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Identification and hit-to-lead exploration of a novel series of histamine H4 receptor inverse agonists

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ABSTRACT

The identification and hit-to-lead exploration of a novel, potent and selective series of histamine H_4 receptor inverse agonists is described. The initial hit, **3A** (IC_{50} 19 nM) was identified by means of a ligand-based virtual screening approach. Subsequent medicinal chemistry exploration yielded **18I** which possessed increased potency (*R*-enantiomer IC_{50} 1 nM) as well as enhanced microsomal stability.

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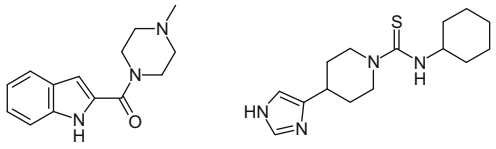
Since its relatively recent discovery, the histamine H4 receptor (H_4R) has been the focus of much attention.[1] The H_4R is primarily expressed on cells that are involved in inflammation and immune responses and there is mounting evidence that targeting this receptor may provide a valuable approach in the treatment of various allergic diseases such as allergic airway inflammation and pruritis.[2] Additionally, it is believed that modulating the H_4R may have potential in the treatment of autoimmune disorders.[3] Thus, the identification of novel ligands for the H_4R is a topic of considerable research and clinical interest.

We have previously reported the successful application of ligand-based virtual screening to the search for novel MCH-1 receptor antagonists.[4] In this work, a similar approach was adopted based on the structures of two H_4R antagonists that had been reported at the time the work was commenced (Fig. 1).[5]

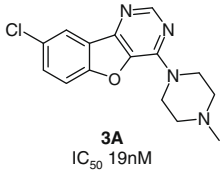
A database of commercially available screening compounds was compiled from the catalogues of various suppliers and then interrogated using these two query compounds and a variety of virtual screening techniques. A set of 1177 compounds was initially selected from the results of these searches. Following removal of duplicates and assessment by an experienced medicinal chemist, 405 compounds were chosen for purchase. Upon screening,[6] two series of particular interest were identified. Both compounds resulted from a pharmacophore search based on **1**. In terms of their properties, the compounds show good potency at the H_4R together with good selectivity over the closely related H_3R. In addition, the compounds possess attractive lead-like qualities. On the basis of its superior potency, **3A** was selected as the starting point for hit-to-lead exploration (Fig. 2).

Further profiling of compound **3A** indicated that metabolic stability in rat microsomes was very low with only 1% parent compound remaining after 10 min incubation. Although metabolite



1: JNJ 7777120 (K_i = 38nM) **2:** Thioperamide (K_i = 27 nM)

Figure 1. H_4R antagonists used as queries for ligand-based virtual screening. K_i values are taken from Ref. 5.



3A
IC_{50} 19nM

Figure 2. Selected hit compound from ligand-based virtual screening.

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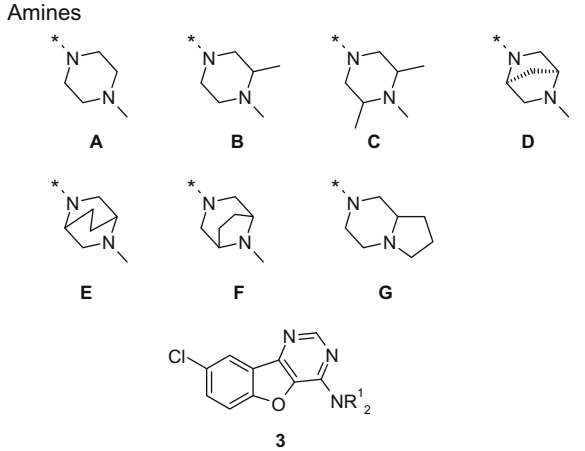
identification was not carried out, we considered that N-demethylation was most likely to be a major contributor to the poor stability. Thus the initial focus of the optimisation programme was aimed at modifications of the amine designed to increase the metabolic stability while retaining potency. The first approaches were designed to increase the steric bulk around the piperazine nitrogen in the hope that this might reduce the propensity for N-demethylation. The results obtained for some of the compounds identified in the original library had suggested that groups larger than methyl on the piperazine nitrogen would result in a loss of potency. This was in agreement with the results reported by Johnson and Johnson around JNJ 7777120.[5] Thus alternative approaches were investigated. These approaches included incorporation of a group adjacent to the *N*-methyl (**B** and **C**), bridging across the piperazine ring (**D** to **F**) and incorporation of the substituent on the nitrogen into a ring (**G**). Of these compounds, the bridged analogues and the bicyclic derivative showed moderate potency, while those analogues with adjacent methyl groups were weakly active or inactive. Compound **3D** showed moderate potency and improved microsomal stability compared to **3A** (Table 1) whereas, surprisingly, the bicyclic derivative **3G** was unstable in microsomes.

