The Histamine H_4 Receptor and Potential Therapeutic Uses for H_4 Ligands

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Abstract: Histamine is a biogenic amine that plays a host of physiological roles and the three major functions for histamine have been largely defined by the activity of three receptors. The inflammatory wheal and flare response is driven by the H_1 receptor [1]. The H_2 receptor controls gastric acid secretion in the gut [2]. The H_3 receptor is involved in neurotransmitter release in the central nervous system [3]. The recent discovery of the histamine H_4 receptor by several groups has lead to the re-evaluation of the physiological role for histamine.

INTRODUCTION

The H_4 receptor is a 390 amino acid protein that is predicted to have seven transmembrane regions. The protein contains motifs that identify it as a member of the biogenic amine family of G-protein coupled receptors (GPCRs) including a DRY sequence at the end of transmembrane 3 and an aspartic acid residue in the second transmembrane domain. The amino acid sequence of the H_4 receptor has low homology to other histamine receptors with the H_3 receptor showing the greatest degree of homology at 35%. Their homology in the transmembrane region is 58% and both receptors have a long third intracellular loop. The homology to H_1 and H_2 receptors is actually lower than to other GPCRs [4].

It has been reported that splice variants of the H_3 receptor exist in several species including humans, although their functional relevance remains somewhat controversial [5-11]. The H_4 receptor has a similar genomic structure to the H_3 receptor in that it contains two introns and three exons [12, 13]. To date no splice variants of the H_4 receptor have been reported. It is worth noting that one of the initial reports [14] of the cloning of the H_4 receptor differs in three amino acids from the other reports and the genomic sequence.

The H_4 receptor expression pattern is distinct from the H_3 receptor. While the expression of the H_3 receptor is mainly restricted to cells in the central nervous system [15], the H_4 receptor seems to be limited to cells of hematopoietic lineage [12-14, 16]. Expression has been shown most convincingly for eosinophils, mast cells, basophils, dendritic cells and T cells [12, 17, 18]. The presence of the H_4 receptor on these cell types suggests that it plays a role in the inflammatory response. In support of this, there is some evidence, based on the promoter region of the gene, that expression may be regulated by inflammatory stimuli such as interferon, TNFα and IL-6 [19].

The H_4 receptor has also been cloned from several species including rat, mouse, pig and guinea pig [20, 21]. The sequences are significantly different from the human H_4 receptor with homologies ranging from 65-72%. Interspecies homology is lower for H_4 than for the other histamine receptors especially the H_3 receptor, which has a greater than 90% homology across species [22]. This interspecies difference is also reflected in its affinity for a range of ligands including histamine [20, 21]. In contrast to the differences in sequence and ligand binding, the expression pattern across the species appears to be similar. The rat, mouse, and guinea pig H_4 receptors show strong expression in bone marrow and spleen [20]. The porcine receptor was found in the lung, spleen and colon (bone marrow was not studied in this work) [21]. In mice and humans expression of the H_4 receptor has been shown in mast cells and eosinophils [17].

The description and characterization of the histamine H_4 receptor occurred in several laboratories [4, 12, 13, 14, 16, 23] with the preliminary pharmacological data appearing almost simultaneously from the individual groups. The initial data reporting the potencies of a range of histaminergic ligands competing with [^{3}H]-histamine binding for the different clones was for the most part consistent between laboratories. However, an exception is the data from Nguyen *et al*. [4] in which [^{3}H]-pyrilamine was used as the competing ligand. The lack of pyrilamine affinity for the H_4 receptor [24] has prompted speculation that these discrepancies are due to the contaminating influence of H_1 receptors [20] and consequently the data from Nguyen *et al*. is excluded from the following tables. The data is tabulated with respect to the class of ligand being evaluated.

For the classical antihistamines, H_1-antagonists, Table **1**, none exhibit affinity for the H_4 receptor. The dual H_1/H_2-agonist, HTMT (**2**) demonstrated very weak affinity without significant functional activity [13].

Histamine H_2-receptor ligands showed somewhat greater affinity, Table **2**, with the dual H_2/H_3-antagonist burimamide (**16**) exhibiting partial agonist activity [13, 16]. The H_2-agonist/H_3 antagonist impromidine (**18**) also behaved as a partial agonist [12]. However, the prototypical histamine H_2-antagonists cimetidine (**19**) and ranitidine (**20**) lacked affinity for the new receptor.

