistently about 0.7 to 0.9 log units higher than with Ala271. For other guanidines, the impact of the amino acid substitution at position 271 was less consistent. These data indicate that the binding of impromidine to $H_2$Rs is considerably more dependent on interaction with Asp271 than the binding of other guanidines to $H_2$R (see modelling section 2.5).

In conclusion, guanidines stabilise an active conformation in $gpH_2R$ more efficiently and potently than in $hH_2R$. Studies on a $hH_2R$-A271D-$G_{s\alpha S}$ fusion protein mutant and also on chimeric $hH_2R$/$gpH_2R$ receptors [92] confirmed that Asp271 accounts for the high potency of the guanidines. However, their high efficacy observed in $gpH_2R$ was not restored by the mutants. The data show that $hH_2R$ and $gpH_2R$ selectively interact with a single class of synthetic agonists, that high agonist potency is mainly due to interaction with a single amino acid and that agonist potency and efficacy are regulated independently of each other. The inverse order of potency of compounds **30a** and **35a** at $hH_2R$ and $gpH_2R$, respectively, indicates that it is possible to develop guanidines with high and selective potency and efficacy at $hH_2R$. Such compounds could be useful for the treatment of cardiac failure, acute myelogenous leukaemia and inflammatory diseases.

### 2.5. Molecular Modelling of the Imidazolylpropyl-guanidine Binding Site of Guinea Pig Histamine $H_2$ Receptor

The crystal structure of bovine rhodopsin [93] has improved the reliability of GPCR models with bound ligands. A model of the seven TM helices of $gpH_2R$ based on the bovine rhodopsin structure in the protein databank (PDB) and on the alignment with the $\beta_2$AR [93] was constructed to suggest the binding mode of imidazolylpropylguanidines and possible reasons for the species selectivity compared to $hH_2R$ [92]. Figure **5** shows the putative binding of impromidine (**7**) and arpromidine (**9**), respectively, to $gpH_2R$. Presumably, the imidazolylpropylguanidine moiety binds to $H_2R$ like histamine in maintaining the key interactions with Asp98 in TM3, the Tyr182/Asp186 couple in TM5, and Phe254 in TM6 (see section 2.1). Disregarding sequence differences deeply within the GPCR core, amino acid exchanges between $hH_2R$ and $gpH_2R$ occur only on the top of TM1 and TM7. The $gpH_2R$ models consistently result in an interhelical TM1-TM7 hydrogen bond between Tyr17 ($hH_2R$: Cys17) and Asp271. In TM7, the Ala271→Asp271 switch is the only non-conserved amino acid exchange between $hH_2R$ and $gpH_2R$, and only Asp271 is capable of directly participating in ligand binding according to the rhodopsin-based alignment of TM7. The model in (Fig. **5a**) suggests that the strong preference of impromidine for $gpH_2R$ relative to $hH_2R$ is due to an H-bond of the $N^{\tau}H$ function to Asp271. For the pyridyl moiety of arpromidine, an ion-dipole interaction with Asp271 may be possible (see Fig. **5b**) in agreement with the 3D QSAR results (cf. section 2.3), indicating that a positive charge around the pyridyl region opposite to the nitrogen increases potency in $gpH_2R$. Ala271 in $hH_2R$ cannot take part in both types of interaction. In the case of highly potent 3-phenyl-3-(2-pyridyl)propyl derivatives (series **30** in Table **1**), the weak ion-dipole interaction with Asp271 might be compensated by better fit of the pyridyl moiety into a pocket of aromatic residues consisting of Tyr78 (TM2), Tyr94 (TM3), Trp275 (TM7) and Tyr278 (TM7). This pocket should restrict ligand bulk as indicated by 3D QSAR results (Fig. **4a**). The $gpH_2R$ model also suggests a salt bridge between Arg257 (TM6) and Glu270 (TM6), positioning both side chains for additional interaction with the phenyl moiety (Fig. **5b**). In agreement with the 3D QSAR (Fig. **4b**), favourable effects of negatively charged *meta* and *para* substituents may be due to ion-dipole interactions or, as anticipated in the case of arpromidine, to a F-HN hydrogen bond with Arg257. The field effect of Arg257 might additionally amplify interactions of Glu270 with positively charged phenyl regions. The aromatic pocket as well as the Arg257/Glu270 couple is present in both $gpH_2R$ and $hH_2R$ so that the moderate species selectivity of the *potency* of arpromidine derivatives becomes plausible.

