Structure−Activity Studies on a Series of a 2-Aminopyrimidine-Containing Histamine H_4 Receptor Ligands

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A series of 2-aminopyrimidines was synthesized as ligands of the histamine H_4 receptor (H_4R). Working in part from a pyrimidine hit that was identified in an HTS campaign, SAR studies were carried out to optimize the potency, which led to compound **3**, 4-*tert*-butyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-ylamine. We further studied this compound by systematically modifying the core pyrimidine moiety, the methylpiperazine at position 4, the NH_2 at position 2, and positions 5 and 6 of the pyrimidine ring. The pyrimidine 6 position benefited the most from this optimization, especially in analogs in which the 6-*tert*-butyl was replaced with aromatic and secondary amine moieties. The highlight of the optimization campaign was compound **4**, 4-[2-amino-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl]benzonitrile, which was potent in vitro and was active as an anti-inflammatory agent in an animal model and had antinociceptive activity in a pain model, which supports the potential of H_4R antagonists in pain.

Introduction

There are presently four known histamine receptors, H_1, H_2, H_3 and H_4.[1] The most recently discovered of these, the histamine H_4 receptor (H_4R),[a,2] has been shown to be present on cells of hematopoietic lineage, such as dendritic cells, mast cells, eosinophils, monocytes, basophils, natural killer cells, and T cells.[3] There is a growing body of evidence that is based on animal models in which the H_4R plays a role in immune and inflammatory responses[3a] and modulates itch responses as well.[4] It has been reported that antagonists of the H_4R are able to block the shape change and chemotaxis of cells such as eosinophils and mast cells; these cell types are involved in modulating many inflammatory processes.[5] Because of these findings and others, agents that interact with the H_4R have been proposed to be useful in the treatment of conditions such as asthma, rheumatoid arthritis, and pruritus.[1c]

Indole piperazine **1** (JNJ7777120) was reported in 2003 as a potent and selective antagonist of the H_4R (Figure 1).[6] This compound has become a standard reference agent for evaluating H_4 activity in many laboratories. For example, compound **1** has been shown to block scratching that is induced by an H_4 agonist in mice,[4a] block the histamine-induced migration of mast cells in the mouse airway,[7] reduce the infiltration of neutrophils in a zymosan-induced peritonitis model,[7] and inhibit histamine-induced shape change and upregulation of adhesion molecules in eosinophils.[5]

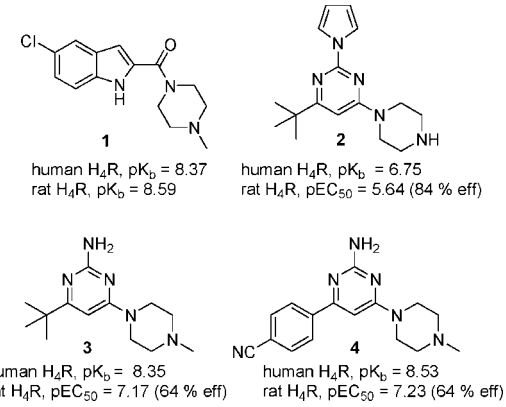

Figure 1. Structures and in vitro potencies of **1**, HTS hit (**2**), and 2-aminopyrimidines **3** and **4**.

With the goal of finding new H_4 ligands of a structural class different from that of **1**, we screened a chemical library for H_4 agonists and antagonists by using a fluorimetric imaging plate reader (FLIPR) cell-based assay that measures changes in the concentration of intracellular calcium. Perhaps not surprisingly, a large proportion of the hits was found to be imidazole-containing compounds, which was likely due to their similarity to the natural ligand histamine that also contains an imidazole moiety. Because imidazole-containing compounds have a higher propensity to inhibit CYPs and because this class is also known to be active at the other receptor subtypes (H_1, H_2, H_3), these hits were viewed to be less interesting than other classes of structures. A small number of the hits were pyrimidines and triazines, which were exemplified by compound **2**. This analog had only very weak potency at the rat H_4R ($pEC_{50} = 5.64$) and somewhat more potency at the human form ($pK_b = 6.75$), although it was anticipated that these might be improved through optimization. Increasing the potency at the rat H_4R was judged

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[a] Abbreviations: BBB, blood−brain barrier; BMMC, bone-marrow-derived mast cell; CHO, Chinese hamster ovary; COX, cyclooxygenase; CYP, cytochrome P_{450} isoenzyme; FLIPR, fluorimetric imaging plate reader; GTPγS, guanosine 5′-O-[gamma-thio]triphosphate; H_4R, histamine H_4 receptor; HEK, human embryonic kidney; MPO, myeloperoxidase; PK, pharmacokinetic; PMN, polymorphonuclear leukocytes; SAR, structure−activity relationship.

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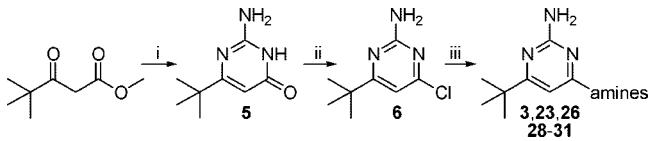
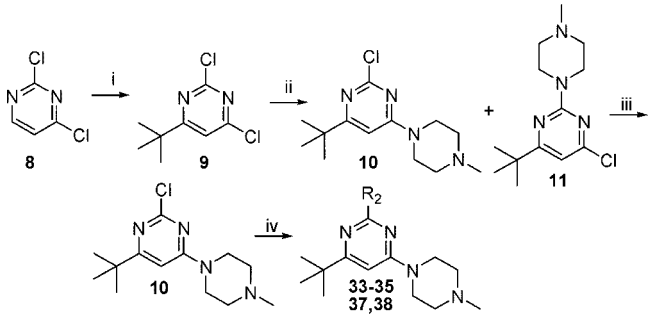
Scheme 1[a]



[a] Reagents and conditions: (i) guanidine hydrochloride, EtONa, EtOH, Δ; (ii) $POCl_3$, Δ; water; and (iii) amine, Δ, EtOH, Et_3N.

Scheme 2[a]



[a] Reagents and conditions: (i) pivalic acid, $AgNO_3$, ammonium persulfate, CH_3CN/H_2O; (ii) *N*-Me-piperazine, Et_3N, EtOH, Δ; (iii) chromatography; (iv) compd **33**: H_2, Pd/C, MeOH; compd **34**: (R_2 = NHMe) 40% aqueous $MeNH_2$, 2-MeOEtOH, Δ; compd **35**: (R_2 = NMe_2): 40% aqueous Me_2NH, 2-MeOEtOH, Δ; compd **37** (R_2 = OH): 1 M HCl, 16 h, Δ; compd **38** (R_2 = OMe): excess NaOMe, MeOH, Δ.

to be the first and most important goal, one reason simply being that compounds would be tested in rodent models.

We found that by simply replacing the pyrrole moiety of **2** with an amino group we dramatically increased the potency, and we found compound **3** to be a moderately potent partial agonist at the rat H_4R (pEC_{50} = 7.17) and a potent H_4 antagonist at the human H_4R (pK_b = 8.35) (Figure 1), which was a potency increase of greater than 30-fold. We envisioned that the lipophilic *t*-Bu group could potentially be replaced with other moieties such as dialkylamines, phenyl groups, and heteroaromatics. This hypothesis was ultimately born out by the 4-CN-phenyl analog (**4**), a compound that was identified early on in the SAR effort as a potent human H_4R antagonist that was active in vivo when profiled in several animal models. We report herein the SAR studies on analogs of compounds **3** and **4**.

