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Benzimidazole- and indole-substituted 1,3′-bipyrrolidine benzamides as histamine H_3 receptor antagonists

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ABSTRACT

Using a focused screen of biogenic amine compounds we identified a novel series of H_3R antagonists. A preliminary SAR study led to reduction of MW while increasing binding affinity and potency. Optimization of the physical properties of the series led to (*S*)-**6n**, with improved brain to plasma exposure and efficacy in both water intake and novel object recognition models.

Histamine-3 receptors (H_3R) are one of four histamine G-protein coupled receptor subtypes ($H_{1-4}R$).[1–3] H_1R and H_2R are postsynaptic receptors involved with allergic responses and gastric acid secretion, respectively, while the H_4R is located on T-cells, neutrophils and monocytes and is implicated in inflammation processes.[4,5] The H_3R is located presynaptically on histaminergic neurons in the CNS, where it acts as an autoreceptor inhibiting the synthesis and release of histamine. H_3R also acts as a heteroreceptor on non-histaminergic neurons inhibiting the release of other neurotransmitters, including acetylcholine, serotonin, noradrenaline and dopamine. Postmortem studies in humans suggest that decreased brain histamine levels may contribute to the cognitive decline in Alzheimer's disease.[6] Furthermore H_3R agonists have been shown to impair memory,[7,8] while H_3R antagonists rescue pharmacologically or genetically induced impairments in various animal models.[9–13] Based on these findings several H_3R antagonists have advanced to clinical trials for cognitive disorders and dementia.[14]

Early H_3R antagonists maintained the histamine imidazole ring, typically connected through an alkyl chain and polar fragment, such as an ether, to a lipophilic moiety (see ciproxifan, **1**[15] and FUB 181, **2**[16] Fig. 1). The presence of the imidazole ring has been implicated in potential pharmacokinetic liabilities[17] and poor brain penetration, so more recent analogs have focused on imidazole ring replacements, typically with a tertiary amine, (see **3**–**5**).[18–20]

Wyeth's H_3R program was initiated with a screen of our focused biogenic amine library using a competitive binding assay[21] with [^{3}H](*R*)-α-methylhistamine and cell membranes from HEK293T

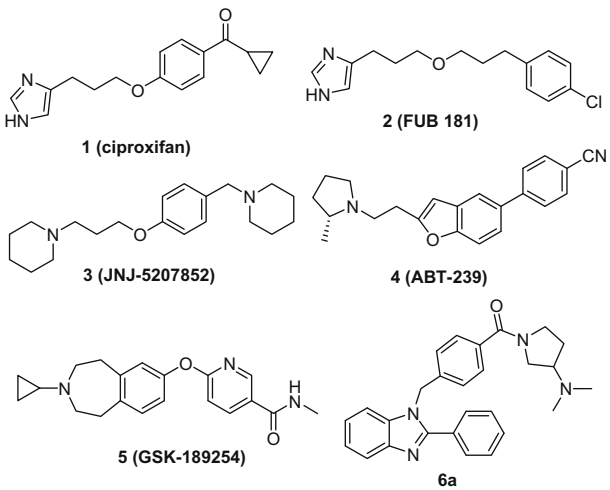

Figure 1. Structures of H_3R antagonists.

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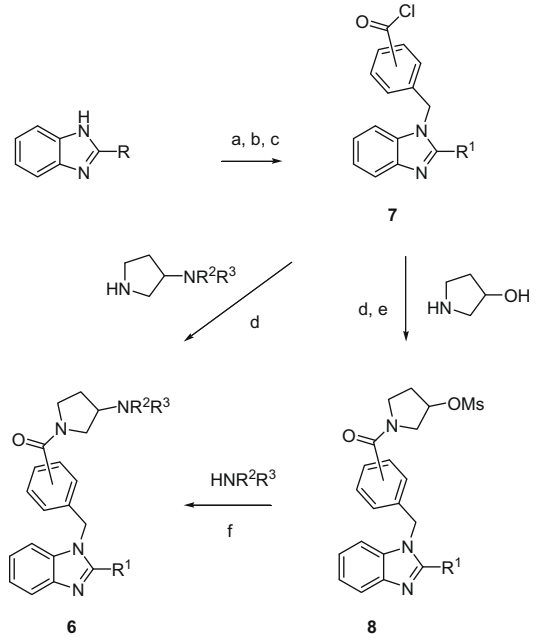
cells over-expressing human H_3 receptors (hH_3R) and led to the identification of advanced hit **6a**. This compound had 102 nM binding affinity for hH_3 receptors and was a potent, full antagonist, inhibiting cAMP production in the hH_3 functional assay[22] with a K_B value of 11 nM. The compound also showed good overall physiochemical properties with excellent solubility in aqueous buffer (>100 μg/mL), good permeability in both PAMPA[23] (4.4×10^{-6} cm/s) and PAMPA–BBB[24] (9.4×10^{-6} cm/s) assays and passive diffusion in a Caco-2 assay ($P_{A>B} = 84.9 \times 10^{-6}$ cm/s, BA/AB = 1.3) suggesting potentially good absorption, permeability and BBB penetration properties. **6a** also had low inhibition of Cyp450 enzymes and moderate to rapid metabolism in human and rat liver microsomes. However, **6a** has a high molecular weight (424) and *c* log *P* (4.43), resulting in relatively low ligand efficiency (0.3) and high LELP (*c* log *P*/LE) (14.8).[25] Therefore, initial efforts were directed at reducing the MW and *c* log *P* of the compounds to more optimal lead-like space, and are described here.

