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# Discovery of a series of potent and selective human H4 antagonists using ligand efficiency and libraries to explore structure–activity relationship (SAR)

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ABSTRACT

We describe the identification of a potent, selective lead series that shows antagonism against the human histamine H4 receptor from thirteen actives identified in an HTS as part of a hit to lead program. By focusing on ligand efficiency and concurrently using a diversity based approach, compounds based around 2,4-diaminopyrimidine were identified with compound **25** being quickly shown to be a good lead. It also had the highest ligand efficiency in the series.

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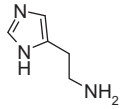
Histamine **1** is a naturally occurring biological amine that affects a variety of functions in the human body. There are four subtypes of histamine receptor, H1–H4, H4 is a 390 amino acid G-protein coupled receptor and the newest member of the histamine receptor family. It was identified at the turn of the millennium by a number independent research groups. Its predominant expression on inflammatory cells and lymphoid tissues, coupled with an active rolein processes ranging from chemotaxis to cytokine release, suggests it may command an important role in immune and inflammatory responses.[1]



Histamine [1]

Asthma is predominantly characterised by bronchoconstriction that is largely reversible and chronic inflammation of the airways.[2] Although there are many contributing factors to the pathogenesis of the disease, a variety of inflammatory cell types which produce a range of chemical mediators and cytokines in the airways, are thought to be most important for its perpetuation. Repeated acute episodes of inflammation can exacerbate the disease, often in response to specific allergens. Histamine **1** is a well-described mediator of this acute inflammation and is involved in vasodilation, smooth muscle contraction, oedema, mucus hypersecretion, and adhesion molecule up-regulation. Histamine **1** is produced during acute asthmatic episodes and its role in many physiological functions associated with asthma is well characterised. However, until recently it has not been thought to be a significant contributor to the disease because histamine H1 receptor antagonists, effective in symptomatic treatment of allergic rhinitis, have little or no efficacy in asthma. More recent evidence suggests that histamine **1** has a role in inflammation and allergy beyond that traditionally described,[3] potentially through activation of the H4 receptor.

Johnson & Johnson were the first company to publish on a selective histamine H4 antagonist,[4] JNJ 10191584 2 and JNJ 7777120 3 (Fig. 1) have been investigated as orally active antagonists. Early studies into the function of H4 receptors suggested that antagonism of these receptors may be of utility for the treatment of allergic diseases with an eosinophilic and/or mast cell component since histamine binding to this receptor subtype stimulated the chemotaxis of both cell types.[5]

The H4 receptor is primarily expressed on eosinophils, mast cells, dendritic cells, and other leucocytes. The H3 receptor is found in the peripheral and central nervous systems and has been implicated in

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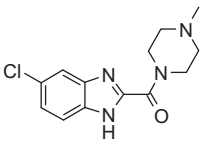
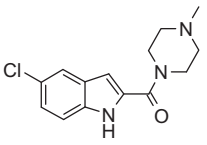


JNJ-10191584 [2]
hH4R Binding Ki 47.7 nM
hH4R Functional Ki 39.6 nM
Highly selective vs H1/H2/H3
LE : 0.52



JNJ-7777120 [3]
hH4R Binding Ki 8.0 nM (lit Ki 4nM)
hH4R Functional Ki 6.8 nM
Highly selective vs H1/H2/H3.
LE : 0.58

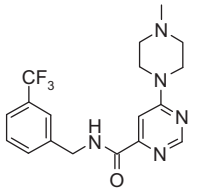
**Figure 1.**

various conditions including loss of arousal, cognition and vigilance. The H1 receptor is expressed in smooth muscles, vascular endothelial cells, the CNS and the heart. H2 receptors are coupled to adenylate cyclase and are highly expressed in gastric cells and cardiac tissue. Selectivity over H1, H2 and H3 was therefore important to avoid any significant adverse effect.

