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Triamino pyrimidines and pyridines as histamine H_4 receptor modulators

Steven P. Meduna *, Brad M. Savall, Hui Cai, James P. Edwards, Robin L. Thurmond, Patricia M. McGovern

Johnson & Johnson Pharmaceutical Research & Development, L.L.C., 3210 Merryfield Row, San Diego, CA 92121, United States

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ABSTRACT

Two series of triamino pyrimidines and a series of triamino pyridines have been synthesized and their structure–activity relationships evaluated for activity at the H_4 receptor in competitive binding and functional assays. Small structural changes in these three hetereoaromatic cores influenced the functional activity of these compounds.

The histamine H_4 receptor is a 390 amino acid G-protein coupled receptor that is implicated in the treatment of inflammatory diseases such as asthma and allergic rhinitis based on the expression of the H_4 receptor on eosinophils, mast cells, dendritic cells and other leukocytes.[1,2] Previous reports from these laboratories have described a series of H_4 receptor selective antagonists that include JNJ 7777120 (**1**) and JNJ 10191584 (**2**).[3,4] Both ligands possess a basic amine linked to a heterocycle, a motif prevalent in known histamine ligands.[5] In an effort to find other heteroaromatic replacements for (**1**) and (**2**), we began investigating triamino pyrimidines[6] (**3**) with diamine sidechains. After surveying a series of diamines, the more active piperazine and aminopyrrolidine diamines became the focus of additional SAR. Small structural changes led to divergent functional activity within a series. Furthermore, other triamino pyrimidine regioisomers[6] and triamino pyridines were explored to evaluate the placement and role of the ring nitrogen (Fig. 1).

Scheme 1 describes the synthetic route used to make the triamino 1,3 pyrimidine core. Commercially available 2-amino-4,6-dichloropyrimidine (**4**) was used as the starting material. Symmetry allowed for either the desired primary or secondary amine or a diamine (*N*-methylpiperazine or (*R*)-3-methylaminopyrrolidine) to be incorporated first. In practice, adding 1 equiv of the primary or secondary amine, followed by the diamine was the preferred sequence, as the primary amines were difficult to add to the deactivated diamino substituted chloropyrimidine.

Scheme 2 outlines the syntheses of the triamino 1,5 pyrimidine and triamino pyridine cores. In the case of 4-amino-2,6-dichloro-

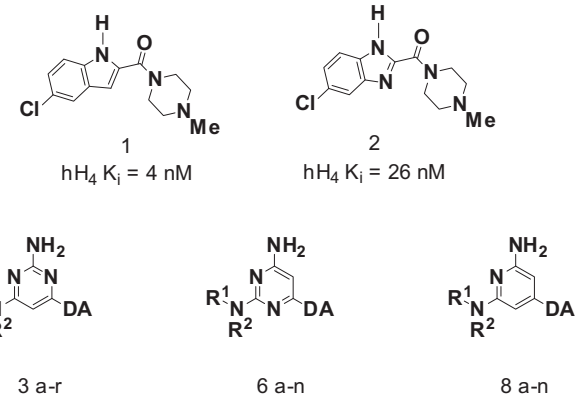
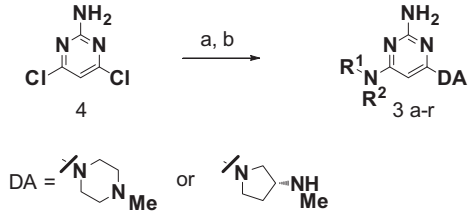


Figure 1. H_4 receptor selective antagonists. R^1R^2N and DA are defined by Tables 1–4.



Scheme 1. Reagents and conditions: (a) R^1R^2NH, DIEA, IPA, 100–160 °C, 1–2 h microwave (μW); (b) diamine, DIEA, IPA, 100–160 °C, 1–2 h (μW), 10–74% combined a and b steps.

* Corresponding author.
E-mail address: smeduna@its.jnj.com (S.P. Meduna).