The *efficacy* of the guanidines could be affected by the putative hydrogen bond between Tyr17 and Asp271, a couple of residues only present in the $gpH_2R$. Neither the investigated mutants nor the $hH_2R$ can form this interaction. As consequence, it may be speculated that the Tyr-Asp interaction stabilises the agonistic conformation of the $gpH_2R$. The high efficacy of guanidines, which directly bind to TM7, would then depend on a preformed, relatively rigid position of TM7, *vs.* TM1.

## 3. HISTAMINE $H_2$ RECEPTOR ANTAGONISTS

### 3.1. Compounds Derived from Early $H_2R$ Antagonists

Burimamide (**40**) was the first histamine receptor antagonist that showed selectivity for $H_2R$ vs. $H_1R$ [7]. It exhibits rather low affinity for the $H_2R$ and, since the discovery of the $H_3R$, it is known that burimamide is considerably more potent at the $H_3R$ than at the $H_2R$ ($pA_2$: 7.2 ($H_3R$) *vs*. 5.1 ($H_2R$) [1]). Moreover, a novel class of non-opioid analgesics (prototypical compound improgran) was derived from burimamide [94,95]. For the treatment of gastric and duodenal ulcer, more potent $H_2R$ antagonists were chemically derived from burimamide resulting into the development of metiamide (**41**) [96] and the first $H_2R$ antagonist launched onto the market, cimetidine (**42**, $pA_2$ 6.1) [8,9,97]. Subsequently, ranitidine (**43**, $pA_2$ 7.2) [98], tiotidine (**45**) ($pA_2$ 7.7) [99], famotidine (**46**, $pA_2$ 7.8) [100], nizatidine (**44**, $pA_2$ 7.1, rat uterus) [101], ebrotidine (**47**,$pA_2$ 7.1) [102-105], roxatidine acetate (**51**, $pA_2$ 6.6) [106-108] and mifentidine (**48**, $pA_2$ 7.6) [109,110] were developed ($pA_2$ values on the guinea pig right atrium unless otherwise indicated). The pharmacochemistry of $H_2R$ antagonists was extensively reviewed (see, for example, refs. [1,15,18]). Representative chemical structures are shown in (Fig. **6**).

Most of the known compounds are characterised by three structural moieties: (1) an imidazole ring, which may be replaced by an aryl or heteroaryl ring bearing a basic substituent (e.g. dimethylaminomethylfurane, guanidinothiazole, piperidinomethylphenyl); (2) a so-called urea equivalent, i. e., a planar, dipolar group, which is uncharged at physiological pH and capable of undergoing H-bonding,