Approximately 30% of the full Pfizer compound collection was screened in a [$^3$H]histamine ligand binding assay. Triage of the initial hits focused on compounds that had been made in parallel arrays for file enrichment. This gave 13 compounds that were active in both functional and binding assays.

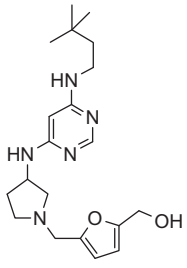
These 13 actives were classed into four series; aminopyrimidine amide **4**, diaminopyrimidine **5**, tertiary alcohol **6** and the pyrimidone **7** series (Fig. 2). Since all compounds were made using parallel chemistry file enrichment, an abundance of close analogues to each of these four series were available.

This Letter looks at the hit to lead optimisation and of library chemistry around the diaminopyrimidine series and how HTS actives were transformed into a potent and selective series of H4 antagonists.



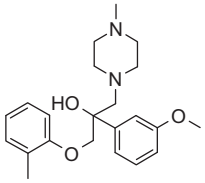
**4**

cLogP 3.3
mw: 379
hH4 func 57%@15μM
hH4 Ki : 34.9μM
LE: <0.22



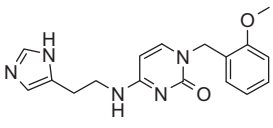
**5**

cLogP 3.8
mw: 374
hH4 func 73%@15μM
hH4 Ki : 2.11μM
LE: 0.286



**6**

cLogP 2
mw: 374
hH4 func 60%@15μM
(ki>2μM)
hH4 binding Ki 3.24μM
H3 Ki : 2.5μM
LE: 0.28



**7**

cLogP 0.9
mw: 325
hH4 func 65%@15μM
(ki>2μM)
hH4 binding Ki 1.6μM
LE: 0.33

**Figure 2.**

The initial hit from the pyrimidone series **7** showed good potency in the binding assays. This was not surprising as all active examples tested featured the histamine side chain. The risk of Cytochrome P450 inhibition by the unsubstituted imidazole group meant this series was not followed up because of the potential for drug–drug interactions.[6]

The tertiary alcohol series, exemplified by **6**, was active in both functional and binding assays. Nearest neighbour searches identified several close analogues for screening and the initial SAR showed that an *ortho*-substituent on the phenyl ether was important. However, later studies showed low selectivity over H3 and Herg activity of these compounds and so no further work was done.

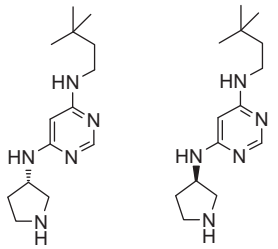
The 2-aminopyrimidine-4-amide **4** was weakly active in the binding assay and inactive in the functional assay. Nearest neighbour searches uncovered several close in analogues, but these compounds were similar in potency.

The fourth series, substituted 4,6-diaminopyrimidine **5** was potent (human H4 $K_i$ 2.11 μM) and also had a ligand efficiency (LE)[7] of 0.286 which was a good starting place. Ligand efficiency is defined as the binding energy of a ligand per heavy atom.[8] So compounds with high ligand efficiency are those where binding has been optimised.[9]

Based on the LE and apparent activity and because it had fewer selectivity issues, the 2,4-diaminopyrimidine series **5** was followed up. The key pharmacophore for known H4 antagonists is a basic amine coupled to a heterocycle.

Our strategy for optimising these hit compounds into potent and selective H4 antagonist were to use diversity based drug design and file mining based approaches in parallel. Special focus on improving the ligand efficiency at each design stage was made. The diaminopyrimidine **5** had a ligand efficiency of 0.286 and this was a good starting place, but there was room for improvement (0.4–0.5 ideally). As both JNJ-10191584 2 and JNJ-7777120 3, contained *N*-methyl piperazines, it was rationalised that the furan portion did not contribute to binding and the diamino-pyrimidine was the key pharmacophore. Synthesis of both stereoisomers of the aminopyrrolidine deriviatives **8** and **9** showed that the (*S*)-enantiomer 8 was 2-fold more potent and had a higher LE than the enantiomer **9** (Fig. 3).