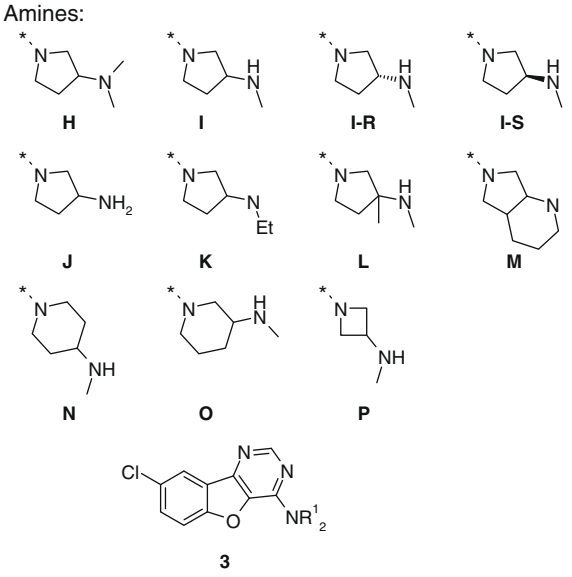
The second approach involved moving the basic centre exocyclic to the ring. Thus a series of 3-amino-pyrrolidines was prepared (**3H**–**3M**, Table 2). The NH-methyl analogue **3I** was the most potent of the compounds prepared and also showed some improvement in microsomal stability. It is interesting to note that either increasing the size of the alkyl group on the nitrogen (**3K**) or incorporating an α-methyl group (**3L**) resulted in a loss in potency, suggesting that the binding pocket for the basic centre is quite narrow and confirming the earlier observations from the library compounds. The primary amine (**3J**) was also potent and had increased microsomal stability. Potency was retained if the ring size was reduced to four atoms (**3P**) but completely lost when the ring size was increased to six atoms (**3N** and **3O**). The two individual enantiomers of compound **3I** were also prepared. It was found that the *R*-enantiomer (**3I-R**) was more active than the *S*-enantiomer (**3I-S**). However, the *S*-enantiomer was found to be more stable in rat microsomes with a half-life of 73 min compared to a half-life of 19 min for the *R*-enantiomer.

Table 1
Analogues designed to block N-demethylation



Compd	IC_{50} (nM) H_4R	% Remaining at 10 min rat microsomes
3A	19	1
3B	1900	1
3C	10,000	nt
3D	111	75
3E	169	66
3F	440	62
3G	236	0

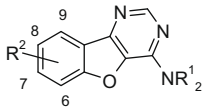
Table 2
Analogues with exocyclic basic centre



Compd	IC_{50} (nM) H_4R	% Remaining at 10 min rat microsomes
3H	21,000	18
3I	95	80
3I-R	33	92
3I-S	285	103
3J	126	107
3K	>10,000	—
3L	1400	—
3M	1400	—
3N	>10,000	—
3O	>10,000	—
3P	87	89

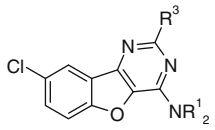
With improvements identified in the stability by modification to the amine, our attention switched to investigation of the substituent on the benzene ring (compounds **4**–**10**, Table 3). It was found that activity was retained with small lipophilic groups in the 8-position of the benzene ring (e.g., **5I**) but no improvement was observed in the microsomal stability. Larger (e.g., **6I**) or more polar groups (e.g., **7I** to **9I**) generally provided a further increase in stability but resulted in a loss in potency. Moving the chlorine atom to the 7-position (**10I-R**) also resulted in a reduction of potency.

