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Discovery and SAR of 6-Alkyl-2,4-diaminopyrimidines as Histamine H_4 Receptor Antagonists

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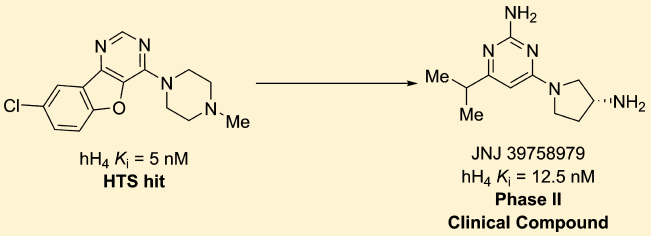
ABSTRACT: This report discloses the discovery and SAR of a series of 6-alkyl-2-aminopyrimidine derived histamine H_4 antagonists that led to the development of JNJ 39758979, which has been studied in phase II clinical trials in asthma and atopic dermatitis. Building on our SAR studies of saturated derivatives from the indole carboxamide series, typified by JNJ 7777120, and incorporating knowledge from the tricyclic pyrimidines led us to the 6-alkyl-2,4-diaminopyrimidine series. A focused medicinal chemistry effort delivered several 6-alkyl-2,4-diaminopyrimidines that behaved as antagonists at both the human and rodent H_4 receptor. Further optimization led to a panel of antagonists that were profiled in animal models of inflammatory disease. On the basis of the preclinical profile and efficacy in several animal models, JNJ 39758979 was selected as a clinical candidate; however, further development was halted during phase II because of the observation of drug-induced agranulocytosis (DIAG) in two subjects.



INTRODUCTION

Histamine receptors are a class of GPCR targets with a successful history of therapeutic pharmacological intervention.[1,2] The classical anti-histamines target the H_1 receptor, and this drug class provides relief to many allergy sufferers worldwide.[3] Likewise, the role of H_2 antagonists in attenuating gastric acid secretion has aided many patients with GI disorders.[4] More recently, high-affinity histamine receptors, H_3,[5] and H_4,[6] were discovered, and ongoing work by several groups is dedicated to determining the mechanism of action and therapeutic potential of these novel histamine receptors.[7] The H_3 receptor is expressed predominantly in the CNS[8] and is implicated in the sleep−wake cycle[9] and in memory formation and alertness,[10,11] whereas the histamine H_4 receptor (H_4R) is expressed predominantly on cells of the immune system.[1,12,13] Preclinical animal studies demonstrated that antagonists of H_4R could be useful in treating diseases such as asthma, rheumatoid arthritis, and atopic dermatitis as well as other immune-driven inflammatory disorders.[7,14] Significant efforts from both industry and academia have targeted H_4R with the goal of discovering new medicines for immune-mediated inflammatory processes.[15−17]

The prototypical histamine H_4 antagonist, JNJ 7777120 (**1**),[18] is one of the most studied compounds in the histamine H_4 field and has been referred as a state-of-the-art[19] ligand that continues to be used to study H_4R. However, JNJ 7777120 has a short half-life because of rapid demethylation of the terminal *N*-methyl piperazine, therefore limiting the potential of JNJ 7777120 as a therapeutic agent. Over the past few years, reports from several laboratories disclosed the discovery of alternative histamine H_4R antagonists, with many focusing on amino-substituted heterocycles.[19−27] Beginning with independent screening hits, several laboratories have converged on the amino pyrimidine template.[15] In 2011, we disclosed a class of tricyclic aminopyrimidine[21] antagonists (**4**) followed by results on triaminoazine-derived[20] antagonists. In the course of our work, we discovered a series of 6-alkyl-2,4-diaminopyrimidine H_4R antagonists, culminating in the discovery of JNJ 39758979 (**5**), which advanced into human clinical trials. This report provides a brief description of our SAR around this template and focuses on the preclinical characterization of JNJ 39758979 and related analogues.

RESULTS AND DISCUSSION

Lead Identification and Analogue Design. Initial high-throughput screening (HTS) of the H_4 receptor identified several promising series (Figure 1), the most tractable of which are the three structurally distinct series: indole/benzimidazole carboxamides typified by JNJ 7777120 (**1**),[28] 2-aryl-benzimidazoles (**2**),[29−31] and tricyclic pyrimidines (**3**). Our first report on the pyrimidine series of H_4R antagonists focused on the discovery of the tricyclic aminopyrimidines, resulting in JNJ 40279486 (**4**) as a novel and highly selective H_4 receptor ligand with desirable pharmacokinetic (PK) properties and selectivity over the hERG channel.[21]

We previously demonstrated that the tricyclic series (**3**) served as a constrained version of the carboxamides (**1**) (Figure 2a), where alignment of the distal nitrogen atoms provides for overlap of the aryl rings. Additionally, chlorine substitution on the aromatic ring increased affinity in this series, a trend shared with the carboxamides. Moreover, we learned that the 2-

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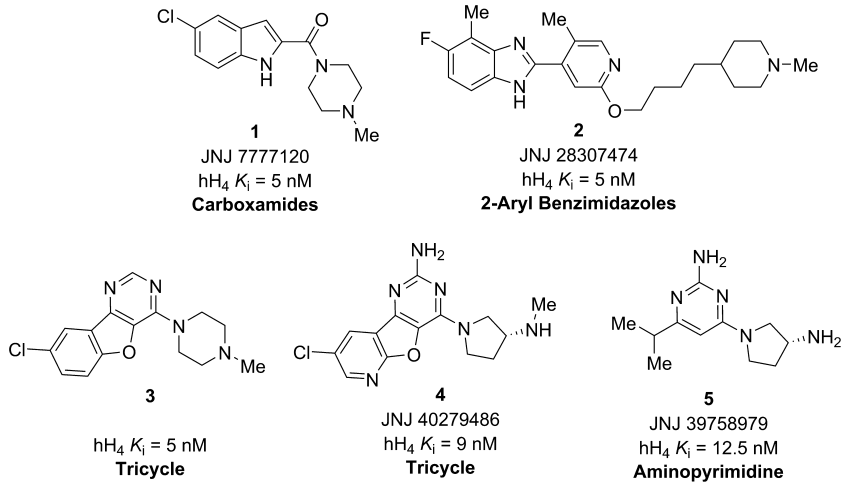

Figure 1. Lead series of histamine H_4 receptor antagonists.

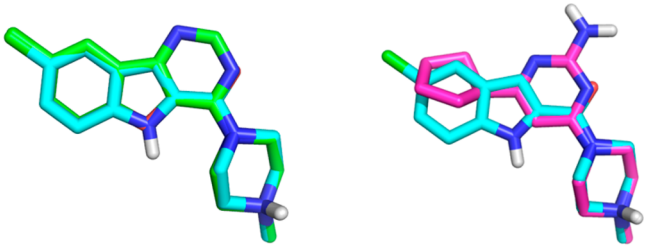

Figure 2. Overlap of carboxamide (**1**; cyan) with tricycle (**3**; green) and 6-alkyl-2-aminopyrimidine (**6**; magenta).

aminopyrimidines (**6**) could tolerate a much wider range of diamines versus their analogous 2-*des*-amino counterparts. This increased diamine tolerance hinted that the 2-aminopyrimidine chemotype (**6**) may be interacting with the receptor in a unique fashion relative to the carboxamide series (**1**), especially because the 2-amino group does not directly map onto any of the atoms of the carboxamide series (e.g., Figure 2b).

During the course of our benzimidazole/indole carboxamide work, we discovered that compounds containing a saturated ring (e.g., **9** and **10**) in place of the aryl ring (**1** and **7**) maintained significant affinity for the H_4 receptor (Figure 3). The increased diamine tolerance of the 2-aminopyrimidines coupled with the receptors tolerance for alkyl groups in that region prompted us to investigate the 6-alkyl-2-amino-pyrimidines series of H_4 modulators represented by compound **11**.

