Histamine H_3 Receptor Agonists

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Abstract: The SAR of H_3 ligands has been difficult to evaluate because of species differences, multiple isoforms and constitutive activity, among other complicating factors. A review is given of the sometimes-conflicting affinity, activity and efficacy data of H_3 agonists that has been described in literature to date.

Keywords: Histamine, H_3, GPCR, agonist, isoform, activity, efficacy, affinity.

INTRODUCTION

Histamine (**1**) mediates its actions *via* four receptor subtypes, the postsynaptic (or hormonal) H_1 and H_2 receptors, the presynaptic H_3 receptor and the recently discovered H_4 receptor. The endogenous ligand has the highest affinity for the H_3 receptor, thus being marginally selective for the receptor subtype that has a regulatory role in the synthesis and release of the neurotransmitter [1, 2]. Whereas the first two histamine receptor subtypes have shown to be targets for blockbuster drugs, the H_3 receptor has not proven to be an amenable drug target yet. Since the discovery of the H_3 receptor in 1983 [1], many interesting effects of H_3 ligands have been demonstrated *in vivo* (for a thorough review, the reader is referred to the literature [3]). These findings have led to speculations about therapeutic applications of H_3 agonists and antagonists, which have recently been reviewed [4].

THE TARGET(S)

For almost two decades, the H_3 receptor was studied by measuring distinct pharmacological effects under influence of H_3 compounds. Only recently has the gene that encodes the human H_3 receptor been cloned [5]. This important breakthrough in 1999 by Lovenberg and co-workers has initiated more molecular approaches that help to unravel the true complexity of H_3 pharmacology. Since then, the gene that encodes the H_3 receptor in various other species has been cloned and considerable species differences have been revealed [6, 7]. Species differences seem to affect the binding of antagonists only. No significant differences can be found when measuring the affinities of H_3 agonists using recombinant H_3-expressing cells [6].

In addition to species differences, multiple isoforms have been revealed. These have been named *H3A*, *H3B* and *H3C* in rat [8], and *H3L* and *H3S* in guinea pig [7] There are at least six distinct human H_3 receptor isomers, of which three encode functional proteins [9]. The most abundant, the unspliced human *isoform 1*, has been shown to correspond to the rat *H3A* and the guinea pig *H3L* isoforms [10]. Two short human H_3 isoforms having deletions in the third intracellular loop have been described (referred to as *isoform 2* and *isoform 4*). These specific deletions seem to increase the affinity of the receptor for the cognate G-protein. Hence, the functional activity and efficacy of H_3 ligands is effected. Although the rank order of agonist potencies is the same for all investigated isoforms (*1* and *2*), the potency of the tested agonists is 20 times lower on the unspliced *isoform 1* than *on isoform 2*. In addition, the efficacy of the ligands seems to be higher on *isoform 2*. For the unspliced isoform, no significant species differences in distribution patterns have been revealed. However, studies have shown that the rat and human splice isoforms display differential expression patterns, thereby introducing another layer of complexity. In another recent development, cell lines expressing recombinant H_3 receptors are in use to screen H_3 compounds [6, 11]. Using these assays, it has been shown that the H_3 receptors have a high degree of constitutive activity. Studying the effect of H_3 ligands on this spontaneous activity has led to a reclassification of many H_3 ligands as either agonists, partial agonists, neutral antagonists or inverse agonists. However, it has to be noted that recombinant systems do not represent an intact tissue and the results must be interpreted carefully.

THE LIGANDS; SAR OF H_3 AGONISTS

Since the discovery of the H_3 receptor in 1983, ligands for this GPCR have been assessed and classified by various functional and binding assays using rodent tissues such as guinea-pig ileum and rat cortex. Comparison of the biological data obtained using these different pharmacological assays has been troublesome, as stunning differences in activity, affinity and efficacy of ligands are using different assays have been reported. Only recently, are cell lines expressing human recombinant H_3 receptors in use to screen compounds. To date, literature data regarding these assays is sparse. In the following subsections, all the relevant screening data concerning H_3 agonists will be summarised.