In contrast to H_1 and H_2-receptor ligands, a wide range of imidazole based H_3 ligands showed robust affinity for the H_4 receptor.

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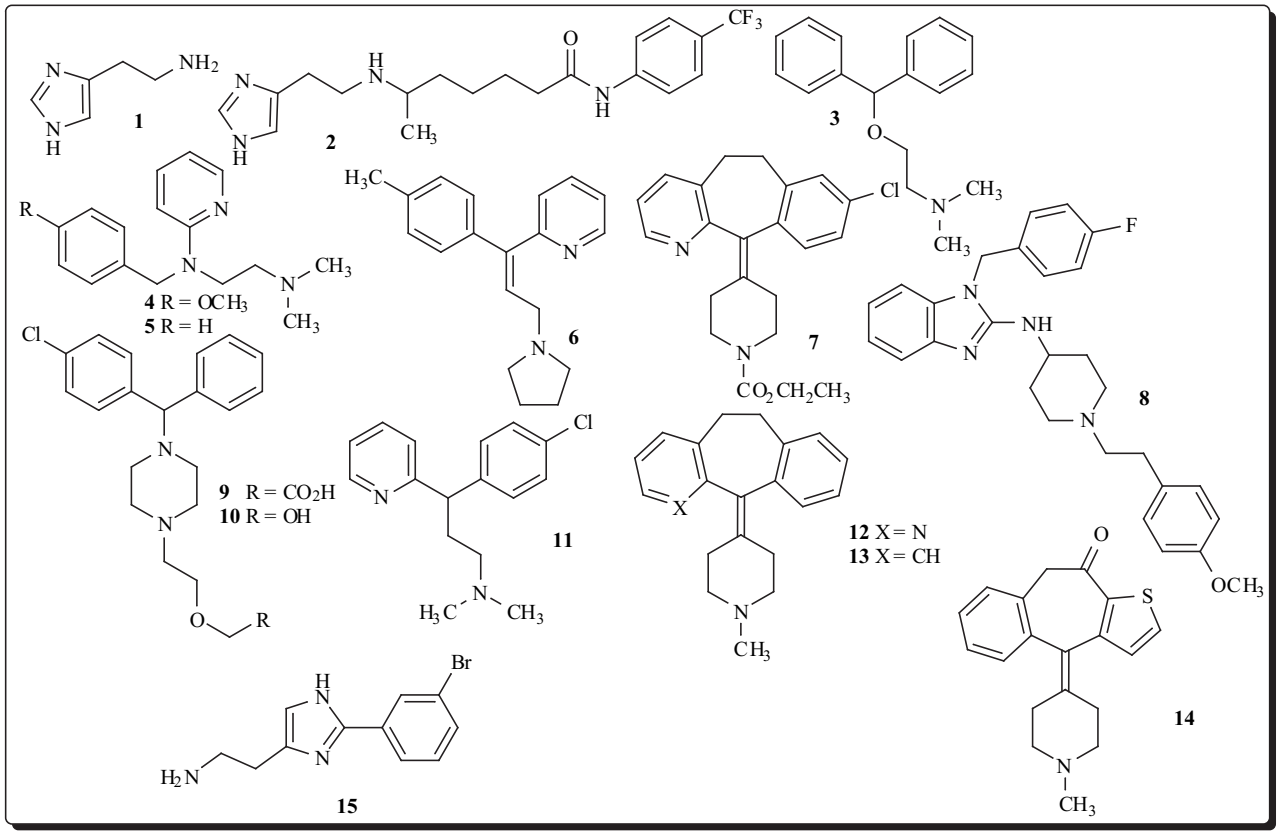
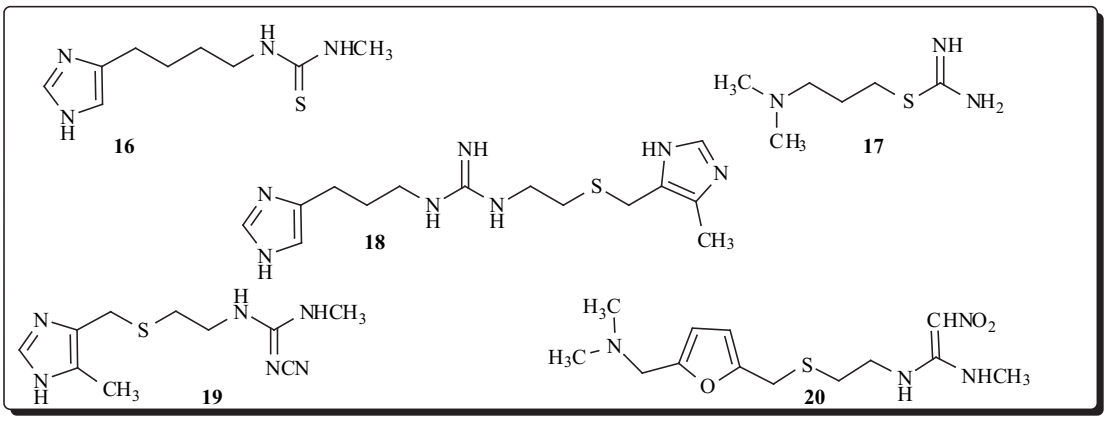