Chemistry and SAR Relationships. Synthetically, the amino pyrimidines were assembled by condensation of appropriately functionalized *β*-keto esters with guanidine hydrochloride in the presence of base to furnish the 4-hydroxypyrimidines (Scheme 1). Conversion of the hydroxypyrimidine (**12a**) to the chloride (**13a**) with neat $POCl_3$ was complicated by the formation of 2-aminophosphonic acid derivatives (**14a**). Fortunately, the use of Et_4NCl as an exogenous chloride source with $POCl_3$ in acetonitrile resulted in very clean formation of the desired chloro intermediates (e.g., **13a**).[32] Optimization of the stoichiometry of $POCl_3$ showed that excess $POCl_3$ or increasing temperature was detrimental to the yield of the desired product. Ultimately, a 20% excess of $POCl_3$ in acetonitrile delivered the desired chloride (**13a**) in >90% yield with none of the chlorophosphamide (**14a**) detected by standard analytical techniques (Figure 4). Alternately, the amines could be condensed with the pyrimidinone using (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) as a dehydrating reagent (Scheme 2).[33] Analogues containing a C-6 methyl group were prepared from commercially available 2-amino-4-chloro-6-methyl pyrimidine. In cases where BOC-protected

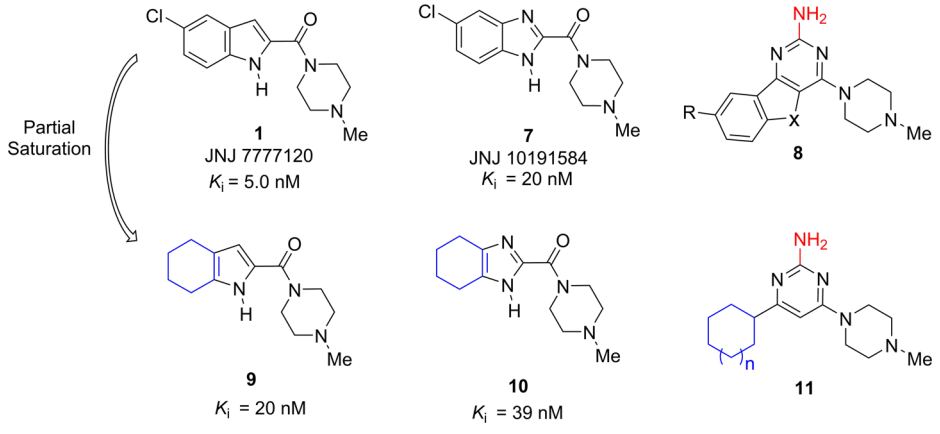

Figure 3. Alkyl substitution.

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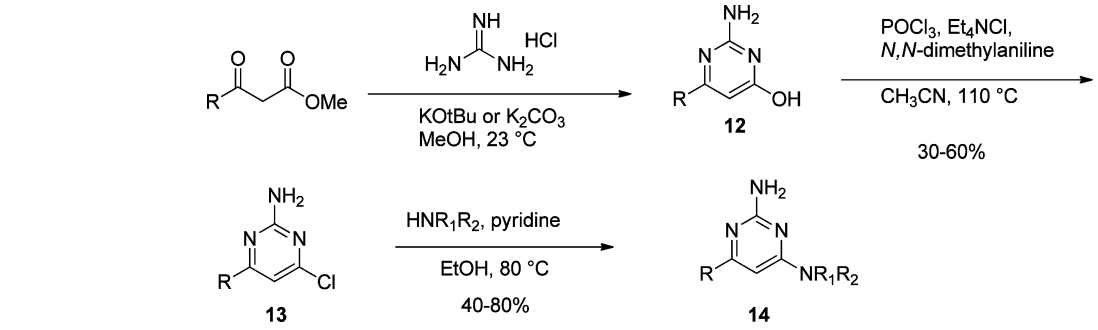
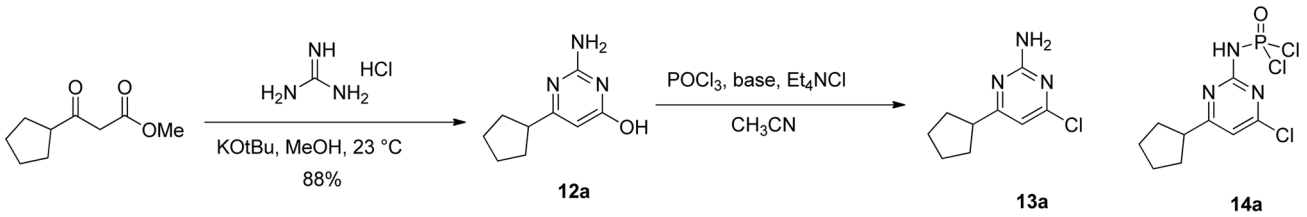
Scheme 1. Synthesis of 2-Amino-6-alkylpyrimidines

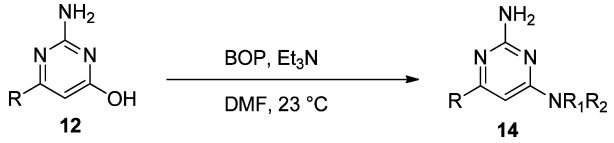




$POCl_3$	Base (eq)	Et_4NCl	Temp °C	Time (min)	Yield 13a	Yield 14a
6.0	*i*Pr_2NEt (1.0)	2.0	110	10	0%	52%
6.0	*N*,*N*-diethylaniline (1.0)	2.0	110	12	37%	51%
1.2	*N*,*N*-diethylaniline (1.0)	2.0	23 - 90	60	49%	30%
2.0	*N*,*N*-diethylaniline (1.0)	2.0	50	60	70%	10%
1.2	*N*,*N*-diethylaniline (0.1)	2.0	40	90	92%	N/A

Figure 4. Optimization of chlorination of 2-aminopyrimidines.

Scheme 2. Synthesis of 2-Amino-6-alkylpyrimidines with BOP Coupling



amines were used, the BOC group was removed using an excess of HCl(aq) in formic acid.

With a straightforward synthesis of the pyrimidine chloride/hydroxypyrimidines, we were able to scan both the 4-position diamine and the 6-position alkyl group efficiently. Table 1 shows a scan of several amines and their activities, with piperazines (**6** and **15**), (*R*)-3-amino pyrrolidines (**16** and **18**), and bicyclic 3-amino pyrrolidine (**20** and **21**) derivatives providing affinities ranging from 0.9−2.9 nM, whereas the (*S*)-3-amino pyrrolidines (**17** and **19**) were less potent than the (R) enantiomer. Reducing the ring size to 2-amino cyclobutylamine (**20**) showed a decrease in affinity (K_i = 5.5 nM) relative to the (*R*)-aminopyrrolidine, whereas increasing the ring size to the 3-amino-piperidine (**21**) reduced affinity further (K_i = 257 nM). Both the 5,6- and the 5,5-fused aminopyrrolidine derivatives (**22** and **23**) were potent antagonists, whereas the symmetrical 5,5-derivative was 1 order of magnitude less potent for the *N*-Me derivative (**24**) and more than 2 orders of magnitude less potent for the NH analogue (**25**). The spirocyclic amine (**26**) was tolerated, whereas oxygenation of the amine portion reduced affinity (**27**−**29**).

Similar to the carboxamide JNJ 7777120, the in vitro metabolism assays indicated that the *N*-methyl piperazine (**6**) was a metabolic liability on the amino pyrimidine template, especially in rodents (data not shown). However, because the equipotent (*R*)-3-amino pyrrolidine derivatives (**16** and **18**) were more stable in in vitro metabolic assays, those diamines were used for subsequent SAR studies of the various alkyl derivatives surveyed in the 6 and 5 positions (Table 2).

A variety of linear and cycloalkyl groups were scanned, with the 6 position relatively tolerant of substitution from methyl to *t*-butyl (**30**−**43**) and even adamantyl (**44**) retaining good affinity, although medium-sized alkyls were preferred. With methyl substitution at the 6 position, addition of a methyl group to the 5 position (**45**) caused a loss of affinity. Likewise, a fused 5,6-cyclopentyl (**46**) was less active compared to the unfused cyclopentyl (**39**). However, when the ring size was increased to a fused 5,6-cyclohexyl ring (**47**), affinity was regained. The incorporation of electron-withdrawing substituents such as chloro (**48**) and trifluoromethyl (**49**) groups at the 6 position were not tolerated, which may allude to a role of the basicity of the pyrimidine nitrogen for affinity.