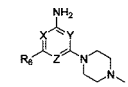
Chemistry

Schemes 1−3 illustrate the routes that were used to make the majority of the compounds described herein. Analogs with various amine groups at the 4 position of the pyrimidine ring were synthesized from chloro intermediate **6**, as shown in Scheme 1. The final product structures are displayed in Table 2. Compounds that evaluate the 2 position of the pyrimidine (Table 3) were synthesized from chloro intermediate **10**, as depicted in Scheme 2. Derivatives that evaluate the SAR of the 5 and 6 position (Table 4) of the pyrimidine ring were generated from chloro analogs **14a**−**d** and **46** (Scheme 3). The more reactive iodo intermediate **15** was used to make a few of the products via a copper-catalyzed coupling to amines (the Ullmann reaction). The Experimental Section contains representative reaction conditions for the syntheses of these derivatives. Detailed experimental procedures for all of the derivatives have been included in the Supporting Information.

Results

The analogs were assessed in vitro in a cell-based Ca^{2+}-flux assay (FLIPR), which allowed an assessment of the potency and of the functional activity (agonism and antagonism) in H_4R-transfected cell lines. For this, HEK-293 cells that stably expressed either the human or the rat H_4R with a coexpressed chimeric G protein (G_{qi5}) were used.[8] For the purpose of describing the in vitro functional profiles of compounds in this assay system, antagonists were defined as having between 0 and 25% efficacy when tested alone and as having the ability to block receptor activation by added histamine, and potencies are expressed as pK_b values. Partial agonists were defined as compounds showing an efficacy between 25 and 74% (relative to the histamine, which was set at 100%), and potencies were expressed as pEC_{50} values. Agonists were defined as compounds having an efficacy of >75% relative to histamine, and potencies were expressed as pEC_{50} values. Tables 1−4 display both pEC_{50} values and pK_b values in the columns. If a percent efficacy is reported, then the compound was found to be a partial agonist or an agonist, and the FLIPR potency that is reported in the tables is the pEC_{50} of the compound. If no percent efficacy is reported, then the compound was found to be an antagonist, and the pK_b is reported. In examining the functional activity of the compounds, we found almost all of the new analogs to be antagonists of the human H_4R. Interestingly, many of the same compounds showed partial or full agonism of the rat H_4 receptor in this assay system. On average, the potencies of these analogs as partial agonists for the rat H_4R was about 8 times weaker than their potencies as antagonists for the human H_4R. These variations in potency and efficacy between the rat and human H_4Rs could be a reflection of the reported interspecies differences in amino acid sequences of the receptor, in which there is only a 69% homology between the rat and human H_4Rs.[9]

Table 1. Heterocyclic Variations of the Core Ring of Compounds **3** and **4**



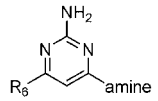
					FLIPR (pK_b or pEC_{50})[a]			
cmpd	R_6	X	Y	Z	human	%eff	rat	%eff
17	t-Bu	N	N	N	7.04±0.20		6.60±0.06[b]	70
18	t-Bu	CH	N	N	5.82±0.38		5.10±0.00[b]	82
19	t-Bu	N	CH	N	6.28±0.04		5.33[c]	50
20	4-CN-Ph	CH	N	CH	5.63±0.35	56	5.39±0.10[b]	
21	4-CN-Ph	N	CH	CH	6.86±0.06		5.73[c]	84
22	4-CN-Ph	CH	CH	N	5.05±0.14[b]		5.14±0.15[b]	

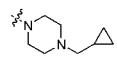
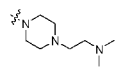
[a] $n \geq 3$; pK_b or pEC_{50} ± the standard deviation (SD) are reported. [b] n = 2. [c] n = 1.

We systematically modified the central core ring by replacing the carbon and nitrogen atoms to generate the analogs that are shown in Table 1. These analogs of compounds **3** and **4** were lower in potency (over a 10-fold loss in potency at the human H_4R). The results of the study suggest that having a nitrogen located at position X in the ring is important for H_4 potency because compounds **17**, **19**, and **21** (wherein X is nitrogen) are more potent than compounds **18**, **20**, and **22** (wherein X is CH).

We investigated the SAR of the 4 position of the pyrimidine ring by making analogs of compounds **3** and **4**. (See Table 2.) All of the examined modifications led to a loss of potency compared with the parent compounds that contained the methylpiperazine moiety. Both nitrogens of the piperazine appear to be required for activity because the replacement of either nitrogen with carbon (examples **23** and **24**) led to a 300- to 1500-fold loss of potency. Even so, a small change such as merely removing the *N*-methyl group from the piperazine, as

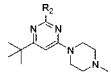
Table 2. Modification of Amine of Compounds **3** and **4**



cmpd	R_6	amine	FLIPR (pK_b or pEC_{50})[a] human	%eff	rat	%eff
23	t-Bu		5.20±0.23		4.63[c]	64
24	t-Bu		5.87±0.31		5.44±0.15[b]	55
25	4-CN-Ph		8.08±0.08		6.88±0.12	67
26	t-Bu		7.86±0.05		6.61±0.27	55
27	4-CN-Ph		5.53±0.06[b]		4.54±0.52[b]	
28	t-Bu		6.44±0.20		5.62±0.26[b]	66
29	t-Bu		6.91±0.09		5.75±0.34[b]	39
30	t-Bu		5.71±0.34		5.95±0.37[b]	55
31	t-Bu		5.13±0.40		5.01[c]	43
32	t-Bu		7.02±0.28		6.52±0.21[b]	67

[a] $n \geq 3$; pK_b or pEC_{50} ± SD are reported. [b] $n = 2$. [c] $n = 1$.

Table 3. Modification of the 2 Position of Pyrimidine Ring of Compound **3**

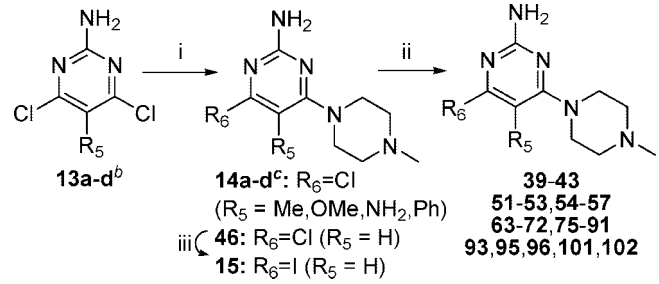


cmp	R_2	FLIPR (pK_b or pEC_{50})[a] human	%eff	rat	%eff
33	H	7.07±0.56		6.03±0.30[b]	79
34	NHMe	6.08±0.34		5.29±0.19[b]	46
35	NMe_2	4.94±0.36[b]	100	4.74[c]	88
10	Cl	5.31±0.87[b]	42	5.06±0.03[b]	75
37	OH	4.39±0.65		4.64[c]	35
38	OMe	5.92±0.18[b]	43	5.33±0.12[b]	90

[a] $n \geq 3$; pK_b or pEC_{50} ± SD are reported. [b] $n = 2$. [c] $n = 1$.

in compound **25**, resulted in a 2- to 3-fold loss of activity at both the human and rat H_4Rs. The replacement of the *N*-methyl group of the piperazine with larger alkyl groups, as in compounds **26** and **27**, or an additional amine, as in compound **28**, was likewise detrimental to potency. The straight-chained diamines as well as the oxygen-linked amines, compounds **29**−**32**, resulted in a reduction in potency. Other diamines have been reported to allow H_4R activity, but direct comparisons to the new compounds await investigation.[10]

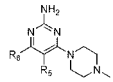
Scheme 3[a]



[a] Reagents and conditions: (i) *N*-Me-piperazine, Et_3N, EtOH, Δ; (ii) Compds **39**−**43**, catalytic hydrogenation: H_2, Pd/C, MeOH; Compounds **51**−**53** and **55**−**57**: intermediate **46**, amine, 2-ethoxyethanol, Hunig's base, 110−140°C; Compds **63**−**72** and **75**−**91**, Suzuki conditions: intermediates **14a**−**d** or **46**, $R_6B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, 1,2-dimethoxyethane, Δ; Compds **93**, **95**, **86**, **101**, and **102**, Ullmann conditions: intermediate **15**, heterocycle, CuI, K_2CO_3, DMF, 130°C; and (iii) NaI, 47% aqueous HI, 80°C, 98%. [b] **13a** (R_5=Me), **13b** (R_5=OMe), **13c** (R_5=NH_2), and **13d** (R_5=Ph). [c] **14a** (R_5=Me), **14b** (R_5=OMe), **14c** (R_5=NH_2), and **14d** (R_5=Ph).

