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Identification of 2-arylbenzimidazoles as potent human histamine H_4 receptor ligands

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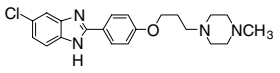
Abstract—A series of 2-arylbenzimidazoles was synthesized and found to bind with high affinity to the human histamine H_4 receptor. Structure–activity relationships were investigated through library preparation and evaluation as well as traditional medicinal chemistry approaches, leading to the discovery of compounds with single-digit nanomolar affinity for the H_4 receptor.

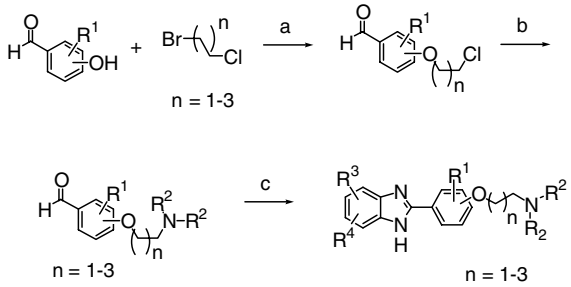
Histamine plays a critical role in a number of physiological processes through interaction with four G-protein coupled receptors: H_1, H_2, H_3, and H_4. The H_1 receptor is involved in allergic and inflammatory responses,[1] the H_2 receptor participates in gastric acid secretion,[2] and the H_3 receptor mediates neurotransmitter release in the central nervous system.[3] Most recently identified, the H_4 receptor is mainly expressed in dendritic cells, eosinophils, and mast cells.[4,5] The preferential expression of the H_4 receptor in immune cells suggests that this receptor is involved in the regulatory functions of histamine during the immune response. Studies have shown that the H_4 receptor controls the release of inflammatory mediators and facilitates leukocyte chemotaxis.[6–10] Modulation of H_4 receptor activity provides an opportunity for treating inflammatory and allergic conditions. Recently, we reported indole, benzimidazole, and thienopyrrole piperazine carboxamides as potent H_4 receptor ligands.[11,12]

In this paper, we describe a series of 2-arylbenzimidazoles with high affinity for the human H_4 receptor. The initial lead compound for this program, compound **1**, was identified as a HTS hit from our corporate compound collection and exhibited moderate affinity (K_i = 124 nM) for the H_4 receptor. An efficient, modular synthetic route for the preparation of **1** enabled rapid variation of substituents on different regions of the molecule.



1: K_i = 124 nM

Analogs of compound **1** were synthesized by the coupling of commercially available phenylenediamines with aromatic aldehydes in the presence of $Na_2S_2O_5$ (Scheme 1).[13] Most aldehyde inputs were synthesized using a two-step procedure: alkylation of the hydroxybenzaldehyde followed by chloride displacement with the desired amine.



Scheme 1. Reagents and conditions: (a) K_2CO_3, CH_3CN, 60 °C, 18–24 h; (b) HNR^2R^2, Na_2CO_3, KI, *n*-BuOH, 95 °C, 18–36 h, ~50% over two steps; (c) phenylenediamine, $Na_2S_2O_5$, DMA, 80 °C, 6–42%.

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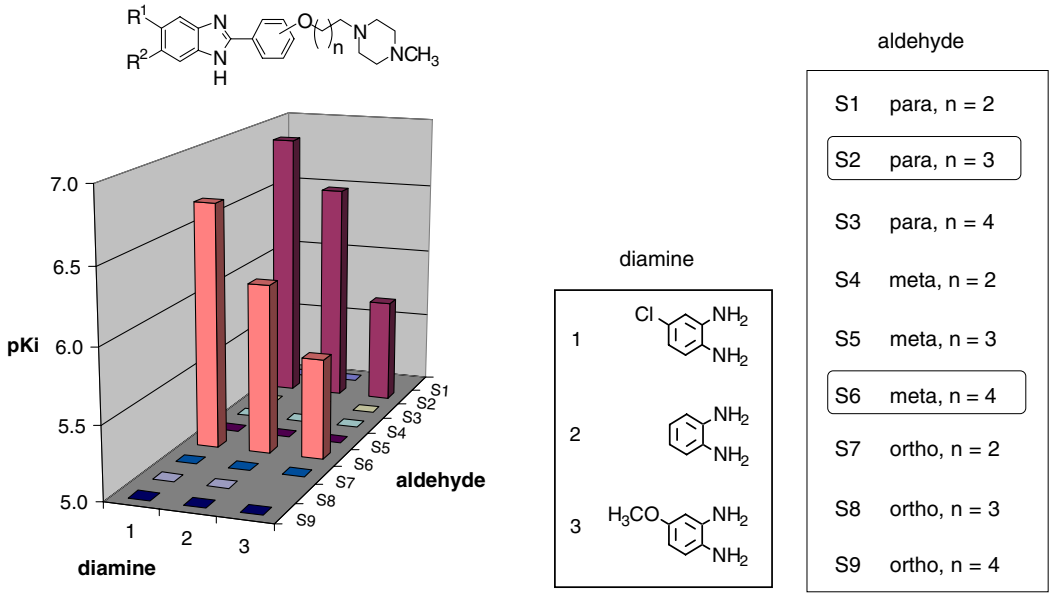
The initial analogs were designed to examine the importance of linker length and position about the central benzene ring (Graph 1). These compounds were examined in a recombinant human histamine H_4 competitive binding assay using [^{3}H]histamine as the radioligand.[14] The SAR investigation revealed a dramatic dependence of binding affinity upon the linker length as well as the linker position about the central aryl ring. Of the nine series that were synthesized and tested for activity, only two sets (S2 and S6) demonstrated $pK_i > 5$. This suggests that the position of the terminal nitrogen relative to the central ring is essential for good receptor binding.[15]

