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Synthesis of novel histamine H4 receptor antagonists

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ABSTRACT

This letter describes the discovery and synthesis of a series of octahydropyrrolo[3,4-*c*]pyrrole based selective histamine hH4 receptor antagonists. The amidine compound **20** was found to be a potent and selective histamine H4 receptor antagonist with moderate clearance and a high volume of distribution.

The fourth human histamine receptor H4 was disclosed in 2000 as a 390 amino acid transmembrane G-protein-coupled-receptor.[1] The receptor is predominantly expressed in cells of the hematopoietic lineage, including eosinophils, basophils and mast cells, although recent reports in the literature show evidence for expression on non-hematopoietic cells[2] and in the CNS.[3]

The majority of the pharmacological research that has been published in the literature has utilised compound **1** (JNJ-7777120);[4] however, more recently a number of other selective tool compounds have been described including A-943931[5] and A-987306,[6] along with numerous patent applications.[7] Efficacy for these small molecule tool compounds has now been demonstrated in multiple pre-clinical models of inflammation and pain.[5,8] These data in conjunction with data from H4 knockout mice[9] support the position that there is potential that selective H4 receptor antagonists represent a new class of therapy to treat a wide range of diseases,[10,11] including asthma and allergic rhinitis. Compound **1** was the first reported potent non-imidazole selective H4 antagonist and is reported to bind the human H4 receptor (hH4) with a K_i of 4 nM and more than 1000-fold selectivity over the other histaminergic receptors. In our hands, the compound also shows high affinity for the hH4 receptor (hH4 Binding K_i 8.0 nM,[12] hH4 Functional K_i 6.8 nM[13]). A limitation of compound **1** is its relatively high intrinsic clearance (Cl 968 ml/min/kg) which results in a short $T_{1/2}$ (0.7 h) in rat.[14]

In this Letter we report on our efforts to identify a selective hH4 antagonist with good intrinsic pharmacokinetics. Given the high ligand efficiency of **1** (LE 0.58 kcal mol^{-1})[15] we perceived it to be an attractive starting point for the program.[16] Using the central amide bond as the key disconnection, SAR of the 'acid' and 'amine' fragments were investigated. A number of alternative 5,6- and 6,6-fused carboxylic acid systems were coupled with *N*-methyl piperazine. Heteroatoms were introduced into the ring system in an attempt to reduce lipophilicity and hence increase metabolic stability; however, all of the analogues prepared showed reduced activity relative to the original lead with representative examples shown in Table 1.

An alternative system prepared with reasonable levels of potency was the benzimidazole analogue **2** (JNJ 10191584).[8] For the imidazo-pyrazine and -pyridine analogues (**3** and **4**, respectively) it is possible that the decrease in activity is driven in part by removal of the H-bond donor, which has potential to bias the conformation of the amide through an internal hydrogen bond. The importance of this NH is supported by the fact that N-methylation has been shown to significantly reduce hH4 activity.[4] However, it is not trivial to deconvolute whether this decrease in activity is due to an intramolecular interaction of the methyl (and subsequent conformational change), a negative interaction of the methyl with the receptor, or disruption of an H-bond network. Azaindoles, exemplified by **5**, showed reduced activity relative to the indole **6** despite retaining the NH; however, the ring's π system is less electron-rich than the indole which may negatively impact on any π-stacking interactions the ligand might be making.

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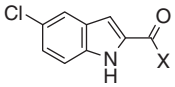
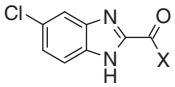
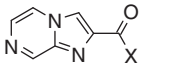
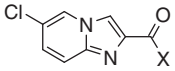
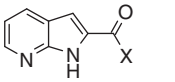
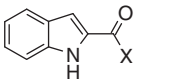
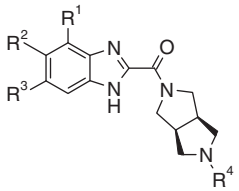
Table 1
Structure and activity for compounds **1**–**6**

Compd	Structure	hH4 bind K_i (nM)	hH4 func. K_i (nM)	hH3 bind K_i (nM)	*c*log*P*
1		8.0	6.8	15,300	2.5
2		47.7	39.6	20,000	1.9
3		20,100	>20,000	nd	−0.1
4		3170	>11,400	28,600	1.7
5		3100	>29,900	36,600	0.7
6[4]		118	46	>20,000	1.7

Where X = *N*-methyl piperazine.
(nd = not determined).

