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Rigidified 2-aminopyrimidines as histamine H_4 receptor antagonists: Effects of substitution about the rigidifying ring

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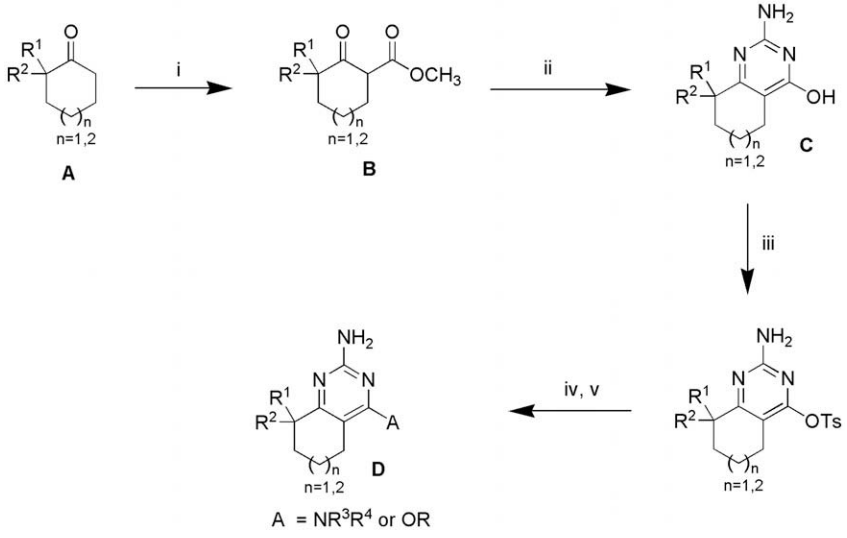
ABSTRACT

Three novel series of histamine H_4 receptor (H_4R) antagonists containing the 2-aminopyrimidine motif are reported. The best of these compounds display good in vitro potency in both functional and binding assays. In addition, representative compounds are able to completely block itch responses when dosed ip in a mouse model of H_4-agonist induced scratching, thus demonstrating their activities as H_4R antagonists.

The histamine H_4 receptor (H_4R) has attracted considerable interest from both academia and industry since its cloning several years ago.[1] H_4R has been found to be expressed in lymphocytes, mast cells, and dendritic cells,[2] and H_4 antagonists have been shown to block histamine-mediated shape change and chemotaxis of eosinophils and mast cells.[3] Thus, it has been proposed that antagonists of H_4R could be used to treat conditions arising from immune and inflammatory responses.[1c] Indeed, H_4 antagonists



Scheme 1. General preparation of compounds. Reagents and conditions: (i) NaH, dimethyl carbonate, reflux, 2 h; (ii) guanidine, DMF, 120 °C, overnight; (iii) TsCl, DMAP, Et_3N, CH_2Cl_2, rt, overnight; (iv) diamine or Boc-protected diamine, Et_3N, CH_3CN, reflux, 48 h; or hydroxylamine, KO*t*Bu, 0 °C to rt, THF, 48 h; (v) TFA, CH_2Cl_2, rt, 1 h.

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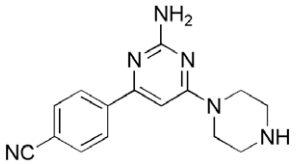
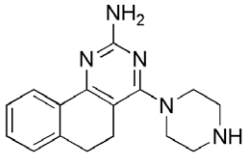
have been reported to be active in in vivo models of H_4-agonist induced itch[4] and ovalbumin-induced airway inflammation.[5] In addition, the antinociceptive activity of H_4 antagonists in models of inflammatory, postsurgical, osteoarthritic, and neuropathic pain has been reported by ourselves[6] and others.[7]

We recently disclosed some results of our studies of H_4R ligands containing the 2-aminopyrimidine moiety. We found that 6-aryl-2-aminopyrimidines, exemplified by **I**, can be potent antagonists of H_4 at both human and rat receptors but tend to suffer from problems with metabolism and off-target activity.[6a] Rigidification of the structure by including a new ring junction, such as in compound **II**, largely overcomes these issues while retaining much of the original potency, with the best potencies being obtained when the rigidifying ring is six- or seven-membered.[6b] We have also demonstrated that the aryl ring of **II** could be appended rather than fused to the rigidifying ring (e.g., **1**) with only a modest reduction in potency.



I
human H_4R pK_b = 8.53
rat H_4R pEC_{50} = 7.23 (64% eff.)