Table 1. H_1 Receptor Ligands and their H_4 Receptor Affinities

Ligand	pK_i	pIC_{50}	Ref.
Histamine (1)	8.13 ± 0.18		[12]
	7.79 ± 0.01		[13]
		8.6	[14]
	8.02 ± 0.04		[16]
	8.36 ± 0.04		[23]
HTMT (2)	5.91 ± 0.05		[13]
Diphenhydramine (3)	< 5		[12]
	< 5		[25]
Pyrilamine (4)		< 4	[13]
	< 4		[23]
Tripelenamine (5)	< 5		[25]
Tripolidine (6)	< 5		[25]
Loratidine (7)	< 5		[25]
Astemizole (8)	< 5		[25]
Cetirizine (9)	< 5		[25]
Hydroxyzine (10)	< 5		[25]
Chlorpheniramine (11)	< 5		[25]
Azatidine (12)	< 5		[25]
Cyproheptadine (13)	< 5		[12]
	< 5		[25]
Ketotifen (14)	< 5		[25]
2-(3-Bromophenyl)histamine (15)	< 5		[25]



With the exception of thioperamide (**21**), which behaved as an inverse agonist [12] or an antagonist [13], these ligands were partial agonists (clobenpropit (**22**) [12, 16]; iodophenpropit (**26**) [13]; imetit (**27**) [16]) or agonists (*R*-α-methylhistamine (**23**) [13, 16]; N-methylhistamine (**25**) [13]). Homologs of histamine, (**29** – **31**) also behave as weak agonists [25]. In contrast to the behavior of the imidazole based H_3-ligands, several non-imidazole based histamine H_3 receptor antagonists **32** [12], **33** [26], **34** [27] and **35** [27] appear devoid of H_4 receptor affinity.

The atypical antipsychotic clozapine (**36**), which was previously reported to be a H_3 antagonist [28], is a low affinity (K_i = 510 ± 110 nM [12]) agonist of the H_4 receptor [12, 13, 14, 25]. Improgan (**37**), a novel imidazole containing antinociceptive agent related to the H_2-antagonist cimetidine (**19**) but without affinity for H_1, H_2 or H_3 receptors, exhibited only weak affinity (K_i = 6000 nM, [13]).

Data obtained for existing histamine receptor ligands has already demonstrated that structures similar to the endogenous ligand exhibit the greatest affinity for the H_4 receptor. Given the low homology between the histamine receptor subtypes, one would not expect non-imidazole ligands to exhibit high affinity across the subtypes. The identification of histamine-based agonists and antagonists is reminiscent of the early structure activity relationships (SARs) for the H_3 receptor and should permit the design and synthesis of selective imidazole-based compounds. However, selectivity issues with respect to the H_3 receptor are already apparent with the observation that the prototypical H_3 antagonist, thioperamide (**21**) is also an H_4-antagonist (inverse agonist). Likewise several H_3-agonists are also H_4-agonists or partial agonists.