CATALYST[16] was used to generate pharmacophores using an example of each linker (S1–S9). Unfortunately, these pharmacophores were unable to discriminate between active and inactive linkers, presumably because of the similarity of the molecules and because inactivity of some of the linkers is not due to lack of particular features, but probably results from conformational considerations. It was hypothesized that the benzimidazole moiety was binding in the same way for all of the analogs, and that the inactive linkers were unable to properly position and orient the positively charged piperazine at low conformational energies. In order to elucidate the bioactive conformation, stochastic conformation searches of an example of each of the linkers S1–S9 using MOE[17] with the MMFF94 force field were carried out. For the active linkers (S2 and S6), a limit of 5 kcal/mol above the global minimum was set. For the inactive linkers, up to 10 kcal/mol energy above the global minimum was allowed. All of the conformations for all of the linkers were aligned using the benzimidazole moiety. The position of the terminal nitrogen of the piperazine was plotted as a sphere in three-dimensional space. A simplified version of the Active Analog Approach[18] was used. Figure 1 illustrates the procedure.



Graph 1. Displacement of [^{3}H]histamine from recombinant histamine H_4 receptor.

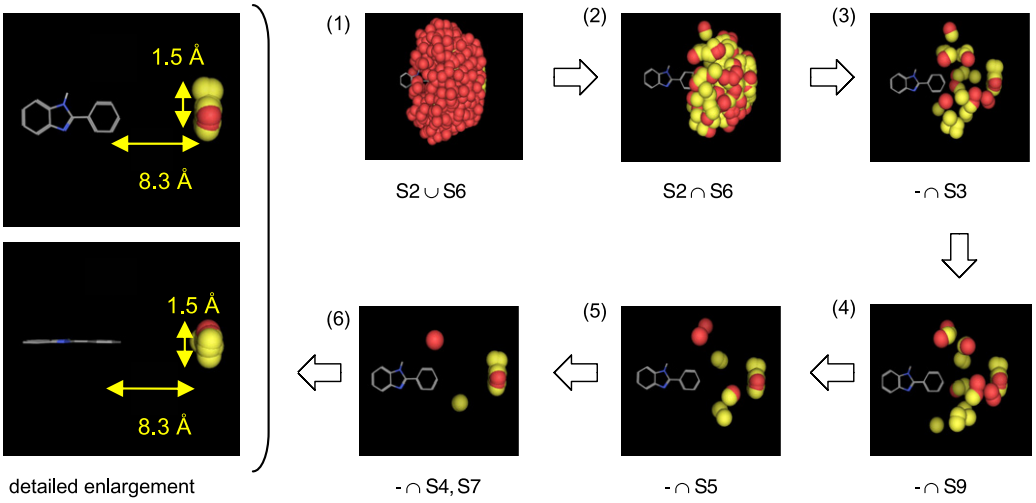


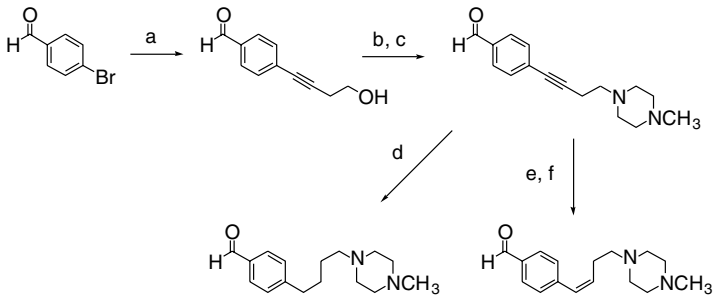
Figure 1. Overlay of lowest-energy conformations of benzimidazoles in graph 1: active minus inactive binders.

