The First Potent and Selective Non-Imidazole Human Histamine H_4 Receptor Antagonists

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Abstract: Following the discovery of the human histamine H_4 receptor, a high throughput screen of our corporate compound collection identified compound **6** as a potential lead. Investigation of the SAR resulted in the discovery of novel compounds **10e** and **10l**, which are the first potent and selective histamine H_4 receptor antagonists to be described.

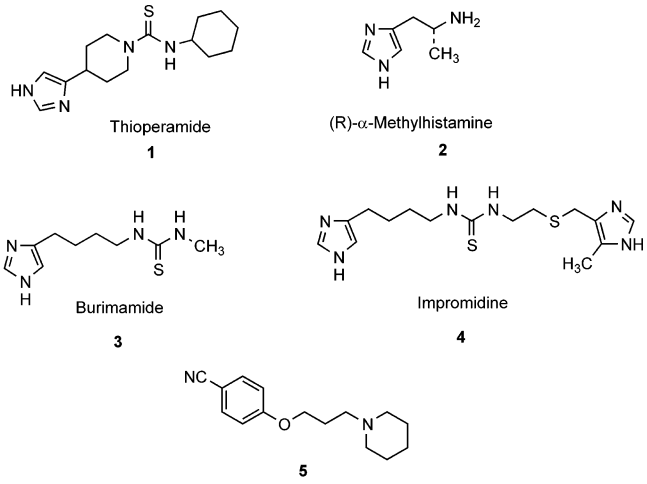
Introduction. Histamine[1] has been shown to play a critical role in several diverse physiological processes.[2−4] It is a key component in the inflammatory response via activation of the histamine H_1 receptor,[5] gastric acid secretion via the histamine H_2 receptor,[6] and mediation of neurotransmitter release in the central nervous system via the histamine H_3 receptor.[7] Recently a fourth histamine receptor, the histamine H_4 receptor, [8−13] was identified.

The histamine H_4 receptor is a 390 amino acid, seven-transmembrane G-protein-coupled receptor. It is expressed mainly on eosinophils and mast cells and has been shown to be involved in chemotaxis of both cell types.[9,10,13−15] The receptor has also been implicated in the release of IL-16 from $CD8^+$ T cells.[16] These data indicate that the histamine H_4 receptor may play a role in the inflammatory response.

The existence of a fourth histamine receptor was postulated by Raible and co-workers as a result of experiments in which histamine produced an increase in cytosolic calcium in eosinophils.[15,17] This calcium influx was not blocked by the known H_1 or H_2 antagonists, pyrilamine or cimetidine, respectively. Interestingly, the known H_3 antagonist thioperamide (**1**) blocked the calcium influx. However, the potent H_3 agonist (R)-α-methylhistamine (**2**) only weakly activated a calcium response in eosinophils. Thus, it was concluded that the increase in cytosolic calcium in eosinophils was the action of a novel receptor. The recent cloning of the histamine H_4 receptor and subsequent experiments[8−13] confirmed the pharmacology described by Raible et al.[15,17]

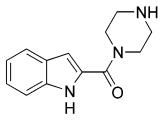
Among the histamine receptors, the H_4 receptor exhibits the highest degree of homology to the H_3 receptor at 40%. Many of the imidazole-based ligands that exhibit binding affinity for the H_4 receptor also show significant affinity for the H_3 receptor as shown in Chart 1.[8] A high affinity non-imidazole H_3 antagonist, 4-(3-piperdin-1-ylpropoxy)benzonitrile (**5**) [18] is devoid of activity at the H_4 receptor, demonstrating that specificity between the two receptors could be achieved.

Chart 1. Binding Affinity of Histamine Ligands to the Human Histamine H_3 and H_4 Receptors[8]



	H_4 Receptor K_i (nM)	H_3 Receptor K_i (nM)
Thioperamide (**1**)	27 ± 13	25 ±7
(R)-α-Methylhistamine (**2**)	146 + 68	0.7 ± 0.3
Burimamide (**3**)	180 ± 40	84 ± 20
Impromidine (**4**)	12.3 ± 4.0	67 ± 16
5	>10,000	25 ± 10

Following the discovery of the H_4 receptor, we set out to identify potent, selective, non-imidazole histamine H_4 ligands. We began with a high throughput screen of our corporate compound collection, which produced several lead compounds including indolylpiperazine **6**.[19] Based on these leads, a medicinal chemistry program was initiated to evaluate the structure−activity relationships (SAR) for indole **6**. The SAR for this series and the biological evaluation of selected analogues will be discussed.