Table 2. H_2 Receptor Ligands and their H_4 Receptor Affinities

Ligand	pK_i	pIC_{50}	Ref.
Burimamide (**16**)	6.76 ± 0.1		[12]
	6.8 ± 0.1		[13]
	7.01 ± 0.05		[16]
Dimaprit (**17**)	6.47 ± 0.2		[12]
	6.17 ± 0.05		[13]
	6.43± 0.08		[16]
	5.5		[25]
Impromidine (**18**)	7.93 ± 0.15		[12]
	7.6		[25]
Cimetidine (**19**)	< 5		[12]
		< 4	[13]
	5.03 ± 0.11		[23]
	< 5		[25]
Ranitidine (**20**)	< 5		[12]
		< 4	[13]
	< 5		[25]

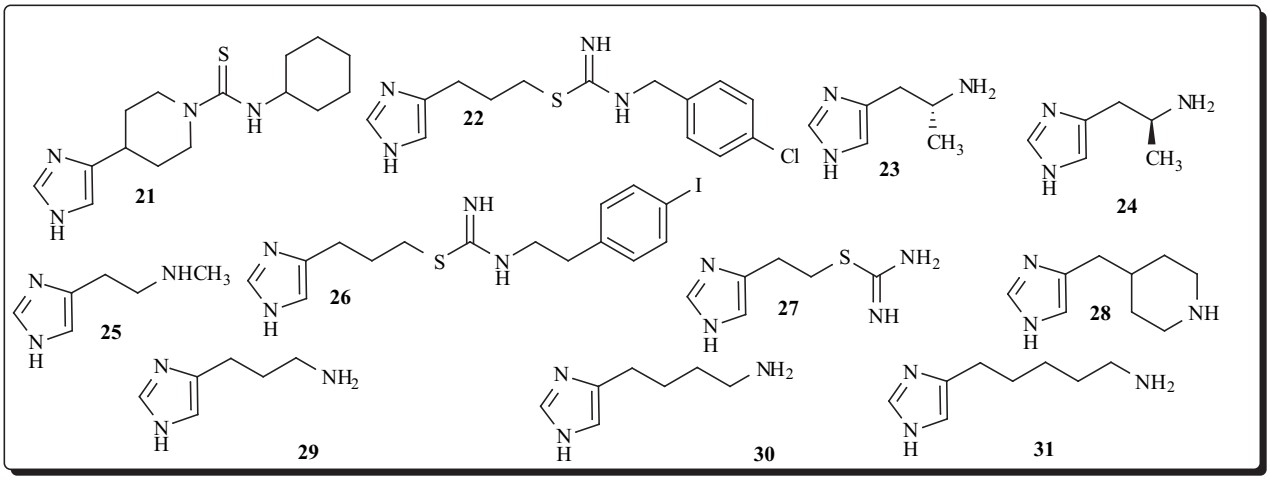
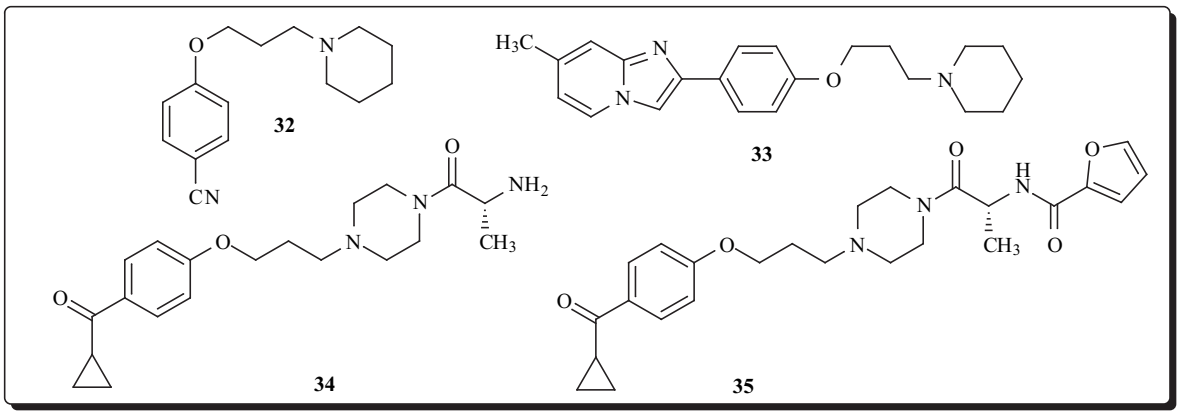