For the endogenous ligand histamine (**1**), pD_2=7.4 has been established using rat cortex tissue (Table **1**) [12]. As mentioned before, no significant species differences can be found and pK_i=7.8 has been reported using human recombinant H_3-expressing cells [6]. The activity of histamine has also been assessed by measuring the inhibition of forskolin-induced cAMP levels using human recombinant H_3-expressing cells (pK_i=8.6) and using the FLIPR assay (pEC_{50}=7.8) [13]. Using a different cell based functional assay named Receptor Selection and Amplification Technology (R-SATTM), the observed EC_{50}

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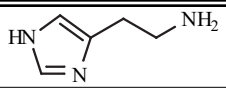
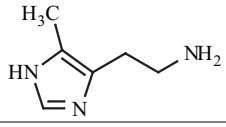
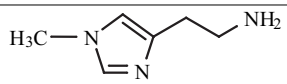
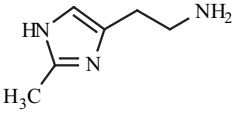
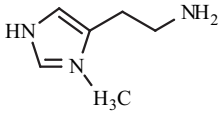
1389-5575/04 $45.00+.00

Table 1. H_3 Activity of Histamine and Imidazole Ring Methylated Analogues

No.	Compound	Structure	pD_2[a]	pK_i[b]
1	histamine		7.4	7.8
2	4(5)-methylhistamine		< 3.0	
3	N^{τ}-methylhistamine		< 6.0	
4	2-methylhistamine		< 4.3	
5	N^{π}-methylhistamine		< 6.0	

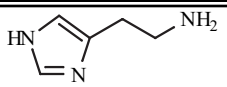
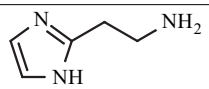
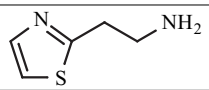
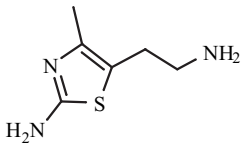
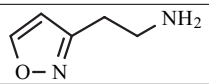
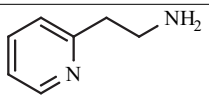
[a]K^+-stimulated [^{3}H]-histamine release from rat cortex.
[b]Displacement of [^{3}H] N^{α}-methylhistamine from human recombinant H3-expressing cells.

of histamine at human unspliced *isoform 1* is 510 nM and at the spliced *isoform 2* (*vide supra)* 30 nM [9].

In the search for more potent and selective H_3 agonists, histamine has been used as a lead structure. It has been shown that the 4-substituted imidazole moiety is essential for agonistic activity. Whereas additional substitution on the imidazole ring of histamine can lead to interesting H_2 (*e.g.*, 4(5)-methylhistamine (**2**)) and H_1 (*e.g.*, 2-methylhistamine (**4**)) agonists, such modifications result in complete lost of H_3 activity.

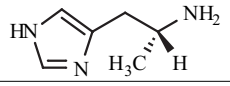
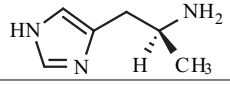
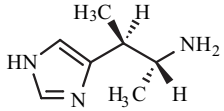
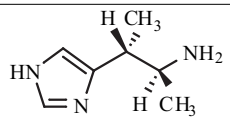
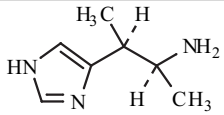
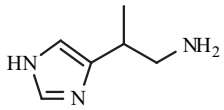
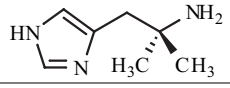
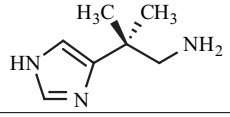
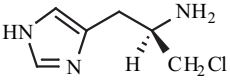
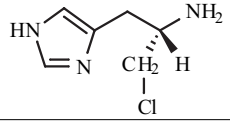
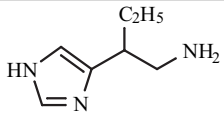
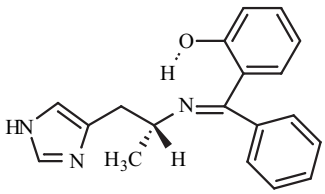
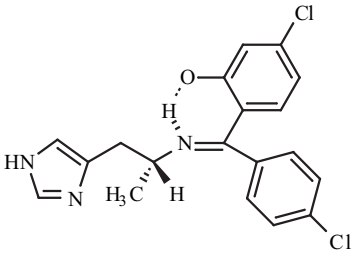
Furthermore, replacement of the imidazole moiety of histamine by other heterocycles or other potential bioisosteric (for H_1 or H_2) replacements is not allowed for H_3 agonism, as is illustrated in Table **2** [12, 14]. This indispensable role of the imidazole ring implies that the interaction of this moiety with the receptor site is highly compulsory.