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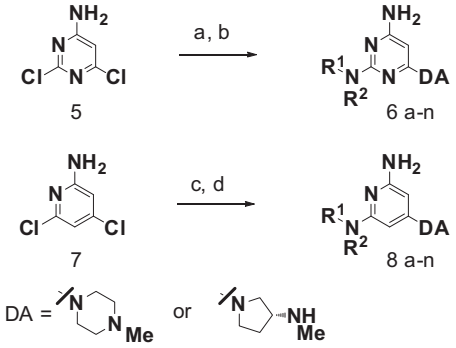
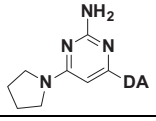


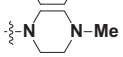
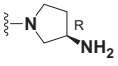
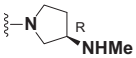
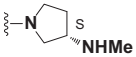
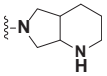
Scheme 2. Reagents and conditions: (a) R^1R^2NH, DIEA, IPA, 180 °C, 1 h (μW); (b) diamine, IPA, 100–180 °C, 1 h (μW), 13–50% combined a and b steps; (c) R^1R^2NH (neat), 160 °C, 8–15 h (μW); (d) diamine, $Yb(OTf)_2$, IPA, 160 °C, 2 h (μW), 3–30% combined c and d steps.

pyrimidine (**5**), initial substitution occurred predominately in the 2 position, so the primary or secondary amine was driven in first, followed by the diamine. Substitution of 2-amino-4,6-dichloropyridine (**7**) with primary amines required microwave heating in the neat amine for several hours. The diamine addition to the disubstituted chloropyridine required Lewis acid activation by adding 1.3 equiv of ytterbium triflate to obtain better yields.

The structure–activity relationship of the triamino 1,3 pyrimidine series is shown in Tables 1 and 2. A number of diamines were screened in the triamino 1,3 pyrimidine series, but the focus of this disclosure will be on molecules derived from the *N*-methylpiperazine and (*R*)-3-methylaminopyrrolidine.The initial compound made in this series contained a pyrrolidine ring and the *N*-methylpiperazine moiety (previously determined as a potent amine terminus in the indole and benzimidazole series)[1,2] on the pyrimidine core (**3b**, K_i = 6 nM). Further analogs indicated that switching from a tertiary amine to a secondary amine resulted in increased activity (**3g** vs **3h**). In this series, the *N*-methylpiperazine diamine displayed up to 9 fold better H_4 binding over the 3-methylaminopyrrolidine diamine, regardless of the 6-positon substitution.

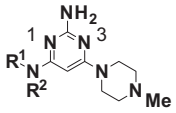
Table 1
Diamine scan on triamino 1,3 pyrimidine core

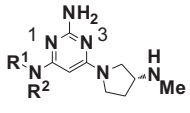


Compd #[f]	DA	hH_4 K_i[a,e] (nM)	EC_{50} nM (α)[b]	pA2[b,c,d]
3a		8	>10,000	N/D
3b		6	>10,000	8.7
3c		64	N/D	N/D
3d		36	>10,000	8.1
3e		83	N/D	N/D
3f		40	N/D	N/D

[a] Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[8]
[b] Compounds with K_i >100 nM not tested in functional assays.
[c] Compounds with α > 0.40 were not tested in the pA2 assay.
[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor.
[e] Unless noted chiral compounds were tested as racemates. Detailed experimental for EC_{50} and pA_2 determinations included in Ref. 9. N/D = not determined.
[f] All compounds were at least 95% pure with the majority being greater than 98% pure.

Table 2
Triamino 1,3 pyrimidine series


3b, 3g-3l


3d, 3m-3r

R^1R^2N	#[b]	hH_4 K_i[a] (nM)	#	hH_4 K_i [a] (nM)
	**3g**	3	**3m**	3
	**3h**	24	**3n**	51
	**3i**	2	**3o**	4
	**3b**	6	**3d**	36
	**3j**	1	**3p**	9
	**3k**	20	**3q**	58
	**3l**	25	**3r**	44

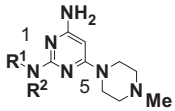
[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[8]
[b] All compounds were at least 95% pure with the majority being greater than 98% pure.