The yellow spheres represent conformations of the S2 linker, and the red spheres represent conformations of the S6 linker. Positions in space that were occupied by both an S2 representative and an S6 representative are illustrated in the second panel of Figure 1, and are candidates for the bioactive conformations of those linkers. Positions that were also occupied by representatives of inactive linkers were systematically removed (panels 3–6). This led to a focused region that is 1.5 Å in diameter and 8.3 Å away from the central benzene ring as the most likely region whose occupancy or unoccupancy by a piperazine nitrogen could explain the data in Graph 1. Therefore, it was theorized that this distance of 8.3 Å between the aryl ring and distal nitrogen of the terminal piperazine is optimal for potent H_4 receptor binding. Note that there were multiple distinct conformations of S2 and S6 that were positioned in this region, and therefore it was not possible to further specify the preferred binding conformation beyond this pharmacophore.

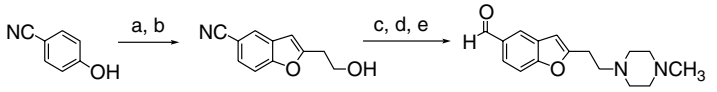
The identification of the likely piperazine binding region suggested the possibility of creating constrained analogs that would have increased potency. Several 48-membered libraries were designed to test this model through the incorporation of rigidified linkages that would span this 8.3 Å distance. For example, the alkyl linker was replaced with a benzene ring, an alkyne or a *trans*-alkene. Although these linkers are in some cases quite different than those described in Graph 1, they all have low-energy conformations in which the terminal nitrogen of the piperazine ring is located within the region identified above. The *cis*-alkene, which does not span this distance, was also included as a linker serving as a negative control for the model. In addition, the aryl ether was constrained as a benzofuran. As before, the *N*-methylpiperazine region of the molecule was not altered. Other benzimidazoles were synthesized using aldehydes with additional substitution on the benzene ring or with a carbon analog of the ether linker. All necessary library aldehyde inputs were synthesized using routes shown in Schemes 1–4.[19]

Benzimidazole formation was conducted in a library format using a Bohdan 48-well MiniBlock system. Sodium metabisulfite was added to the individual reaction tubes prior to the addition of the aldehyde and phenylenediamine inputs as stock solutions in DMF. Upon reaction completion, as monitored by HPLC, polymer-supported hydrazine was added to the reaction tubes to remove excess aldehyde starting material. Filtration of the resultant reaction mixtures was followed by purification through reverse phase preparative HPLC. Select data for these benzimidazoles are shown in Graph 2.

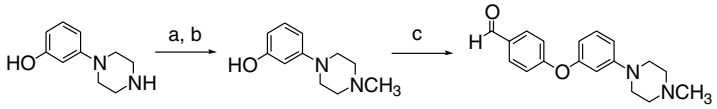
An inspection of Graph 2 clearly suggests that the conformational model described above is incomplete. Failure of the rigidified linkers to improve H_4 receptor