The SAR of the 2 position of the pyrimidine ring was investigated. (See Table 3.) The replacement of the NH_2 group with a hydrogen atom (**33**) was the most well-tolerated change, but even this resulted in a 10-fold loss of potency relative to that of compound **3**. Methylation of the NH_2 group resulted in a further loss of activity, as did the replacement with Cl, OH, or OMe. In summary, all of the compounds retained at least

Table 4. Modification of the 5 and 6 Positions of the Pyrimidine Ring



FLIPR (pK_b or pEC_{50})[a]

cmp	R_6	R_5	human	%eff	rat	%eff	cmp	R_6	R_5	human	%eff	rat	%eff
39	H	H	7.36±0.17	42	6.94±0.26[b]	87	**71**	4-Cl-Ph	H	8.32±0.28		7.43±0.31	74
40	H	Me	7.07±0.17		7.18±0.18	81	**72**	3-Cl-Ph	II	8.24±0.24		7.50±0.27	50
41	H	OMe	6.90±0.17		6.20±0.21[b]	65	**73**	4-I-Ph	H	7.83±0.18		6.56±0.15[b]	58
42	H	NH_2	7.47±0.13	38	7.37±0.22	88	**74**	3-I-Ph	H	8.27±0.26		6.05±0.29[b]	61
43	II	Ph	5.32±0.04[b]		5.08[c]	72	**75**	4-CN-Ph	Me	7.36±0.08		7.23±0.14	63
44	Me	II	7.20±0.51		6.44±0.62	77	**76**	4-CN-Ph	OMe	6.04±0.10		5.29±0.03[b]	51
3	t-Bu	H	8.35±0.45		7.17±0.13	64	**77**	4-CN-Ph	Ph	5.69±0.30[b]		5.29±0.12[b]	
45	adamant-1-yl	H	7.59±0.40		6.50±0.54	61	**4**	4-CN-Ph	H	8.53±0.34		7.23±0.19	64
46	Cl	II	4.75±0.40		4.77±0.05[b]	65	**78**	3-CN-Ph	H	8.06±0.07		6.63±0.38	48
15	I	H	6.33±0.15		5.69[c]	67	**79**	2-CN-Ph	H	6.75±0.11		5.92[c]	73
48	OH	H	4.44±0.10[b]		5.83±0.18[b]	92	**80**	4-Ph-Ph	II	6.29±0.15		6.03±0.43[b]	
49	OMe	H	5.53±0.40		4.98±0.02[b]	55	**81**	3-Ph-Ph	H	6.85±0.06		6.38±0.24[b]	
50	NH_2	H	6.37±0.48		6.66±0.01[b]	71	**82**	2-Ph-Ph	H	6.79±0.39		5.72±0.80[b]	
51	NHMe	H	8.28±0.16		6.31±0.15[b]	93	**83**	naphth-2-yl	H	7.02±0.20		6.76±0.25[b]	41
52	NMe_2	H	6.85±0.08		5.52±0.28[b]	59	**84**	naphth-1-yl	H	7.81±0.34		7.27±0.36	
53	NEt_2	H	7.55±0.40		6.45±0.32[b]	90	**85**	pyridin-4-yl	H	7.38±0.12		6.64±0.10[b]	63
54	pyrrolin-1-yl	H	8.41±0.22		7.16±0.20	79	**86**	pyridin-3-yl	H	7.30±0.41		6.68±0.33	70
55	3-(NMe_2)-pyrrolidin-1-yl	H	5.76±0.14[b]		5.44[c]	62	**87**	2,6-difluoropyridin-3-yl	II	8.27±0.07		7.81±0.12	82
56	piperidin-1-yl	II	8.05±0.21		7.33±0.12	73	**88**	2-OMe-pyridin-3-yl	H	7.57±0.62	32	7.03±0.23[b]	72
57	morphonlin-1-yl	H	7.16±0.35		6.47±0.43[b]	87	**89**	6-OMe-pyridin-3-yl	II	7.39±0.15		6.63±0.22	76
58	4-Me-piperazin-1-yl	H	6.84±0.35		5.87±0.23[b]	68	**90**	pyrimidin-5-yl	H	5.84±0.29		5.10±0.14[b]	61
59	Ph-NII-	II	8.18±0.03		6.93±0.31[b]	81	**91**	2-OMe-pyrimidin-5-yl	H	5.91±0.19		5.49±0.09[b]	53
60	Ph-NMe-	II	6.96±0.20		6.10±0.48		**92**	Ph-S-	H	5.99±0.36		5.05[c]	68
61	Ph-CH2-NH-	H	7.19±0.21	56	6.31[c]	87	**93**	imidazol-1-yl	II	5.60±0.39[b]	31	5.10[c]	56
62	Ph-CH2-NMe-	H	6.77±0.07		6.47±0.43		**95**	4-Ph-imidazol-1-yl	H	5.68±0.05[b]		5.74[c]	
63	Ph	II	8.39±0.39		7.40±0.12	79	**96**	benzimidazol-1-yl	H	6.75±0.27	82	6.46[c]	
64	4-Me-Ph	H	8.06±0.34		7.25±0.35	81	**97**	imidazol-4-yl	H	7.79±0.04	41	6.41±0.33[b]	77
65	3-Me-Ph	II	8.62±0.34		7.37±0.09	59	**98**	1-Me-imidazol-4-yl	H	8.05±0.18		6.99±0.18	91
66	2-Me-Ph	H	8.07±0.09		6.75±0.28[b]	60	**99**	pyrazol-4-yl	H	8.38±0.14		5.74±1.70[b]	53
67	4-OMe-Ph	H	8.08±0.19		6.94±0.22	71	**100**	1-Me-pyrazol-4-yl	H	7.31±0.05		6.66±0.06[b]	78
68	3-OMe-Ph	H	8.12±0.16		6.99±0.18	69	**101**	imidazolidin-2-one-1-yl	H	5.38±0.13[b]		4.91[c]	27
69	2-OMe-Ph	H	8.86±0.52		7.36±0.16	68	**102**	pyridin-2-one-1-yl	H	5.41±0.52[b]	27	4.85[c]	33
70	2,6-di-OMe-Ph	H	6.81±0.19		6.71±0.15[b]	81							

[a] $n \geq 3$; pK_b or $pEC_{50} \pm$ SD are reported. [b] $n = 2$. [c] $n = 1$.

some activity at the receptor, although none approached the activity of the parent **3**; therefore, such changes can be judged as being detrimental to H_4 activity.

The SAR of the 5- and 6 positions of the pyrimidine ring was quite variable, and diverse analogs showed a wide range of potencies on the order of low nanometers to low micrometers (Table 4). Unsubstituted analog **39** had significant potency, although it was less than that of **3** or **4**. Analogs that were substituted at the 5 position with Me, OMe, or Ph (**40**, **41**, **43** and **75**−**77**) were active but were of lower potency at the human H_4R. Interestingly, compounds **40** and **42** maintained activity for the rat H_4R, which resulted in comparable potencies for both the rat and human H_4Rs. A high degree of receptor activation (agonism) was seen at the rat H_4R for compounds **39**, **40**, and **42**, all of which lacked a substitution at position 6.