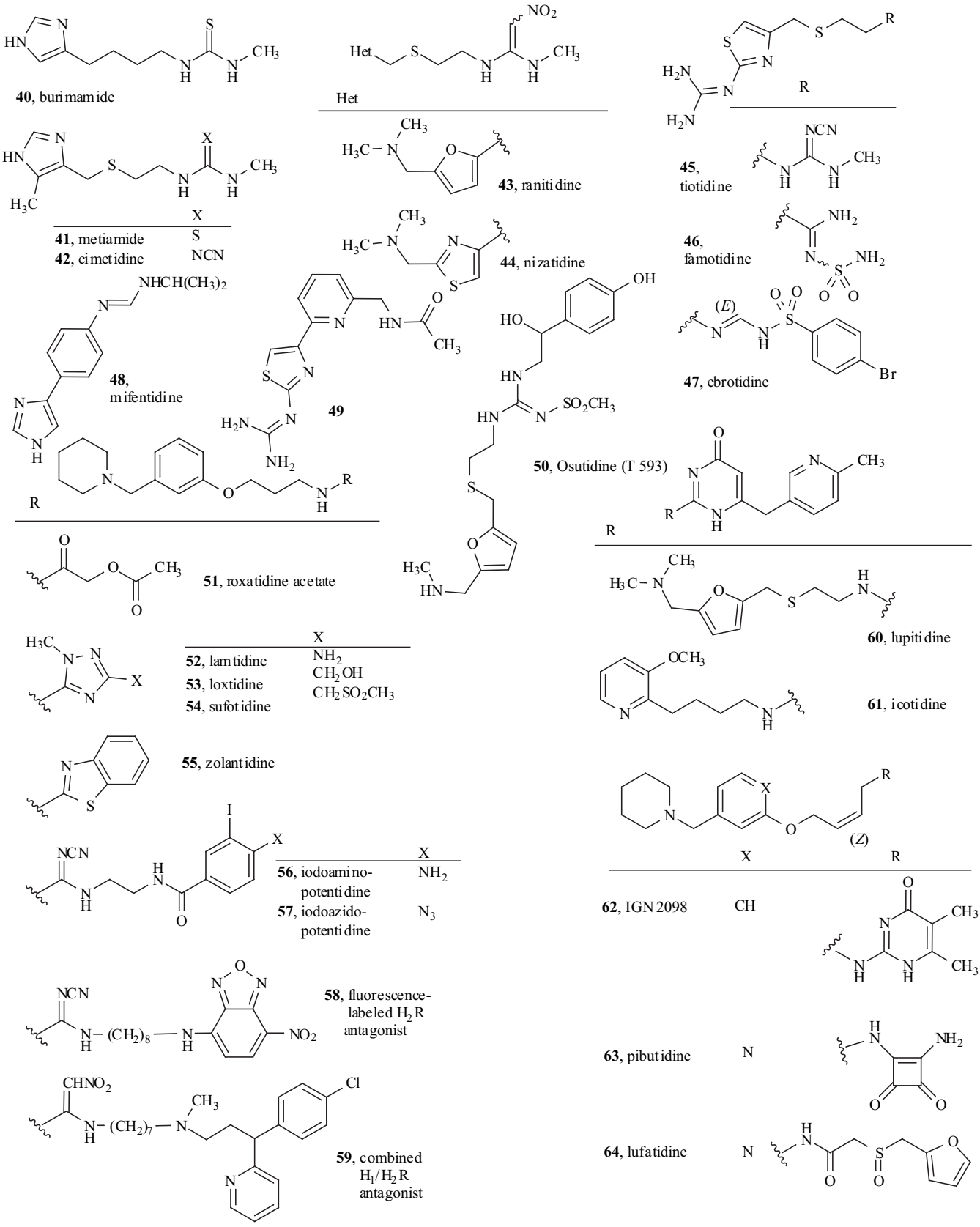


**Fig. (6).** Characteristic structures of classical and several more recently developed $H_2R$ antagonists.

for instance, thiourea, guanidine derivatives with electron-withdrawing substituents (e.g., cyano or sulfonyl), nitroethenediamine, sulfonyl or sulfamoyl amidine groups, amide functions as well as ring-integrated dipolar groups such as isocytosine; and (3) a flexible chain connecting both π-electron systems. $H_2R$ antagonistic activity has also been

found in conformationally constrained compounds with an aryl ring instead of the chain, e.g. the imidazolylphenylformamidine, mifentidine (**48**) [109,111], or analogues having different heterocyclic rings instead of imidazole and phenyl, for example pyridyltriazoles [112] and 2-guanidinothiazoles with 4-furyl [113,114], 4-phenyl (see DA 4643 [111]), 4-(4-imidazolyl) (e.g., zaltidine [115,116]) or 4-(2-pyridyl) substituents (e.g., **49** [117]).

*In vitro* mutagenesis studies [29] indicated that the $H_2R$ binding of these antagonists overlaps with the binding of histamine, sharing at least two interactions: [Methyl-$^3$H]tiotidine affinity for Asp98Ala was abolished and reduced for an Asp186Ala or Asp186Asn mutant. A third interaction with Thr190, which probably does not belong to the histamine binding site (see section 2.1) may play a role for antagonists as concluded from the missing affinity of pibutidine (**63**) for Thr190Ala-mutated $H_2R$ [118].

Attempts to increase the potency and the duration of the antisecretory effects of $H_2R$ antagonists were very successful. However, the enthusiasm to develop long-acting $H_2R$ blockers such as lupitidine (**60**, $pA_2$ 7.8, guinea pig atrium) [119], or the insurmountable, i.e. non-competitive, antagonists lamtidine (**52**) [120-122] and loxtidine (**53**) as drugs, was dampened to some extent due to the occurrence of gastric carcinoids tumours in rodents after long-term administration of **60** [123,124] and **53** [125,126]. Possibly, gastric enterochromaffin-like (ECL) cell hyperplasia and carcinoid tumours are a consequence of prolonged achlorhydria [124,127-129].