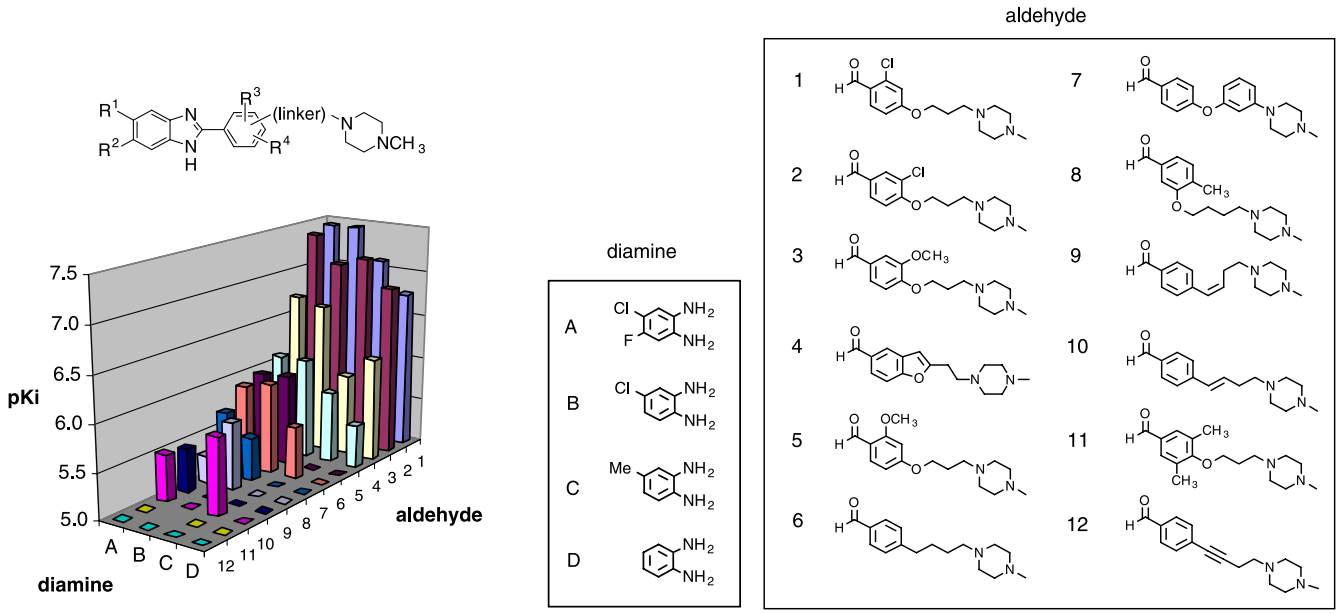
Scheme 2. Reagents and conditions: (a) homopropargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, rt, quant.; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C to rt, quant.; (c) *N*-methylpiperazine (10 equiv), EtOH, rt, 43%; (d) H_2 (1 atm), Lindlar catalyst, 1:1 EtOAc/EtOH, 3 h, 80%; (e) i—9-BBN, THF, 50 °C, 7 h; ii—HOAc, 60 °C, 14 h; iii—6 N $NaOH/H_2O_2$, 40 °C, 4 h, 50% conversion (1:1 alcohol/aldehyde); (f) Dess–Martin periodinane, CH_2Cl_2, 2 h, quant.



Scheme 3. Reagents and conditions: (a) I_2, satd $NaHCO_3$, 5 °C to rt, 18 h, 33%; (b) homopropargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, rt, 15 h, quant.; (c) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C, 1.5 h, quant.; (d) *N*-methylpiperazine (10 equiv), CH_3CN, rt, 16 h, 46%; (e) DIBAL, CH_2Cl_2, −60 °C to rt, 6 h, 56%.



Scheme 4. Reagents and conditions: (a) $(Boc)_2O$, 1:1 THF/H_2O, rt, 18 h, 99%; (b) LAH, Et_2O, reflux, 64%; (c) 4-fluorobenzaldehyde, Cs_2CO_3, DMF, 90 °C, 18 h, 76%.



Graph 2. Displacement of [^{3}H]histamine from recombinant histamine H_4 receptor.

affinity suggests that some aspects of the compounds in Graph 1 are not adequately represented in the conformational model or in the rigidified compounds. The chosen rigid linkers may be unable to accommodate unknown structural requirements of the H_4 receptor that can be satisfied by a flexible linker, which can more easily adapt its conformation. As mentioned above, unambiguous determination of the bioactive conformations of the compounds shown in Graph 1 was not possible due to the existence of multiple conformations satisfying the pharmacophore. The ether oxygen, present in all of the linkages in Graph 1 but absent in many of the subsequent linkages, may also play a role. Interestingly, the benzofuran compound, perhaps the most structurally similar constrained analog to the compounds in Graph 1, does have modest activity. Finally, the conformation of the linker can be influenced by the substitution patterns on the central aromatic ring, potentially introducing unfavorable steric interactions between the benzene ring substituent and the linker. This can prevent optimal positioning of the piperazine for receptor binding, as exemplified by the poor activity of the compounds incorporating aldehyde **8** (Graph 2).

