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Agonist/antagonist modulation in a series of 2-aryl benzimidazole H_4 receptor ligands

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ABSTRACT

The present work details the transformation of a series of human histamine H_4 agonists into potent functional antagonists. Replacement of the aminopyrrolidine diamine functionality with a 5,6-fused pyrrolopiperidine ring system led to an antagonist. The dissection of this fused diamine led to the eventual replacement with heterocycles. The incorporation of histamine as the terminal amine led to a very potent and selective histamine H_4 agonist; whereas incorporation of the constrained histamine analog, spinacamine, modulated the functional activity to give a partial agonist. In two separate series, we demonstrate that constraining the terminal amino portion modulated the spectrum of functional activity of histamine H_4 ligands.

The histamine H_4 receptor is a 390 amino acid G-protein coupled receptor that is implicated in inflammatory diseases such as asthma and allergic rhinitis based on the expression of the H_4 receptor on haematopoietic cells such as eosinophils, mast cells, dendritic cells, and other leukocytes.[1,2] Furthermore, several groups have reported the diverse pharmacological effects of inhibition of the H_4 receptor in animal models of asthma, arthritis, pruritus, colitis and pain.[3,4] We initially reported on the prototypical H_4 receptor antagonist JNJ 7777120 (**1**) and have also recently disclosed the structurally distinct 2-arylbenzimidazole series (**2**) of H_4 ligands (Fig. 1).[5,6] This report further details our work on this 'extended' series of H_4 ligands; particularly, our efforts to turn these from agonists to antagonists of the H_4 receptor. In our efforts to transform these agonists into antagonists we were conscious that subtle structural changes in compound type can impact functional behavior in GPCRs.[7–11] We therefore initiated a scan of various diamines in order to probe the effect this would have on the functional activity of the 2-aryl benzimidazole series.

SAR around one of our originally reported H_4 ligands (**3**) demonstrated that deconstruction of the piperazine was not beneficial for binding activity in this series (Fig. 2), but increasing the ring size to a homopiperazine (**4**) did show a modest increase in activity. Although the *N*-methylpyrrolidine (**7**) was less active than the

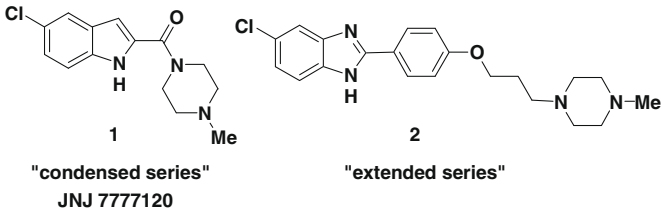

Figure 1. Representative histamine H_4 chemotypes.

starting piperazine (**3**), it did encourage us to further investigate other diamines on more active core structures.

We focused our efforts on the 4-fluoro-5-methyl benzimidazole coupled with methyl substitution on the central aromatic ring as it provided a boost in affinity in several of the series that were being prosecuted.[13] Consequently, we modified[12] our original synthesis[6] to introduce the diamine in the last step as opposed to the middle of the synthesis. As we continued to investigate other amines on the more potent series (Table 1), we were encouraged that the 3-aminopyrrolidine was a partial agonist with a pA2 of 7.3 (**9**). Mono or dialkylation of the terminal nitrogen, or enlarging the ring size to 3-aminopiperidine maintained or increased the partial agonism (**10–13**). One of the more intriguing results came from the incorporation of the racemic 5,6-pyrrolopiperidine, which provided a full antagonist (**14**). Resolution of the enantiomers showed that the (*R,R*) eutomer was 50-fold more potent than the (*S,S*) distomer (**16 and 17**).