We note some interesting SARs by comparing the influence of these small substituents at position 5 versus position 6 of the pyrimidine core. When we used unsubstituted analog **39** as a comparator molecule, the introduction of NH_2 and OMe at position 5 did not have much of an effect on the potency (compound **41** and **42**); however, when similar changes were applied to pyrimidine position 6 (compounds **49** and **50**), there was an obvious detrimental effect on the potency. The 6 position was preferred over the 5 position as the attachment site when it was probed with the large lipophilic phenyl group; that is, the 5-Ph analog (**43**) had 5 μM potency, whereas the 6-Ph analog (**63**) had 4 nM potency, which was 10 times more potent than unsubstituted analog **39**. Because of these intriguing findings, we investigated the SAR at position 6 in much greater detail.

Table 5. PK of Compounds **1** and **4** in Rat and Mouse[a]

	iv				po				
compd	dose	$V\beta$	Clp	C_{max}	$t_{1/2}$	dose	C_{max}	t_{max}	$t_{1/2}$	F
4, rat	1	7.1	10.3	0.16	0.4	1	0.032	0.4	n.d.[b]	31
4, mouse	2.5	3.9	15.3	0.57	0.2	2.5	0.092	0.25	0.6	41
1, rat	10	37	12.6	1.14	1.7	10	0.22	0.25	2.5	47
1, mouse	5	24	23.2	0.56	0.7	20	0.14	1.33	1.4	24

[a] Units: dose (mg/kg), $V\beta$ (L/kg), Clp (L/h/kg), C_{max} (μg/mL), t_{max} (h), $t_{1/2}$ (h). [b] Not able to determine.

Table 6. Potencies of Compounds **1**, **4**, and **25** at H_3 and H_4 Subtypes

	H_4 FLIPR (pK_b or pEC$_{50}$)[a]					H_4 binding[b]		H_3 binding[c]	
compd	human	rat	% eff	mouse	% eff	human	rat	human	rat
1	8.37 ± 0.02	8.59 ± 0.02		8.62 ± 0.05		7.92 ± 0.22	8.57 ± 0.10	5.65 ± 0.05	5.88 ± 0.09
4	8.53 ± 0.12	7.23 ± 0.08	64	6.91 ± 0.20	54	7.46 ± 0.06	6.56 ± 0.06	5.89 ± 0.04	6.61 ± 0.03
25	8.08 ± 0.05	6.61 ± 0.16	67	6.99 ± 0.05	53	7.38 ± 0.11	6.47 ± 0.02	6.03 ± 0.08	6.92 ± 0.07

[a] $n \geq 3$; pK_b or pEC$_{50}$ ± SEM are reported. [b] $n \geq 3$; pK_i ± SEM are reported. [c] $n = 2$.

Relative to compound **3** containing a *t*-butyl at position 6, the bulky lipophilic adamantyl analog, **45**, was 10 times less potent. The substitution with chlorine at position 6 resulted in a compound (**46**) that appeared to be devoid of H_4 activity (K_b > 10 μM), whereas the iodo analog (**15**) maintained moderate H_4R activity. It is possible that the size of the lipophilic group is critical; adamantyl is too large, and *t*-butyl, aryl, and iodo are more optimal.

Relative to compound **39** (unsubstituted at R_5 and R_6), compounds **51**−**62** with primary and secondary amines at the 6 position had comparable or higher H_4 potencies. A trend was seen in which primary amine substituents with a free NH adjacent to the pyrimidine ring (compounds **51**, **59**, and **61**) had higher potencies than secondary amines with no free NH (compounds **52**, **60**, and **62**). Pyrrolidine **54** was more potent than diethyl amine **53**, which perhaps points to a potential benefit for cyclic amines as compared with acyclic amines. Molecules with diamines on both sides, as in **55** and **58**, were less potent than similar 6-substituted amines, compounds **54** and **56**, respectively.

The finding that phenyl derivative **63** was equipotent with compound **3** prompted a more in depth investigation of the effects of substitutions to the phenyl ring. For the majority of the new compounds (**64**−**74** and **78**−**84**), high potency was retained, although most did not show a significant boost in potency. An exception was 2-OMe derivative **69**, which showed a 3-fold boost in potency relative to **63**. However, an additional methoxy group in diortho-OMe analog **70** showed a 100-fold reduced potency relative to **63**. Biphenyl and naphthyl analogs **80**−**84** were also active but were not improved in potency compared with **63**, although the biphenyl analogs and the 1-naphthyl derivative were in vitro antagonists in the rat.

The effect of heterocyclic substituents at the 6 position of the pyrimidine was examined next. Regardless of the position of the nitrogen, the pyridyl analogs (**85** and **86**) showed slightly reduced potencies relative to **63**. The more lipophilic and less basic difluoropyridine, **87**, retained the high potency of **63**. The incorporation of an ortho methoxy moiety on the pyridine ring, as in compounds **88** and **89**, did not result in a boost in potency, in contrast with the influence of an ortho methoxy group on the phenyl ring (**69**). Imidazole (**93**) and benzimidazole (**96**), which were both attached to the pyrimidine ring through the nitrogen atom, reduced the potency. Furthermore, full agonism was noted at the human H_4R in the case of **96**. However, other imidazoles (**97** and **98**), which were both connected to the pyrimidine ring via the carbon at the 4 position of the imidazole, maintained high potency. Pyrazol-4-yl derivative **99** was equipotent with phenyl analog **69** for the human H_4R, but it lost potency in rat. Two analogs with polar heterocycles, imidazolidinone (**101**) and pyridinone (**102**), lost a substantial amount of receptor potency.

The H_4R is known to be expressed in cells of the immune system, and H_4R antagonists have been reported to be active in different in vivo models of inflammation. Of the new compounds described here, **4** was selected to be tested in vivo.

Compound **4** was assessed for its PK properties in rat and mouse (Table 5). In the rat, at 1 mg/kg, compound **4** had a $t_{1/2}$ of 0.4 h after iv administration. After it was orally administered at 1 mg/kg, compound **4** plasma levels reached a C_{max} of 32 ng/mL at 0.4 h and an oral bioavailability (F) of 31%. Similar results were found in PK studies in the mouse, wherein the compound dosed at 2.5 mg/kg iv had a $t_{1/2}$ of 0.2 h, and the compound dosed po had a $t_{1/2}$ of 0.6 h, with maximum plasma levels reaching a C_{max} of 92 ng/mL at 0.25 h and a oral bioavailability of 41%. Compound **1** was also tested in PK studies, and the results were comparable to those that were previously reported.[7] Both **1** and **4** have high clearance values (Clp) in rat and mouse. The $t_{1/2}$ of compound **1** is longer than that of **4** in rat and mouse, which is probably due to its higher volume of distribution ($V\beta$). Despite this, compound **1** and **4** are adequate for allowing profiling in acute inflammation and pain models. We hypothesized that the methyl group of the *N*-methyl piperazine of **4** might be labile in vivo, and indeed this was confirmed by testing. During the PK studies, a metabolite of compound **4** was observed in plasma and was identified as the des-methyl analog, compound **25**.