The effect of substituents on the central ring is itself quite interesting. Generally small lipophilic substituents (e.g., chloro) maintained or improved binding efficiency (Graph 2, aldehydes **1** and **2**); however, increased substitution, especially in the case of the di-methyl substituted analog (Graph 2, aldehyde **11**), led to a drastic loss in H_4 receptor affinity. Modeling of the low energy conformations of substituted benzene rings elucidates a possible reason for this observation (Fig. 2). For example, bis-substitution (Fig. 2D) or the use of a carbon analog for the ether linkage (Fig. 2C) appears to twist the linker into an orthogonal position with respect to the central benzene ring, leading to compounds with poor receptor affinity. On the other hand, mono-substitution (Fig. 2B) allows the alkyl ether linker to lie in the plane of the central ring, enabling potent receptor binding.

In general, the SAR revealed in this library study does not provide sufficient evidence to validate or invalidate the proposed binding site of the piperazine component. It may be that the model proposed above is sufficient to explain the activity differences observed between the set of closely related molecules in Graph 1, but unable to account for the larger variations introduced by the rigid-

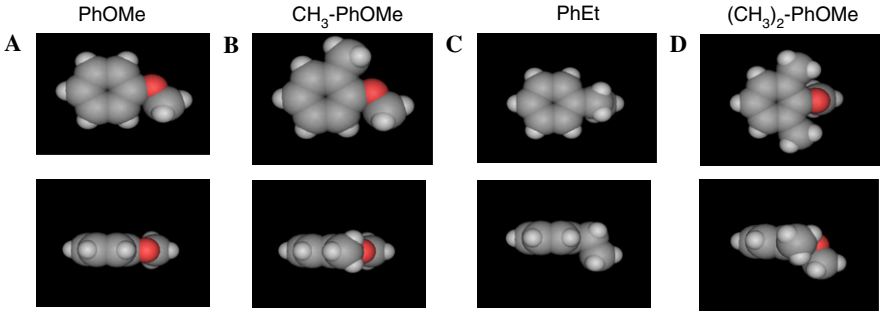
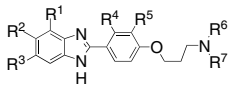


Figure 2. Modeling of low-energy conformations of simple substituted benzene rings.

Table 1. Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor



Entry	R^1	R^2	R^3	R^4	R^5	NR^6R^{7a}	K_i (nM)
1	CH_3	CH_3	H	Cl	H	A	46
2	CH_3	H	CH_3	Cl	H	A	22
3	H	$C(CH_3)_3$	H	Cl	H	A	93
4	–CH=CH–CH=CH–		H	Cl	H	A	28
5	H	$C(CH_3)_3$	H	H	Cl	B	250
6	H	$C(CH_3)_3$	H	H	Cl	C	26
7	H	$C(CH_3)_3$	H	H	Cl	A	65
8	H	$C(CH_3)_3$	H	H	CH_3	A	26
9	CH_3	CH_3	H	Cl	H	C	9
10	CH_3	H	CH_3	Cl	H	C	1

[a] A = *N*-methylpiperazine; B = 3-dimethylaminopyrrolidine; C = *N*-methylhomopiperazine.

ified molecules. Empirically it can be reasonably concluded that compounds comprised of a flexible linker which can adopt the preferred conformation as a result of appropriate choice of phenyl substituents will likely result in potent H_4 receptor ligands.

With these criteria in mind, additional analogs were investigated in a non-library format. Substitution at the 4-position of the benzimidazole, in addition to the 5- and 6-positions, was found to be tolerated (Table 1, entries 1 and 2). Larger groups, such as a *tert*-butyl group or a fused benzene ring, could be displayed on the benzimidazole ring without much adverse effect (Table 1, entries 3 and 4). The *N*-methylpiperazine was replaced with pyrrolidine and homopiperazine isosteres. The former demonstrated a significant loss in activity, whereas the latter displayed a slight increase in H_4 receptor affinity with respect to the parent compound (entries 5–7). Further analysis of small lipophilic substitution on the central aromatic ring revealed the methyl group as a suitable alternative to the previously identified chloro- and methoxy-groups (entry 8).

Based on this expanded SAR, 4-substituted benzimidazoles with lipophilic central ring substitution and the homopiperazine terminal diamine were prepared. These compounds demonstrate single-digit nanomolar activity (entries 9 and 10), with up to 100-fold improvement over initial lead compound **1**.

In summary, we have examined 2-arylbenzimidazoles and identified compounds with low nanomolar affinity for the H_4 receptor. A pharmacophore was developed to explain the sharp activity differences observed between different linker lengths and positions in otherwise closely related compounds. Through library preparation and analysis, this method was examined and the SAR of three regions of the molecule was simultaneously explored. Additional analog preparation through traditional medicinal chemistry approaches facilitated further SAR expansion.