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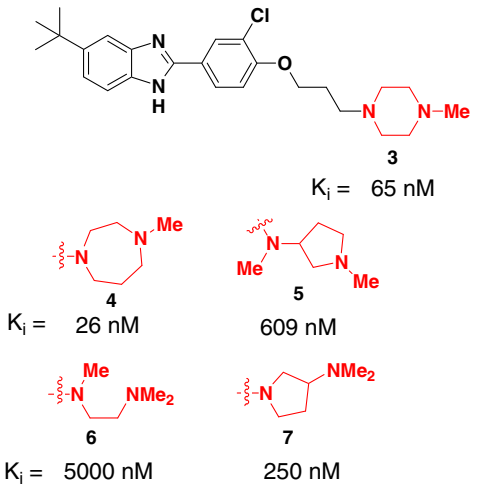
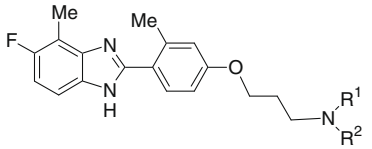


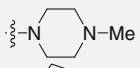
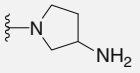
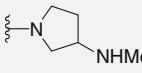
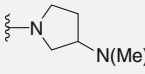
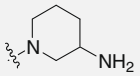
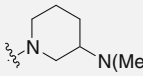
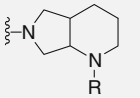
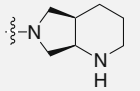
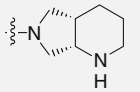
Figure 2. Initial diamine scan.

In a related series, we had noted a difference in functional activity between piperazine and piperidine,[14] the latter being a complete antagonist. We had hypothesized that the distal basic nitrogen in the piperazine ring is interacting with Asp^{94} in TM3,[15] while the proximal nitrogen in the piperazine might be making a second interaction in the receptor, resulting in a conformation change and subsequent agonist behavior. The piperidine, lacking the proximal nitrogen, would still be able to interact with Asp^{94}, but be unable to make the putative 'agonist interaction'. We further speculated that the 5,6-pyrrolopiperidine might be adopting a low-energy conformation in which the basic piperidine nitrogen is interacting with both Asp^{94} and with the nitrogen in the pyrrolidine ring, preventing it from making the agonist interaction with the receptor. It is interesting to note that the N-Me version of this 5,6-pyrrolopiperidine, which would be unable to adopt such a conformation, is significantly less potent at the H_4 receptor (**15**, K_i = 1000 nM), which may support this possibility. However, attempts to quantify this behavior based on analysis of low-energy conformations and calculations of inter-nitrogen distances of these and other diamines did not yield any descriptors capable of predicting agonist/antagonist behavior.

Encouraged by our ability to modulate functional activity by the incorporation of the constrained 3-aminopyrrolidine, we initiated two routes to further probe the agonist modulation. The first route consisted of scanning a series of various ring sizes (Table 2) and the second route involved deconstructing the 5,6-pyrrolopiperidine to further dissect the effects of structural changes on the functional activity of this unique system (Fig. 3). Initially, we decided to probe the effect of ring size and orientation of the distal nitrogen through various fused ring systems (Table 2). Both the 5,6- and 5,5-fused

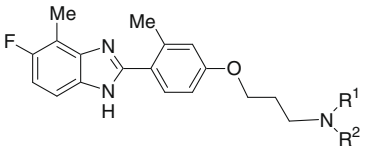
Table 1
Diamine scan on preferred core

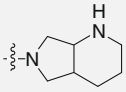
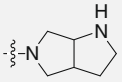
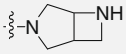
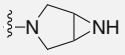
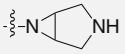
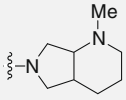
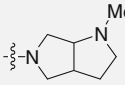
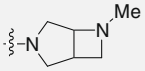
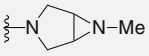
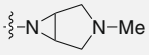


Compd #	NR^1R^2	hH_4 K_i[a,e] (nM)	EC_{50} nM[b] (α)	pA2[b,c,d]
8		32	50 (0.65)	N/A
9		80	470 (0.47)	7.3
10		20	62 (0.59)	N/A
11		84	150 (0.55)	N/A
12		72	331 (0.81)	
13		2000	NA	NA
14 15		R = H; 42 R = Me; 1000	>10,000 NA	7.3 NA
16		14 (R,R)	>10,000	7.6
17		691 (S,S)	N/A	N/A