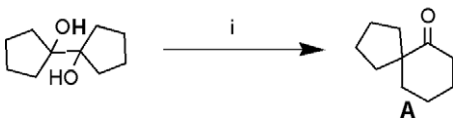


II
human H_4R pK_b = 7.58
rat H_4R pEC_{50} = 7.62 (52% eff.)

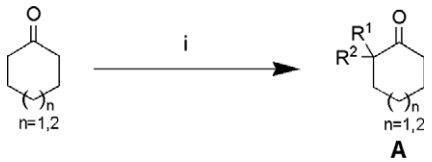
Intrigued by the in vitro results obtained with **1**, and in an effort to further understand the SAR of such compounds, we prepared a series of similar rigidified analogs with substitution(s) about the rigidifying ring. The results of this campaign are presented in this communication.

Synthesis of the compounds in this study proceeded according to the general synthetic scheme in Scheme 1. Ketone **A**, available commercially or prepared according to Scheme 2 or 3, was converted to the corresponding 1,3-dicarbonyl **B** with sodium hydride and dimethyl carbonate. Reaction of **B** with guanidine with heating in DMF afforded hydroxypyrimidine **C**, which upon tosylation and reaction with a diamine or hydroxylamine yielded compound **D**. For those diamines that were Boc-protected, a final deprotection step with TFA removed the Boc group.

Ketone **A** with *spiro*-cyclopentyl substitution alpha to the carbonyl was prepared via pinacol rearrangement of bi(cyclopentane)-1,1′-diol, as outlined in Scheme 2. With other *spiro*-cycloalkyl substitution, or with bis-alkyl or bis-benzyl substitution, the required ketones were prepared according to Scheme 3.[8]



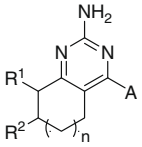
Scheme 2. Synthesis of *spiro*-cyclopentyl compounds **A**. Reagents and conditions: (i) $(CH_3O)_3CH$, BF_3–OEt_2, −20 °C to rt, 2 h.

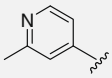
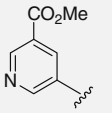
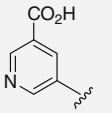


Scheme 3. Synthesis of compounds **A** with other *spiro*-cycloalkyl substitution or with bis-alkyl or bis-benzyl substitution. Reagents and conditions: (i) KO*t*Bu, electrophile, *t*BuOH, rt, overnight (electrophiles: 1-chloro-5-iodopentane, MeI, EtI, BnBr, or 1,2-bis(bromomethyl)benzene).

The in vitro results at H_4R are shown in Tables 1–3, with the functional assays being run as previously described.[6] In an earlier publication,[6b] we demonstrated that compounds with a rigidifying cycloalkyl ring are potent at H_4, with six- or seven-membered rings being preferred and roughly equivalent to each other in terms of

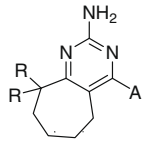
Table 1
Summary of in vitro potency at histamine H_4 receptors[a]



Compd	R^1	R^2	n	A	Human H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)	Rat H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)
1	Ph	H	2		7.24 ± 0.02	7.26 ± 0.07
2	H	H	2		6.57 ± 0.02	6.46 ± 0.11 (80%)
3	Ph	H	2		7.88 ± 0.27	8.24 ± 0.24
4	Ph	H	1		7.52 ± 0.11	7.28 ± 0.21 (49%)
5	Ph	H	1		8.04 ± 0.06	7.61 ± 0.10 (47%)
6	Ph	H	1		7.34 ± 0.03	<7.34[b]
7	Ph	H	2		7.88 ± 0.05	7.51 ± 0.08
8	Ph	H	2		7.57 ± 0.05	7.26 ± 0.06 (84%)
9	Ph	H	2		7.59 ± 0.08	7.97 ± 0.20
10		H	2		6.31 ± 0.05	6.37 ± 0.02
11		H	2		5.63 ± 0.07	<5.44[b]
12		H	2		5.37 ± 0.005	<4.91[b]
13	H	Ph	2		7.17 ± 0.06	7.37 ± 0.13
14	H	Ph	2		6.61 ± 0.05	6.84 ± 0.16
15	H	Ph	2		7.15 ± 0.09	7.73 ± 0.15

[a] $n \geqslant 2$.
[b] $n = 1$.