The H_1 through H_4 activities of compound **4** and its metabolite, compound **25**, were compared with those of compound **1**. Table 6 displays the FLIPR H_4R potencies in human, rat, and mouse as well as the binding potencies of these agents at the human and rat H_3Rs and H_4Rs. Compound **1** was nearly equipotent and was an antagonist in the human, rat, and mouse H_4Rs and was highly selective over the other histamine subtypes (data not shown). Compound **4** was a potent antagonist at the human H_4R but was less potent and a was partial agonist in rat and mouse. The similarity in the in vitro potencies and efficacies of the rat and mouse subtypes is consistent with the relatively high homology of 84% between the rat and mouse H_4Rs at the protein level.[9] In binding studies, compound **4** was 30 times more selective for human H_4R over H_3R, but it was nonselective for rat H_4R over H_3R. Although compounds **1** and **4** have similar potencies in human H_4R binding, compound **4** is significantly weaker in binding at the rat H_4R (K_i = 275 nM) than is compound **1** (K_i = 3 nM). The metabolite of **4**,

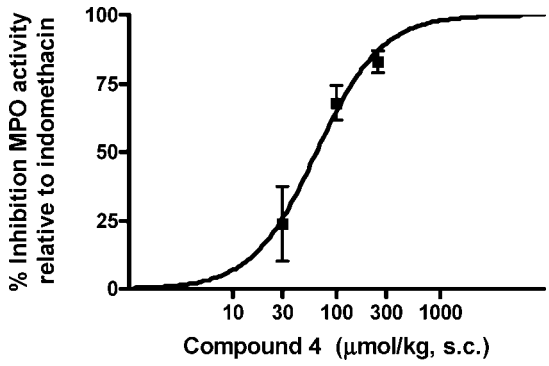


Figure 2. Inhibition by compound **4** of MPO activity, a biomarker for PMN infiltration, in the mouse zymosan peritonitis model.

compound **25**, was also active at H_4R and had potencies that were similar to those of compound **4** across the H_3 and H_4 receptors in binding and functional studies. Although mouse H_4R affinity studies were not conducted, compounds **4** and **25** would be expected to have binding potencies that are similar to that of the rat H_4R. This is based on the observation that for compounds for which both rat and mouse H_4R binding affinities have been reported, including compound **1**, the majority have a less than 2-fold difference in potencies.[3a,7,9] Compounds **4** and **25** were selective over the human H_1 and H_2 sites, wherein pK_i values were <5.2.

The zymosan peritonitis model in mice has been used to assess acute inflammatory responses in vivo. The pro-inflammatory properties of zymosan are attributed to complement pathway activation as well as chemokine and cytokine release from neutrophils, mast cells, and macrophages, which leads to an accumulation of polymorphonuclear leukocytes (PMN) as a characteristic feature. The activity of the enzyme myeloperoxidase (MPO), which is found in granules of PMN, has been shown to correlate well with the number of PMNs in tissue samples, and it is consequently used as a biomarker for PMN infiltration.[11] The H_4R has been shown to partially mediate the zymosan-induced recruitment of neutrophils.[7,12]

Compound **4** was tested in a model of zymosan peritonitis. Briefly, vehicle or compound **4** was dosed sc in BALB/c male mice (22−25 g) 30 min prior to zymosan injection. Zymosan (0.25 mg/mouse in 0.5 mL of saline, ip) was then injected, and after 2 h, peritoneal lavages were collected, and the MPO activity was determined. Compound **4** was found to decrease the level of MPO activity in the lavages with an ED_{50} of 64 μmol/kg (see Figure 2). The efficacy of compound **4** was determined relative to that for indomethacin (10 mg/kg). Compound **1** has been reported to be active in this type of inflammation model[7] and was found to inhibit the activity of MPO with an ED_{50} of 61 μmol/kg, and it is almost identical in potency to the new pyrimidine analog **4**.

To profile compound **4** in in vivo models of H_4 activity further, we evaluated compound **4** in a mouse itch model.[13] This is a relevant model because antagonists of the H_4R such as thioperamide and compound **1** have been shown to reduce H_4 agonist-mediated itch in mice.[4a,14] When it was dosed ip, compound **4** was found to reduce the itch that was induced by the H_4R agonist clobenpropit in a dose-dependent manner with an ED_{50} of 34 μmol/kg. Compound **1** was also active in this model as an antagonist with an ED_{50} of 25 μmol/kg. To validate the H_4 antagonist activity of compound **4** in this model further, we tested compound **4** for H_4 agonist activity. Compound **4**, in doses ranging from 0.1 to 10 μmol, was injected into the scruff of the neck of mice and did not induce a scratch response, which indicates that this compound does not function as an H_4 agonist in this model.

The in vivo profile of compound **4** in mice supports the thought that this compound is an antagonist by demonstrating potencies that are comparable to those of compound **1**. This is in conflict with the FLIPR data, in which compound **4** demonstrated partial agonist activity in both rat and mice. However, compounds that are partial agonists may induce or stabilize receptor conformations that have lower affinities for G proteins compared with receptors that are stimulated by full agonists.[15] In addition, partial agonists can also act as antagonists by competing for receptor binding with full agonists.[16] Therefore, a compound, such as **4**, that is a partial agonist in vitro may function as an antagonist in vivo.

The similarity of the in vivo potencies of compounds **1** and **4** is not well predicted by the in vitro potencies of compounds **1** and **4** in the rat and mouse H_4R, wherein compound **1** is over 100 times more potent than compound **4**. To investigate the activities of compounds **1** and **4** in the zymosan and itch models further, we evaluated plasma levels in mouse. Compounds **1** and **4** were dosed at $\sim$25 μmol/kg ip, and their plasma levels at 30 min postdosing were found to be 860 $\pm$ 90 and 1150 $\pm$ 130 ng/mL, respectively. The higher plasma level of compound **4** supports the fact that compound **4** is indeed less potent than compound **1** in both the zymosan and itch models, although by less than or equal to a factor of 2 and not to the full extent that was predicted by the rat binding studies in which compound **4** was over 100 times less potent than compound **1**.

To probe their H_4R functional activity further, we tested compounds **1** and **4** in a rat GTPγS assay[17] that used the same cell line that was used in the FLIPR assay. Compounds **1** and **4** were both antagonists with pK_b values of 7.19 $\pm$ 0.01 and 6.21 $\pm$ 0.04, respectively. Tested in the agonist mode, compound **1** was a potent partial agonist ($pEC_{50} = 7.37 \pm 0.07$) with low efficacy (23%), but compound **4** was a weak but full agonist with a pEC_{50} of 5.33 $\pm$ 0.61. This in vitro pharmacology of compound **1** motivated us to more closely investigate assays of native H_4R function relevant to its in vivo behavior.

An important point here is that the FLIPR and GTPγS studies were run in an artificial system that used non-native tissue and a chimeric G protein (G_{qi5}). To address the profile in a more relevant in vitro system, we tested compound **4** in an assay by using native tissue that contains the H_4R. In methodology similar to that reported for eosinophils,[5a] a mouse bone-marrow-derived mast cell (BMMC) shape-change assay was used. Compound **4** was found to inhibit histamine-induced shape change in BMMCs with a pK_b of 6.56 ($n = 3$). Compound **1** was also tested in this assay and was found to inhibit shape change in BMMCs with a very similar potency (pK_b) of 6.23. In general, the sum total of the data indicates that compound **4** acts as an antagonist in vivo.