[a] Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[16]
[b] Compounds with K_i >100 nM not tested in functional assays.
[c] Compounds with α >0.40 were not tested in the pA2 assay.
[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor.
[e] Unless noted chiral compounds were tested as racemates. Detailed experimental for EC_{50} and pA2 determinations included in references.[18]

Table 2
Fused diamine scan on preferred core



Compd #	NR^1R^2	hH_4 K_i[a,e] (nM)	EC_{50}[b] nM (α)	pA2[b,c,d]
14		42	>10,000	7.3
18		26	>10,000	7.9
19		>10,000	N/A	N/A
20		>10,000	N/A	N/A
21		>10,000	N/A	N/A
15		1000	N/A	N/A
22		232	N/A	N/A
23		971	N/A	N/A
24		89	>10,000	6.8
25		77	710 (0.29)	7.18

[a] Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[16]
[b] Compounds with K_i >100 nM not tested in functional assays.
[c] Compounds with α >0.40 were not tested in the pA2 assay.
[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor.
[e] Unless noted chiral compounds were tested as racemates. Detailed experimental for EC_{50} and pA2 determinations included in references.[18]

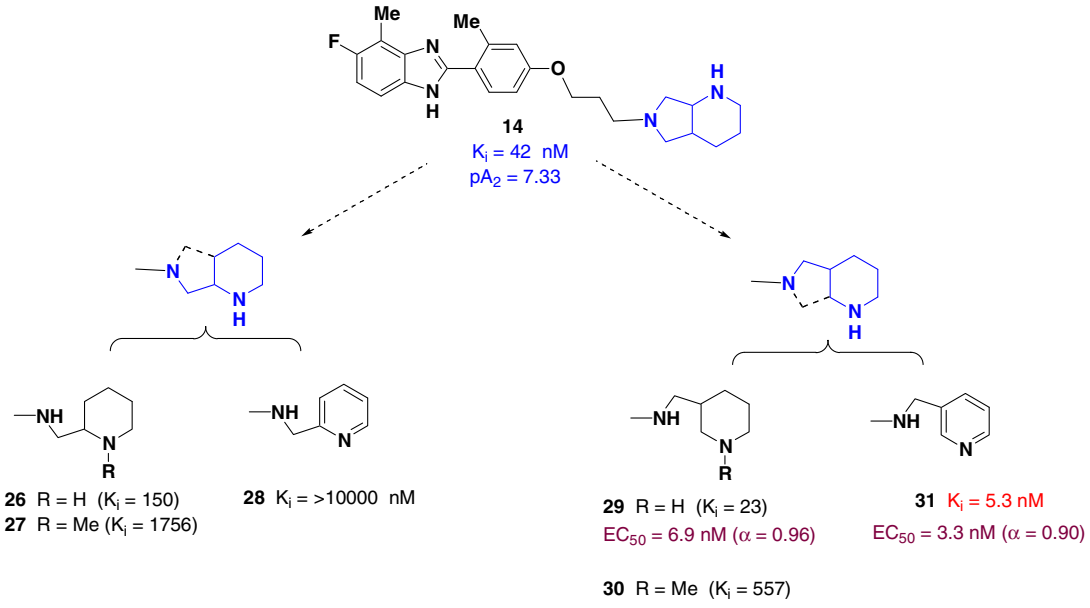
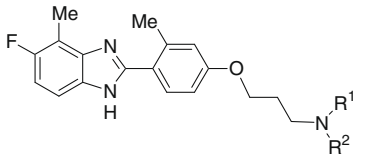
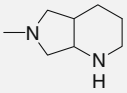
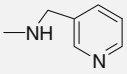
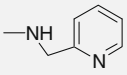
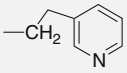
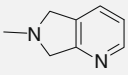
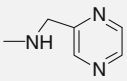
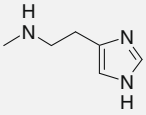
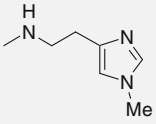
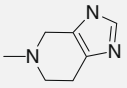