Investigations of an alternate triamino pyrimidine regioisomer (herein referred to as the triamino 1,5 pyrimidines) led to a differentiated series (Table 3). Unlike the triamino 1,3 pyrimidine series, the triamino 1,5 pyrimidine series favored the 3-methylaminopyrrolidine diamine over the *N*-methylpiperazine diamine in H_4 binding affinity. The trend of more favorable activity of the substituted secondary amines over tertiary amines continued in this series (**6a** vs **6b**), though generally this series was less active. Such divergent SAR in closely related series suggests that the two series may have different binding modes.[7]

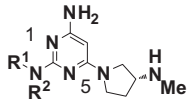
The role of the pyrimidine nitrogen was further explored by making a series of substituted triamino pyridines (Table 4). Relative to the triamino pyrimidines, the H_4 binding affinity of the triamino pyridines was comparable or better. The other pyridine regioisomer, displaying a ring nitrogen in the two position in reference to the diamine, resulted in an inactive compound (data not shown). Again, as in the triamino 1,5 pyrimidine series, the 3-methylaminopyrrolidine diamine was favored over the *N*-methylpiperazine diamine. As in both triamino pyrimidine series, substituted secondary amines displayed higher receptor affinity over tertiary amines.

The differences between the three series were not only evident in the H_4 binding affinities; they were also present in the functional activity of these compounds. Tables 5 and 6 show a comparison of functional activity of several triamino 1,3 pyrimidines, triamino 1,5 pyrimidines and triamino pyridine compounds as measured by the EC_{50} and pA2 data. All compounds containing the 3-methylaminopyrrolidine diamine tested in each of the three series behaved as functional antagonists against the H_4 receptor

Table 3
Triamino 1,5 pyrimidine series



6a-6g

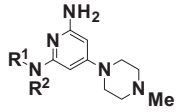


6h-6n

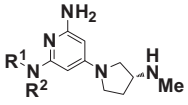
R^1R^2N	# [b]	hH_4 K_i [a] (nM)	#	hH_4 K_i [a] (nM)
	**6a**	162	**6h**	6
	**6b**	853	**6i**	140
	**6c**	15	**6j**	1
	**6d**	132	**6k**	127
	**6e**	10	**6l**	6
	**6f**	1076	**6m**	69
	**6g**	1155	**6n**	255

[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[8]
[b] All compounds were at least 95% pure with the majority being greater than 98% pure.

Table 4
Triamino pyridine series



8a-g



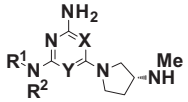
8h-n

R^1R^2N	# [b]	hH_4 K_i [a] (nM)	#	hH_4 K_i [a] (nM)
	**8a**	28	**8h**	3
	**8b**	189	**8i**	29
	**8c**	2	**8j**	0.2
	**8d**	100	**8k**	77
	**8e**	3	**8l**	0.9
	**8f**	148	**8m**	50
	**8g**	0.3	**8n**	0.04

[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[8]
[b] All compounds were at least 95% pure with the majority being greater than 98% pure.

(Table 5). The *N*-methylpiperazine substituted diamines, however, did display variable functional activity (Table 6). All of the secondary amine substituted triamino 1,5 pyrimidines (e.g., **6c** and **6e**) and triamino pyridines (e.g., **8a**, **8e**, and **8c**) displayed partial agonist activity. The only triamino pyridine with *N*-methylpiperazine substitution that behaves as a full antagonist was the tertiary

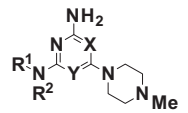
Table 5
Functional activity of the 3-methylaminopyrrolidine compounds



#[e]	R^1R^2N	X	Y	K_i [a] (nM)	EC_{50} [b] (nM)	α [c]	pA2 [d]
8h		CH	CH	3	>10,000	––	8.8
3m		N	CH	3	>10,000	––	8.0
6h		CH	N	6	>10,000	––	7.9
8l		CH	CH	0.9	>10,000	––	*
3p		N	CH	9	>10,000	––	9.4
6l		CH	N	6	>10,000	––	7.9
8j		CH	CH	0.2	>10,000	––	8.8
3o		N	CH	4	>10,000	––	9.3
6j		CH	N	1	>10,000	––	8.8
8k		CH	CH	77	>10,000	––	7.8
3d		N	CH	36	>10,000	––	8.1

[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[8]
[b] Compounds with K_i >100 nM not tested in functional assays.
[c] values determined relative to histamine as a control with histamine assigned α = 1.0. Compounds with α >0.40 were not tested in the pA2 assay.
[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor. Detailed experimental for EC_{50} and pA_2 determinations included in Ref. 9.
[e] All compounds were at least 95% pure with the majority being greater than 98% pure.
* Displays possible insurmountable antagonism.