6

K_i = 38 nM

Chemistry. Indolylpiperazines **6** and **8a**−**f** were synthesized as shown in Scheme 1. Commercially available indole-2-carboxylic acid (**7a**) was coupled to a range of piperazines using standard amide coupling conditions to provide analogues **8a**−**d**. The Boc-protected compound **8a** was converted to lead compound **6** by deprotection with trifluoroacetic acid in dichloromethane. Coupling of 1-methylindole-2-carboxylic acid (**7b**) with *N*-methylpiperazine provided compound **8e**, and reduction of **8b** with $LiAlH_4$ gave methylene analogue **8f**.[20]

N-Methylpiperazine analogues **10a**−**t** were prepared in an analogous manner from the corresponding substituted indole carboxylic acids (**9**) as shown in Scheme 2.[21]

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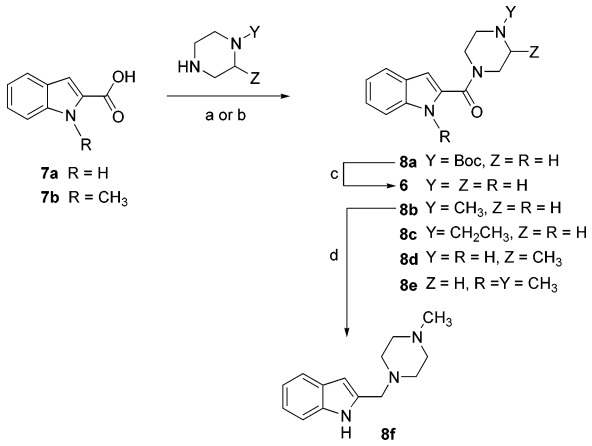
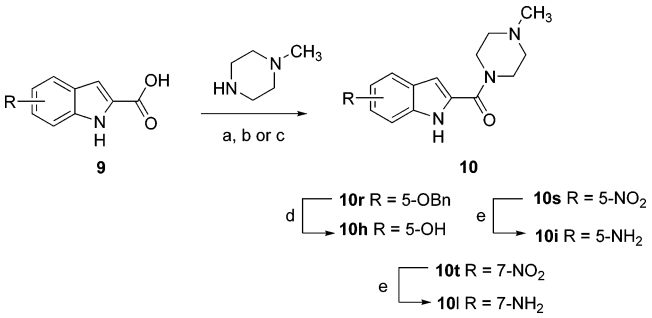
Scheme 1[a]



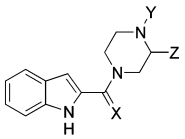
[a] Reagents: (a) HATU, HOAT, DIPEA, DMF; (b) CDI, THF; (c) TFA, DCM; (d) $LiAlH_4$, THF.

Scheme 2[a]



[a] Reagents (a) HATU, HOAT, DIPEA, DMF; (b) CDI, THF; (c) EDC, (HOBT) DCM; (d) 10% Pd/C, H_2, EtOH, EtOAc; (e) 10% Pd/C, NH_4CO_2H, CH_3OH, reflux.

Table 1. Binding Affinity of Piperazine Amide Analogues[a]



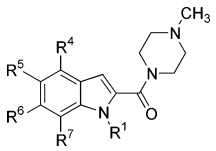
	X	Y	Z	K_i (nM)[a]
6	O	H	H	38 ± 1
8b	O	CH_3	H	17 ± 1
8c	O	CH_3CH_2	H	260 ± 32
8d	O	H	CH_3	202 ± 2
8f	H_2	CH_3	H	10 000
8g	O	CH_2CH_2Ph	H	7000

[a] Displacement of [^{3}H] histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean ± SEM of three or more independent determinations and calculated according to Cheng and Prusoff.[22]

Compound **10h** was prepared by catalytic hydrogenation of benzyloxy compound **10r**, whereas the amino analogues **10i** and **10l** were prepared by reduction of the corresponding nitro derivatives **10s** and **10t**, respectively.

Results. The initial investigation of the indolylpiperazines focused on modifications to the piperazine amide portion of lead compound **6** as shown in Table 1. A slight increase in binding affinity was observed upon methylation of the piperazine nitrogen, as seen in compound **8b** (K_i = 17 nM). However this portion of the molecule proved to be sensitive to steric effects as suggested by

Table 2. Binding Affinity of Indole-Substituted Analogues[a]



	R^4	R^5	R^6	R^7	R^1	K_i (nM)[a]
8b	H	H	H	H	H	17 ± 1
8e	H	H	H	H	Me	>10 000
10a	Br	H	H	H	H	32 ± 2
10b	H	Br	H	H	H	8 ± 1
10c	H	H	Br	H	H	147 ± 23
10d	H	H	H	Br	H	61 ± 5
10e	H	Cl	H	H	H	4 ± 1
10f	H	F	H	H	H	15 ± 1
10g	H	CH_3	H	H	H	46 ± 5
10h	H	OH	H	H	H	23 ± 2
10i	H	NH_2	H	H	H	15 ± 2
10j[23]	H	OCH_3	H	H	H	3000 ± 10
10k	H	H	H	Cl	H	19 ± 1
10l	H	H	H	NH_2	H	8 ± 1
10m	H	H	H	CH_3	H	7 ± 1
10n	H	Cl	H	Cl	H	11 ± 1
10o	Cl	Cl	H	H	H	5 ± 3
10p	H	CH_3	H	CH_3	H	31 ± 1
10q	H	F	H	F	H	14 ± 1