We have recently reported that H_4 antagonists are active in a variety of different pain models. Surprisingly, H_4 antagonists are active in models that assess not only inflammatory pain but also noninflammatory pain.[18,19] Compound **4** was tested in a dose−response fashion in a model of inflammatory pain, the carrageenan-induced thermal hyperalgesia model.[20,21] (See Figure 3.) Compound **4** was administered ip in rats and was found to increase the paw withdrawal latency relative to vehicle by 36% at 30 μmol/kg and by 100% at 100 μmol/kg. The ED_{50} for efficacy in this model was 32 μmol/kg. Plasma levels were measured and were found to be 212 $\pm$ 36, 836 $\pm$ 217, and 2468 $\pm$ 350 ng/mL at 10, 30, and 100 μmol/kg, respectively. Since the protein plasma binding of compound **4** in rat is 67%

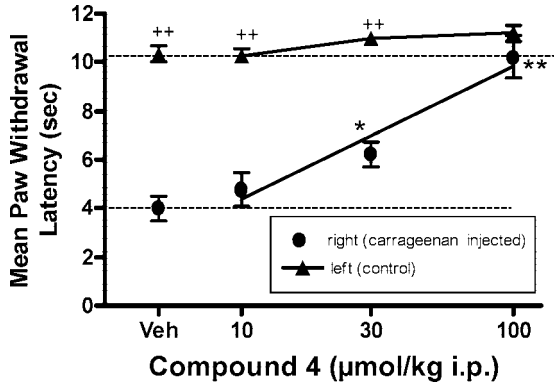


Figure 3. Inhibition of carrageenan-induced hyperalgesia by compound **4**.

(33% free), the free fraction of compound **4** at the 30 μmol/kg dose was 280 ng/mL, which corresponds to a concentration of 950 nM. The total plasma level for metabolite **25** at the 30 μmol/kg dose was 201 $\pm$ 36 ng/mL, which indicates that $\sim$20% of compound **4** was being metabolized.

Compound **1**, which was also tested in this carrageenan model, was administered ip in rats and was found to increase the paw withdrawal latency relative to vehicle by 49% at 100 μmol/kg and by 96% at 300 μmol/kg. The ED_{50} of compound **1** for efficacy in this model was 80 μmol/kg. Plasma levels were measured and were found to be 432 $\pm$ 55, 1454 $\pm$ 295, and 4135 $\pm$ 633 ng/mL at 30, 100, and 300 μmol/kg, respectively. The approximate concentration of compound **1** at a dose of 80 μmol/kg is 1200 ng/mL. The protein plasma binding of compound **1** in rat of 72% (28% free) was taken into consideration, and the free fraction of compound **1** at the 80 μmol/kg dose was approximately 340 ng/mL, which corresponds to a concentration of 1200 nM.

The estimated EC_{50} plasma free fraction potencies of compounds **1** (340 ng/mL) and **4** (200 ng/mL) in the rat carrageenan model do not correspond with what was observed in the rat binding studies wherein compounds **1** is over 100 times more potent than compound **4**. A point of consideration is the activity of compound **4** at the H_3R. Reports have indicated roles for both agonists[22] and antagonists[23] of the H_3R in pain. Compounds **1** and **4** were evaluated in GTPγS studies for their functional activity at the H_3R. Compounds **1** and **4** were found to be antagonists with pK_b values of 5.70 $\pm$ 0.04 and 6.50 $\pm$ 0.03, respectively. If the results are taken in isolation, then the finding that compound **4** has antagonism of the H_3R opens the possibility that some of the antinociceptive activity of compound **4** could be due to the contribution of both the H_3R and the H_4R. However, additional results are relevant: (1) compound **1** is a highly selective H_4R (compared with the H_3R) antagonist and is active in pain models and (2) we have found that structurally distinct (and selective vs the H_3R) H_4R antagonists are comparable to, or more active in pain than, compounds **1** and **4**.[24]

The ancillary activity of compound **4** was evaluated across a panel of receptors, kinases, and ion channels, including opioid receptors and COX enzymes (CEREP and in-house).[25] At 10 μmol, compound **4** inhibited the specific binding of competitive ligands (percent inhibition in parentheses) at α_2 (92%) and at several serotonin sites, namely, 5-HT_{1A} (99%), 5-HT_{1B} (86%), 5-HT_{2A} (71%), and 5-HT_7 (53%). Compound **4** was retested in-house, and we found that it bound to the following sites with the following potencies (K_i, μM): 5-HT_{1A} (0.042), 5-HT_{1D} (0.12), 5-HT_{2A} (4.1), 5-HT_{2C} (3.0), 5-HT_7 (5.9), α_{2A} (0.074), and α_{2C} (0.29).[26] Because compound **4** bound with moderate potency to the 5-HT_{1A} site, we examined it for functional activity in a GTPγS binding assay. Compound **4** was found to be a weak (pEC_{50} = 5.79 $\pm$ 0.08) but full agonist (92% efficacy relative to serotonin) in CHO cell membranes that express the 5-HT_{1A} subtype.[27]

Compound **4** was examined for its ability to cross the blood−brain barrier (BBB). In a PK study, rats were given an ip dose of 30 μmol/kg of compound **4**. After 30 min, plasma and brain samples were evaluated. The plasma levels of compound **4** were similar to those that were seen in the carrageenan rat model, namely 600 $\pm$ 20 ng/mL. Surprisingly, compound **4** was found to enter the brain readily. The brain levels were 12.1 $\pm$ 1.1 μg/g of brain tissue, which was 20 times higher than the plasma levels. The levels of metabolite **25** in the plasma and brain were 140 $\pm$ 10 ng/mL and 610 $\pm$ 40 ng/g, respectively. The concentration of compound **4** in the brain was 19 times higher than the concentration of the metabolite, which indicates that compound **25** crosses the BBB less effectively than does compound **4** and that any central effects from the administration of compound **4** are probably due to the parent compound and not the metabolite.

As displayed in Figure 4, we examined compound **4** for motor side effects in rat by using a rotorod model to assess motor coordination and a locomotor activity model to assess spontaneous exploratory behavior.[28] Compound **4** had no effect at up to 300 μmol/kg in rotorod performance (10 times greater than the ED_{50} in the carrageenan model). At 100 μmol/kg, 3 times greater than the ED_{50} in the carrageenan model, a significant effect was seen in the locomotor activity of compound **4**. These results indicate that there is a moderate separation between the efficacy in the carrageenan model for compound **4** and the motor side effects.

Conclusions

A series of 2-aminopyrimidines containing H_4R antagonists was discovered from pyrimidine containing high-throughput screening hits. SARs on compound **3** revealed that the *t*-Bu group was most amenable to substitution with a variety of nonpolar substituents such as aryl and amino groups. From this series, compound **4** was further evaluated and was found to be active in a zymosan peritonitis model of inflammation as well as a murine itch model with potencies similar to those of compound **1**. Compound **4** was also found to increase the paw withdrawal latency in an inflammatory model of pain, the rat carrageenan model. Compound **1** has also been found to attenuate the hyperalgesic response to thermal and mechanical stimuli in carrageenan-treated rats significantly.[19,29] Others have also indicated a role for the H_4 receptor beyond the immune system. For example, the murine itch response has been proposed to involve histamine that acts on peripheral terminals of sensory neurons through the activation of H_4 receptors.[4a] Further evaluation of the role of the H_4R in pain is warranted.

Experimental Section

General. Proton NMR spectra were obtained on a Varian Mercury Plus 300 or Varian UNITY Plus 300 MHz instrument with chemical shifts (δ) that are reported relative to tetramethylsilane as an internal standard. Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses were performed by Quantitative Technologies and Robertson Microlit Laboratories. Column chromatography was carried out on silica gel 60 (230−400 mesh). Thin-layer chromatography (TLC) was performed by the use of 250 mm silica gel 60 glass-backed plates with F254 as the indicator.