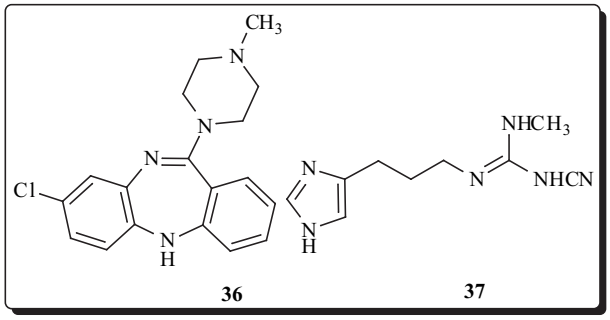
Table 3. H_3 Receptor Ligands and their H_4 receptor affinities

Ligand	pK_i	pIC_{50}	Ref.
Thioperamide (**21**)	7.63 ± 0 23		[12]
	6.29± 0.08		[13]
		6.6	[14]
	6.7 ± 0.11		[16]
	7.26 ± 0.06		[23]
	6.92		[25]
Clobenpropit (**22**)	7.9 ± 0.1		[12]
		8.5	[14]
	8.14± 0.03		[16]
	8.18 ± 0.02		[23]
	8.10		[25]
R-α-Methylhistamine (**23**)	6.89± 0.22		[12]
	6.47 ± 0.08		[13]
	6.86± 0.04		[16]
	6.76 ± 0.05		[23]
	6.62		[25]
S-α-Methylhistamine (**24**)	5.47± 0.04		[16]
N-Methylhistamine (**25**)	7.64± 0.4		[12]
	6.86 ± 0.13		[13]
	7.2 ± 0.01		[16]
	6.53		[25]
Iodophenpropit (**26**)	7.8		[13]
	7.87 ± 0.04		[23]
	7.8		[25]
Imetit (**27**)	8.6 ± 0.1		[12]
	7.8 ± 0.1		[13]
	8.5 ± 0.1		[16]
Imipip (**28**)	8.05 ± 0.05		[12]
	7.65 ± 0.05		[13]
	7.66		[25]
Homohistamine (**29**)	7.46		[25]
Imbutamine (**30**)	6.47		[25]
Impentamine (**31**)	6.12		[25]



32 **33** **34** **35**

The first formal disclosure of selective H_4 antagonists occurred recently [29] from Johnson and Johnson with the presentation of data for JNJ 7777120 (**38**), which followed the publication of the first patent application describing histamine H_4 receptor ligands from the same group [30]. Thus the series of indolylpiperazines were identified following a high throughput screening campaign, which afforded the weak lead **39** [31]. A series of modifications were then made to both the piperazine and indole fragments. Initial examination of the piperazine component demonstrated the favorable effect of a small alkyl substituent (**40**) on nitrogen and the need for an amide linkage (**41**).

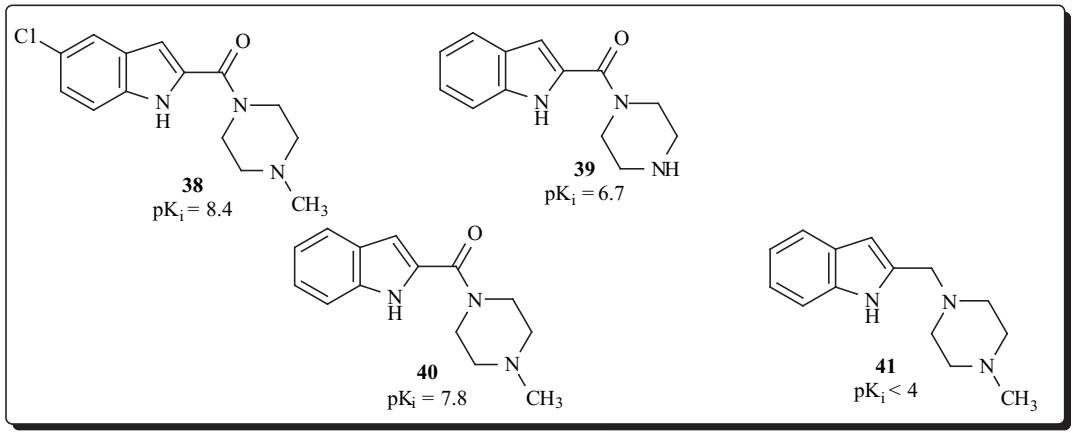


36 **37**

A detailed investigation of substituents on the indole nucleus indicated that a range of groups were tolerated, Table **4**, with alkylation of the indole nitrogen being a notable exception. Further evaluation of **38** confirmed that it behaved as an antagonist in a recombinant system exhibiting a $pA_2 = 8.14$ and that it possessed > 1000 fold selectivity with respect to other histamine receptors.