For decades the guinea pig right atrium was used as a standard *in vitro* model of predictive value for the pharmacological characterisation of $H_2R$ antagonists to be developed as antiulcer agents. Indeed, by means of the GPCR-$G_\alpha$ fusion protein technique (see section 2.4) it has recently been shown [92] that $H_2R$ antagonists, unlike the guanidine-type agonists, are not selective for $gpH_2R$ relative to $hH_2R$ since the affinities of several examined compounds (cimetidine, ranitidine, famotidine, tiotidine zolantidine and aminopotentidine) was nearly identical at $hH_2R$-$G_{s\alpha S}$ and $gpH_2R$-$G_{s\alpha S}$. However, it cannot be excluded that $H_2R$ antagonists chemically distinct from the aforementioned compounds exhibit species-selective affinities for the $H_2R$.

Previous studies had shown that the $hH_2R$ is constitutively active, *i.e.* undergoes agonist-independent isomerisation from an inactive R state to an active (R*) state, resulting in an increase in the basal $G_s$- and adenylyl cyclase activity [130,131]. Certain $H_2R$ antagonists were found to act as inverse agonist, i.e., to decrease the activity of the agonist-free $hH_2R$, thereby diminishing basal adenylyl cyclase activity [132]. Ranitidine (**43**) and even more prominently aminopotentidine had a consistent inverse agonist effect at $hH_2R$-$G_{s\alpha S}$ [92]. At $gpH_2R$-$G_{s\alpha S}$, ranitidine showed the greatest inverse agonist effect among the antagonists studied which was also significantly greater than at $hH_2R$-$G_{s\alpha S}$. In contrast, aminopotentidine was considerably more efficient as an inverse agonist at $hH_2R$-$G_{s\alpha S}$ than at $gpH_2R$-$G_{s\alpha S}$. These data show that both antagonists differentially stabilise an inactive conformation in $hH_2R$ and $gpH_2R$. The absolute inverse agonist activities of aminopotentidine at $hH_2R$-$G_{s\alpha S}$ and of ranitidine at $gpH_2R$-$G_{s\alpha S}$, respectively, were similar, indicating that both GPCRs exhibit a similar degree of constitutive activity. In comparison, the rat $H_2R$ is less constitutively active than the $hH_2R$, i.e., the inhibitory effects of antagonists on basal adenylyl cyclase activity are smaller in cells expressing rat $H_2R$ compared to cells expressing $hH_2R$ [132].

### 3.2. $H_2R$ Antagonists with Additional Pharmacological Properties

Since the advent of the $H^+/K^+$-ATPase blockers and with increasing success of these drugs in therapy, the search for new $H_2R$ antagonists as antiulcer agents decreased although the established drugs are still valuable and safe standard therapeutics. This is reflected by the fact that cimetidine and ranitidine are now available as over-the counter drugs in several countries. Interestingly, some recent reports suggest a potential therapeutic value of $H_2R$ antagonists with additional gastroprotective properties. Structural variations of the early $H_2R$ antagonists, such as incorporation of bulky residues were tolerated over a wide range in the guanidinothiazole (e.g. **47**, **49**), the aminoalkylfurane (e.g. **50**) [133] and, in particular, in the piperidinomethylphenoxypropylamine (see **51-59**) as well as the analogous (*Z*)-configured piperidinomethylpyridyloxybutenylamine series (see pibutidine, **63**; lufatidine, **64**) [134]. In the latter two series potent $H_2R$ antagonists were obtained by the introduction of various heterocycles and other relatively small dipolar groups as substituents at the amino group, leading to aminotriazoles as in **52-54**, a benzothiazole as in the centrally active $H_2R$ antagonist zolantidine (**55**) [135], pyrimidine derivatives as in **62** [136], thiadiazoles [137], ureas [138], guanidine derivatives, nitroethenediamines and amides including ring systems like squareamides (e.g., pibutidine, **63** [139]). Some effort has been spent on the design of hybrid molecules combining $H_2R$ antagonistic activity with a second pharmacological action considered useful in the treatment of ulcer patients, for instance, with gastrin receptor antagonism [140,141], activity against *Helicobacter pylori* [113,114,142,143] or gastroprotective activity [104,117,144-146], e.g., by incorporation of NO-donating functional groups [147-149]. The "symbiotic approach" was, however, not restricted to drugs acting on gastric secretion. Dual $H_1R$ and $H_2R$ antagonistic activity was, for instance, found in pyridylbutyl-substitued isocytosines such as icotidine (**61**) [150]. In this case the balance between both qualities of action may be strongly shifted towards $H_1R$ by introducing lipophilic substituents (e.g., methyl and Br as in temelastine [151]) at the pyridine nucleus [18]. Well-balanced combined $H_1/H_2R$ antagonists could be useful to prevent life-threatening anaphylactoid reactions in anaesthesia and surgery [152,153]. In search for such compounds the prototypic substructures of $H_1R$ and $H_2R$ antagonists were combined via cyanoguanidine, urea, or nitroethenediamine groups [154-158]. Whereas the loss of the strongly basic side-chain nitrogen resulted in a decrease of $H_1R$ affinity compared to single reference $H_1R$ and $H_2R$ antagonists, moderately to highly potent combined $H_1/H_2R$ antagonists were obtained when a spacer group was used to connect both the basic amino group of the $H_1R$ antagonist and the "urea equivalent" of the $H_2R$ antagonist portion as shown in Fig. **6** for compound **59** ($pA_2$ values: $H_2$, guinea pig atrium 7.1; $H_1$, guinea pig ileum: 7.82) [158].