Table 3
Modifications to the aromatic substitution



Compd	R^2	IC_{50} (nM) H_4R	% Remaining at 10 min rat microsomes
4I-R	8-Me	86	74
5I-R	8-CF_3	30	59
6I	8-Ph	>10,000	97
7I	8-CO_2H	>30,000	80
8I	8-CN	6200	95
9I	8-CONHMe	>30,000	—
10I-R	7-Cl	171	—

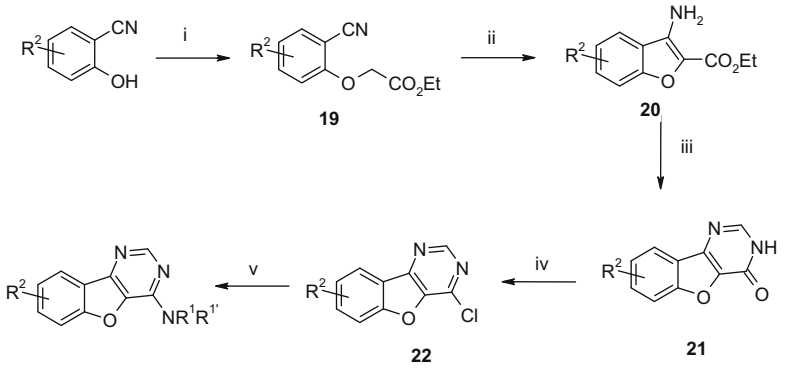
Table 4
Modifications to the 2-position



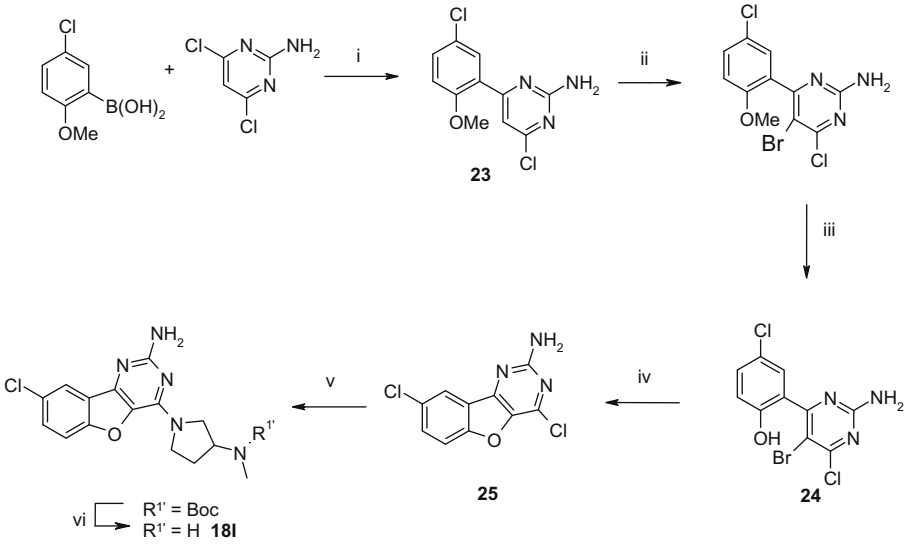
Compd	R^3	IC_{50} (nM) H_4R	% Remaining at 10 min rat microsomes
11A	2-Me	37	0
11I	2-Me	1600	95
12A	2-Ph	>30,000	40
13A	2-CF_3	83	7
13I-R	2-CF_3	14200	—
14A	2-CycloPr	310	8
15A	2-CO_2H	>10,000	106
16A	2-OH	1000	83
17A	2-OMe	90	13
18I-R	2-NH_2	1	62
18I-S	2-NH_2	13	68