Table 2. Histamine H_3 Receptor Activity of Imidazole Substituted Histamine Analogues

No.	Compound	Structure	pD_2[a]
1	histamine		7.4
6	2-(2-aminoethyl)imidazole [H_2 agonist]		< 4.0
7	2-(2-aminoethyl)-thiazole [H_2 agonist]		< 3.0
8	amthamine [H_2 agonist]		4.7[b]
9	3-(2-aminoethyl)-isoxazole [H_1 agonist]		< 4.0
10	2-(2-aminoethyl)-pyridine [H_1 agonist]		< 4.0

[a]K^+-stimulated [^{3}H]-histamine release from rat cortex.
[b]Electrically evoked contractions of guinea-pig ileum.

Table 3. H_3 Activity of Ethylene Side Chain Substituted Histamine Analogues

No.	Compound	Structure	pD_2[a]	pK_i[b]
1	Histamine	-	7.4	7.8
11	(R)-α-methylhistamine		8.4	8.7
12	(S)-α-methylhistamine		6.3	
13	(R)-α,(S)-β-dimethylhistamine		8.5	
14	(S)-α,(R)-β-dimethylhistamine		6.5	
15	(±)-threo-α,β-dimethylhistamine		6.7	
16	(±)-β-methylhistamine		7.7	
17	α,α-dimethylhistamine		7.6	
18	β,β-dimethylhistamine		5.8	
19	(R)-α-chloro-methylhistamine		4.7	
20	(S)-α-chloro-methylhistamine		5.9	
21	(±)-β-ethylhistamine		5.0	
22	BP 2.94			
23				

[a]K^+-stimulated [^{3}H]-histamine release from rat cortex.
[b]Displacement of [^{3}H] N^{α}-methylhistamine from human recombinant H_3-expressing cells.

Whereas alteration of the imidazole unit is not allowed, modification of the side chain can result in very potent and selective H_3 agonists. Methylation of the α–position leads to enantiomeric compounds that reveal the stereoselectivity of the H_3 receptor (Table **3**). Whereas the eutomer (most active enantiomer) (R)-α-methylhistamine (**11**) has an activity that is about ten times higher than the endogenous ligand (**1**), the distomer (S)-α-methylhistamine (**12**) is about 15 times less active than histamine [6, 13, 15]. (R)-α-methylhistamine (**11**) is often used as a standard agonist for pharmacological studies involving H_3 receptors, as it is not only very potent but also very selective (about 20,000 times more potent on H_3 than on the H_1 and H_2 receptors and more than 200 times more potent than on the structurally closely related H_4 receptor). Again, it has been shown that (R)-α-methylhistamine (**11**) is about twenty times less active on the human isoform 1 than on isoform 2 [9].

It is allowed to methylate both the α and β-position of the ethylene side chain [16]. (R)α,(S)β-Dimethylhistamine (**13**) is a potent and very selective agonist as it is about 100,000 times more active at the H_3 receptor than at the H_1 and H_2 receptor. Methylation of the β-position is synthetically difficult and the resolution of β-methylhistamine (**16**) has not yet been described. Pharmacological data using the racemic mixture **16** indicates that a methyl group can be accommodated in this position [17]. The histamine derivative with two methyl groups in the α-position (**17**) is also a potent agonist. However, double methylation at the β-position results in a very weak agonist **18** [17]. Increasing the Van Der Waals radius of the substituents on either the α- or β-position of the ethylene side chain is not allowed (**19**-**21**) [12].

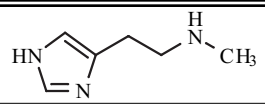
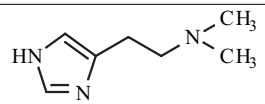
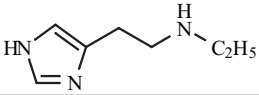
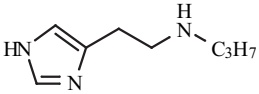
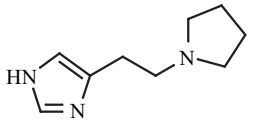
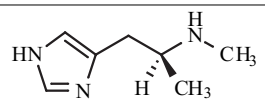
Krause and co-workers have developed very effective azomethine prodrugs of (R)-α-methylhistamine (**11**) [18]. These prodrugs have significantly improved bioavailability. In addition, the azomethines are not as easily catabolised by N-methyltransferase, resulting in a longer half-life. BP 2.94 (**22**) has been shown to act mainly in the periphery. Dicholoro analogue **23** is very efficient for delivering high levels of (R)-α-methylhistamine (**11**) in the CNS. These compounds are very useful as a pharmacological tool and may become H_3 histaminergic drugs for therapeutic use as anti-inflammatory and antinociceptive agents [19].