Most recently the first disclosure of selective H_4-receptor agonists, **42** and **43**, has occurred [32] following an examination of the behavior of a series of analogs of the H_3 agonist imfuramine **44.** Replacement of the amino group of the tetrahydrofuran with a cyanoguanidine led to a reduction in H_3 affinity concomitant with an increase in H_4 affinity, particularly for **42** and **43**, which behaved as agonists in a functional assay.

There have only been a few reports in the literature referring to the biological function of the H_4 receptor. In retrospect, hints to the existence of a fourth histamine receptor were evident as early as 1975 when Clark *et al*. [33] showed that over a narrow concentration range, 0.3 to 1.25 μM, histamine could induce eosinophil chemotaxis. This effect could not be blocked by H_1 or H_2 receptor antagonists, which were the only two histamine receptors known at the time. At higher concentrations of histamine it appeared that H_2 receptor activation inhibited eosinophil chemotaxis [33]. In 1994 Raible *et al.* [34] described a novel histamine receptor on eosinophils that was not H_1, H_2 or H_3 based on the action of known ligands. They showed that the histamine-mediated calcium mobilization in eosinophils could be blocked by thioperamide (**21**), burimamide (**16**) and impromidine (**18**). Although the antagonist data supported a role for the H_3 receptor in this process, (*R*)-α-methylhistamine (**23**) was a less potent agonist than was histamine, which was inconsistent with H_3 receptor pharmacology. The authors suggested the existence of a new histamine receptor. Based on what is now known about the pharmacology and expression profile of the H_4 receptor, it is clear that this receptor was H_4. Recently [23] it has been

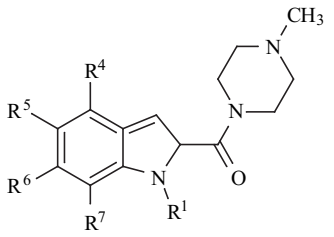


38 $pK_i = 8.4$ **39** $pK_i = 6.7$ **40** $pK_i = 7.8$ **41** $pK_i < 4$

suggested that eosinophil chemotaxis is mediated via the H_4 receptor due to the fact that it can be inhibited by thioperamide (**21**) consistent with the finding of Clark *et al*. [33]. The authors also show that imetit (**27**) does not induce chemotaxis and that clobenpropit (**22**) acts as an antagonist, which may be consistent with the fact that both compounds are partial agonists of the human H_4 receptor in transfected systems.

Hofstra *et al*. [17] have uncovered a role for the H_4 receptor in mast cell function. Mast cells are one of the first lines of defense in the innate immune response, but can also play a pathological role in allergy and other inflammatory diseases. One of the main characteristics of mast cells is that they are able to degranulate after IgE receptor cross-linking in response to allergens. This triggers the release of histamine and other inflammatory mediators. The H_4 receptor does not appear to play a major role in this function since mast cells derived from H_4 receptor knockout mice show no defects in degranulation [17]. However, histamine can induce calcium influx and chemotaxis in mast cells as it does for eosinophils. These effects are attributed to the H_4 receptor since they can be antagonized by thioperamide (**21**) and do not occur in mast cells derived from H_4 receptor knockout mice [17]. The chemotaxis of mast cells to histamine may play a role in allergic rhinitis and allergy where increases in mast cell numbers are found [35-39]. In addition, it is known that in response to antigens there is a redistribution of mast cells to the epithelial lining of the nasal mucosa [40, 41]. It is possible that some of the redistribution that is seen in allergic conditions may be mediated by histamine via the H_4 receptor.