Figure 3. Dissection of 5,6-pyrrolopiperadine. [a]Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[16] [b]Compounds with K_i >100 nM not tested in functional assays. [c]Compounds with α >0.40 were not tested in the pA2 assay. [d]Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor. [e]Unless noted chiral compounds were tested as racemates. Detailed experimental for EC_{50} and pA2 determinations included in references.[18]

Table 3
Heterocycle scan



Compd #	NR^1R^2	K_i[a] (nM)	pA2/EC_{50}[b,c,d,e]
14		42	pA2 = 7.3
31		5.3	EC_{50} = 3.3 nM (α = 0.90)
28		>10,000	
32		152	
33		228	
34		>10,000	
35		0.21	EC_{50} = 0.56 nM (α = 0.96)
36		133	
37		3.3	EC_{50} = 3.3 nM (α = 0.61)

[a] Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[16]
[b] Compounds with K_i >100 nM not tested in functional assays.
[c] Compounds with α >0.40 were not tested in the pA2 assay.
[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor.
[e] Unless noted chiral compounds were tested as racemates. Detailed experimental for EC_{50} and pA2 determinations included in references.[18]

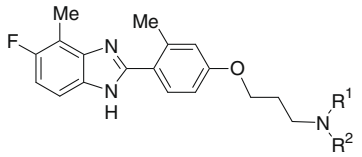
diamines were reasonably potent antagonists at the H_4 receptor (**14, 18**), while further contraction of the fused ring resulted in a loss in affinity (**19–21**). Of further interest was that methylation of these diamines often resulted in a reversal of SAR relative to the des-methyl analog (**22–25**). In the case of the 5,3-fused diamine, methylation of either regioisomer not only restored affinity, but also resulted in varying degrees of antagonism (**20 and 21, 24, and 25**). This contrasts with the 5,6-fused system which lost affinity upon methylation (**14, 15**).

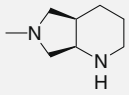
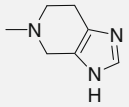
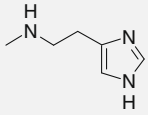
In our attempt to understand the SAR of the antagonism imparted by the 5,6-diamine, deconstruction of the 5,6-diamine revealed some interesting SAR (Fig. 3). Breaking the five-membered ring at either position demonstrated that the 3-aminomethyl piperidine derivative (**29**) was not only a better ligand than the 2-aminomethylpiperidine derivative (**26**), but was also a fairly potent agonist relative to histamine (histamine K_i = 60 nM). Additionally, in both cases methylation was detrimental to activity (**27, 30**). These results demonstrated that although a 3-aminomethyl piperidine was a better mimic of the 5,6-pyrrolopiperidine in a binding sense, the effects on functional activity were more complex.

We also looked at the corresponding pyridine derivatives: 2-aminomethyl pyridine is an anticipated isostere for a 1,2-diamine[17] but use of this motif led to loss of activity (**28**). In contrast, the 3-aminomethyl pyridine derivative (**31**) is a low nanomolar ligand that is a log more efficacious than histamine in the functional assay. The increased potency and efficacy in the functional assay of the 3-aminopyridine derivatives relative to the 5,6-pyrrolopiperidine and 2-aminomethylpyridine was intriguing, and we set off to examine the effect of additional heterocycles on functional activity (Table 3).

Replacement of the proximal nitrogen with carbon (**32**) led to a decrease in activity as did constraining the proximal nitrogen into a ring (**33**); although neither change led to a complete loss in activity. Replacement of the pyridine ring with a non-basic pyrazine ring resulted in significant reduction of H_4 affinity (**34**). However, the most interesting results occurred when we incorporated known H_4 ligands as the diamine component. The incorporation of histamine resulted in a very potent functional agonist (**35**), while the *N*-methyl histamine derivative (**36**) was almost three orders of magnitude less potent than the histamine derivative. The incorporation of the constrained histamine analog spinac-

