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Novel substituted pyrrolidines are high affinity histamine H_3 receptor antagonists

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ABSTRACT

Pre-clinical characterization of novel substituted pyrrolidines that are high affinity histamine H_3 receptor antagonists is described. These compounds efficiently penetrate the CNS and occupy the histamine H_3 receptor in rat brain following oral administration. One compound, (2*S*,4*R*)-1-[2-(4-cyclobutyl-[1,4]diazepane-1-carbonyl)-4-(3-fluoro-phenoxy)-pyrrolidin-1-yl]-ethanone, was extensively profiled and shows promise as a potential clinical candidate.

The modulation of histamine H_3 receptor function is of significant interest to neuroscientists. Numerous recent reports have demonstrated that histamine H_3 receptors regulate the sleep–wake cycle in pre-clinical models and have demonstrated the potential utility of histamine H_3 antagonists for the treatment of various conditions including ADHD, narcolepsy and Alzheimer's disease.[1] Towards that end, a number of companies have initiated clinical trials for some of these indications and several reviews covering the topic have appeared in the recent literature.[2–4]

Our foray into the field of H_3 receptor antagonists include the chemotypes shown in Figure 1: propyloxy-piperidines (**1**),[5] 2-aryloxymethyl-morpholines (**2**),[6] and (4-aminobutyn-1-yl)benzylamines (**3**).[7]

We have also reported the discovery that benzyl amine-based compounds, such as **4**, are dual acting histamine H_3 receptor antagonists and serotonin transporter (SERT) inhibitors.[8] Each of these chemotypes share structural features consisting of a basic amine linked by four atoms to a central aromatic core, which is also substituted by a *meta*- or *para*-benzylamine moiety.

The literature suggests that the most important feature of these histamine H_3 receptor antagonists is the tri-substituted basic amine linked by four atoms to an aromatic ring.[2–4] We were particularly interested in compounds that retained the piperazine-type amide moiety and aryloxy portion of **4** with replacement of the central aromatic ring with other linkers as these groups showed promise in our earlier efforts to prepare dual acting histamine H_3 antagonists/SERT inhibitors.

Therefore we began investigating the properties of compounds with the general structure **5** (Fig. 2), which contains an amide linked through a heterocyclic ring (**A**) to an aromatic ring. We now report one aspect of these efforts in which the A ring is a pyrrolidine ring derived from proline as in compound **6**,

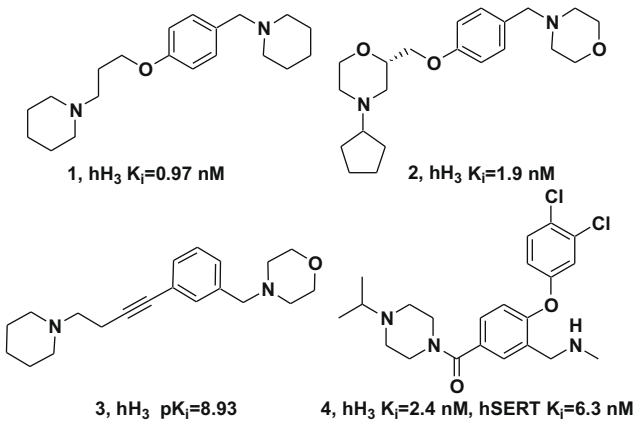

Figure 1. Histamine H_3 antagonist chemotypes.

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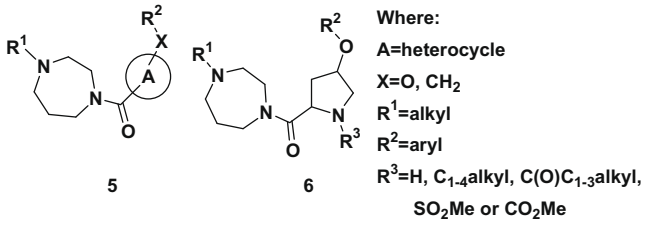


Figure 2. Substituted pyrrolidines.

wherein we examined the stereochemistry of the proline ring, nitrogen substitutions for both R_1 and R_3 as well as aryl group substitution, R_2.