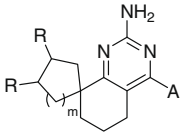
Table 2
Summary of in vitro potency at histamine H_4 receptors[a]



Compd	R	A	Human H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)	Rat H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)
16	Me		7.60 ± 0.22	7.04 ± 0.18 (62%)
17	Me		7.09 ± 0.20	<6.26[b] (64%)
18	Et		7.11 ± 0.08	<6.81[b] (37%)
19	Bn		4.57 ± 0.004 (86%)	4.60 ± 0.03 (129%)

[a] $n \geqslant 2$.
[b] $n = 1$.

Table 3
Summary of in vitro potency at histamine H_4 receptors[a]



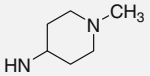
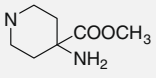
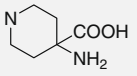
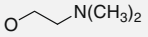
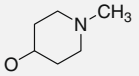
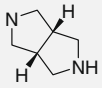
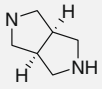
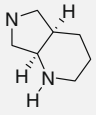
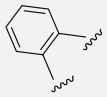
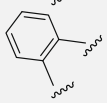
Compd	R	m	A	Human H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)	Rat H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)
20	H	1		8.01 ± 0.02	<5.32[b] (77%)
21	H	1		8.07 ± 0.18	<6.60[b] (35%)
22	H	1		7.66 ± 0.07	6.90 ± 0.03 (84%)
23	H	1		4.90 ± 0.02	4.77 ± 0.07 (52%)
24	H	1		<4.67	<4.68
25	H	1		<4.16	4.63 ± 0.05 (48%)
26	H	1		8.11 ± 0.08	<6.00[b] (88%)
27	H	1		7.70 ± 0.08	<5.48[b] (86%)
28	H	1		8.56 ± 0.05	5.42 ± 0.28 (87%)
29	H	1		8.54 ± 0.08	7.48 ± 0.06 (58%)

Table 3 (*continued*)

Compd	R	m	A	Human H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)	Rat H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)
30	H	1		8.34 ± 0.07	7.60 ± 0.13 (46%)
31	H	1		8.57 ± 0.09	6.76 ± 0.31 (71%)
32	H	1		7.28 ± 0.10	6.87 ± 0.02 (63%)
33	H	1		5.96 ± 0.06	5.13 ± 0.05 (53%)
34	H	1		5.99 ± 0.05	5.65 ± 0.10 (48%)
35	H	2		7.32 ± 0.06	<4.86[b] (105%)
36	H	2		7.33 ± 0.01	<4.96[b] (84%)
37	H	2		7.00 ± 0.10	5.81 ± 0.1 (50%)
38		1		4.57 ± 0.0006 (49%)	4.86 ± 0.24 (135%)
39		1		6.30 ± 0.02	6.09 ± 0.08

[a] $n \geqslant 2$.
[b] $n = 1$.

potency. We had also demonstrated that such structural rigidification was helpful in increasing oral bioavailability and drug-likeness, presumably by reducing the number of rotatable bonds. For compounds **1**–**15** (Table 1), the marked effect of including an appended phenyl group alpha to the pyrimidine ring is evident in comparing the potencies of compounds **1** and **2**, suggesting that some substitution is beneficial about the rigidifying cycloalkyl ring. However, potencies decrease when the phenyl group is placed at the beta position instead of the alpha position (potencies of **14** vs **1**). Furthermore, it appears that a less lipophilic substitution at this position may not be optimal, as clearly diminished potencies are observed for the alpha-pyridyl compounds **10**, **11**, and **12**. As has been noted earlier with related chemical series,[6a] many of the compounds showing antagonism in the human H_4R FLIPR assay show partial agonism in the rat H_4R FLIPR assay. These results may be due to the substantial difference in sequences between the human and rat H_4Rs (69% homology).[9]

Evaluation of three of the most potent compounds from Table 1 in H_4R binding assays, performed as previously described,[6] confirmed that these compounds indeed display moderately good potencies at both the human H_4R (**1**: pK_i = 7.40 ± 0.11; **9**: pK_i = 8.15 ± 0.32; **15**: pK_i = 7.31 ± 0.006) and rat H_4R (**1**: pK_i = 8.35 ± 0.05; **9**: pK_i = 8.57 ± 0.05; **15**: 8.49 ± 0.18).