Table 4
Profile of selective H_4 agonist/antagonists



Compd #	NR^1R^2	hH_1 K_i[f] (nM)	hH_2 K_i[g] (nM)	hH_3 K_i[h] (nM)	hH_4 K_i[a] (nM)	hH_4 pA2/EC_{50}[b,c,d,e]	mH_4 K_i[a] (nM)	mH_4 pA2/EC_{50}[b,c,d,e]
16		>10,000	>10,000	119	14	pA2 = 7.6	1197	pA2 = 3.6
37		>10,000	>10,000	9000	3.3	EC_{50} = 3.3 nM (α = 0.61)	548	pA2 = 4.8
35		>10,000	131	377	0.21	EC_{50} = 0.56 nM (α = 0.96)	12	EC_{50} = 39 nM (α = 0.75)

[a] Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[16]
[b] Compounds with K_i >100 nM not tested in functional assays.
[c] Compounds with α >0.40 were not tested in the pA2 assay.
[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor.
[e] Unless noted chiral compounds were tested as racemates. Detailed experimental for EC_{50} and pA2 determinations included in references.[18]
[f] Human recombinant H_1 receptor expressed in SK-N-MC cells was determined by competitive radioligand binding using [^{3}H]-pyrilamine, as the radioligand.
[g] Human recombinant H_2 receptor expressed in CHO cells was determined by competitive radioligand binding using [^{125}I]APT as the radioligand.
[h] Human recombinant H_3 receptor expressed in SK-N-MC cells was determined by competitive radioligand binding using [^{125}H]-iodoproxyphan as the radioligand.

amine (**37**), attenuated the functional activity to provide a partial agonist relative to the histamine derivative. It is interesting that in two very different terminal amines, we see that constraining the diamine portion into a bicyclic system resulted in turning potent agonists into partial or full antagonists (vide supra).

Table 4 summarizes the human and mouse activities of the two cyclic diamine full and partial antagonists and the histamine derived agonist. The pyrrolopiperidine antagonist **16** has good selectivity over H_1 and H_2, and moderate (~ninefold) over H_3 while the spinacamine derived antagonist **37** is a highly selective human H_4 receptor partial agonist. The histamine derived **35** is a notably selective human H_4 receptor agonist with >600-fold affinity over H_2 and >1700-fold affinity over the H_3 receptor while having no affinity for the H_1 receptor.

In conclusion: with dimethylaminopyrrolidine as a starting diamine, incremental changes led to the discovery of a 2-aryl benzimidazole partial agonist. Further exploration around the aminopyr rolidine template led to the discovery of a 5,6-pyrrolopiperadine containing 2-aryl benzimidazole as the first full antagonist in this novel series of H_4 modulators. Through the deconstruction of the 5,6-pyrrolopiperidine template we were able to demonstrate the ability to access potent agonists and antagonists of the H_4 receptor.

References and notes

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18. *Details for EC_{50} and pA2 assays*: Mouse and Human H4 were cloned into the pCINeo mammalian expression vector and transfected into the human neuroblastoma SK-N-MC cell line. The construct contains the reporter gene B galactosidase under the control of cyclic AMP responsive element. In the EC_{50} assay, the compounds are added to the media and allowed to incubate for 10 min at room temperature before the addition of forskolin (5 μM final concentration). In the pA2 assay, the cells are preincubated with compound for 10 min, then incubated with agonist for 10 minutes before the addition of forskolin. After a 6 h incubation (for both assays) at 37 °C, the media is aspirated and the plates are stored at −40 °C overnight. Cells are lysed with 25 μL of 0.1× assay buffer (10 mM Na phosphate, pH 8, 0.2 mM $MgSO_4$, 0.01 mM $MnCl_2$) and incubated at room temperature for 10 min. Cells are then incubated with 100 μL of 1× assay buffer including 0.5% Triton X-100 and 40 mM B mercaptoethanol for 10 min. Substrate solution (25 μL) (1 mg/ml chlorophenolred B–D galactopyranoside) was added and the color was quantitated at an absorbance of 570 nm. Alpha values are calculated using the full agonist (positive control) as the standard of 1.