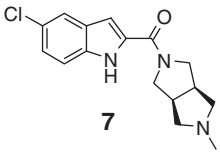
Table 2
Structure and hH4/hH3 activity for compounds **7**–**16**



Compd	R^1	R^2	R^3	R^4	hH4 bind K_i (nM)	hH4 func. K_i (nM)	hH3 bind K_i (nM)
7[a]	—	—	—	—	16	27	8750
8	H	Cl	H	Me	129	218	nd
9	Me	F	H	Me	52	97	nd
10	F	F	H	Me	74	134	nd
11	H	F	H	Me	83	145	15,500
12	H	H	H	Me	102	189	nd
13	H	F	F	Me	128	236	17,900
14	H	Cl	H	Et	1960	3300	nd
15	H	Cl	H	*i*Pr	2670	4490	nd
16	H	Cl	H	H	5080	8180	nd

(nd = not determined).
[a] Compound **7** is indole shown previously.

Previous literature data[4,17] suggested that the pendant basic centre is a key pharmacophoric element. As a consequence, our primary focus was to look for alternative diamine fragments to replace the piperazine. Substructure searching within the Pfizer file and literature identified about 100 diverse low MWT (<200) diamine fragments which had potential to mimic the piperazine of **1**. A library was prepared using 5-chloroindole-2-carboxylic acid as the coupling partner. One of the most promising leads to emerge from this library work was compound **7**.



7

Replacing the piperazine with the bicyclic octahydropyrrolo[3,4-*c*]pyrrole moiety gave a compound **7** which retained selective hH4 activity (Table 2) and showed increased metabolic stability in both rat and human in vitro (Table 5). Molecular modelling clearly shows the potential of the fused system to mimic piperazine, particularly if the bicyclic system adopts a 'boat-like' conformation as shown in Figure 1. The fused system demonstrated a significant increase in pK_a relative to the piperazine (8.28 vs 6.86), which resulted in a log unit drop in Log*D* for direct analogues.

A key risk for the indole analogues was the fact that the compounds consistently gave positive results in a reactive metabolite screen which is used as an early indicator for potential of idiosyncratic toxicity.[18] The exact nature of the reactive metabolite was not elucidated; however, indoles have the potential to form reactive metabolites via epoxidation of the C2–C3 bond, or to generate quino-methide type intermediates following metabolism of the C-5 position. We were thus keen to re-evaluate alternative acid fragments. Based on the precedence from **2** we prepared the benzimidazole analogue **8** (Table 2). Compound **8** was negative in the reactive metabolite assay and retained good metabolic stability; however, there was a approximately 10-fold decrease in hH4 activity.

We were keen to explore the SAR around **8**; however, simple amide formation from the benzimidazole acids was precluded because of the facile decarboxylation of the corresponding parent carboxylic acids. Instead the required compounds were accessed from the nitro aniline precursors as shown in Scheme 1.[18,12a] Unfortunately, despite preparing over 90 analogues varying the substitution on the benzimidazole, we were never able to match the potency that had been seen with the indoles. SAR of the terminal substituent on the amino group was also very limited, with sharp decrease in activity seen for both the desmethyl compound **16** and homologues **14** and **15**. The highest potency achieved was for compound **9**; however this compound showed reduced metabolic stability in vitro and so was not pursued further (see Table 5).

Three key azaindole analogues **17**, **18** and **19** were also prepared (Table 3). The 5-chloro analogue **18**, gave a 10-fold potency increase relative to the 5-H compound **17**, whereas the 6-chloro analogue **19** gave a threefold decrease in activity. These compounds provided some of the strongest evidence that the C-5 substituent of the 6,5-system was making a specific interaction with the receptor, as opposed to binding through a non-specific lipophilic interaction. Further variation of the substitution was limited by the synthetic access to the template.

During the purification of the 5,6-difluoro benzimidazole analogue **13**, a low yield of the amidine analogue **20** was also isolated. The exact mechanism for formation of the amidine is not known; however, we hypothesise that it was formed during the chromatographic purification of the compounds with an eluent system containing ammonia. Surprisingly, the amidine **20**[19] showed a significant potency advantage over the amides, retaining good metabolic stability. Given the potency of the amidines, an optimised route was developed making use of the 2-cyano benzimidazoles as the key intermediate (Scheme 2).[12a,20]

The SAR for aromatic substitution of the amidines mirrored that of the corresponding amides (Table 4), with all compounds showing at least a fivefold potency increase relative to the amide analogue. In-silico pK_a calculations suggested that the amidines were likely to sit as the zwitterionic species, protonated on the amidine and deprotonated on the benzimidazole. Support for this was later