The amino group of histamine has been alkylated as well (Table **4**). N^{α}-methylhistamine (**24**) is a very potent H_3 agonist. It is also selective, compound (**24**) is about 40 times more active on the H_3 receptor than on the closely related H_4 receptor [20].

Double methylation of the amino group, leading to **25** is also allowed for H_3 activity [21]. As was found for substituents on the ethylene spacer, larger substituents on the amino group of histamine results in diminished H_3 activity. (**26**-**28**) [22], indicating that the available space in the agonistic binding site is very limited. Remarkably, it has been reported that (R)α,N^{α}-dimethylhistamine (**29**) has a low H_3 activity, although both (R)α-methylhistamine (**11**) and N^{α}-methylhistamine (**24**) are very potent agonists [23]. These findings have not yet been rationalised.

Both (R)-α-methylhistamine (**11**) and N^{α}-methylhistamine (**24**) have been tritiated and used to characterise the histamine H_3 receptor in binding assays. However, the involvement of G-protein coupling in agonist binding, and hence, the putative presence of two affinity states hampers straightforward use of agonists in these

Table 4. Histamine H_3 Activity of Nα-Alkyl Substituted Histamine Analogues

No.	Compound	Structure	pD_2[a]	pK_i[c]
24	N^{α}-methylhistamine		7.8[b]	8.6
25	N^{α},N^{α}-dimethylhistamine		7.6	
26	N^{α}-ethylhistamine		7.1	
27	N^{α}-propylhistamine		< 5.2	
28	N-[2-(1H-imidazol-4-yl)ethyl]-pyrrolidine		6.2 (α=0.6)	
29	(R)α,N^{α}-dimethylhistamine		5.8	

[a]K^+-stimulated [^{3}H]-histamine release from rat cortex.

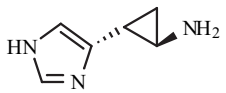
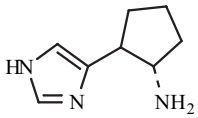
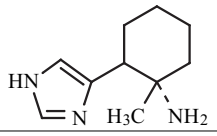
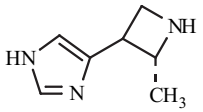
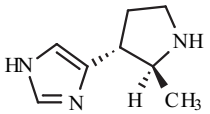
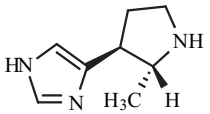
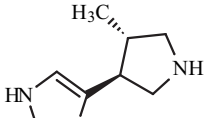
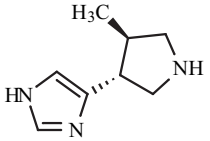
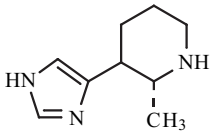
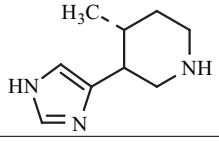
[b]Electrically evoked contractions of guinea-pig ileum.

[c]Displacement of [^{3}H] N^{α}-methylhistamine from human recombinant H_3-expressing cells.

studies [24]. To circumvent these problems, labelled antagonists have been developed subsequently.

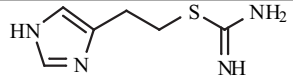
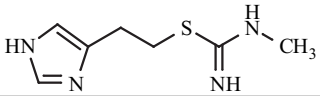
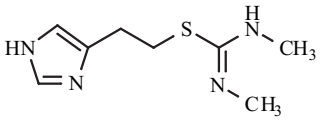
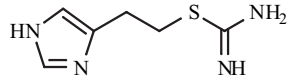
Several histamine analogues have been reported in which the flexible side chain is incorporated in a ring structure (Table **5**). (1S,2S)-Cyclopropylhistamine (**30**) is considerably more active than its enantiomer (1R,2R)-cyclopropylhistamine (**31**) [25]. Incorporation of the α and β carbon atoms of the ethylene linker of histamine in larger ring systems leads to the low activity compounds **32**-**33** (both tested as racemic mixtures of their respective *trans*-isomers) [26]. Incorporation of the basic amino group in side chain ring systems can lead to potent H_3 agonists. It has been reported in patent literature that the racemic mixture of *trans*-substituted azetidine **34** has a high H_3 affinity [27].