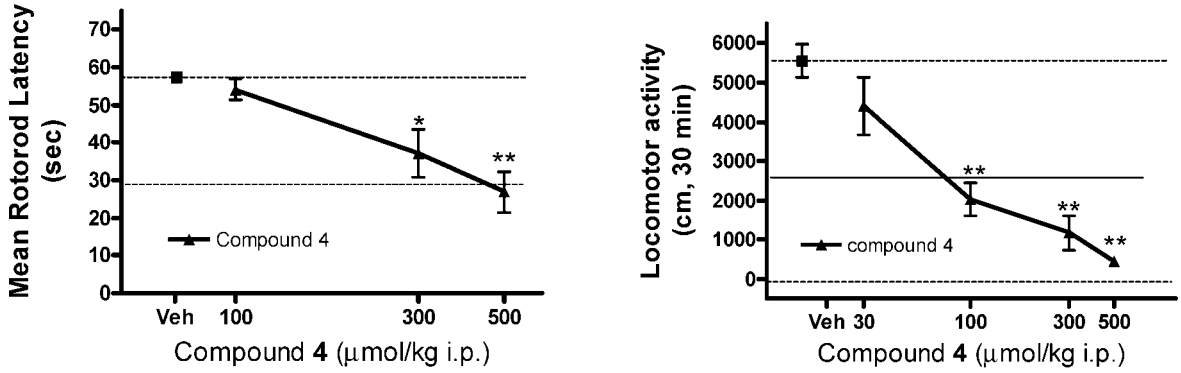


Figure 4. Compound **4** in rat rotorod model and rat locomotor model.

4-*tert*-Butyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-ylamine (3). A mixture of compound **6** (130 mg, 0.70 mmol), 1-methylpiperazine (77 mg, 0.77 mmol), and Et_3N (0.75 mL, 5.4 mmol) in EtOH (1.5 mL) was heated in a sealed vial to 80 °C for 16 h, was cooled, was treated with 1 M NaOH (10 mL), and was extracted with CH_2Cl_2 (2 × 25 mL). The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, concentrated, and chromatographed on silica gel by elution with gradients of 2, 3.5, and 5% MeOH/concd NH_4OH (9:1) in CH_2Cl_2 to provide 0.17 g (95%) of the title compound. ^1H NMR ($CDCl_3$, δ): 1.24 (s, 9H), 2.33 (s, 3H), 2.45 (m, 4H), 3.60 (m, 4H), 4.66 (bs, 2H), 5.94 (s, 1H). MS (DCI/NH_3) *m/z*: 250 $(M + H)^+$. Anal. Calcd for $(C_{13}H_{23}N_5 \cdot 0.2CH_3OH \cdot 0.08CH_2Cl_2)$: C, H, N.

4-[2-Amino-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl]benzonitrile Maleate (4). Compound **46** (11.2 g, 49 mmol) was treated with 4-cyanophenylboronic acid (10.8 g, 74 mmol), 1,2-dimethoxyethane (150 mL), and 2 M Na_2CO_3 (54 mL, 108 mmol), and the atmosphere of the reaction was purged with nitrogen. The mixture was treated with $Pd(PPh_3)_4$ (4.5 g, 3.9 mmol), was heated to 90 °C overnight under a nitrogen atmosphere, was cooled to ambient temperature, was treated with 1 M NaOH (125 mL), and was extracted with CH_2Cl_2 (250 mL, followed by 3 × 100 mL). The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, concentrated, and chromatographed on silica gel by elution with gradients of 2 and 3.5% MeOH/concd NH_4OH (9:1) in CH_2Cl_2 to provide 12.7 g (88%) of the title compound as a free base. This free base (12.7 g, 43 mmol) was suspended in acetone (100 mL) and was treated with a solution of maleic acid (5.0 g, 43 mmol) in acetone, and the salt started to fall out of solution. This mixture was heated under reflux for 10 min with mixing and was allowed to stand at ambient temperature overnight. The solid was collected by filtration, was washed with acetone, and was dried overnight under vacuum to provide 15.6 g of the title compound. mp 187−190 °C (dec). ^1H NMR (DMSO-d_6, δ): 2.79 (s, 3H), 3.20 (bs, 4H), 3.31 (bs, 4H), 6.04 (s, 2H), 6.39 (s, 2H), 6.83 (s, 1H), 7.95 (d, J = 8.48 Hz, 2H), 8.24 (d, J = 8.82 Hz, 2H). MS (DCI/NH_3) *m/z*: 258 $(M + H)^+$. Anal. Calcd for $(C_{16}H_{18}N_6 \cdot C_4H_4O_4)$: C, H, N.

4-*tert*-Butyl-6-chloropyrimidin-2-amine (6). 2-Amino-6-*tert*-butylpyrimidin-4(3*H*)-one (**5**)[30] (2.7 g, 16 mmol) was treated with $POCl_3$ (15 mL), was heated under reflux for 2 h, was cooled, was concentrated to a solid, and was treated with a mixture of ice and saturated NH_4OH. After standing for 1 h, the solid was collected by filtration and was washed with water. The solid was dissolved in CH_2Cl_2, dried ($MgSO_4$), filtered, and concentrated to provide the title compound. ^1H NMR (DMSO-d_6, δ): 1.21 (s, 9H), 6.64 (2, 1H), 6.95 (bs, 2H). MS (DCI/NH_3) *m/z*: 186 $(M + H)^+$.

4-*tert*-Butyl-2,6-dichloropyrimidine (9). A mixture of 2,4-dichloropyrimidine (3.0 g, 20 mmol), pivalic acid (6.2 g, 60 mmol) and silver nitrate (6.8 g, 40 mmol) in 1:1 CH_3CN/H_2O (60 mL) was degassed with N_2, heated to 80 °C, treated with a 1.2 M aqueous solution of ammonium persulfate (34 mL, 40 mmol), heated to 80 °C under N_2 for 2 h, cooled, treated with a solution of concentrated NH_4OH (15 mL) in H_2O (60 mL) and extracted twice with CH_2Cl_2. The combined CH_2Cl_2 layers were washed with $NaHCO_3$ sln, dried ($MgSO_4$), filtered, concentrated, and chromatographed by elution with 50:1, followed by 20:1, hexane/EtOAc to provide 0.57 g (14%) of the title compound. ^1H NMR ($CDCl_3$, δ): 1.35 (s, 9H), 7.27 (s, 1H). MS (DCI/NH_3) *m/z*: 205 $(M + H)^+$.

4-*tert*-Butyl-2-chloro-6-(4-methylpiperazin-1-yl)pyrimidine (10) and 4-*tert*-Butyl-6-chloro-2-(4-methylpiperazin-1-yl)pyrimidine (11). The substitution of compound **9** (300 mg, 1.4 mmol) for compound **6** in the procedure for compound **3** provided a mixture of **10** and **11**, which were separated by chromatography by the use of a gradient of EtOAc/HCOOH/H_2O (28:1:1, 16:1:1, 13:1:1, and 5.5:1:1) to provide, after concentration, the formic acid salts of **11** as the less polar product and **10** as the more polar product. Each product was individually converted to the free base by chromatography by elution with gradients of 2 and 3.5% MeOH/concd NH_4OH (9:1) in CH_2Cl_2 to provide 80 mg (21%) of compound **11** and 250 mg (65%) of compound **10**. Compound **11**: ^1H NMR ($CDCl_3$, δ): 1.26 (s, 9H), 2.35 (s, 3H), 2.47 (t, J = 5.1 Hz, 4H), 3.86 (t, J = 5.1 Hz, 4H), 6.52 (s, 1H). MS (DCI/NH_3) *m/z*: 269 $(M + H)^+$. Compound **10**: ^1H NMR ($CDCl_3$, δ): 1.28 (s, 9H), 2.36 (s, 3H), 2.50 (m, 4H), 3.68 (m, 4H), 6.34 (s, 1H). MS (DCI/NH_3) *m/z*: 269 $(M + H)^+$. Anal. Calcd for $(C_{13}H_{21}ClN_4 \cdot 0.25H_2O)$: C, H, N.