Compared to the indole carboxamide series, typified by JNJ 7777120, the 6-alkyl-2,4-diaminopyrimidines tolerated a range of diamines as well as a range of 6-alkyl substituents. Consequently, this series provided a good selection of potent compounds that possessed a range of affinity and acceptable ADME parameters, which made them suitable as in vivo candidates. Several of the compounds were worthy of more advanced profiling, and the remainder of this report will highlight these efforts with a focus on JNJ 39758979 (**5**).

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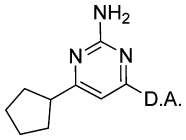
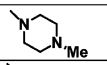
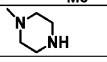
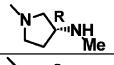
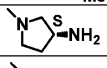
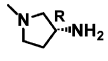
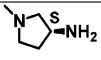
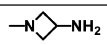
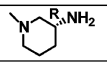
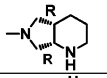
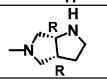
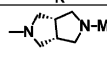
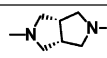
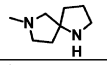
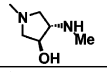
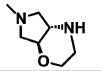
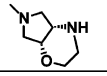
Table 1. Diamine Scan SAR of 2-Amino-6-cyclopentyl Pyrimidine

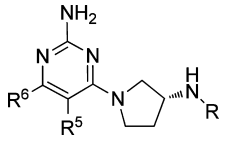


Entry	Diamine	hH4 K_i[a]	hH4 EC_{50}[b]	Alpha[c]	hH4 pA_2[d]
6		1.4	>10000	N/D	9.9
15		1.2	>10000	N/D	9.5
16		1.1	>10000	N/D	9.6
17		25	>10000	N/D	N/D
18		0.92	>10000	N/D	9.2
19		36	>10000	N/D	N/D
20		5.5	1300	0.14	7.6
21		257	N/D	N/D	N/D
22		1.8	>10000	N/D	>10
23		2.9	>10000	N/D	10.0
24		25	>10000	N/D	7.7
25		328	N/D	N/D	N/D
26		11.2	>10000	N/D	8.6
27		30	>10000	N/D	8.0
28		56	>10000	N/D	6.9
29		520	N/D	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 were calculated according to Cheng and Prusoff.[34] [b]Compounds with K_i > 100 nM were not tested in functional assays. [c]Compounds with α > 0.30 were not tested in the pA_2 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. N/D = not determined.

In Vivo Profiling of Lead Molecules. Table 3 shows a comparison of several of three of the more extensively profiled molecules. One of the first compounds that progressed into PK studies was compound **15**, a potent inhibitor with a hK_i = 1.2 nM and a corresponding pA_2 = 9.5. The compound was stable in metabolic assays with a $t_{1/2}$ >100 min in human microsomes and 86 min in rat microsomes and showed no interaction with

Table 2. Left-Side Substituent SAR of 2-Amino-6-alkyl Pyrimidines



entry	R	substituent (R^5, R^6)	hH4 K_i[a]	hH4 EC_{50}[b]	alpha[c]	hH4 pA_2[d]
30	Me	6-methyl	77	N/D	N/D	N/D
31	Me	6-ethyl	21	>10 000	N/D	8.0
32	H	6-ethyl	21	>10 000	N/D	7.9
33	Me	6-*i*-propyl	12	>10 000	N/D	7.9
5	H	6-*i*-propyl	12.5	>10 000	N/D	7.9
34	Me	6-butyl	1.5	>10 000	N/D	8.4
35	Me	6-benzyl	36	>10 000	N/D	8.0
36	H	6-benzyl	100	N/D	N/D	N/D
37	Me	6-*c*-propyl	28	>10 000	N/D	8.1
38	Me	6-*c*-butyl	2.4	>10 000	N/D	9.4
39	Me	6-*c*-pentyl	1.1	>10 000	N/D	9.6
40	H	6-*c*-pentyl	0.92	>10 000	N/D	>10
41	Me	6-*c*-hexyl	2.3	>10 000	N/D	>10
42	Me	6-*t*-butyl	8.0	>10 000	N/D	8.6
43	H	6-*t*-butyl	9.4	>10 000	N/D	8.3
44	Me	6-adamantyl	1.7	>10 000	N/D	>10
45	Me	5,6-dimethyl	357	N/D	N/D	N/D
46	Me	5,6-*c*-pentyl	159	N/D	N/D	N/D
47	Me	5,6-*c*-hexyl	43	>10 000	N/D	7.6
48	Me	6-Cl	>10 000	N/D	N/D	N/D
49	Me	6-CF_3	>10 000	N/D	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 were calculated according to Cheng and Prusoff.[34] [b]Compounds with K_i > 100 nM were not tested in functional assays. [c]Compounds with α > 0.30 were not tested in the pA_2 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. N/D = not determined.

the hERG channel in a binding assay. There was a slight (21%) reduction in the delayed rectifier current (I_{Kr}) at 3 μM in hERG-transfected HEK293 cells, but there were no notable effects on multiple parameters of the isolated rabbit Purkinje fibers. When dosed in rats, this compound demonstrated a moderate C_{max} (0.3 μM), an oral $t_{1/2}$ of 9 h with a volume of distribution at the steady state (V_{ss}) of 2.7 l/kg/h, and bioavailability of F = 20%. The moderate C_{max} and low bioavailability were not predicted by the microsomal data, although alternate routes of clearance were not investigated at this point. Although compound **15** was a suitable lead molecule, it had two liabilities: it had appreciable affinity at the H_3 receptor (K_i = 24 nM) and it was a partial agonist in mouse (EC_{50} = 7.3 nM, α = 0.57), the species in which several efficacy studies would be run. Profiling of additional compounds (Table 3) provided a range of half-lives and distribution behaviors providing a suitable range of properties to profile these molecules using mouse in vivo efficacy models.

A comparison of compound **31** to JNJ 39758979 (**5**) indicates that **31** is superior to JNJ 39758979 from a PK standpoint when comparing i.v. and p.o. $t_{1/2}$, AUC, C_{max}, clearance, and bioavailability. Despite **31** having better PK parameters, JNJ 39758979 showed an increased PD effect in inhibition of LPS-induced TNFα release (Figure 5). As seen in

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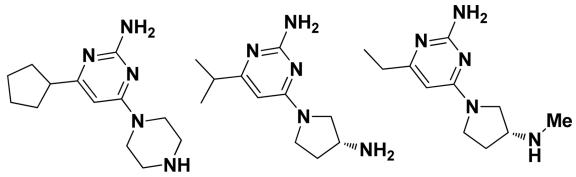
Table 3. Comparison of Lead Molecules



Assay	15	JNJ 39758979 (5)	31
Human H_4 K_i (nM)	1.2	12.5	21
Human H_4 pA_2	9.5	7.9	8.0
Mouse H_4 K_i (nM)	20	5.3	62
Mouse H_4 pA_2	8	8.3	7.3
Metabolism t ½ (Min)	hu >100 rat 86	hu >180 rat 61	hu >120 rat 63
Human H_3 K_i (nM)	24	1043	670
Human H_1		>1000	>1000
hERG PC (μM)	>10	>10	>10
CaCo (cm/s*10^6) A->B/B->A	32 / 16	23 / 22	54 / 14
PPB %free (h/r/m)	ND	81/83/76	26/ND/ND
Solubility (pH=7/pH=2)	>0.4 / >0.4	>0.4 / >0.4	ND / ND
Rat PK			
i.v. (2 mg/kg)			
$t_{1/2}$ (h)	1.1	2.1	5.3
AUC (μM*h)	5	1.4	11.0
Cl (L/h)	0.63	2.2	0.3
Vss (L/kg)	2.7	19.9	6.3
p.o. (10 mg/kg)			
%F	20	36	50
$t_{1/2}$ (h)	9	7.5	9.7
Cmax (μM)	0.31	0.3	1.5