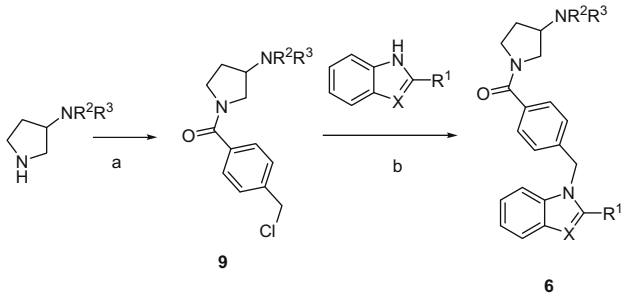
The synthesis of **6a** and related compounds begins with the deprotonation of benzimidazoles followed by coupling with methyl 4-(bromomethyl)benzoate (Scheme 1). Hydrolysis of the ester followed by acid chloride formation generates intermediates **7**, which are coupled with readily available *N*,*N*-dialkylpyrrolidin-3-amines to afford the desired products **6**. Alternatively, coupling of the acid chlorides with 3-pyrrolidinol followed by mesylate formation leads to intermediate **8**, and displacement with secondary amines gives the desired products **6**. Displacement of the mesylate prepared from (*R*)- or (*S*)-3-pyrrolidinol with inversion of the asymmetric center is used for a stereospecific synthesis of analogs. Initial examination of the connectivity requirement around the central phenyl ring was accomplished using both 2- and 3-(bromomethyl)benzoate (Scheme 1, step a).

To vary the benzimidazole portion of the series, 3-amino substituted pyrrolidines are coupled with 4-chloromethyl benzoyl chloride affording the chloromethyl derivative **9**, which is subsequently reacted with benzimidazoles and indoles to give the desired targets **6** (Scheme 2). The 1,3′-bipyrrolidines **10** are prepared from 3-aminopyrrolidines by *N*-Boc protection of the secondary amine, bis-alkylation of the primary amine and deprotection (Scheme 3).

All of the compounds were evaluated for their ability to displace [^{3}H](*R*)-α-methylhistamine binding to cloned human H_3 receptors

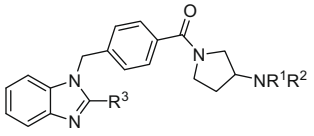


Scheme 1. Reagents and conditions: (a) NaH, methyl 2-, 3- or 4-(bromomethyl)benzoate, THF/DMF (5:1), rt 16 h; (b) LiOH, MeOH/water (2:1), rt, 16 h; (c) oxalyl chloride (2 M soln. in DCM), DMF (2 drops), DCM rt, 2 h; (d) cyclic amine, DIEA, THF, rt, 2–8 h; (e) Et_3N, MsCl, DCM 0 °C to rt, 16 h; (f) amine, DMF, rt, 16 h.



Scheme 2. Reagents and conditions: (a) DIEA, 4-chloromethyl benzoyl chloride, THF, rt, 2–8 h; (b) NaH, THF/DMF (5:1), rt, 16 h.

Table 1
Binding affinity and functional activity of **6** analogs



Compd	NR^1R^2	R^3	hH_3 K_i[a] (nM)	hH_3 K_b[b] (nM)
6a	NMe_2	Ph	100	11
6b	NMeEt	Ph	91	
6c	Pyrrolidine	Ph	10	4
6d	NMeEtOH	Ph	1200	
(*S*)-**6a**	NMe_2	Ph	65	16
(*R*)-**6a**	NMe_2	Ph	440	108
6e	NMe_2	2-pyridyl	235	
6f	NMe_2	H	390	3
6g	NMe_2	Me	17	
6h	Pyrrolidine	H	23	
(*S*)-**6h**	Pyrrolidine	H	10	0.5
(*R*)-**6h**	Pyrrolidine	H	27	
(*S*)-**6i**	Pyrrolidine	Me	5	0.3
(*R*)-**6i**	Pyrrolidine	Me	3	2

[a] Displacement of [^{3}H](*R*)-α-methylhistamine binding to cloned hH_3 receptors stably expressed in HEK293T cells.