Table 5. Histamine H_3 Activity and Affinity of Conformationally Restrained Histamine Analogues

No.	Compound	Structure	pD_2[a]	pK_i[b]
30	(S)α,(S)β-cyclopropylhistamine		7.1 (α=0.71)	7.6[c]
31	(R)α,(R)β-cyclopropylhistamine		5.8 (α=0.64)	8.7[c]
32				6.1
33				< 5.7
34				8.2
35	SCH 49647 (2S,3R)			7.5
36	SCH 49648 (2R,3S)		7.1	8.5
37	SCH 50972 (3S,4S)			7.5
38	SCH 50971 (3R,4R)		7.5	8.6
39	(±)			< 5.7
40∂	(±)			6.4

[a]Electrically evoked contractions of guinea-pig ileum.
[b]Displacement of [^{3}H] N$^\alpha$-methylhistamine from guinea-pig brain tissue.
[c]Displacement of [^{3}H] N$^\alpha$-methylhistamine from rat cortex.

Table 6. Histamine H_3 Receptor Activity of Imetit and Analogues

No.	Compound	Structure	pD_2[a]	pK_i[c]
41	imetit		8.1 9.0 [b]	9.2
42	VUF 8621		7.8[b]	
43	VUF 8973		pA_2=7.3[b]	
44	SKF 91606		9.0	

[a]Electrically evoked contractions of guinea-pig ileum.
[b]K^+-stimulated [^{3}H]-histamine release from rat cortex.
[c]Displacement of [^{3}H] N^{α}-methylhistamine from human recombinant H_3-expressing cells.

Increasing the ring size from a four to five-membered ring leads to the stereospecific pyrrolidine analogues **35**-**37** [26, 27]. These compounds were prepared as rigid analogues of (R)-α-methylhistamine (**11**) and give valuable information about the biologically active conformation of H_3 ligands (*vide infra*). SCH49648 (**36**) and SCH50971 (**38**) are very potent H_3 agonists with excellent *in vivo* receptor selectivity [28]. Interestingly, these substituted pyrrolidine containing ligands can be considered as (R)-α-methyl analogues with ethyl substituents on the β-position (or, alternatively, on the N^{α}-position). Although these substituents are not allowed on the flexible side chain analogues (see **21** and **26**, respectively), incorporation of larger substituents in a ring system does not hinder receptor binding. Further enlargement of the ring results in the low affinity compounds **39** and **40** [27], indicating that these ligands cannot adopt the desired conformation or, alternatively, that these bulkier structures cannot be accommodated in the binding site.

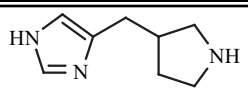
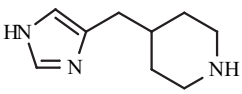
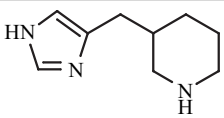
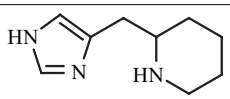
Imetit Derivatives

Substitution of the amino group of histamine by an isothiourea moiety resulted in the highly potent and selective agonist imetit (**41**) (Table **6**) [6, 29-32]. This compound is about three times less active on the human *isoform 1* than on *isoform 2* [9]. In contrast to methylation of the amino group of histamine (**1**), which is allowed for activity (see **24**), methylation of isothiourea moiety drastically reduces activity (**42**) [30, 32] Double methylation leads to the antagonist **43**. The sulphur atom of imetit does not seem to be important for receptor binding as the amidine analogue SKF91606 (**44**) is even more potent than imetit (**41**) [30]. As with histamine (**1**), replacement of the imidazole ring of these highly potent imetit derivatives results in analogues that lack H_3 activity.