References and notes

1. Ash, A. S. F.; Schild, H. O. *Br. J. Pharmacol.* **1966**, *27*, 427.
2. Black, J. W.; Duncan, W. A. M.; Durant, C. J.; Ganellin, C. R.; Parsons, E. M. *Nature (London)* **1972**, *236*, 385.
3. Arrang, J. M.; Garbarg, M.; Schwartz, J. C. *Nature (London)* **1983**, *302*, 832.
4. de Esch, I. J. P.; Thurmond, R. L.; Jongejan, A.; Leurs, R. *Trends Pharmacol. Sci.* **2005**, *26*, 462.
5. Fung-Leung, W.-P.; Thurmond, R. L.; Ling, P.; Karlsson, L. *Curr. Opin. Investig. Drugs* **2004**, *5*, 1174.
6. O'Reilly, M.; Alpert, R.; Jenkinson, S.; Gladue, R. P.; Foo, S.; Trim, S.; Peter, B.; Trevethick, M.; Fidock, M. *J. Recept. Signal Transduct. Res.* **2002**, *22*, 431.
7. Hofstra, C.; Desai, P. J.; Thurmond, R. L.; Fung-Leung, W.-P. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 1212.
8. Buckland, K. F.; Williams, T. J.; Conroy, D. M. *Br. J. Pharmacol.* **2003**, *140*, 1117.
9. Thurmond, R. L.; Desai, P. J.; Dunford, P. J.; Fung-Leung, W.-P.; Hofstra, C. L.; Jiang, W.; Nguyen, S.; Riley, J. P.; Sun, S.; Williams, K. N.; Edwards, J. P.; Karlsson, L. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 404.
10. Ling, P.; Ngo, K.; Nguyen, S.; Thurmond, R. L.; Edwards, J. P.; Karlsson, L.; Fung-Leung, W.-P. *Br. J. Pharmacol.* **2004**, *142*, 1.
11. Venable, J. D.; Cai, H.; Chai, W.; Dvorak, C. A.; Grice, C. A.; Jablonowski, J. A.; Shah, C. R.; Kwok, A. K.; Ly, K. S.; Pio, B.; Wei, J.; Desai, P. J.; Jiang, W.; Nguyen, S.; Ling, P. L.; Wilson, S. J.; Dunford, P. J.; Thurmond, R. L.; Lovenberg, T. W.; Karlsson, L.; Carruthers, N. I.; Edwards, J. P. *J. Med. Chem.* **2005**, *48*, 8289.
12. Jablonowski, J. A.; Grice, C. A.; Chai, W.; Dvorak, C. A.; Venable, J. D.; Kwok, A. K.; Ly, K. S.; Wei, J.; Baker, S. M.; Desai, P. J.; Jiang, W.; Wilson, S. J.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P.; Lovenberg, T. W.; Carruthers, N. I. *J. Med. Chem.* **2003**, *46*, 3957.
13. Arienti, K. L.; Brunmark, A.; Axe, F. U.; McClure, K.; Lee, A.; Blevitt, J.; Neff, D. K.; Huang, L.; Crawford, S.; Pandit, C. R.; Karlsson, L.; Breitenbucher, J. G. *J. Med. Chem.* **2005**, *48*, 1873.
14. Binding assay conditions are described in Ref. 9. Compounds were tested in triplicate to generate K_i values.
15. In addition, a compound displaying a piperidine in place of the *N*-methylpiperazine moiety, 5-chloro-2-[4-(3-piperidin-1-yl-propoxy)-phenyl]-1*H*-benzoimidazole, was prepared and found to display $pK_i < 5.5$ for the human H_4 receptor.

16. Accelrys Inc., Catalyst, Release 4.7, San Diego: Accelrys Inc., 2002.
17. Molecular Operating Environment (MOE), version 2001.01; Chemical Computing Group Inc.: Montreal, Canada, 2001.
18. Marshall, G. R.; Barry, C. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. In *Computer-Assisted Drug Design*; Olsen, E. C., Christoffersen, R. E., Eds.; American Chemical Society: Washington D.C., 1979; Vol. 112, pp 205–226.
19. Alkene isomerization was observed under the benzimidazole formation conditions used to access the *trans*-alkene benzimidazoles. The *trans*- and *cis*-isomers were isolated using reverse phase preparative HPLC, and the individual products were evaluated for H_4 receptor binding.