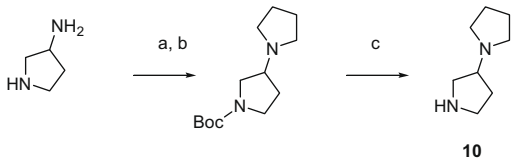
[b] Inhibition of cAMP production in HEK293T cells stably transfected with hH_3 receptors. Mean of three determinations with a standard error of ±30%.

stably expressed in HEK293T cells. Compounds demonstrating suitable affinity were assayed for inhibition of cAMP production, and shown to be full antagonists. The results are summarized in Tables 1–3.

Variations of the basic amine group (NR^1R^2) present in **6a** led to the identification of the pyrrolidino group (**6c**, Table 1) with a 10-fold improvement in binding affinity. Interestingly, the enantiomeric pairs (e.g., (*R*)- and (*S*)-**6a**) showed only a sevenfold eudismic ratio with respect to both affinity and functional activity in favor of the eutomer (*S*)-**6a**.

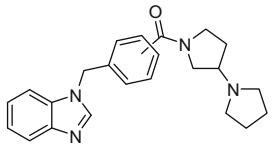
In order to reduce the MW and *c* log *P*, a series of analogs were prepared in which the 2-phenyl group in **6a** is replaced with more polar and/or smaller groups. We were gratified to see that indeed this group could be replaced by the 2-pyridyl group (**6e**) with only a slight reduction in potency, whereas replacement with the obviously smaller methyl group (**6g**) provides a significant (sixfold) increase in affinity.

Combining the smaller 2-substituents (i.e., H and Me) with the preferred pyrrolidine tertiary amine leads to **6h–6i**. Again in general the (*S*)-isomer has slightly higher affinity and functional activity than the distomer with the eutomers exhibiting sub-nanomolar inhibition of cAMP production. Analog **6k** (Table 2) with 1,3 connectivity loses ~20-fold in binding affinity, while the 1,2-con-



Scheme 3. Reagents and conditions: (a) $(Boc)_2O$, MeOH, rt, 16 h; (b) 1,4-dibromobutane, K_2CO_3, DMF, 110 °C, 16 h; (c) 2 N HCl in dioxane, rt, 3 h.

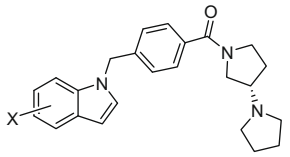
Table 2
Binding affinity of **6** analogs



Compd	Connectivity	hH_3 K_i (nM)
6h	1,4	23
6k	1,3	400
6l	1,2	4000

Displacement of [^{3}H]-(*R*)-α-methylhistamine binding to cloned hH_3 receptors stably expressed in HEK293T cells. Mean of three determinations with a standard error of ±30%.

Table 3
Binding affinity and functional activity of **6** analogs



Compd	X	hH_3 K_i (nM)	hH_3 K_b (nM)
(*S*)-**6m**	H	15	2.4
(*S*)-**6n**	7-Cl	2.5	0.3
(*S*)-**6o**	6-Cl	150	2.1
(*S*)-**6p**	5-Cl	130	0.9

Displacement of [^{3}H]-(*R*)-α-methylhistamine binding to cloned hH_3 receptors stably expressed in HEK293T cells. Mean of three determinations with a standard error of ±30%.

nected analog **6l** is nearly 200-fold less potent, showing a strict requirement for the 1,4 substitution pattern among these ligands.

The pharmacokinetic profile of (*S*)-**6h** in rats indicates high clearance, moderate volume of distribution and half-life and excellent bioavailability (Table 4). Brain and plasma exposure measured after a 10 mg/kg ip injection show AUCs of 231 and 1039 h ng/mL respectively, indicating a brain to plasma ratio of 0.22. The low brain exposure is somewhat surprising given the low MW, *c* log *P* and polar surface area (PSA) of (*S*)-**6h** (see Table 5).[26,27]

Replacing the benzimidazole with an indole ((*S*)-**6m**) and chloro-substituted indoles ((*S*)-**6n–p**) results in compounds which are calculated to have a slightly higher log *P* and lower PSA. While the solubility of these analogs is slightly reduced the permeability in both PAMPA and PAMPA–BBB is improved significantly, suggesting potentially improved brain to plasma ratios. The unsubstituted indole analog (*S*)-**6m** demonstrates comparable activity to the corresponding benzimidazole ((*S*)-**6h**) however the 7-chloro derivative (*S*)-**6n** has sixfold increased affinity and functional activity at the hH_3R (Table 3).