The (*S*)-enantiomer **8** was potent in both the functional and binding assays as well as being selective over H1, H2 and H3. This compound **8** was a good starting point as it had a high ligand efficiency and selectivity over H3.



**8**

LE: 0.45
hH4 bind Ki: 502nM
hH4 func Ki: 572nM
H3 Ki : 8.22μM
H1 Ki : >40μM
H2 Ki : 8.82μM
mw 263, log$D_{7.4}$ 0.6

**9**

LE: 0.43
hH4 bind Ki: 939nM

H3 Ki 13.6μM

**Figure 3.**

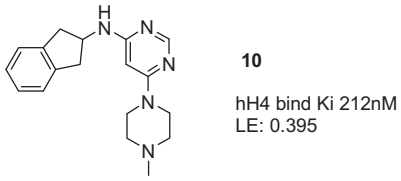



**Figure 4.**

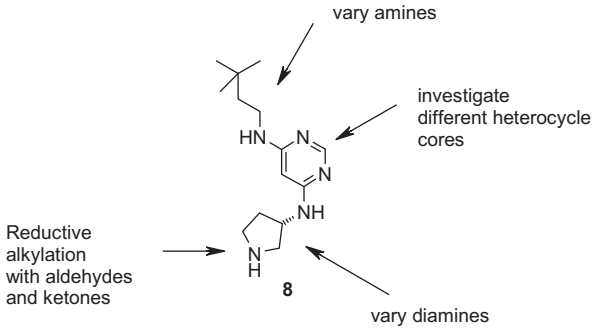



**Figure 5.** Library strategy.

Nearest neighbour searches of the Pfizer compound collection around compound **8** uncovered the 2-aminoindan derivative **10** (hH4 binding $K_i$: 212 nM) which was more potent than the original active **5**, it also had a much higher ligand efficiency (LE) of 0.395 (Fig. 4).

Concurrently the HTS hit **5** was followed up with library chemistry. With four points of diversity around the diaminopyrimidine **5**, SAR was probed using parallel chemistry (Fig. 5). Our first library was designed to explore alternatives to the furan group by reductive alkylations on the pyrrolidine amine with a set of aldehydes and ketones.

Reductive amination of the amine **8** was carried out by reaction with the aldehyde/ketone and sodium triacetoxyborohydride in dichloroethane (DCE) at room temperature overnight. After purification, 80 out of a possible 81 compounds were isolated giving a success rate of 98%. Biological testing of the compounds in the H4 binding screen revealed only 2 actives (Fig. 6) with very weak potency ($K_i$ 20 μM).

This library demonstrated that alkylating the amine with large groups was not tolerated. From the known SAR around JNJ-10191584 and JNJ-7777120 as well as the *N*-methylpyrrolidine derivative **13**, this showed that methyl was the optimum size.

Our second library was designed to look at SAR around the diamine and also at how small changes to the alkyl chain on the

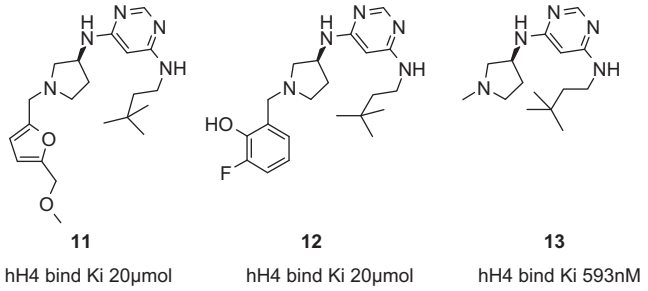



**Figure 6.** First library modifying pyrrolidine N-substitution.

aminoindan derivative **10** were tolerated. Three alkylated 2-amino, 4-chloro-pyrimidines templates were synthesised by reductive amination of 3-amino-6-chloropyrimidine **14** with three aldehydes (cyclopropylaldehyde, pivaldehyde and amyl aldehyde) (Fig. 7), then *ipso*-displacement of the pyrimidine-chlorine with a series of Boc-protected diamines.