Table 6
Functional activity of the *N*-methylpiperazine compounds



#[e]	R^1R^2N	X	Y	K_i [a] (nM)	EC_{50} [b] (nM)	α [c]	pA2 [d]
8a		CH	CH	28	533	0.6	––
3g		N	CH	3	12	0.57	––
6a		CH	N	162	––	––	––
8e		CH	CH	3	11	0.73	––
3j		N	CH	1	101	0.46	––

(*continued on next page*)

Table 6 (*continued*)

#[e]	R^1R^2N	X	Y	K_i[a] (nM)	EC_{50}[b] (nM)	α[c]	pA2[d]
6e		CH	N	10	16	0.63	––
8c		CH	CH	2	50	0.51	––
3i		N	CH	2	>10,000	––	8.9
6c		CH	N	15	86	0.71	––
8d		CH	CH	100	>10,000	––	7.7
3b		N	CH	6	>10,000	––	––
3h		N	CH	24	>10,000	––	––

[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor. K_i values are the geometric mean of three or more independent determinations and calculated according to Cheng and Prusoff.[8]

[b] Compounds with K_i >100 nM not tested in functional assays.

[c] α values determined relative to histamine as a control with histamine assigned $\alpha = 1.0$. Compounds with α >0.40 were not tested in the pA2 assay.

[d] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor. Detailed experimental for EC_{50} and pA_2 determinations included in Ref. 9.

[e] All compounds were at least 95% pure with the majority being greater than 98% pure.

amine (**8d**). The triamino 1,3 pyrimidines, on the other hand, displayed either antagonism (e.g., **3b**, **3h**, and **3i**) or partial agonism (e.g., **3g** and **3j**) again setting this series apart from the other two.

Comparison of the three heteroaromatic cores demonstrated a rank order potency (triamino pyridines > triamino 1,3 pyrimidines > triamino 1,5 pyrimidines) among the three cores. Additionally the relative potency of 3-methylaminopyrrolidine versus *N*-methylpiperazine was dependant on the core, where *N*-methylpiperazine was favored for the triamino 1,3 pyrimidines and *N*-methylpyrrolidine was favored for the triamino 1,5 pyrimidine and the triamino pyridine cores. However, the functional activity against the H_4 receptor was dependant on both the nature of the diamine and the heteroaromatic core. The use of 3-methylamino pyrrolidine resulted in antagonists across all three cores. Finally, with *N*-methyl piperazine the pyridines and triamino 1,5 pyrimidines behaved as partial agonists while the triamino 1,3 pyrimidines showed a mix of partial agonists and antagonists.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.017.

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6. For simplicity between the pyrimidine regioisomers, compounds where the NH_2 group is between the ring nitrogens are referred to as triamino 1,3 pyrimidines while analogues of the other regioisomer are referred to as triamino 1,5 pyrimidines.
7. (a) Jojart, B.; Kiss, R.; Viskolcz, B.; Keseru, G. M. *J. Chem. Inf. Model.* **2008**, *48*, 1199–1210; (b) Igel, P.; Geyer, R.; Strasser, A.; Dove, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2009**, *52*, 6297.
8. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, 22.
9. *Details for EC_{50} and pA_2 assays*: Mouse and human H4 were cloned into the pCINeo mammalian expression vector and transfected into the human neuroblastoma SK-N-MC cell line. The construct contains the reporter gene B galactosidase under the control of cyclic AMP responsive element. In the EC_{50} assay, the compounds are added to the media and allowed to incubate for 10 min at room temperature before the addition of forskolin (5 μM final concentration). In the pA2 assay, the cells are preincubated with compound for 10 min, then incubated with agonist for 10 min before the addition of forskolin. After a 6 h incubation (for both assays) at 37 °C, the media is aspirated and the plates are stored at −40 °C overnight. Cells are lysed with 25 μL of 0.1× assay buffer (10 mM Na phosphate, pH 8, 0.2 mM $MgSO_4$, 0.01 mM $MnCl_2$) and incubated at room temperature for 10 min. Cells are then incubated with 100 μL of 1× assay buffer including 0.5% Triton X-100 and 40 mM B mercaptoethanol for 10 min. 25 μL of substrate solution (1 mg/mL chlorophenolred B–D galactopyranoside) was added and the color was quantitated at an absorbance of 570 nm. α values are calculated using the full agonist (positive control) as the standard of 1.