Immepip Derivatives

Elongating the imidazole side chain of histamine and incorporating it into a ring structure results in potent H_3

Table 7. Histamine H_3 receptor activity of immepip and analogues

No.	Compound	Structure	pD_2[a]	pK_i[b]
45	(±)-VUF 4864		7.3 (α=0.8)	
46	immepip		8.0	8.8
47	VUF 4858		pA_2=6.5	
48	VUF 4888		pA_2< 5.0	

[a] Electrically evoked contractions of guinea-pig ileum.
[b] Displacement of [^{3}H] N^{a}-methylhistamine from human recombinant H_3-expressing cells.

agonists (Table **7**). The racemic mixture of (±)-3-(1*H*-imidazol-4-yl-methyl)pyrrolidine (VUF4864, **45**) has considerable H_3 agonistic activity [33]. Replacement of the pyrrolidine moiety by a piperidine ring results in the potent and selective H_3 agonist immepip (**46**) [6, 34]. In this compound, the imidazole ring and the basic amino group are separated by four methylene units, but, nevertheless, the ring system is able to adopt the proper conformation for binding to the agonistic binding site [35]. Replacement of the 4-piperidine ring of immepip (**46**) by a 3-piperidine ring or a 2-piperidine ring (leading to **47** and **48**, respectively), results in diminished activity[33].

Imifuramine and Stereoisomer

Harusawa and co-workers have described a novel H_3 agonist [36]. Four stereoisomers (**49**-**52**) of a tetrahydrofuran-containing compound were synthesised and their respective H_3 pharmacology was studied using *in vivo* (rat) brain microdialysis (Table **8**). Imifuramine (**51**) was identified as a H_3 receptor agonist, as it decreases histamine release, an effect that could be blocked by selective H_3 antagonists.

It has been noted by the authors that imifuramine (**51**), having the *trans*-configuration, is not able to form an intramolecular hydrogen-bond between the cationic amino group and the imidazole moiety, thereby indicating that such a hydrogen bond is not important for H_3 agonism [37].

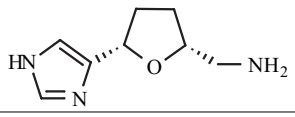
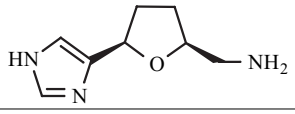
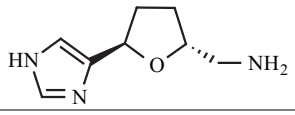
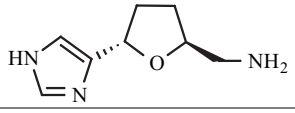
Impentamine Derivatives

The compound impentamine (**53**) was first described as a potent H_3 antagonist as the compound is unable to activate the H_3 receptor pathways in the guinea pig ileum [38]. However, using cell lines expressing human H_3 receptors coupled to Gαi, it was revealed that impentamine reduces cAMP production, thereby revealing agonistic activity. Wieland and co-workers have reported a range of functional activities for impentamine and its analogues, varying from agonist to neutral antagonist and inverse agonist (Table **9**). The authors obtained comparable results using rat H_3 receptors.

There has been an increase in the number of studies using such cell lines expressing recombinant H_3 receptors [27]. The use of recombinant receptors has led to the reclassification of several well-worked ligands. As a result, not only impentamine but also burimadine has been reclassified [28]. A similar reclassification was needed for iodoproxyfan (**60**) and analogues. Originally described as H_3 antagonists [39], these compounds are in fact agonists [6, 40]. Interestingly, the suggested reclassification of these compounds is still a matter of much debate as seemingly conflicting data is still being published. For example, Uveges and co-workers have found that in a FLIPR assay, impentamine (**53**) was unable to stimulate the recombinant wild-type human H_3 receptor and mutation variants, except one: E206A [13]. In the FLIPR assay, the receptors are coupled to the phosphoinositide pathway through a chimera of Gαq. In general, the H_3 agonist potencies determined in the FLIPR are relatively low. Uveges *et al*. have proposed that the receptor couples more efficient to the α-subunit of the native Gi protein than to the chimeric Gαq subunit. Compared to agonists like histamine, impentamine (**53**) seems to induce a different receptor conformation that has a lower intrinsic potency, an effect that is only apparent in the coupling with the Gαq protein. A weaker agonist-receptor complex could also explain why impentamine (**53**) is unable to activate the H_3 receptor pathways in the guinea pig ileum, although in this case a different receptor subtype cannot be ruled out.