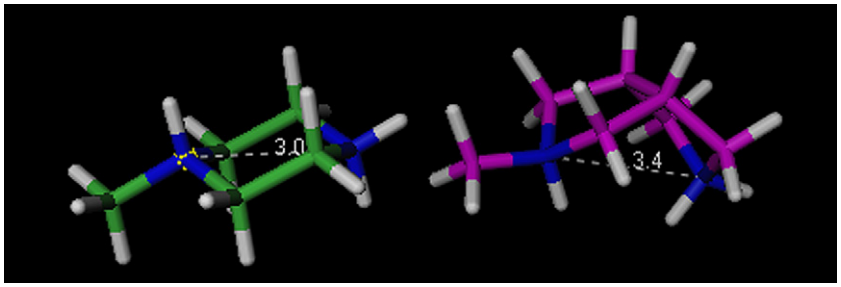
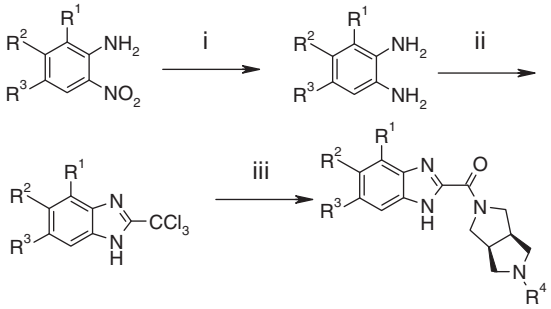
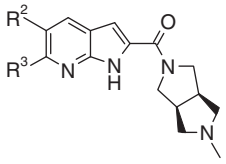


Figure 1. Molecular modelling showing 'boat' conformation of piperazine and bicyclic system. Distances are marked in angstroms.



Scheme 1. Synthesis of benzimidazole amides (**8**–**16**). Reagents and conditions: (i) Fe/HCl, EtOH, H_2O, reflux; (ii) $Cl_3CC(NH)OMe$, AcOH, rt; (iii) K_2CO_3, $MeCN/H_2O$, amine, rt.

Table 3
hH4 binding data for compounds **17**–**19**



Compd	R^2	R^3	hH4 bind K_i (nM)
17	H	H	973
18	Cl	H	83
19	H	Cl	2090

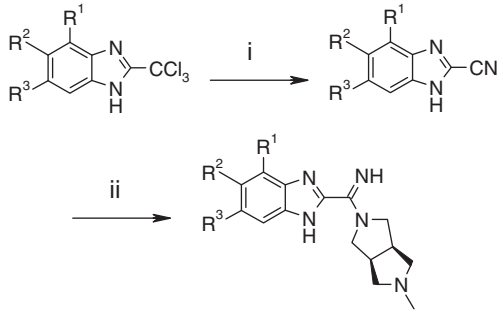
Table 4
Structure and hH4 Binding activity for compounds **20**–**26**

Compd	R^1	R^2	R^3	hH4 bind K_i (nM)	hH4 func. K_i (nM)	hH3 Bind K_i (nM)
20	H	F	F	9.5	17.2	3090
21	H	Cl	H	26	56	nd
22	Me	F	H	4.6	9.3	nd
23	F	F	H	49	24	nd
24	H	F	H	4.6	9.1	1190
25	F	F	F	70	20	>20,000
26	H	H	H	6.5	12.4	3810

(nd = not determined).

regarding the role that the NH plays in driving activity. However, the single crystal structure does not directly support this hypothesis since the crystallised molecule was a methanol solvate, which may have had significant impact on conformation in the solid state.

Whilst solid samples of the amidines were stable, further profiling of the compounds indicated some level of chemical instability in certain solvents (e.g., methanol). Stability in biological assay media was unaffected; however, we were aware that the instability would hamper further development of the compound. Cyclic amidines were hypothesised to offer increased chemical stability and a model compound **27** was prepared. No evidence of chemical instability was observed; however, as anticipated, the compound was devoid of hH4 activity. Based on this we designed compound **28**. Chemical stability was retained, but unfortunately the compound was inactive against hH4.