4-Iodo-6-(4-methylpiperazin-1-yl)pyrimidin-2-ylamine (15). A mixture of compound **46** (2.3 g, 10 mmol), NaI (7.5 g, 50 mmol), and 47% hydroiodic acid (54 mL) was heated to 80 °C for 16 h and was allowed to cool to room temperature and stand for 1 h. The solid was collected by filtration, washed with water, and partitioned between 0.5 M NaOH (50 mL) and CH_2Cl_2 (50 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, and concentrated to provide 2.78 g (87%) of the title compound. ^1H NMR ($CDCl_3$, δ): 2.33 (s, 3H), 2.44 (t, J = 5.1 Hz, 4H), 3.57 (t, J = 5.1 Hz, 4H), 4.76 (bs, 2H), 6.40 (s, 1H). MS (DCI/NH_3) *m/z*: 320 $(M + H)^+$. Anal. Calcd for $(C_9H_{14}IN_5)$: C, H, N.

[4-*tert*-Butyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl]methylamine (34). A mixture of compound **10** (30 mg, 0.11 mmol), 40% aqueous H_2NMe (5 drops), and 2-methoxyethanol (1 mL) was heated in a sealed tube to 110 °C for 16 h and was cooled, treated with 1 M NaOH (10 mL), and extracted with CH_2Cl_2 (2 × 25 mL). The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, concentrated, and chromatographed by elution with gradients of 2, 3.5, and 5% MeOH/concd NH_4OH (9:1) in CH_2Cl_2 to provide 27 mg (94%) of the title compound. ^1H NMR ($CDCl_3$, δ): 1.25 (s, 9H), 2.33 (s, 3H), 2.46 (t, J = 5.4 Hz, 4H), 2.94 (d, J = 4.7 Hz, 3H), 3.62 (m, 4H), 4.64 (bs, 1H), 5.87 (s, 1H). MS (DCI/NH_3) *m/z*: 264 $(M + H)^+$. Anal. Calcd for $(C_{14}H_{25}N_5 \cdot 0.09CH_2Cl_2)$: C, H, N.

4-Chloro-6-(4-methylpiperazin-1-yl)pyrimidin-2-ylamine (46). 2-Amino-4,6-dichloropyrimidine (15 g, 91 mmol) was treated with 1-methylpiperazine (10.1 g, 100 mmol), Et_3N (90 mL, 0.64 mol), and EtOH (185 mL), was heated to 80 °C overnight, was cooled, and was concentrated and partitioned between CH_2Cl_2 (100 mL) and 1 M NaOH (125 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (4 × 100 mL). The

combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, concentrated, and crystallized from EtOAc to provide 14.9 g (71%) of the title compound. ^{1}H NMR ($CDCl_3$, δ): 2.34 (s, 3H), 2.45 (m, 4H), 3.60 (m, 4H), 4.82 (s, 2H), 5.96 (s, 1H). MS (DCI/NH_3) *m/z*: 228 $(M + H)^+$. Anal. Calcd for $(C_9H_{14}ClN_5 \cdot 0.06CH_2Cl_2)$: C, H, N.

Example of Suzuki Conditions. 4-(4-Methylpiperazin-1-yl)-6-*p*-tolylpyrimidin-2-ylamine (64). A vial containing compound **46** (0.10 g, 0.44 mmol) was treated with 4-methylphenylboronic acid (90 mg, 0.66 mmol), $Pd(PPh_3)_4$ (51 mg, 0.044 mmol), 1,2-dimethoxyethane (1.3 mL), and 2 M Na_2CO_3 (0.48 mL, 0.97 mmol) and was degassed with a stream of N_2 for 1 min. The vial was sealed and was heated to 90 °C for 16 h, was cooled, was treated with 1 M NaOH, and was extracted with CH_2Cl_2 (2 × 25 mL). The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by preparative HPLC on a Waters Symmetry C8 column (40 × 100 mm^2, 7 μm particle size) by the use of a gradient of 10−100% acetonitrile and 0.1% aqueous TFA over 12 min (15 min run time) at a flow rate of 70 mL/min. The purified compound was converted to the free base by chromatography on silica gel by elution with 2, 3.5, and 5% MeOH/concd NH_4OH (9:1) in CH_2Cl_2 to provide 94 mg (75%) of the title compound. ^{1}H NMR ($CDCl_3$, δ): 2.34 (s, 3H), 2.39 (s, 3H), 2.48 (t, J = 5.4 Hz, 4H), 3.68 (t, J = 5.1 Hz, 4H), 4.83 (bs, 2H), 6.33 (s, 1H), 7.23 (d, J = 8.1 Hz, 2H), 7.80 (d, J = 8.5 Hz, 2H). MS (DCI/NH_3) *m/z*: 284 $(M + H)^+$. Anal. Calcd for $(C_{16}H_{21}N_5 \cdot 0.06CH_2Cl_2)$: C, H, N.

Example of Ullmann Conditions. 4-(4-Methylpiperazin-1-yl)-6-(4-phenylimidazol-1-yl)pyrimidin-2-ylamine (95). A mixture of compound **15** (160 mg, 0.5 mmol), 4-phenylimidazole (87 mg, 0.6 mmol), CuI (25 mg, 0.13 mmol), and K_2CO_3 (90 mg, 0.65 mmol) in DMF (0.5 mL) was heated to 135 °C overnight, was cooled, was treated with 1 M NaOH (10 mL), and was extracted with CH_2Cl_2 (3 × 25 mL). The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by preparative HPLC on a Waters Symmetry C8 column (40 × 100 mm^2, 7 μm particle size) by the use of a gradient of 10−100% acetonitrile and 0.1% aqueous TFA over 12 min (15 min run time) at a flow rate of 70 mL/min. The purified compound was converted to the free base via chromatography (2, 3.5, and 5% MeOH/NH_4OH (9:1) in CH_2Cl_2) to provide 87 mg (52%) of the title compound. ^{1}H NMR ($CDCl_3$, δ): 2.36 (s, 3H), 2.50 (t, J = 5.1 Hz, 4H), 3.69 (t, J = 4.7 Hz, 4H), 4.88 (bs, 2H), 5.93 (s, 1H), 7.27 (m, 1H), 7.40 (t, J = 7.5 Hz, 2H), 7.81−7.87 (m, 3H), 8.34 (d, J = 1.4 Hz, 1H). MS (DCI/NH_3) *m/z*: 336 $(M + H)^+$. Anal. Calcd for $(C_{18}H_{21}N_7 \cdot 0.25CH_2Cl_2 \cdot 0.5CH_3OH)$: C, H, N.

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Supporting Information Available: Combustion analysis, full synthetic experimental section, and description of biological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Compound **4** was screened against a panel of 26 kinomes and was found to be inactive (IC_{50} > 10 μM). Compound **4** was also found to not interact with opioid receptors and COX enzymes, human and mouse P_2X_7 channels (IC_{50} > 100 μM), T-type voltage-gated calcium channels (IC_{50} > 10 μM), NMDA receptor (IC_{50} > 100 μM), neuronal nicotinc receptor (K_i > 100 μM), and cannabinoid (K_i > 10 μM).

(26) Statisical analysis: 95% confidence intervals for 5-HT sites and upper and lower limits based on the SEM for the α_2 subtypes. 5-HT_{1A}: 0.042 (0.037−0.047), 5-HT_{1D}: 0.123 (0.11−0.14), 5-HT_{2A}: 4.08 (3.8−4.4), 5-HT_{2C}: 2.97 (1.7−5.2), 5-HT_7: 5.88 (5.6−6.2), α_{2A}: 0.074 (0.086, 0.064), α_{2C}: 0.288 (0.398, 0.209).

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