All the libraries were designed using Pfizer's internal design tool PGVL hub[10] and the design was guided using Lipinski's rule of 5.[11] After filtering, the second library consisting of 234 compounds was designed. The reductive amination step was high yielding and the three templates (Fig. 7) were then used directly in the *ipso* substitution reaction. Each reaction was carried out on 100 μmol scale with 10 equiv of diamine and excess Hunig's base in DMSO. The reactions were heated at 150 °C over 16 h. After work-up and purification only 25 compounds were isolated in sufficient purity and quantity (>1 mg) giving a library success rate of 11%. This low success rate was attributed to poor reactivity of the diamines and instability of the Boc-amines at high temperatures. Analysis of the LC–MS data at each synthetic step showed that the majority of compounds were lost in synthesis (61%) and the remainder in purification. Table 1 shows the most active compounds from this library (Fig. 8).

The *N*-methyl piperazine derivative **15** was the most potent compound and also had the highest LE. However, it was active in the binding assay[12] but not in the functional assay and it also had lower selectivity over H3. The dimethylaminopyrrolidine derivative **16** was potent in both functional and binding assays but was almost equipotent against H3. The remaining three analogues were less potent.

Library 3 was designed to modify the second alkyl amine, keeping the diamine (*N*-methylpiperizine) constant again using *ipso*-displacement chemistry.

After work-up and purification 187 compounds were isolated (99% success rate; 187 compounds in the design). 32 compounds from this library showed weak potency in the 2–4 μM range with the bicycloamine derivative **20** (Fig. 9) being the most potent with

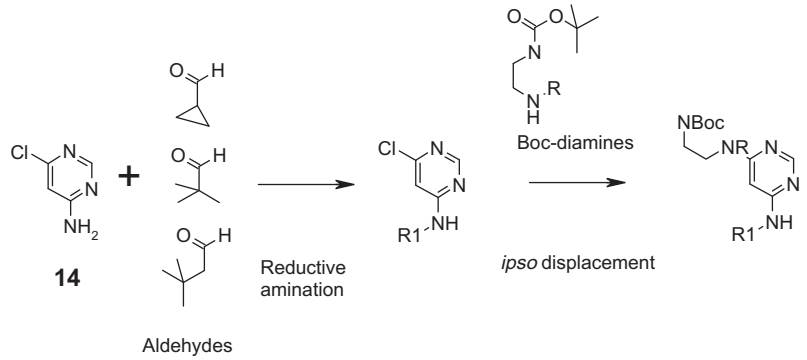



**Figure 7.** Second library exploring diamines.

**Table 1**
Activity of most potent library compounds

| Compound | hH4 bind $K_i$[a] (nM) | hH3 bind $K_i$ (nM) | Ligand efficiency (LE)[b] | Log$D_{7.4}$ |
|---|---|---|---|---|
| **15** | 23.6 | 825 | 0.54 | 1.75 |
| **16** | 58.9 | 54 | 0.49 | 1.09 |
| **17** | 201 | — | 0.48 | 1.40 |
| **18** | 235 | — | 0.46 | 0.96 |
| **19** | 601 | — | 0.35 | 3.84 |

[a] Displacement of [$^3$H]histamine from the recombinant human histamine H4 receptor. $K_i$ was calculated according to Cheng and Prussoff and are the geometric mean of three or more independent determinations.
[b] LE calculated using $K_i$ binding.