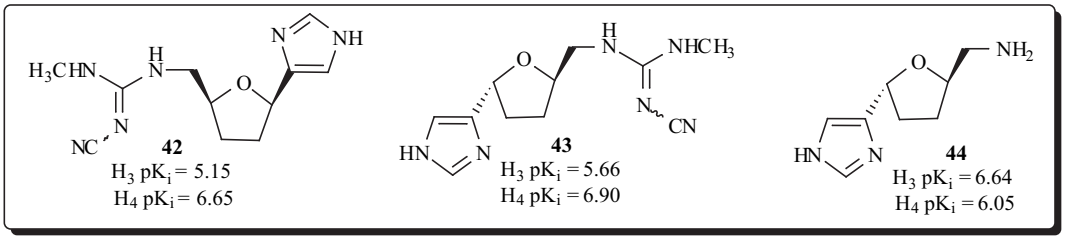
Table 4. H_4 Receptor Affinity of Indolylpiperazines

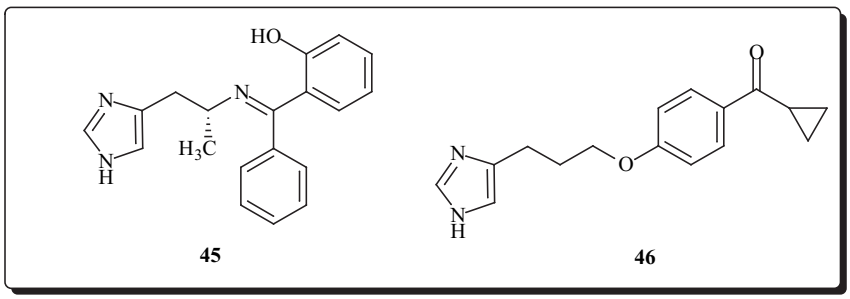


R_4	R_5	R_6	R_7	R_1	pK_i
H	H	H	H	H	7.8
H	H	H	H	CH_3	<4
Br	H	H	H	H	7.5
H	Br	H	H	H	8.1
H	H	Br	H	H	6.8
H	H	H	Br	H	7.2
H	Cl	H	H	H	8.4
H	F	H	H	H	7.8
H	CH_3	H	H	H	7.3
H	OH	H	H	H	7.6
H	NH_2	H	H	H	7.8
H	OCH_3	H	H	H	5.5
H	H	H	Cl	H	7.7
H	H	H	NH_2	H	8.1
H	H	H	CH_3	H	8.2
H	Cl	H	Cl	H	8.0
Cl	Cl	H	H	H	8.3
H	CH_3	H	CH_3	H	7.5
H	F	H	F	H	7.9

A role for the H_4 receptor in $CD8^+$ T cells has also been described. Gantner *et al*. [18] showed that both H_4 and H_2 receptors control histamine-induced IL-16 release from $CD8^+$ T cells. IL-16 is found in the bronchoalveolar fluid of allergen- or histamine-challenged asthmatics [42, 43] and is thought to be important in $CD4^+$ T cell migration. Both cimetidine (**19**) and thioperamide (**21**) were able to completely block the histamine-induced IL-16 release. The authors conclude that the thioperamide (**21**) effect was due to the H_4 receptor since they showed the presence of H_4 receptor but not H_3 receptor mRNA by RT-PCR.

Some of the signaling pathways associated with the H_4 receptor have been elucidated. All histamine receptors initiate signaling by activating specific G-proteins. The H_1 receptor acts through $G\alpha_q$ G-proteins resulting in calcium mobilization, H_2 receptors signal through $G\alpha_s$ G-proteins leading to cAMP increases, while H_3 receptors signal through $G\alpha_{i/o}$ G-proteins and inhibition of cAMP [22]. Several groups have shown that histamine could inhibit forskolin-stimulated cAMP increases in cells transfected with the H_4 receptor [12-14, 44]. Oda *et al*. [14] showed that this could be blocked by pretreating the cells with pertussis toxin, which specifically blocks $G\alpha_{i/o}$ G-proteins. An increase in intracellular calcium in response to histamine can be observed if the H_4 receptor is cotransfected with promiscuous or chimeric G-proteins like $G\alpha_{qi5}$, $G\alpha_{qi1/2}$, $G\alpha_{qi3}$, $G\alpha_{15}$ or $G\alpha_{16}$ [13, 14, 16, 20]. In addition to changed in cAMP and calcium, the activation of the H_4 receptor can induce MAP kinase phosphorylation in transfected cells. The phosphorylation can be inhibited by pertussis toxin implying that this effect is downstream of G-protein activation [16]. Taken together, this data indicates





45 **46**

that in transfected systems the H_4 receptor is able to couple to $G\alpha_{i/o}$ G-proteins, which can be linked to several second messenger pathways such as calcium, cAMP and MAP kinase signaling cascades.