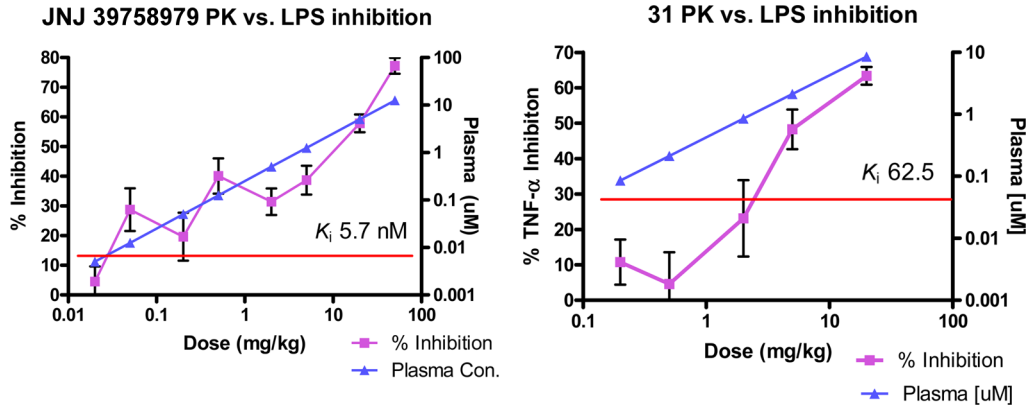


Figure 5. Comparison of JNJ 39758979 and **31** in LPS-induced TNFα inhibition to blood levels in the mouse.

Figure 5, JNJ 39758979 showed inhibition when plasma concentrations were at or above the mouse K_i, whereas **31** had plasma concentrations that were above the mouse K_i during then entire dose range but only showed inhibition at the high doses. The only parameter that we measured that could explain this difference is that JNJ 39758979 had a significantly higher volume of distribution relative to **31**. It should be noted that for **31** the volume of distribution was measured only in the rat and dog and in both cases is lower than for JNJ 39758979. After significant in vitro and in vivo characterization, JNJ 39758979 was found to be most efficacious in a range of in vivo models and was selected to move forward as the clinical candidate.

Characterization of JNJ 39758979. JNJ 39758979 is a selective, high-affinity histamine H_4 receptor antagonist with a K_i of 12.5 nM versus the human H_4 receptor and functionally antagonizes histamine-induced cAMP inhibition with a pA_2 of 7.9 in transfected cells. At the mouse H_4R, the K_i = 5.3 nM and the pA_2 = 8.3. At the monkey H_4R, the K_i = 25 nM and the pA_2 = 7.5. The affinity for the rat (K_i = 188 nM, pA_2 = 7.2) and guinea pig H_4R (K_i = 306 nM) is moderate, and JNJ 39758979 has little if any affinity for the dog H_4R ($K_i \geq 10$ μM). The compound is highly selective for H_4R, as it exhibits low affinity for the H_1, H_2, and H_3 receptors. In addition, JNJ 39758979 was screened through a broad panel of kinases, receptors, and channels without any significant cross-reactivity.[35] Furthermore, the compound was also selective for the mouse H_4 receptor over mouse H_1 and H_3 receptors (the mouse H_2 receptor was not studied). JNJ 39758979 was negative in the Ames test and showed no inhibition of CYP450 3A4, 2C9, 2D6, or 1A2 at concentrations of 50−100 μM. The compound did not inhibit astemizole binding to the hERG channel at concentrations up to 30 μM and showed no inhibition of the hERG-mediated K^+ current in transfected cells at concentrations up to 10 μM.

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JNJ 39758979 is metabolically stable ($t_{1/2}$ > 120 min) when incubated in vitro with human, rat, dog, or monkey liver microsomes. This compound exhibited excellent exposure, bioavailability, and half-life in mouse, rat, and dog (Figure 6 and

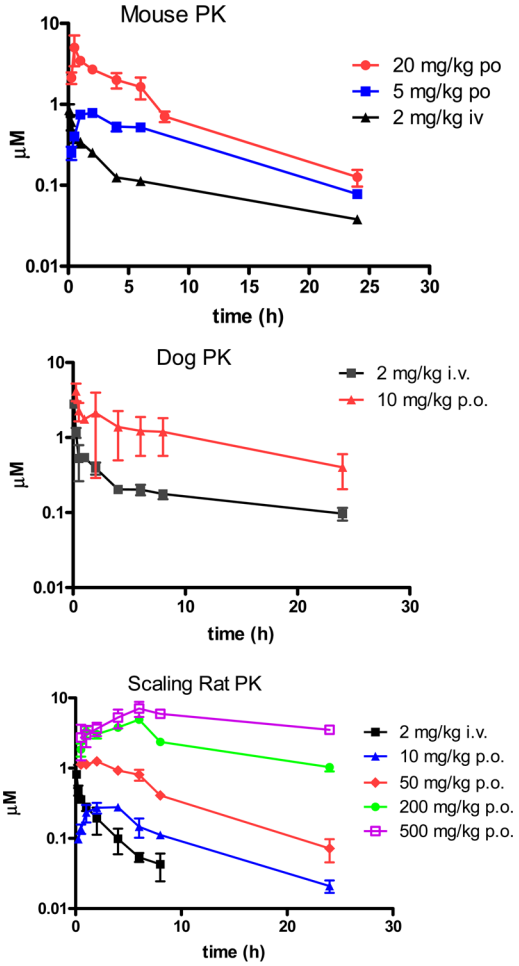

Figure 6. JNJ 39758979 PK curves in mouse and dog and scaling PK in rat.

Table 4). There was also no difference in pharmacokinetic properties between Balb/c mice (shown) and C57/Bl6 mice (data not shown). JNJ 39758979 showed dose-proportional PK in the rat in the range of 2−500 mpK (Figure 6).

The tissue distribution of JNJ 39758979 was determined following a single 5 mg/kg oral dose in mice. The brain, liver, and kidneys from three mice per time point were surgically removed up to 96 h after dosing to determine JNJ 39758979 concentrations with a liquid chromatography coupled to tandem mass spectrometry (LC−MS/MS) assay (lower limit of quantitation = 1 ng/g). JNJ 39758979 rapidly reached the kidneys and liver (mean t_{max} = 2.0 h). In comparison, distribution to the brain was slow (mean t_{max} = 6.0 h). The elimination of JNJ 39758979 was slow from the brain, liver, and kidneys, with mean $t_{1/2}$ values of 42.5, 22.3, and 20.5 h, respectively. The highest exposure (based on C_{max} and AUC_{0-inf} values) was observed in the liver followed by the kidney and brain. Tissue-to-plasma ratios for liver and kidney ranged from 23.2 to 95.8; the tissue-to-plasma ratios in brain increased with time from 0.256 to 22.7 up to 48 h after dosing.

The pruritic effect of histamine is well-documented, and recent reports have described the contribution of the H_4R to the itch induced by histamine and other pruritogens.[36] JNJ 39758979 was able to inhibit histamine-induced itch at doses of 5 and 20 mg/kg in mice to a similar extent as JNJ 7777120 (Figure 7). There is some indication that efficacy in this model requires that the compound reach the central nervous system. Although JNJ 39758979 crosses the blood-brain barrier, at the end point of the itch study, the brain concentration relative to the plasma concentration is low because of the delay in C_{max} for the brain, which may explain the shift in efficacy relative to the LPS-induced TNFα model.

Preclinical efficacy has also been reported for H_4R antagonist in models of arthritis.[37] JNJ 39758979 exhibits dose-dependent inhibition of the clinical score in a mouse collagen-induced arthritis model (Figure 8). The efficacy of a second H_4R antagonist, JNJ 28307474, is shown for comparison and is similar to that previously reported.[37]

In summary, JNJ 39758979 is a potent and selective H_4 receptor antagonist that shows good anti-inflammatory and antipruritic activity in vivo. In tolerability studies, JNJ 39758979 was well-tolerated in rats and dogs and no systemic toxicity risks were identified.