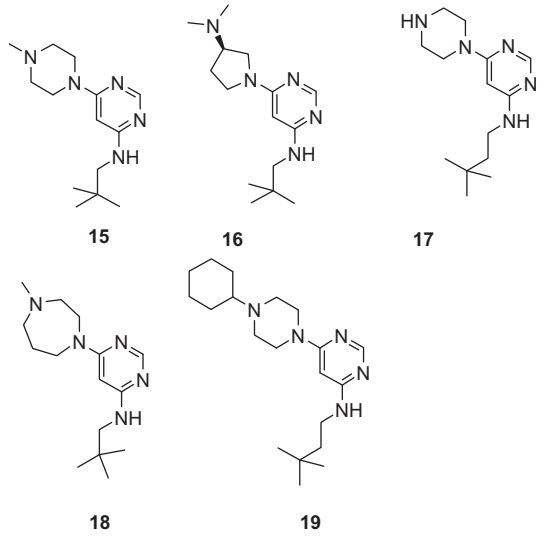



**Figure 8.** Most potent diamino pyrimidines from library 2.

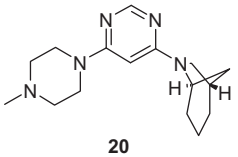



**Figure 9.**

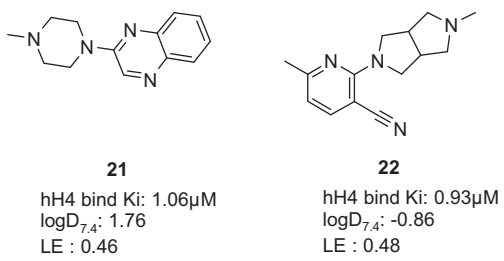



**Figure 10.**

a $K_i$ of 860 nM in the binding assay but there was divergent activity in the functional assay. Biological testing showed that, in general the SAR was very tight and large groups were not tolerated on the amine.

Library 4 was designed to attempt to identify alternative heterocyclic cores and consisted of a two step synthetic route involving *ipso* diplacement of a chloroheteroaromatic with a Boc-protected diamine, followed by acid mediated deprotection. There were 289 compounds in the design and 288 were purified and tested against H4 (success rate of 99%). From this library two compounds were identified, the aminoquinoxaline drivative **21** and bicyclo-diamine **22** (Fig. 10). Both compounds were small with high LE's and reasonable potencies in the binding assays, but they were inactive in the functional assay.

Library 5 was designed to vary both the diamines and amines to explore a wider set of amines and diamines and so it was the largest in terms of numbers. The synthesis involved sequential *ipso*-displacement of 4,6-dichloropyrimidine with a series of diamines and then displacement with a set of low molecular weight amines. The library design was filtered using a *c*log*P* range of 0.5–3 and *c*log*D* range of −1 to 3, then with in-silico HLM and RLM models to help filter down the library size to a few hundred compounds (586). Synthesis was carried out and the first *ipso* displacement with 5 equiv of amine at 70 °C and then 10 equiv of the diamine at 150 °C for 30 h.[13] After purification, 585 compounds were isolated giving a success rate of 99% reflecting how well the chemistry has worked. It is noteworthy that Boc-protected diamines were not used in this library, hence the higher success rate as compared with library 2. In total 15 compounds were found to be active against human H4 in this library. Two key compounds, **23** and **24** were identified as they were the most potent and had very good LE's. **23** had a LE of 0.6, the highest of all compounds but it was inactive in the functional assay and the microsomal stability (RLM $Cl_{int}$: 143 μl/min/s) needed improving (Fig. 11).

RLM was measured because for the majority of compounds within the pyrimidine series, RLM had been higher than HLM and it was the aim to get enough exposure in animal species to test the safety of the compounds.

The library work and file mining efforts were followed up by synthesis of discrete single compounds. Based on the emerging SAR, *N*-methyl piperazine and 2-methylbutylamine appeared to be the optimum groups and so the compound **25** with these opti-

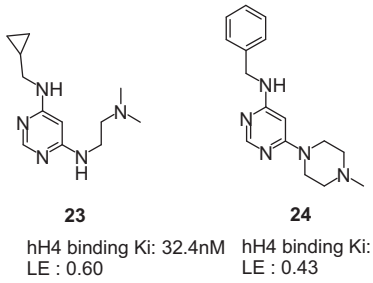



**Figure 11.** Displacement of [$^3$H]histamine from the recombinant human histamine H4 receptor. $K_i$ was calculated according to Cheng and Prussoff and are the geometric mean of three or more independent determinations.