We briefly investigated geminal di-substitution at the alpha position (compounds **16–19**, Table 2), but these compounds were less potent than the corresponding alpha-phenyl derivatives. The weakest of these compounds (**19**) even showed agonist activity in both the human and rat H_4 FLIPR assays, for reasons that are not clear. However, it was found that inclusion of a spiroalkyl ring at the alpha position (in effect, 'tying back' the ethyl groups of **18**) was tolerated and in fact led to some quite potent compounds, such as **28**, **29**, **30**, and **31** (Table 3). Importantly, such spiro-cyclopentyl compounds retain the favorable physicochemical properties of the appended compounds[10] but with generally higher potencies at H_4. It was found that compounds with a spiro cyclohexyl ring or spiro indenyl group at the alpha position were consistently less potent than the corresponding cyclopentyl analogs (e.g., **36** or **39** vs. **31**), in one case even exhibiting weak agonism at both human and rat H_4R (**38**). Evaluation of two of the most potent compounds from Table 3 in H_4R binding assays confirmed that these compounds were potent at H_4Rs of both human (**29**: pK_i = 8.70 ± 0.18; **30**: pK_i = 8.82 ± 0.23) and rat (**29**: pK_i = 8.58 ± 0.05; **30**: pK_i = 8.84 ± 0.02).

H_4 antagonists are known to reduce H_4 agonist-induced itch in mice.[4] In order to assess the in vivo effects of the present classes of H_4 antagonists, the functional activity of compounds **1, 3**, and **31** was determined in a mouse H_4 FLIPR assay, then the compounds were tested in a mouse model of H_4 agonist (clobenpropit)-induced scratching, according to the method of Bell et al.[4d] As can be seen in Table 4, compound **31**, which acts as a partial agonist in the rat H_4 FLIPR assay, acts as an antagonist in the mouse H_4 FLIPR assay. This is a surprising result, as there is a fairly high homology (84%) be-

Table 4
In vitro and in vivo results of selected H_4 antagonists in a model of H_4-agonist induced itch

Compound	Mouse H_4 FLIPR $pK_b \pm SEM$[a]	Blockade of scratching in mouse, ED_{50}, ip[b] (μmol/kg)
1	7.34 ± 0.11	10
3	7.90 ± 0.05	3
31	7.82 ± 0.07	1

[a] $n \geqslant 2$.
[b] At ED_{50}, the responses of drug-treated animals were significantly ($p < 0.05$) different from responses of vehicle-treated animals.

Table 5
Summary of in vitro potency for selected compounds at histamine H_3 receptors[a]

Compd	Human H_3 binding potency $pK_i \pm SEM$	Rat H_3 binding potency $pK_i \pm SEM$
1	5.65 ± 0.08	6.18 ± 0.02
15	6.65 ± 0.13	6.98 ± 0.03
29	6.98 ± 0.03	7.14 ± 0.04
30	7.20 ± 0.05	8.17 ± 0.13
31	6.53 ± 0.03	7.10 ± 0.03

[a] $n \geqslant 2$.

tween the rat H_4R and the mouse H_4R at the protein level.[9] The in vivo assay, meanwhile, revealed that all three of the compounds studied were able to completely block itch responses after ip administration and displayed ED_{50} values of 10 μmol/kg or less.

In addition to displaying H_4 antagonist activity, a few of the studied compounds also showed modest antagonist activity at the H_3 receptor (Table 5), with the binding assay being performed as described previously.[11] Both H_4 and H_3 receptor antagonists have been proposed for the treatment of pain,[1c,12] so the therapeutic potential of combining these two activities in a single molecule is intriguing. The data obtained for such compounds as **30** suggest that achieving such dual activity may be possible.

In summary, we have designed three novel series of drug-like H_4R antagonists containing the 2-aminopyrimidine motif. In general, such compounds with an alpha-spiro moiety are more potent than the corresponding alpha-substituted or alpha-*gem*-disubstituted analogs. The best of these compounds display good in vitro potency in both functional and binding assays. Finally, representative members of these series show ED_{50} values of 10 μmol/kg or less in a mouse model of itch, thus demonstrating their in vivo efficacies as H_4R antagonists.