CONCLUSIONS

Taking a cue from two classes of H_4R antagonists, the saturated carboxamide derivatives and the tricyclic aminopyrimidines, we arrived at the 6-alkyl-2,4-diaminopyrimidine class of H_4R antagonists. The 6-alkyl-2,4-diaminopyrimidine class of antagonists is particularly noteworthy in the field of H_4 receptor pharmacology because they behave as antagonists at both the human and the rodent H_4 receptor. During the course of these investigations, we selected JNJ 39758979 as a clinical candidate on the basis of the desirable in vitro and in vivo profile in several animal PK/PD models. JNJ 39758979 was studied in phase II clinical trials in asthma and atopic dermatitis; however, further clinical progression was halted during phase II because of the observation of idiosyncratic drug-induced agranulocytosis (DIAG) in two subjects (NCT01497119). DIAG is an idiosyncratic drug reaction occurring across many classes of drugs due to off-target effects.[38] The mechanisms are still not completely understood for any of the drugs that cause DIAG, but it is postulated that this is due to off-target effects of JNJ 39758979 such as the formation of reactive metabolites and or intermediates, as has been shown for other drugs that cause agranulocytosis.[39]

EXPERIMENTAL SECTION

Histamine-Induced Itch in Mice. The model of histamine-induced scratching in C57/bl6 mice (n = 6−8 per group) was used to judge the antipruritic effects of JNJ 39758979 as previously described.[36] The compound was given p.o. in 20% hydroxypropyl-β-cyclodextran 30 min before an intradermal injection of histamine (100 μg). Bouts of scratching were calculated using an automated system. Immediately after histamine injection, mice were placed in containers above a solenoid, and magnets previously placed on the mouse ear generated scratch-specific signals that were counted over a 20 min time span.

LPS-Induced TNFα in Mice. The effect of JNJ 39758979 on LPS-induced TNFα production was studied as previously described.[40] In each experiment, mice (Balb/c, n = 7−9 per group) were dosed with JNJ 39758979 p.o. in 20% hydroxypropyl-β-cyclodextran 30 min before on i.p. administration of 200 μL of 0.1 mg/mL LPS. Plasma was collected 2 h later, and the levels of TNFα were quantitated by ELISA.

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Table 4. Pharmacokinetic Data

compound	species	route (dose)	t_{max} (h)	C_{max}[a] (μM)	AUC (h μM)	Cl (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	% F
JNJ 39758979 (**5**)	Balb/c mice	i.v. (2 mg/kg)		1.02	3.27	2.4	31.00	7.8	
		p.o. (5 mg/kg)	2	0.79	9.69			6.8	84
		p.o. (20 mg/kg)	0.50	5.03	24.60			4.7	75
	Sprague-Dawley rats	i.v. (2 mg/kg)		1.1	1.4	5.5	19.9	2.1	
		p.o. (10 mg/kg)	2.4	0.3	2.6			5.7	38
		p.o. (10 mg/kg)	2.00	0.26	2.43			9.41	35
		p.o. (50 mg/kg)	2.00	1.25	13.39			5.45	38
		p.o. (200 mg/kg)	8.00	4.91	71.25			6.16	51
		p.o. (500 mg/kg)	8.00	7.07	184.15			12.00	53
	Beagle dogs	i.v. (2 mg/kg)		4.22	6.7	1.4	19.9	10.2	
		p.o. (10 mg/kg)	0.8	3.9	31.6			11.2	95
15	Sprague-Dawley rats	i.v. (2 mg/kg)		25	20.55	0.422	3.1	2.2	
		p.o. (10 mg/kg)	2	0.6	8.2			8.7	20.7
	Beagle dogs	i.v. (2 mg/kg)		1.76	5.38	21.19	24.42	10.77	
		p.o. (10 mg/kg)	4	1.49	30.8			9.01	114.5
35	Balb/c mice	p.o. (20 mg/kg)	0.5	8.5	60			5.2	NA
	Sprague-Dawley rats	i.v. (2 mg/kg)		19	11	0.33	6.3	5.3	
		p.o. (10 mg/kg)	5	1.6	27.8			9.3	50
	Beagle dogs	i.v. (2 mg/kg)		2.5	8.1	15.5	15.1	9.3	
		p.o. (10 mg/kg)	0.6	4.2	45			10	112

[a] C_0 for i.v.

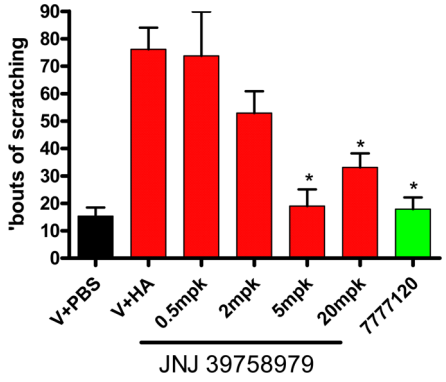

Figure 7. JNJ 39758979 inhibits histamine-induced itch.

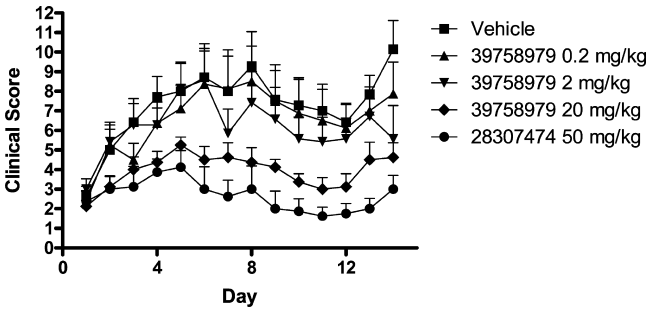

Figure 8. JNJ 39758979 is efficacious in a mouse collagen-induced arthritis model.

Collagen-Induced Arthritis Model. The effect of JNJ 39758979 on collagen-induced arthritis was studied as previously described.[37] The compounds were dosed orally in 20% hydroxypropyl-β-cyclodextran. Eight mice were used per group.

In Vitro Pharmacology. Histamine receptor K_i, EC_{50}, and pA_2 determinations were carried out using transfected cells as previously described.[41] All assays were carried out in triplicate.

In Vivo Pharmacokinetics. In vivo pharmacokinetic data are given in Table 4.

Chemistry. *Chemicals and Reagents.* Solvents and reagents were purchased from commercial suppliers and used as received. The diamines used were obtained from commercial sources, with the exception of the oxygenated diamines used in the synthesis of compounds **27**–**29**, which were synthesized according to procedures in the literature.[42,43] Silica gel was used for all chromatographic purification unless otherwise noted. Unless otherwise specified, reaction solutions were stirred at room temperature (rt) under a nitrogen atmosphere. Where solutions are dried, they are generally dried over a drying agent such as Na_2SO_4 or $MgSO_4$. Where mixtures, solutions, and extracts were concentrated, they were typically concentrated on a rotary evaporator under reduced pressure.

The purity of all tested compounds was >95% by HPLC, performed by analytical HPLC. Reversed-phase HPLC was performed on a Hewlett-Packard HPLC Series 1100 with a Phenomenex ONYX monolithic C18 (5 μm, 4.6 × 100 mm) column. Detection was done at λ = 230, 254, and 280 nm. The flow rate was 1 mL/min. The gradient was 10 to 90% acetonitrile/water (20 mM NH_4OH) over 5.0 min. NMR spectra were obtained on Bruker model DRX spectrometers. The format of the ^1H NMR data is as follows: chemical shift in ppm downfield of the tetramethylsilane reference (multiplicity, coupling constant, J, in Hz, integration). Mass spectra were obtained on an Agilent series 1100 MSD using electrospray ionization (ESI) in either positive or negative mode as indicated. Calculated mass corresponds to the exact mass. Enantiomeric purity was determined on an Agilent 1260 infinity series analytical SFC using a Chiralpak IC column (5 μm, 250 × 4.6 mm, Chiral Technologies) with a mobile phase of 30% ethanol containing either 0.2% isopropylamine or 0.2% triethylamine in CO_2 at a flow rate of 2 mL/min. Column temperature was set to 40 °C, and backpressure regulator was set to 60 °C and 150 Bar. Unless otherwise noted, the optical purity of all compounds tested was greater than 98.5%.