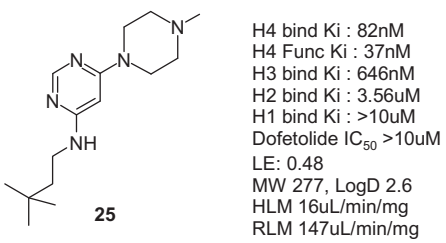



**Figure 12.**

mum groups was synthesised. The diaminopyrimidines were nominated as the lead series after biological, drug metabolism and safety studies had been carried out on the lead compound **25**. This series was taken into lead optimisation. The diaminopyrimidine **25** showed good selectivity against H1, H2. It was 5-fold selective over H3 and exhibited encouraging metabolic stability in the human liver microsomal assay (HLM), and moderate stability in the rat liver microsomal assay (RLM). So the key goals in lead optimisation were to improve metabolic stability, selectivity over H3 and potency. In addition, the LE was comparable with the J&J H4 antagonists (LE 0.52 and 0.58) (Fig. 12).

When tested against a panel of over 40 receptor targets representing the major classes of biogenic amine receptors, ion channels binding sites, neuropeptide receptors and transporters, these compounds showed no off-target activity.

By focusing on the average contribution to binding that each atom makes (LE) in this program, we were able to take an active **5** from the HTS, remove excess molecular weight and look at the key binding elements and hence find a leaner more potent compound (**8**) by file mining of the Pfizer compound collection. With the help of parallel chemistry to make analogues of **8** and hence generate data for novel SAR, we were able to identify that the 2,6-diaminopyrimidine series had the potential to deliver a potent and selective human H4 antagonist. At each stage LE increased and **25** which had a LE of 0.48 is a good example of the lead series which was taken into lead optimisation.