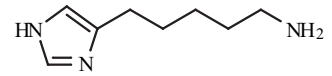
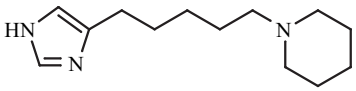
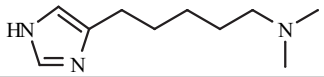
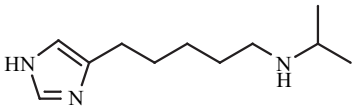
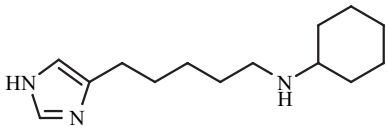
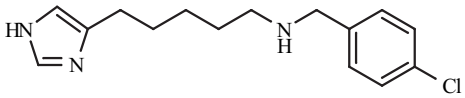
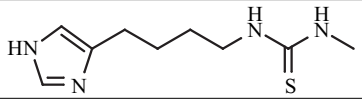
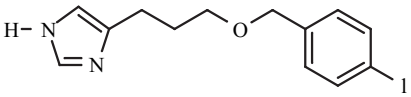
An alternative explanation is that the basal activity is changed by artificially coupling a G protein to activate the Gq signally pathway. The concept of basal activity is illustrated in (Fig. **1**). In this model, the receptor exists in equilibrium between two functionally distinct states: the inactive state (R) and the active state (R*) [41-42]. The constitutive level of receptor activity is governed by the equilibrium between R and R*. It is this equilibrium that might be effected by artificially coupling a G protein to activate the Gq signally pathway. As a result, the receptor

Table 8. Histamine H_3 Receptor *In Vivo* Activity of Imifuramine and Stereoisomers

No.	Structure	Stereochemistry	Reduction of histamine release[a]
49		2S,5R	0%
50		2R,5S	0%
51 imifuramine		2R,5R	70%
52		2S,5S	0%

[a]Effect after administration of 10μM compound.

Table 9. Histamine H_3 Receptor Activity of Impentamine and Analogues

No.	Compound	Structure	pK_i	pEC_{50}	α
53	impentamine		8.3	8.6	0.9
54	VUF 5300		8.0	8.7	1.0
55	VUF 5207		7.8	7.9	0.7
56	VUF 4904		7.9		-0.1
57	VUF 4903		8.0	8.1	-0.6
58	VUF 5202		8.6	8.7	-0.9
59	Burimamide		7.1	6.7	0.8
60	iodoproxyfan				

would become promiscuous in choice of signalling pathway. The generation of such recombinant systems may lead to increased constitutive activity, structural instability and/or enhanced conformational flexibility, as seen for other receptor types [41]. The observation that the mutant E206A is stimulated by impentamine (**53**) supports the idea that the structural conformation of the H_3 receptor is important.

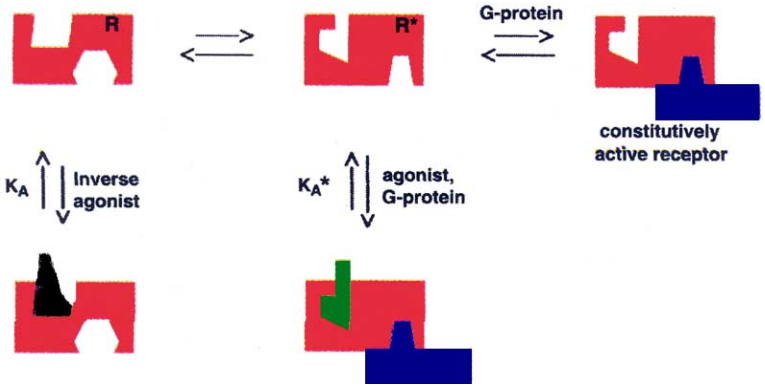


Fig. (1). Simplified model for G protein activation. Receptor in red, G-protein in blue, inverse agonist in grey, agonist in green. **R** represents inactive receptor state, **R*** represents active receptor. The G protein can only bind to **R***. Usually, the equilibrium constant L, which is defined as L=[**R**]/[**R***], is large, *i.e.*, the vast majority of the receptors are in the inactive state. Ligands (Lig) have different affinities for the two states of a receptor; the affinities for **R** and **R*** are characterised by K_A and K_A*, respectively (K_A=[R][Lig]/[R·Lig] and K_A*=[R*][Lig]/[R*·Lig].

Yet another explanation for the differences in ligand characteristics is that variation in tissue types as well as species differences could contribute to structural variations and a change to the constitutive level of activation, resulting in a significant change in the observed response of the ligand.

It is clear that further structural studies are required to determine the changes induced by different classes of ligands as well as species differences, in order to clarify the current discrepancies in ligand classification.

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