In primary mouse mast cells histamine induces calcium mobilization from intra-cellular stores as well as an increase in cAMP [17]. The cAMP response can be completely blocked by ranitidine (**20**), a H_2 receptor antagonist, but not by thioperamide (**21**), a dual H_3/H_4 antagonist. Conversely, thioperamide (**21**) blocks the calcium response whereas antagonists selective for the other histamine receptors have no effect [17]. Final proof that activation of the H_4 receptor leads to this increase in calcium comes from the fact that the response was abolished in mast cells from H_4 receptor knockout mice [17]. As in the transfected cells, the calcium response in mast cells could be blocked by pertussis toxin. A phospholipase C inhibitor, U73122, also effectively inhibited this calcium response [17]. . Thus, the activation of H_4 receptors on mast cells as well as eosinophils results in calcium mobilization. In mast cells activation of G proteins triggers the activation of phospholipase C, which leads calcium release from the endoplasmic reticulum probably via the formation of inositol-1,4,5-triphosphate.

The function of the H_4 receptor in mast cells, eosinophils and T cells indicate that it may play a major role in the inflammatory response. Indeed it has been reported that H_4 receptor antagonists are active in several mouse peritonitis models [45, 30]. In addition, the possible anti-inflammatory activity of H_4 receptor ligands can be taken from the literature based on the activity of thioperamide (**21**) and (*R*)-α-methylhistamine (**23**) on the H_4 receptor. For example, it has been reported that a prodrug of (*R*)-α-methylhistamine (**23**), BP 2-94 (**45**), can block zymosan-induced paw edema in mice and this can be reversed by thioperamide (**21**) [46]. Also BP 2-94 (**45**), (*R*)-α-methylhistamine (**23**) and ciproxifan (**46**) are efficacious in models of experimental colitis [47, 48]. In addition (*R*)-α-methylhistamine can block carrageenan-induced paw edema in rats [48, 49]. These findings indicate that H_4 ligands may be useful in treating a variety of inflammatory conditions in humans.

Histamine is released in many inflammatory situations. One of the most well known is during an allergic reaction, and H_1 receptor antagonists are useful in reducing the symptoms associated with this release. However, in conditions such as allergic rhinitis H_1 antagonists, whilst useful, only reduce the symptoms by about 40-50%. In particular, histamine-induced nasal blockage cannot be completely inhibited by either H_1 or H_2 antagonists [50], which suggests a role for the H_4 receptor. Support for this arises both from the known involvement of mast cells in allergic rhinitis, together with the observation that mast cells express H_4 receptors.

Asthma is another condition where histamine is released. However, H_1 receptor antagonists have only modest effects and are not used as a first line treatment in the clinic, but may have a use as adjunct therapy [51-53]. Mast cells, eosinophils and T cells are all involved in the etiology of asthma, and since the H_4 receptor has functions in all of these cell types, antagonists for the receptor may be useful for the treatment of asthma.

Itching associated with conditions like atopic dermatitis and uticaria is also thought to be partially mediated by histamine [54]. Although H_1 antagonists are useful in the treatment of uticaria, they are ineffective in other conditions like atopic dermatitis [55]. This may suggest the importance of other mediators or to the involvement of other histamine receptors like H_3 and H_4.

The function and expression of the H_4 receptor in dendritic cells and T cells suggest that it may play an important role in autoimmune diseases like rheumatoid arthritis, multiple sclerosis, type I diabetes and systemic lupus erythematosus. Over the past few years it has become clear that mast cells and histamine play a role in these autoimmune conditions [56-58] and point to the potential for the use of H_4 antagonists for their treatment.

The discovery, of the fourth histamine receptor by several laboratories was quickly followed by an investigation of its' genomic structure, expression pattern and pharmacology. Thus the new receptor shows greatest homology to the histamine H_3 receptor with an expression pattern limited to cells of the immune system; basophils, eosinophils, mast cells and dendritic cells. Consequently this has suggested roles for H_4 ligands in the treatment of autoimmune, inflammatory and allergic disorders, including rheumatoid arthritis, asthma and allergic rhinitis. The availability of both selective H_4 agonists and antagonists will aid in delineating the role for this new target as an opportunity for therapeutic intervention.

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