Unfortunately, the rat pharmacokinetic profile of (*S*)-**6n** shows rapid clearance, low exposure and oral bioavailability (Table 4).

Table 4
Pharmacokinetic properties for (*S*)-**6h** and (*S*)-**6n** in rats

	(*S*)-**6h**	(*S*)-**6n**
iv dose (mg/kg)	2	2
Clp (mL/min/kg)	56	112
Vss (L/kg)	3.6	7.3
po dose (mg/kg)	10	10
C_{max} (ng/mL)	1751	22
T_{max} (h)	0.4	1.2
AUC (h ng/ml)	2736	63
$t_{1/2}$	1.3	1.9
F%	93	5
ip dose (mg/kg)	10	30
C_{max} brain/plasma (b/p) (ng/mL)	92/388	6536/1086
C_{max} brain (nM)	245	16019
%Fu (brain)	13,8	3.5
T_{max} b/p (h)	1.0/1.0	0.5/0.5
AUC b/p (h ng/ml)	231/1039	15007/2182
b/p ratio	0.2	6.9

Table 5
Calculated and measured properties of selected **6** analogs

Compd	MW	*c* log *P*	*p* log *D*	PSA[a]	Pe[b]	BBB[c]	aq sol.[d]
6a	424.5	4.2	3.4	41.3	4.4	9.4	>100
(*S*)-**6h**	374.5	2.6	1.4	41.4	0.3	3.7	51
(*S*)-**6i**	388.5	2.9	1.6	41.4	0.3	4.0	32
(*S*)-**6m**	373.5	3.6	2.3	28.5	8.3	9.4	28
(*S*)-**6n**	407.9	4.4	3.0	28.5	6.5	7.0	18
(*S*)-**6o**	407.9	4.4	3.0	28.5	7.3	8.6	13
(*S*)-**6p**	407.9	4.4	3.0	28.5	7.8	8.1	10

[a] PSA = polar surface area.
[b] Pe = effective permeability measured by PAMPA method ($\times 10^{-6}$ cm/s).[23]
[c] BBB = blood–brain barrier permeability measured by PAMPA–BBB method ($\times 10^{-6}$ cm/s).[24]
[d] Aqueous solubility μg/mL at pH 3.1.

However, the brain and plasma exposure measured after a 30 mg/kg ip injection in rats shows a significant improvement with AUCs of 15007 and 2182 h ng/mL for brain and plasma, respectively, and a brain to plasma ratio of 6.9. The fraction of (*S*)-**6n** unbound in brain is 3.5%, giving a free drug concentration of 229 ng/mL or 562 nM at T_{max}, approximately 25-fold greater than the rH_3 affinity (rH_3 K_i = 21.7 nM). Compound (*S*)-**6n** demonstrates >1000-fold selectivity for hH_3R over hH_1R and hH_2R receptors and >100-fold selectivity over various serotonin ($5\text{-}HT_{2a,2c,6,7}$), dopamine (D_2) and alpha adrenergic ($\alpha_{1,2a}$) receptors in binding assays.

The selective H_3R agonist (*R*)-α-methylhistamine has been shown to induce a dose dependent increase in water consumption in rats mediated by H_3R within the CNS.[28,29] This increased water intake effect was blocked by (*S*)-**6n** dosed intraperitoneally (ip) at both 10 and 30 mg/kg (Fig. 2).

Furthermore, H_3R antagonists have been shown to reverse delay-dependent deficits in rodent novel object recognition by enhancing memory encoding and/or retention.[30,31] In the novel object recognition model, rats previously exposed to identical objects can discriminate between a novel object and a familiar, previously experienced object, during a subsequent trial. Figure 3 shows a statistically significant differential exploration of the novel and familiar object for rats dosed with (*S*)-**6n** (minimally efficacious dose = 0.3 mg/kg) prior to the learning trial. The preferential exploration of the novel object demonstrates enhanced object recognition memory in rats dosed with (*S*)-**6n** relative to those dosed with vehicle and represents efficacy as a cognitive enhancer.