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## References and notes

1. Liu, C.; Ma, X.-J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N. I.; Lovenberg, T. W. *Mol. Pharmacol.* **2001**, *59*, 420; Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E., Jr.; Greenfeder, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W.; Shin, N.; Gustafson, E. L.; Qiao, X.; Wang, S.; Hendrick, J. A.; Greene, J.; Bayne, M.; Monsma, F. J., Jr. *J. Pharmacol. Exp. Ther.* **2001**, *293*, 1058; Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L.; O'Dowd, B. F. *Mol. Pharmacol.* **2001**, *59*, 427; Zhu, Y.; Michalovich, D.; Wu, H.; Tan, K. B.; Dytko, G. M.; Mannan, I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X.; Herrity, N. C.; Vawter, L.; Sarau, H. M.; Ames, R. S.; Davenport, C. M.; Hieble, J. P.; Wilson, S.; Bergsma, D. J.; Fitzgerald, L. R. *Mol. Pharmacol.* **2001**, 59; 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.
2. Cohn, L.; Elias, J. A.; Chupp, G. L. *Annu. Rev. Immunol.* **2004**, *22*, 789–815.
3. Dy, M.; Schneider, E. *Cytokine Growth Factor Rev.* **2004**, *15*, 393; Jutel, M.; Blaser, K.; Akdis, C. A. *J. Investig. Allergol. Clin. Immunol.* **2005**, *15*, 1.
4. 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.; Nguyen, S.; Ling, P. *J. Med. Chem* **2003**, *46*, 3957; Coruzzi, G.; Adami, M.; Guaita, E.; de Esch, I. J.; Leurs, R. E. *J. Pharmacol.* **2007**, *563*, 240.
5. Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. *Nat. Rev. Drug Disc.* **2008**, *7*, 41; Jutel, M.; Blaser, K.; Akdis, C. A. *Chem. Immunol. Allergy* **2006**, *91*, 174.
6. Zhou, Z. W.; Zhou, S. F. *Expert Opin. Drug Metab. Toxicol.* **2009**, *5*, 579.
7. Ligand efficiencies (LE) were calculated using the formula: $LE = (\Delta G)/N$ where $\Delta G = -RT\ln K_i$. $K_i$ is the binding constant and $K_i$ was calculated according to Cheng and Prussoff and are the geometric mean of three or more independent determinations. $N$ is the number of non-hydrogen atoms.
8. Kuntz, I. D.; Chen, K.; Sharp, K. A.; Kollman, P. A. *Proc. Natl. Acad Sci. U.S.A.* **1999**, *96*, 9997; Reynolds, L. H.; Bembenek, S. D.; Touge, B. A. *Biorg. Med. Chem. Lett.* **2007**, *17*, 4258.
9. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
10. Peng, Z.; Yang, B.; Mattaparti, S.; Shulok, T.; Thacher, T.; Kong, J.; Kostrowicki, J.; Hu, Q.; Na, J.; Zhou, J. Z.; Klatte, D.; Chao, B.; Ito, S.; Clark, J.; Sciammetta, N.; Coner, B.; Waller, C.; Kuki, A. *Methods Mol. Biol.* **2011**, *685*, 295; Peng, Z.; Hu, Q. *Methods Mol. Biol.* **2011**, *685*, 321.
11. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Del. Rev.* **2001**, *46*, 3.
12. *H4 binding assay*: Cell pellets from CHO cells expressing the histamine $H_4$ receptor were homogenised in ice-cold 50 mM Tris–HCl/0.5 μM $CaCl_2$ buffer containing a protease inhibitor cocktail (Roche®, United Kingdom) using a ground glass homogeniser. Homogenates were centrifuged at 48,000 rpm for 30 min at 4 °C. The membrane pellet was resuspended in fresh buffer and the centrifugation step was repeated as described above. The membrane pellet was resuspended in 50 mM Tris–HCl in the same volume as the original cell pellet. Aliquots of membrane preparations were stored at −8 °C and were used for [$^3$H]-Histamine binding experiments.
Cell membranes (20–35 μg/well) were incubated for 90 min shaking at room temperature with 3 nM [2,5-$^3$H]Histamine dihydrochloride (30–60 Ci/mmol) in 50 mM Tris–HCl (pH 7.4), with or without competing H4 ligands. The reaction was terminated by rapid filtration through 0.5% polyethyleniminesoaked Unifilter GF/B plates (Packard) followed by three washes with 1 ml ice-cold 50 mM Tris–HCl. Filters were dried for 45 min at 45 °C and bound radiolabel was determined using scintillation counting techniques. Non-specific binding was defined with 10 μM JNJ7777120. For competition binding studies, $K_i$ values were calculated from the $IC_{50}$ value based on an experimentally determined ligand $K_d$ of 5.2 nl and a ligand concentration of 5 nM according to the Cheng–Prussoff equation where; $K_i = (IC_{50})/(1 + ([L]/K_d))$.
13. The amines were dissolved in dimethylsulfoxide (DMSO) to a concentration of 0.5 M. The heteroaryl chlorides were similarly dissolved in dichloromethane (DCM) to a concentration of 0.5 M. An aliquot of the chloride (0.15 ml, 75 μmol, 1 equiv) was dispensed to a Teflon 96 well plate and the solvent was allowed to evaporate. An aliquot of the amine (0.45 ml, 150 μmol, 3 equiv) and *N*,*N*-diisopropylethylamine (DIPEA, 0.1 ml) were added to each well and the plates were sealed with a Teflon sheet using a Combiclamp and heated to 150° for 16 h. The solvent was removed in vacuo (Genevac). The samples were redissolved in a 1:1 mixture of dichloromethane:methanol (0.8 ml). A solution of HCl in 1,4-dioxane (4.0 M, 0.25 ml, 13 equiv) was added and the reaction mixtures were shaken overnight at room temperature. A sample (30 μl) was retained for analysis and the solvent removed in vacuo (Genevac).