Example **14** provides a typical procedure beginning with the β-keto ester all the way through to the final product. Other derivatives were made in an analogous fashion.

*Example **15**: 4-Cyclopentyl-6-(4-methyl-piperazin-1-yl)-pyrimidin-2-ylamine Hydrochloride. Step A: 2-Amino-6-cyclopentyl-3H-pyrimidin-4-one.* To a solution of 3-cyclopentyl-3-oxo-propionic acid ethyl ester (5.0 g, 27.4 mmol) and guanidine hydrochloride (3.1 g, 33.0 mmol) in MeOH (50 mL) at 23 °C was added potassium *tert*-butoxide portionwise (16.7 g. 149 mmol) over 15 min with vigorous stirring, and the reaction was warmed to 60 °C. The reaction was

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cooled to 23 °C and stirred overnight, and the precipitated salt was removed by filtration. The solution was concentrated to ~10 mL, diluted with 10 mL of water, and adjusted to pH 5 by the addition of 6.0 N HCl (6.1 mL). The resulting precipitate was filtered and dried via suction and then vacuum to yield a white solid (4.3 g, 87%) that was used without further purification. ^{1}H NMR (MeOD): 10.71−10.54 (m, 1H), 6.58−6.32 (m, 2H), 5.44−5.37 (m, 1H), 2.66 (p, J = 8.1 Hz, 1H), 1.91−1.73 (m, 2H), 1.72−1.48 (m, 6H).

Step B: 2-Amino-4-chloro-6-cyclopentylpyrimidine. A suspension of 2-amino-6-cyclopentyl-3*H*-pyrimidin-4-one (1.52 g, 8.4 mmol), tetraethyl ammonium chloride (2.8 g 16.9 mmol), and dimethylaniline (1.1 mL, 8.4 mmol) in acetonitrile (16 mL) was treated with phosphorus oxychloride (4.7 mL, 51 mmol) and plunged into a preheated 110 °C bath for 20 min. The resulting solution was cooled to 23 °C, concentrated to a minimum volume, diluted with $CHCl_3$ and ice, and stirred for 30 min. The layers were separated, and the organic was washed with water (3 × 50 mL) and 5% $NaHCO_3$, separated, dried over Na_2SO_4, filtered, and concentrated to yield 2.0 g of crude product that was used without purification.

Step C: 4-Cyclopentyl-6-piperazin-1-yl-pyrimidin-2-ylamine. A solution of crude 2-amino-4-chloro-6-cyclopentylpyrimidine (150 mg, 0.76 mmol), *N*-Boc piperizine (184 mg, 0.99 mmol), and Et_3N (210 uL, 1.5 mmol) in EtOH (2 mL) was heated to 70 °C for 16 h. The reaction was cooled to room temperature and concentrated, and the crude residue was purified (4 g SiO_2, 0 to 10% NH_3/MeOH/CH_2Cl_2) to yield a white solid (34 mg, 11%). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{18}H_{29}N_5O_2$, 347.2; found, 348.3. ^{1}H NMR (MeOD): 6.01 (s, 1H), 3.66−3.58 (m, 4H), 3.53−3.43 (m, 4H), 3.33 (td, J = 3.28, 1.64, Hz, 1H), 2.90−2.75 (m, 1H), 2.05−1.90 (m, 2H), 1.87−1.77 (m, 2H), 1.77−1.62 (m, 4H), 1.53−1.46 (m, 9H).

Step D: 4-Cyclopentyl-6-piperazin-1-yl-pyrimidin-2-ylamine. A solution of 4-cyclopentyl-6-piperazin-1-yl-pyrimidin-2-ylamine (34 mg, 0.10 mmol) in formic acid (3 mL) was treated with 6.0 N HCl (0.1 mL) and stirred for 2 h. The reaction was diluted with MeOH and concentrated. This process was repeated two times to remove the formic acid to yield a white solid (30 mg, 97%). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{13}H_{21}N_5$, 247.2; found, 248.2. ^{1}H NMR (MeOD): 6.45 (s, 1H), 4.34−4.16 (m, 2H), 4.13−3.96 (m, 2H), 3.42−3.34 (m, 4H), 3.08−2.97 (p, J = 8.0 Hz, 1H), 2.22−2.08 (m, 2H), 1.99−1.83 (m, 2H), 1.83−1.65 (m, 4H).

The following examples were prepared in a manner analogous to Example 15.

*Example **5**: (R)-4-(3-Amino-pyrrolidin-1-yl)-6-isopropyl-pyrimidin-2-ylamine Hydrochloride.* MS (ESI): mass calcd for $C_{11}H_{19}N_5$, 221.16; m/z found, 222.2 $[M+H]^+$. ^{1}H NMR (MeOD); mixture of forms): 6.10 (s, 0.67H), 6.08 (s, 0.33H), 4.16-3.68 (m, 5H), 2.89 (sept, J = 6.9, 1H), 2.60−2.50 (m, 0.67H), 2.50−2.42 (m, 0.33H), 2.32−2.22 (m, 0.67H), 2.22−2.14 (m, 0.33H), 1.33 (d, J = 7.0, 6H); $[\alpha]_D^{20}$ = −29.3 (c 1.00, MeOH).

*Example **6**: 4-Cyclopentyl-6-(4-methyl-piperazin-1-yl)-pyrimidin-2-ylamine.* A solution of crude 2-amino-4-chloro-6-cyclopentylpyrimidine (92 mg, 0.47 mmol) and *N*-methyl piperizine (0.15 mL, 1.4 mmol) in EtOH (2 mL) was heated at 70 °C for 2 h. The reaction was cooled to room temperature and concentrated, and the crude residue was purified (12 g SiO_2, 0 to 10% NH_3/MeOH/CH_2Cl_2) to yield an oil (81 mg, 66%). HRMS (ESI) m/z: mass calcd for $C_{14}H_{23}N_5$, 262.2026; found, 262.2020 (−2.4 ppm). ^{1}H NMR ($CDCl_3$): 5.85 (s, 1H), 4.78 (s, 2H), 3.59 (t, J = 5.0 Hz, 4H), 2.81 (q, J = 8.7 Hz, 1H), 2.44 (t, J = 5.0 Hz, 4H), 2.32 (s, 3H), 2.04−1.89 (m, 2H), 1.82−1.59 (m, 6H).

*Example **16**: (R)-4-Cyclopentyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{14}H_{23}N_5$, 262.2026; found, 262.2017 (−3.5 ppm). ^{1}H NMR (MeOD): 5.75 (s, 1H), 3.74−3.51 (m, 2H), 3.52−3.39 (m, 1H), 3.37−3.30 (m, 2H), 2.86−2.75 (m, 1H), 2.41 (s, 3H), 2.27−2.14 (m, 1H), 2.05−1.92 (m, 2H), 1.93−1.83 (m, 1H), 1.84−1.73 (m, 2H), 1.73−1.60 (m, 4H).

*Example **17**: (S)-4-Cyclopentyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{14}H_{23}N_5$, 262.2026; found, 262.2017 (−3.5 ppm). ^{1}H NMR (MeOD): 5.75 (s, 1H), 3.74−3.51 (m, 2H), 3.52−3.39 (m, 1H), 3.37−3.30 (m, 2H), 2.86−2.75 (m, 1H), 2.41 (s, 3H), 2.27−2.14 (m, 1H), 2.05−1.92 (m, 2H), 1.93−1.83 (m, 1H), 1.84−1.73 (m, 2H), 1.73−1.60 (m, 4H).