An investigation was then carried out into the nature of the substituent in the 2-position of the pyrimidine ring (compounds **11**–**18**, Table 4). Early in the investigation it was found that the 2-methyl analogue with *N*-methylpiperazine as the amine (**11A**) was equipotent with the 2-unsubstituted analogue (**3A**). However, when the amine was replaced by the *N*-methylaminopyrrolidine (**I**), the 2-methyl derivative (**11I**) showed a significant loss of potency. A number of other analogues were prepared with either *N*-methylpiperazine or *N*-methylaminopyrrolidine as the amine and a similar pattern was observed. Small lipophilic groups in the 2-position were tolerated with *N*-methylpiperazine as the amine (e.g., **13A** and **14A**) but not with *N*-methylaminopyrrolidine (e.g., **13I**). As was observed for the substituent on the benzene ring, larger and more polar groups were generally not tolerated at all in the 2-position (e.g., **12A**, **15A** and **16A**). However, an amino group in the 2-position was an exception. It was found that this substituent provided extremely good potency with *N*-methylaminopyrrolidine as the amine (**18I-R** and **18I-S**). Indeed, this provided the most potent analogue prepared. It is interesting to see the similarity in structure of these compounds to those recently disclosed by Cowart et al.[7] Both the *R* and *S* isomers of this compound were prepared but in this case little difference in microsomal stability was observed between the enantiomers, with the *R*-enantiomer having a half-life of 13 min compared to a half-life of 21 min for the *S*-enantiomer.

The majority of the compounds under investigation were prepared according to Scheme 1. Reaction of the appropriately substituted cyanophenol with ethyl bromoacetate gave the phenoxyester



Scheme 1. Reagents and conditions: (i) $BrCH_2CO_2Et$, NaH, DMF room temperature; (ii) $KOBu^t$, THF, room temperature; (iii) (a) $HC(OEt)_3$, microwave 200 °C 10 min, (b) 2 M ammonia in MeOH, microwave 140 °C 10 min; (iv) $POCl_3$, microwave 180 °C 15 min; (v) $R^1R^{1'}NH$, Et_2NCH_2–polystyrene, IMS, microwave 120 °C 30 min.



Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$, Cs_2CO_3, DME, microwave 120 °C 10 min; (ii) NBS, acetonitrile, reflux; (iii) BCl_3, DCM; (iv) CuCl, Cs_2CO_3, $Bu^tC(O)CH_2C(O)Bu^t$, toluene, reflux 24 h; (v) *N*-methyl-*N*-Boc-aminopyrrolidine, Et_2NCH_2–polystyrene, IMS, microwave 120 °C 30 min; (vi) TFA, DCM.

Table 5
Functional activities for selected compounds

Compd	IC_{50} (nM) H_4R assay	IC_{50} (nM) GTPγS assay	Max % inhib.	Response
3D	111	70	105	Antagonist
3I-R	33	41	151	Inverse agonist
3I-S	285	292	122	Inverse agonist
3P	87	76	149	Inverse agonist
13A	83	39	100	Antagonist
18I-R	1	3	138	Inverse agonist
18I-S	13	4	170	Inverse agonist

19 which was cyclised to the benzofuran **20** on treatment with base. The pyrimidine ring was formed by the reaction of the aminobenzofuran ester **20** with triethyl orthoformate followed by treatment with ammonia. The pyrimidinone **21** was converted to the chloropyrimidine **22** with phosphorus oxychloride then reaction with the appropriate amine gave the target compounds. In some cases, the amine required boc-protection in order to obtain the desired target. Deprotection was then achieved using trifluoroacetic acid in dichloromethane.