Scheme 2. Synthesis of benzimidazole amidines (**20**–**26**). Reagents and conditions: (i) $NH_{3(aq)}$, EtOH, H_2O, −5 °C to rt; (ii) *N*-acetyl-L-cysteine, MeOH, amine, reflux.

provided by a single crystal X-ray structure of **25** shown in Figure 2.[21] We believe that a potential consequence of the zwiterionic nature would be an increased tendency for the molecule to sit in a planar conformation, which ties in with previous hypotheses

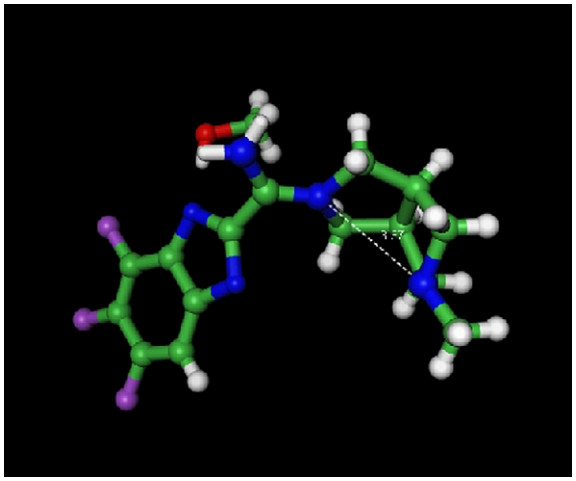
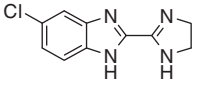


Figure 2. Single-crystal X-ray structure of **25**.

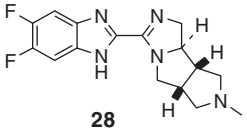
Table 5
The in vitro and in vivo rat PK parameters for key compounds

Compd	HLM (μl/min/mg)	RLM (μl/min/mg)	Cl (ml/min/mg)	*T*½ (h)	Vd (l/kg)
1	28	61	968[a]	0.2[a]	2.3[a]
2	19	nd	700[b]	0.7[b]	23[b]
7	<7	36	nd	nd	nd
8	<7	36	nd	nd	nd
9	<7	214	nd	nd	nd
11	<7	40	161[c]	0.7h[c]	6.1[c]
13	<7	40	58[c]	0.8h[c]	4.1[c]
20	<7	10	31[c]	11h[c]	48[c]

(nd = not determined).
[a] 3 mg/kg subcutaneous.
[b] 10 mg/kg subcutaneous.
[c] 1 mg/kg intravenous.



27



28

Given the excellent levels of potency and metabolic stability, compound **20** was selected for further progression. Rat pharmacokinetics indicated moderate clearance (31 mL/min/kg) and high volume of distribution (48 L/kg) with 20% oral bioavailability (Table 5). The high volume observed for **20** was not unexpected given the dibasic nature of the compound.

In conclusion we identified a metabolically robust piperazine isostere that provided compounds which retained H4 potency and selectivity. The amidines exemplified by **20** represent selective compounds which were suitable for further evaluation. Preliminary results for the in vivo pharmacological characterisation of **20** have been communicated.[22]

Acknowledgments

The authors wish to acknowledge the contributions to this project by numerous colleagues at Pfizer in the departments of Biology, Chemistry and Pharmacokinetic, Dynamics and Metabolism and Pharmaceutical Sciences and in particular Cheryl Doherty of Pharmaceutical Sciences.

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12. Binding affinity to the H4 receptor (hH4 Bind) was assessed using an in vitro radioligand ([^{3}H]-histamine) filtration binding assay in a stable CHO cell line expressing recombinant human H4 receptor with the Ga15 protein. Detailed experimental conditions are provided in (a) 'Preparation of octahydropyrrolo[3,4-*c*]pyrrole derivatives as histamine H4 receptor ligands' Lane, C. A. L.; Price, D. A. U.S. Patent Application US2006111416.
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21. CCDC 850451 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033.
22. This work was first presented at the Gordon Research Conference on Medicinal Chemistry at Colby-Sawyer College, New London, NH, USA, August 12, 2010 in a presentation by Charles E. Mowbray titled: 'The Discovery and Evaluation of PF-3893787: A Novel Histamine H4 Receptor Antagonist'. The compounds described in this letter have been disclosed in Ref. 12a and the findings of in vivo toxicological investigations have been reported in detail by Mowbray, C. E.; Bell, A. S.; Clarke, N. P.; Collins, M; Jones, R. M.; Lane, C. A. L.; Liu, W. L.; Newman, S. D.; Paradowski, M.; Schenck, E. J.; Selby, M. D.; Swain, N. A.; Williams, D. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6596.