*Example **18**: 4-((R)-3-Amino-pyrrolidin-1-yl)-6-cyclopentyl-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{13}H_{21}N_5$, 248.1870; found, 248.1860 (−3.9 ppm). ^{1}H NMR (MeOD): 5.72 (s, 1H), 3.85−3.54 (m, 3H), 3.54−3.32 (m, 1H), 3.32−3.15 (m, 1H), 2.92−2.71 (m, 1H), 2.24 (ddd, J = 12.8, 12.7, 6.42 Hz, 1H), 2.03−1.94 (m, 2H), 1.95−1.75 (m, 3H), 1.74−1.63 (m, 4H).

*Example **19**: 4-((S)-3-Amino-pyrrolidin-1-yl)-6-cyclopentyl-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{13}H_{21}N_5$, 248.1870; found, 248.1860 (−3.9 ppm). ^{1}H NMR (MeOD): 5.72 (s, 1H), 3.85−3.54 (m, 3H), 3.54−3.32 (m, 1H), 3.32−3.15 (m, 1H), 2.92−2.71 (m, 1H), 2.24 (ddd, J = 12.8, 12.7, 6.42 Hz, 1H), 2.03−1.94 (m, 2H), 1.95−1.75 (m, 3H), 1.74−1.63 (m, 4H).

*Example **20**: 4-(3-Amino-azetidin-1-yl)-6-cyclopentyl-pyrimidin-2-ylamine hydrochloride.* HRMS (ESI) m/z: mass calcd for $C_{12}H_{19}N_5$, 234.1713; found, 234.1707 (−2.7 ppm). ^{1}H NMR (MeOD): 10.97−8.73 (br s, 3H), 7.73 (s, 2H), 5.98 (s, 1H), 4.38 (s, 2H), 4.30−4.07 (m, 3H), 2.96−2.84 (m, 1H), 2.11−1.89 (m, 2H), 1.89−1.71 (m, 2H), 1.73−1.52 (m, 4H).

*Example **21**: (R)-4-(3-Amino-piperidin-1-yl)-6-cyclopentyl-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd, 262.2026; found, 262.2020 (−2.4 ppm).

*Example **22**: 4-Cyclopentyl-6-((R,R)-octahydro-pyrrolo[3,4-b]-pyridin-6-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{16}H_{25}N_5$, 288.2183; found, 288.2181 (−0.6 ppm). ^{1}H NMR (MeOD): 5.77−5.68 (m, 1H), 3.65−3.25 (m, 5H), 2.91 (td, J = 12.3, 3.8 Hz, 1H), 2.86−2.74 (m, 1H), 2.66−2.55 (m, 1H), 2.47−2.25 (m, 1H), 2.05−1.89 (m, 2H), 1.88−1.73 (m, 4H), 1.75−1.58 (m, 4H), 1.54−1.44 (m, 1H).

*Example **23**: 4-Cyclopentyl-6-((R,R)-hexahydro-pyrrolo[3,4-b]-pyrrol-5-yl)-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{15}H_{23}N_5$, 273.2; found, 274.2. ^{1}H NMR ($CDCl_3$): 5.63 (s, 1H), 5.36−5.13 (m, 2H), 3.97−3.85 (m, 1H), 3.78−3.63 (m, 1H), 3.64−3.54 (m, 1H), 3.56−3.42 (m, 1H), 3.38−3.22 (m, 1H), 3.20−3.04 (m, 2H), 3.05−2.92 (m, 2H), 2.92−2.75 (m, 2H), 2.11−1.89 (m, 3H), 1.86−1.57 (m, 7H).

*Example **24**: 4-Cyclopentyl-6-(cis-5-methyl-hexahydro-pyrrolo-[3,4-c]pyrrol-2-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{16}H_{25}N_5$, 288.2183; found, 288.2181 (−0.6 ppm). ^{1}H NMR ($CDCl_3$): 5.77 (s, 1H), 3.64−3.56 (m, 2H), 3.46−3.37 (m, 3H), 3.04−2.95 (m, 2H), 2.85−2.75 (m, 2H), 2.44 (dd, J = 9.8, 4.1 Hz, 2H), 2.32 (s, 3H), 2.03−1.92 (m, 2H), 1.84−1.75 (m, 2H), 1.73−1.62 (m, 4H).

*Example **25**: 4-Cyclopentyl-6-(hexahydro-pyrrolo[3,4-c]pyrrol-2-yl)-pyrimidin-2-ylamine hydrochloride.* HRMS (ESI) m/z: mass calcd for $C_{15}H_{23}N_5$, 274.2026; found, 274.2018 (−3.0 ppm).

*Example **26**: 4-Cyclopentyl-6-(cis-1,7-diaza-spiro[4.4]non-7-yl)-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{16}H_{25}N_5$, 287.2; found, 288.2. ^{1}H NMR (MeOD): 5.72 (s, 1H), 3.68−3.27 (m, 4H), 3.07−2.88 (m, 2H), 2.85−2.74 (m, 1H), 2.08−1.92 (m, 4H), 1.93−1.74 (m, 6H), 1.74−1.57 (m, 4H).

*Example **27**: trans-1-(2-Amino-6-cyclopentyl-pyrimidin-4-yl)-4-methylamino-pyrrolidin-3-ol.* HRMS (ESI) m/z: mass calcd for $C_{14}H_{23}N_5O$ 278.1975; found, 278.1970 (−1.9 ppm). ^{1}H NMR ($CDCl_3$): 5.52 (s, 1H), 5.34−5.17 (m, 2H), 4.17−4.07 (m, 1H), 3.75−3.56 (m, 2H), 3.40−3.08 (m, 4H), 3.10−3.02 (m, 1H), 2.78−2.66 (m, 1H), 2.37 (s, 3H), 1.96−1.84 (m, 2H), 1.75−1.63 (m, 2H), 1.63−1.49 (m, 4H).

*Example **28**: 4-Cyclopentyl-6-(trans-hexahydro-pyrrolo[3,4-b]-[1,4]oxazin-6-yl)-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{16}H_{23}N_5O$, 289.2; found, 290.2. ^{1}H NMR (MeOD): 5.62 (s, 1H), 4.72 (s, 2H), 3.99 (dd, J = 11.7, 2.5 Hz, 2H), 3.77 (dt, J = 11.7, 2.9 Hz, 1H), 3.67−3.49 (m, 2H), 3.20 (t, J = 9.8, Hz, 1H), 3.13−3.03 (m, 2H), 2.98 (dd, J = 12.3, 1.9 Hz, 2H), 2.86−2.76 (m, 1H), 2.07−1.91 (m, 4H), 1.83−1.57 (m, 4H).

*Example **29**: 4-Cyclopentyl-6-(cis-hexahydro-pyrrolo[3,4-b][1,4]-oxazin-6-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{15}H_{23}N_5O$, 290.1975; found, 290.1975 (−0.1 ppm).

*Example **30**: 4-Methyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd, 208.1557; found, 208.1559 (1.1 ppm).

*Example **31**: 4-Ethyl-6-R-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{12}H_{20}N_5$, 222.1713; found, 222.1713 (−0.1 ppm). ^{1}H NMR ($CDCl_3$): 5.61 (s, 1H), 5.21 (s, 2H), 3.72−3.12 (m, 5H), 2.55−2.41 (m, 5H), 2.22−2.09 (m, 1H), 1.93−1.77 (m, 1H), 1.27−1.15 (t, J = 7.3 Hz, 3H). $[\alpha]_D^{20}$ = −27.6 (c 0.40, MeOH).

*Example **32**: 4-(R)-(3-Amino-pyrrolidin-1-yl)-6-ethyl-pyrimidin-2-ylamine Hydrochloride.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{10}H_{17}N_5$, 207.3; found, 208.2. ^{1}H NMR (DMSO): 8.85−8.58 (m, 2H), 8.32−7.18 (m, 1H), 6.17−6.10 (m, 1H), 4.04−3.85 (m, 1H), 3.85−3.53 (m, 4H), 2.66−2.53 (q, J = 7.5, 15.1 Hz, 2H), 2.41−2.09 (m, 2H), 1.28−1.19 (t, J = 7.5, 15.1 Hz, 3H)

*Example **33**: (R)- 4-Isopropyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{12}H_{21}N_5$, 236.1870; found, 236.1867 (−1.2 ppm). ^{1}H NMR ($CDCl_3$): 5.66 (s, 1H), 5.00−4.87 (m, 2H), 3.75−3.56 (m, 2H), 3.56−3.43 (m, 1H), 3.43−3.23 (m, 2H), 2.71 (q, J = 6.9 Hz, 1H), 2.53 (s, 3H), 2.22 (dt, J = 13.4, 6.1 Hz, 1H), 1.98−1.81 (m, 1H), 1.26 (d, J = 6.9 Hz, 6H).