Some of the compounds could not be prepared in this manner and required an alternative synthesis. The preparation of the 2-amino derivatives (**18I**) exemplifies the alternative approach as shown in Scheme 2. Suzuki coupling of 5-chloro-2-methoxyphenyl boronic acid with 2-amino-4,6-dichloropyrimidine gave the biaryl derivative **23**. Bromination with NBS followed by demethylation of the methoxy group gave the bromophenol derivative **24** which was cyclised to give the benzofuranopyrimidine core **25**. Reaction with boc-protected aminopyrrolidine followed by treatment with trifluoroacetic acid gave the desired molecule **18I**.

A number of compounds that showed good potency in the primary assay and improved microsomal stability were selected for further studies. Compounds were tested for their functional activity in a GTPγS assay[8] and most were shown to be either antagonists or inverse agonists. Results for selected compounds are shown in Table 5.

Selectivity against other histamine receptors was also determined as well as activity against five isoforms of human CYP450. In addition, selected compounds were tested in a rat PK study. Compound **18I-R** was the most interesting compound carried forward. The compound showed excellent selectivity with IC_{50} values in H_1, H_2 and H_3 binding assays of >30 μM, >30 μM and 5.8 μM respectively. In the CYP450 assay, activity was only observed against CYP1A2 with 45% inhibition at 1 μM. In a rat PK study at an oral dose of 5 mg/Kg, compound **18I-R** was shown to have high clearance (117 ml/min/Kg), a high volume of distribution (7 l/Kg) and an oral bioavailability of 5%. However, in higher species the PK properties were significantly improved. In dog, the compound had an oral bioavailability of 36% and the corresponding value in cynomolgus monkey was 99%. Clearance values of 52 and 62 ml/min/Kg and volumes of distribution of 26 and 25 l/Kg were obtained for the two species, respectively.

In conclusion, we have identified a lead series of histamine H_4R antagonists and inverse agonists using virtual screening. Modifications of the various parts of the structure have resulted in improvements in both potency and stability leading to the discovery of a highly potent and selective histamine H_4R inverse agonist with an acceptable oral PK profile in dog and monkey.

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6. Compound potency was assessed using a [^{3}H]-histamine radioligand binding SPA. Briefly, IC_{50} was determined from a 6-point semi-log dose response curve performed in duplicate wells. All reagents were prepared in 50 mM Tris pH 7.5, 5 mM EDTA, 0.5% BSA and the assay performed in the presence of 1% DMSO (v/v). Compounds were incubated for 2.5 h at room temperature in a clear-bottom, white-walled 96-well isoplate in the presence of 20 nM [^{3}H]-histamine dihydrochloride (GE Healthcare), 25 μg human H4 receptor expressing membranes (Euroscreen) and 0.75 mg SPA beads (GE Healthcare) in a total volume of 200 μl. Non-specific binding was determined in the presence of 4 μM histamine dihydrochloride (Sigma–Aldrich). Plates were counted using a Microbeta scintillation counter (Perkin–Elmer).
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8. Functional activity was determined using a [^{35}S]-GTPγS SPA. All reagents were prepared in 20 mM Hepes pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 20 μg/ml saponin and the assay performed in the presence of 1% DMSO (v/v). Compounds were pre-incubated with 15 μg of H4 receptor expressing membranes (Euroscreen) for 15 min at 30 °C in the presence of 10 μM GDP (Sigma–Aldrich) in a clear-bottom, white-walled 96-well Isoplate. Agonist (150 nM histamine dihydrochloride (Sigma–Aldrich)) was added for 30 min, 30 °C, before addition of 1 nM [^{35}S]-GTPγS (GE Healthcare) and 0.75 mg SPA beads (GE Healthcare) for an additional 30 min. Non-specific binding was determined in the presence of 10 μM GTPγS (Sigma–Aldrich). SPA beads were sedimented by centrifugation and counted using a Microbeta scintillation counter (Perkin–Elmer).