[a] Displacement of [^{3}H] histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean ± SEM of three or more independent determinations and calculated according to Cheng and Prusoff.[22]

the *N*-ethyl analogue **8c**, K_i = 260 nM. This was confirmed by the preparation of the phenethylpiperazine analogue **8g**, which showed low affinity for the H_4 receptor (K_i = 7000 nM). Interestingly, *C*-methyl substitution on the piperazine was tolerated as illustrated by racemic compound **8d** (K_i = 202 nM), while the amide linkage was found to be critical as shown by analogue **8f**.

Having only achieved a slight increase in binding affinity with the *N*-methylpiperazine analogue **8b**, we turned our attention to the indole nucleus as shown in Table 2. Investigation of substitution at the N-1 position provided methylated analogue **8e**, which was devoid of activity at the H_4 receptor. Substitution on the 4, 5, 6, and 7 positions of the indole ring was initially investigated with a bromine substituent due to the availability of corresponding starting materials. The 6-Br analogue (**10c**) showed a slight decrease in affinity (K_i = 147 nM) over the parent **8b**, whereas the 4- and 7-Br analogues were essentially equipotent to **8b**. The 5-Br (**10b**) displayed an encouraging increase in potency (K_i = 8 nM), with similar results obtained for the 5-Cl (**10e**) and 5-F (**10f**) compounds. A variety of substituents at the 5-position were evaluated and all were well tolerated by the H_4 receptor with the exception of the 5-OCH_3 analogue **10j** with K_i = 3000 nM.

Further investigation of the SAR of the indolylpiperazines led to the preparation of compounds **10k**−**q**. The 7-Cl analogue **10k** was slightly less potent then the 5-Cl analogue (K_i = 19 nM vs 4 nM), respectively. In contrast, the methyl-substituted analogue 7-CH_3 (**10m**), showed slightly higher affinity than its 5-substituted counterpart, whereas the 7-NH_2 (**10l**) and 5-NH_2 (**10i**) compounds are essentially equipotent. In light of these trends, several disubstituted analogues were prepared.

The 5,7- and 4,5-dichloroindolylpiperazines (**10n** and **10o**, respectively) displayed high receptor affinity with K_i's = 11 nM and 5 nM, respectively. The 5,7-dimethyl compound **10p** (K_i = 31 nM) was found to be equipotent to the 5-CH_3 analogue **10g** (K_i = 46 nM) but slightly less potent than the 7-CH_3 compound **10m** (K_i = 7 nM). A similar trend was seen for the 5,7-difluoro indolylpiperazine **10q** when compared to its 5-fluoro analogue.

A detailed biological evaluation of **10e** and **10l** was undertaken due to their high affinity for the H_4 receptor. Functional activity versus the human H_4 receptor was determined using SK-N-MC cells stably transfected with the human H_4 receptor.[8] In these cells, addition of histamine induces a decrease in the forskolin stimulated cAMP levels. Compounds **10e** and **10l** produced a rightward shift in the histamine dose response curve yielding a pA_2 = 8.14 and 8.11, respectively, confirming that they function as H_4 receptor antagonists. These compounds also showed high affinity for the rat histamine H_4 receptor[24] (**10e** K_i = 2.4 nM and **10l** K_i = 3.3 nM) and were found to be >1000-fold selective for the H_4 receptor over the other histamine receptors. When tested against a panel of over 50 receptor targets representing the major classes of biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and transporters, these compounds showed minimal biological activity.

Conclusion. After screening our corporate compound collection against the histamine H_4 receptor and identifying lead compound indolylpiperazine (**6**), we began a medicinal chemistry program to improve the biological activity of lead compound **6** and elucidate the SAR for the series. Several general trends can be gleaned from the results presented in Tables 1 and 2. Our SAR investigation suggested that in order to maintain potency less than 100 nM, substitution on the piperazine nitrogen must be limited to a methyl group. In contrast, a variety of substituents about the indole ring were well tolerated. In general, lipophilic groups or compact polar groups increased affinity for the H_4 receptor relative to the unsubstituted analogue. Disubstitution on the indole ring was also tolerated, resulting in compounds with activity comparable to the 5-substituted analogues. Detailed biological evaluation of selected analogues, **10e** and **10l**, demonstrated that these ligands are selective for the histamine H_4 receptor and that they function as receptor antagonists. Thus, we have prepared the first potent and selective non-imidazole histamine H_4 antagonists. Further pharmacological characterization of **10e**, JNJ 7777120, will be reported in due course.

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Supporting Information Available: Experimental procedures and analytical data for target compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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