### 3.3. Radiolabelled and Fluorescent $H_2R$ Antagonists

The piperidinomethylphenoxypropylamine substructure was used as building block for the preparation of selective [$^{125}$I]iodinated $H_2R$ antagonists as radioligands with high affinity and specific activity (superior to [$^3$H]tiotidine [159]) or photoaffinity labelling reagents, respectively [160,161]. Recent data indicate that [$^3$H]tiotidine labels only a subpopulation of the available $H_2R$ molecules [92]. Iodoaminopotentidine (**56**, $pK_i$ 9.15) and Iodoazidopotentidine (**57**, $pK_i$ 8.58) proved to be very useful probes for the detection of $H_2Rs$ in various tissues and for the determination of binding data. Recently, by analogy with this approach, the concept of labelling the *N*-cyano-*N'*-[3-[3-(piperidin-1-ylmethyl)phenoxy]propyl]guanidine partial structure was successfully transferred to the design of fluorescent histamine $H_2R$ antagonists, for example, when a spacer of sufficient length and a fluorophore contributing additional receptor affinity was selected as with the nitrobenzoxadiazole-labelled compound **58**, which is about as active as famotidine in the isolated guinea pig right atrium ($pA_2$ 7.96) [162]. Potent fluorescent $H_2R$ antagonists may facilitate the analysis of ligand/$H_2R$ interactions given several disadvantages of the available radioligands (low specific activity, relatively low affinity, high price and labelling of only distinct receptor molecule populations in case of [$^3$H]tiotidine; high price and short half-life in case of [$^{125}$I]radioligands).

## 4. CONCLUSION

SAR of $H_2R$ agonists and in particular antagonists relying on large congeneric series were extensively analysed in previous reviews. Pharmacophores as well as structural features increasing affinity and/or efficacy are well known. The trend in designing new $H_2R$ antagonists aims at symbiotic antiulcer drugs incorporating additional activities like peptide receptor antagonism, activity against *Helicobacter pylori* or gastroprotection as well as at improved pharmacokinetic profiles. Although the beneficial hemodynamic effects of $H_2R$ stimulation in severe congestive heart failure was already demonstrated in patients about 20 years ago, $H_2R$ agonists are, generally, far from being therapeutically applied. Nevertheless, $H_2R$ agonists have proven as very valuable pharmacological tools to investigate the physiological role of $H_2R$ stimulation and to study binding sites and the molecular basis of species-selectivity. In this respect, successful approaches and results from the $H_2R$ field may be exemplary for and applicable to other GPCRs. The refinement of GPCR models based on the structure of bovine rhodopsin, more detailed *in vitro* mutagenesis studies and new assay techniques like the GPCR-$G_{s\alpha}$ fusion protein approach or the use of fluorescent ligands will further increase the knowledge about the nature of ligand-GPCR interaction and about species differences in receptor conformations, states, flexibility, and activation.

## ACKNOWLEDGEMENTS

The authors' work was supported by grants of the Deutsche Forschungsgemeinschaft (A.B., S.D., S.E.; Graduiertenkolleg GRK 760), Fonds der Chemischen Industrie (A.B. and S.E.), the Deutscher Akademischer Austauschdienst within the International Quality Network "Medicinal Chemistry" (S.E. and A.B.), the National Institutes of Health COBRE award 1 P20 RR15563 and matching support from the State of Kansas and the University of Kansas (R.S.) and a grant from the Army Research Office (DAAD 19-00-1-006) (R.S.).

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