Acknowledgments

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References and notes

1. (a) Engelhardt, H.; Smits, R. A.; Leurs, R.; Haaksma, E.; de Esch, I. J. P. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 628; (b) Akdis, C. A.; Estelle, F.; Simons, R. *Eur. J. Pharmacol.* **2006**, *533*, 69; (c) de Esch, I. J. P.; Thurmond, R. L.; Jongejan, A.; Leurs, R. *Trends Pharmacol. Sci.* **2005**, *26*, 462.
2. (a) Damaj, B. B.; Becerra, C. B.; Esber, H. J.; Wen, Y.; Maghazachi, A. A. *J. Immunol.* **2007**, *179*, 7907; (b) Zhang, M.; Thurmond, R. L.; Dunford, P. J. *Pharmacol. Ther.* **2007**, *113*, 594.
3. (a) Ling, P.; Ngo, K.; Nguyen, S.; Thurmond, R. L.; Edwards, J. P.; Karlsson, L.; Fung-Leung, W. P. *Br. J. Pharmacol.* **2004**, *142*, 161; (b) Buckland, K. F.; Williams, T. J.; Conroy, D. M. *Br. J. Pharmacol.* **2003**, *140*, 1117; (c) O' Reilly, M.; Alpert, R.; Jenkinson, S.; Gladue, R. P.; Foo, S.; Trim, S.; Peter, B.; Trevethick, M.; Fidock, M. *J. Recept. Signal Trans.* **2002**, *22*(1–4), 431.
4. (a) Dunford, P. J.; Williams, K. N.; Desai, P. J.; McQueen, D.; Karlsson, L.; Thurmond, R. *J. Allergy Clin. Immunol.* **2007**, *119*, 176; (b) Rees, J.; Murray, C. S. *Clin. Exp. Dermatol.* **2005**, *30*, 471; (c) Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. *Nat. Rev. Drug Disc.* **2008**, *7*, 41; (d) Bell, J. K.; McQueen, D. S.; Rees, J. L. *Br. J. Pharmacol* **2004**, *142*, 374.
5. Dunford, P. J.; O'Donnell, N.; Riley, J. P.; Williams, K. N.; Karlsson, L.; Thurmond, R. L. *J. Immun.* **2006**, *176*, 7062.
6. (a) Altenbach, R. J.; Adair, R. M.; Bettencourt, B. M.; Black, L. A.; Fix-Stenzel, S. R.; Gopalakrishnan, S. M.; Hsieh, G. C.; Liu, H.; Marsh, K. C.; McPherson, M. J.; Milicic, I.; Miller, T. R.; Vortherms, T. A.; Warrior, U.; Wetter, J. M.; Wishart, N.; Witte, D. G.; Honore, P.; Esbenshade, T. A.; Hancock, A. A.; Brioni, J. D.; Cowart, M. C. *J. Med. Chem.* **2008**, *51*, 6571; (b) Cowart, M. C.; Altenbach, R. J.; Liu, H.; Hsieh, G. C.; Drizin, I.; Milicic, I.; Miller, T. R.; Witte, D. G.; Wishart, N.; Fix-Stenzel, S. R.; McPherson, M. J.; Adair, R. M.; Wetter, J. M.; Bettencourt, B. M.; Marsh, K. C.; Sullivan, J. P.; Honore, P.; Esbenshade, T. A.; Brioni, J. D. *J. Med. Chem.* **2008**, *51*, 6547; (c) Liu, H.; Altenbach, R. J.; Carr, T. L.; Chandran, P.; Hsieh, G. C.; Lewis, L. R.; Manelli, A. M.; Milicic, I.; Marsh, K. C.; Miller, T. R.; Strakhova, M. I.; Vortherms, T. A.; Wakefield, B. D.; Wetter, J. M.; Witte, D. G.; Honore, P.; Esbenshade, T. A.; Brioni, J. D.; Cowart, M. D. *J. Med. Chem.* **2008**, *51*, 7094.
7. (a) Coruzzi, G.; Adami, M.; Guaita, E.; de Esch, I. J. P.; Leurs, R. *Eur. J. Pharmacol.* **2007**, *563*, 240; (b) 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.
8. Bartelt, R. J.; Weisleder, D.; Momany, F. A. *Synthesis* **2003**, 117.
9. Liu, C.; Wilson, S. J.; Kuei, C.; Lovenberg, T. W. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 121.
10. For example, comparison of log *D* values measured at pH 7.4—**1**: 1.73 versus **28**: 2.20; **8**:2.05 versus **30**: 1.60.
11. Esbenshade, T. A.; Krueger, K. M.; Miller, T. R.; Kang, C. H.; Denny, L. I.; Witte, D. G.; Yao, B. B.; Fox, G. B.; Faghih, R.; Bennani, Y. L.; Williams, M.; Hancock, A. A. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 887.
12. Alguacil, L. F.; Perez-Garcia, C. *Curr. Drug Targets CNS Neurol. Disord.* **2003**, *2*, 303.