*Example **34**: 4-Butyl-6-((R)-3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{13}H_{23}N_5$, 250.2026; found, 250.2023 (−1.3 ppm). ^{1}H NMR ($CDCl_3$): 5.57 (s, 1H), 5.22 (s, 2H), 3.75−3.07 (m, 5H), 2.45 (s, 3H), 2.43−2.38 (m, 2H), 2.18−2.08 (m, 1H), 1.88−1.73 (m, 1H), 1.66−1.55 (m, 2H), 1.42−1.30 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H).

*Example **35**: (R)-Benzyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{16}H_{21}N_5$, 283.37; found, 284.2. ^{1}H NMR (400 MHz, $CDCl_3$): 7.18−7.32 (m, 5H), 5.50 (s, 1H), 4.83−4.85 (bs, 2H), 3.78 (s, 3H), 3.2−3.7 (m, 4H), 2.44 (s, 3H), 2.05−2.15 (m, 1H), 1.74−1.84 (m, 1H).

*Example **36**: 4-((R)-3-Amino-pyrrolidin-1-yl)-6-benzyl-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{15}H_{19}N_5$, 269.2; found, 270.12. ^{1}H NMR ($CDCl_3$) (free base): 7.21−7.34 (m, 5H), 5.72 (bs, 2H), 5.45 (s, 1H), 3.83 (s, 2H), 3.60−3.71 (m, 2H), 2.84−3.60 (bm, 5H), 2.04−2.21 (m, 1H), 1.63−1.84 (m, 1H).

*Example **37**: 4-Cyclopropyl-6-(R)-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{12}H_{19}N_5$, 234.1713; found, 234.1710. ^{1}H NMR ($CDCl_3$): 5.58 (s, 1H), 5.30 (s, 1H), 4.73−4.63 (m, 1H), 3.75−3.11 (m, 5H), 2.46 (s, 3H), 2.21−2.10 (m, 1H), 2.04−2.00 (m, 1H), 1.88−1.77 (m, 1H), 1.77−1.67 (m, 1H), 1.00−0.91 (m, 2H), 0.89−0.82 (m, 2H).

*Example **38**: 4-Cyclobutyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{13}H_{21}N_5$, 248.1870; found, 248.1862 (−3.1 ppm). ^{1}H NMR ($CDCl_3$): 5.62 (s, 1H), 4.71 (s, 2H), 3.75−3.52 (m, 2H), 3.50−3.39 (m, 1H), 3.38−3.20 (m, 3H), 2.52−2.44 (s, 3H), 2.33−2.10 (m, 5H), 2.09−1.76 (m, 5H).

*Example **39**: (R)-4-Cyclopentyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{14}H_{23}N_5$, 262.2026; found, 262.2017. ^{1}H NMR (MeOD): 5.75 (s, 1H), 3.74−3.51 (m, 2H), 3.52−3.39 (m, 1H), 3.37−3.30 (m, 2H), 2.86−2.75 (m, 1H), 2.41 (s, 3H), 2.27−2.14 (m, 1H), 2.05−1.92 (m, 2H), 1.93−1.83 (m, 1H), 1.84−1.73 (m, 2H), 1.73−1.60 (m, 4H).

*Example **40**: 4-((R)-3-Amino-pyrrolidin-1-yl)-6-cyclopentyl-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{13}H_{21}N_5$, 247.2; found, 248.2. ^{1}H NMR (MeOD): 5.72 (s, 1H), 3.85−3.54 (m, 3H), 3.54−3.32 (m, 1H), 3.32−3.15 (m, 1H), 2.92−2.71 (m, 1H), 2.24 (ddd, J = 12.8, 12.7, 6.42 Hz, 1H), 2.03−1.94 (m, 2H), 1.95−1.75 (m, 3H), 1.74−1.63 (m, 4H).

*Example **41**: 4-Cyclohexyl-6-(R)-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{15}H_{25}N_5$, 276.2183; found, 276.2178 (−1.7 ppm). ^{1}H NMR ($CDCl_3$): 5.59 (s, 1H), 5.30 (s, 2H), 4.69 (s, 2H), 3.71−3.51 (m, 2H), 3.46−3.38 (m, 1H), 3.37−3.29 (m, 1H), 2.47 (s, 3H), 2.36−2.26 (m, 1H), 2.22−2.12 (m, 1.5H), 1.95−1.77 (m, 5.5H), 1.76−1.68 (m, 1H), 1.47−1.17 (m, 6H).

*Example **42**: 4-tert-Butyl-6-((R)-3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{13}H_{23}N_5$, 249.4; found, 250.2. ^{1}H NMR ($CDCl_3$): 5.71 (s, 1H), 4.80 (s, 1H), 3.72−3.51 (m, 2H), 3.49−3.40 (m, 1H), 3.38−3.22 (m, 2H), 2.47 (s, 3H), 2.21−2.10 (m, 1H), 1.91−1.76 (m, 1H), 1.25 (s, 9H).

*Example **43**: 4-((R)-3-Amino-pyrrolidin-1-yl)-6-tert-butyl-pyrimidin-2-ylamine Hydrochloride.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{12}H_{21}N_5$, 235.3; found, 236.3. ^{1}H NMR (MeOD): 6.11−5.99 (m, 1H), 4.22−3.96 (m, 2H), 3.95−3.70 (m, 3H), 2.64−2.40 (m, 1H), 2.39−2.12 (m, 1H), 1.40 (s, 9H).

*Example **44**: 4-Adamantan-1-yl-6-((R)-3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* MS (ESI) m/z: $[M + H]^+$ calcd for $C_{19}H_{29}N_5$, 327.5; found, 328.4. ^{1}H NMR ($CDCl_3$) 5.92 (s, 1H), 4.71 (s, 2H), 3.71−3.17 (m, 5H), 2.48 (s, 3H), 1.99−1.92 (m, 6H), 1.81−1.72 (m, 6H), 1.38 (s, 1H), 1.15 (s, 1H).

*Example **45**: (R)-4,5-Dimethyl-6-(3-methylamino-pyrrolidin-1-yl)-pyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd, 222.1713; found, 222.1712 (−0.5 ppm).

*Example **46**: 4-(3-Methylamino-pyrrolidin-1-yl)-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{12}H_{19}N_5$, 234.1713; found, 234.1706 (−3.1 ppm). ^{1}H NMR ($CDCl_3$): 4.88 (s, 2H), 3.84−3.74 (m, 2.7H), 3.72−3.61 (m, 1.3H), 3.49−3.41 (m, 2H), 3.27−3.20 (m, 1H), 3.08−2.92 (m, 2H), 2.82−2.61 (m, 2H), 2.46 (s, 3H), 2.14−2.04 (m, 1H), 2.02−1.90 (m, 2H), 1.83−1.74 (m, 2H).

*Example **47**: (R)-4-(3-Methylamino-pyrrolidin-1-yl)-5,6,7,8-tetrahydro-quinazolin-2-ylamine.* HRMS (ESI) m/z: mass calcd for $C_{13}H_{21}N_5$, 248.1870; found, 248.1866 (−1.5 ppm). ^{1}H NMR (MeOD): 3.67−3.55 (m, 2H), 3.53−3.45 (m, 1H), 3.28 (dd, J = 11.0, 5.5 Hz, 1H), 3.06 (p, J = 6.0 Hz, 1H), 2.58−2.45 (m, 2H), 2.38 (t, J = 6.4 Hz, 2H), 2.24 (s, 3H) 2.01−1.90 (m, 1H), 1.68−1.45 (m, 5H).

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Notes

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