In summary, we have identified a potent and selective series of H_3R antagonists. Optimization led to analogs with increased

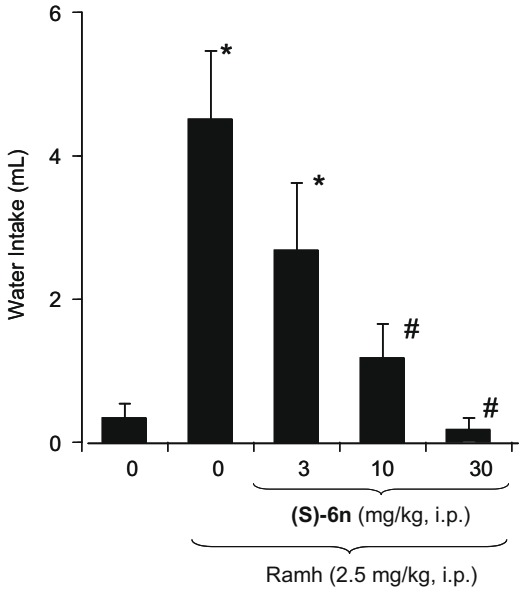


Figure 2. Effect of (*S*)-6n on (*R*)-α-methylhistamine (Ramh)-induced water consumption in rats. $^{*}p$ <0.05 versus veh; $^{\#}p$ <0.05 versus Ramh alone.

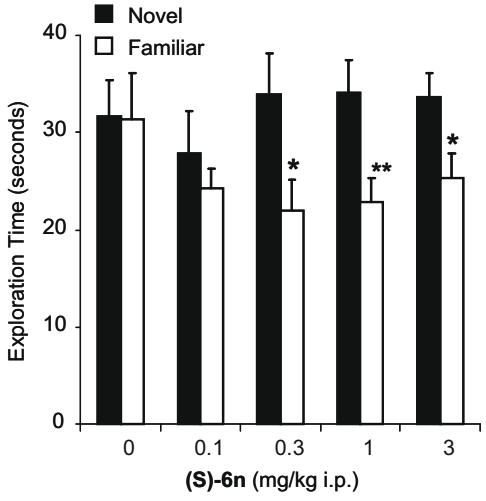


Figure 3. Novel object recognition in rats after ip dosing of the H_3R antagonist (*S*)-**6n**. $^{*}p$ <0.03; $^{**}p$ <0.005.

affinity and potency, and further modulation of the physical properties of the series led to (*S*)-**6n**, which has excellent brain to plasma exposure and demonstrated efficacy in both the water intake and novel object recognition rat models.

Acknowledgments

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21. HEK293T cells expressing the human cloned histamine H3 receptor were harvested in phosphate-buffered saline (PBS) and pelleted by centrifugation at 1000*g* for 5 min at 4 °C and membranes were prepared by homogenizing the cell pellets in ice-cold 50 mM Tris–HCl buffer, pH 7.4 and 0.5 mM EDTA for approximately 30 s using a Polytron homogenizer. The homogenates were centrifuged at 40,000*g* for 30 min at 4 °C, and the resulting pellet was re-homogenized and centrifuged as described above. The membranes were finally resuspended in 50 mM Tris–HCl buffer, pH 7.4 at a concentration of approximately 1 mg protein/mL, and were stored at -70 °C until use. Receptor binding studies were carried out on these membranes in 50 mM Tris–HCl buffer, pH 7.4, at room temperature. Assays consisted of 100 μL of displacing compound (final concentration of 0.05 nM–500 nM) or buffer, 20 μL of [3*H*]R-α-methylhistamine at a final concentration of 5 nM and the reaction was initiated by the addition of 100 mL of human H3 receptor membranes (30 μg of protein/well). Nonspecific binding was determined in the presence of 10 mM clobenpropit. The experiments were terminated by rapid filtration through a GF/B filter plate (presoaked in 0.1% (v/v) polyethyleneimine), and then the filters were washed with ice-cold buffer (50 mM Tris–HCl). Filters were dried and then 25 μL of Microscint 20 was added to each well. Radioactivity was determined by liquid scintillation spectrometry using a Packard TopCount.
22. Functional in vitro activity of the compounds was determined in cAMP accumulation assays. Culture media was removed from the cells and they were washed twice with PBS (Ca^{2+}/Mg^{2+}-free) containing 500 μM IBMX. Cells were detached, by gentle tapping, and resuspended in the same buffer. 2000 cells/well were incubated with 1 μM histamine, 10 μM forskolin and test compounds (0.03 nM–10 μM) in a total volume of 30 μL in 96-well plates for 30 min at 30 °C. Cyclic AMP levels were then measured using the HitHunter cAMP kit (DiscoveRx, Fremont, CA) according to the manufacturer's instructions. Chemiluminescent signals were detected using a Packard TopCount. Data were analyzed using Prizm.
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