The initial synthesis of compounds **11** and **12** is outlined below in Scheme 1.[9] For the synthesis of the 2*R*,4*S* analogs, (2*R*,4*R*)-*cis*-4-hydroxy-pyrrolidine-2-carboxylic acid (**7**) was first treated with $(Boc)_2O$ to form **8**. The *N*-Boc protected amino acid **8** was then converted to the diazepane amides **9** using standard methods. Next, a Mitsunobu reaction was utilized to invert the C-4 stereochemistry to form compounds **10**. Subsequent *N*-Boc deprotection produced the substituted pyrrolidines as described in Scheme 1 where R_4 is defined as the substituents shown in Tables 1 and 2. The same procedure outlined in Scheme 1 was used to prepare compounds **13** and **14** from commercially available *N*-Boc-(2*S*,4*R*)-*trans*-4-hydroxypyrrolidine-2-carboxylic acid using resin bound PPh_3.[10]

Although a number of analogs were made using the synthesis outlined in Scheme 1, the variability in yield and limited scope of the Mitsunobu reaction led us to explore other synthetic routes to prepare the desired compounds **11** and **12**.

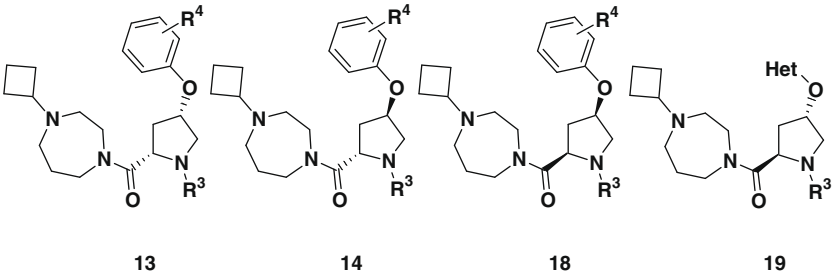
In Scheme 2, (2*R*,4*S*)-*trans*-4-hydroxy-pyrrolidine-2-carboxylic acid (**15**) is converted to **17** as described in Scheme 1 and then **17** is treated with an aryl iodide using copper catalysis to install the aryloxy substituent with retention of configuration at the 4-position.

Compounds **18** were also synthesized using the route outlined in Scheme 2 starting from (2*R*,4*R*)-*cis*-4-hydroxypyrrolidine-2-carboxylic acid. This route allowed for higher, more consistent yields and also allowed for the introduction of heterocyclic aryl rings using the corresponding aryl iodides. Thus, we also prepared compounds **19** using this route.

Table 1
Binding and functional data for the human H_3 receptor for compounds **11** and **12**

Compds	R^1	R^3	R^4	Human H_3 K_i[a] (nM)	Human H_3 pA_2[b]
11a	*c*-Bu	—	H	5.3 ± 2.2	8.09
11b	*c*-Bu	—	2-Me	2.8 ± 0.1	
11c	*c*-Bu	—	3-Me	5.2 ± 1.2	
11d	*c*-Bu	—	4-Me	4.7 ± 0.8	
11e	*c*-Bu	—	3-Me, 4-Cl	1.3 ± 0.5	8.94 ± 0.06
11f	*c*-Bu	—	3-OMe	5.0 ± 1.0	
11g	*c*-Bu	—	4-SMe	2.8 ± 0.1	8.65 ± 0.04
11h	*c*-Bu	—	3-Me, 4-Cl	1.3 ± 0.5	8.94 ± 0.06
11i	*c*-Bu	—	4-CF_3	4.1 ± 0.7	
11j	*c*-Bu	—	3-F	3.4 ± 1.7	8.68 ± 0.09
11k	*c*-Bu	—	4-F	1.6 ± 0.4	8.90
11l	*c*-Bu	—	4-Cl	2.0 ± 0.9	9.02
11m	*c*-Bu	—	3-Cl	2.5 ± 1.5	9.28
11n	*c*-Bu	—	4-CN	2.4 ± 0.8	8.68
11o	*c*-Pent	—	4-F	4.1 ± 3.3	8.80
11p	*c*-Pent	—	4-CN	3.2 ± 1.4	9.15
11q	*c*-Pr	—	4-CN	23 ± 5	
12a	*c*-Bu	Me	4-F	1.8 ± 0.7	9.13
12b	*c*-Bu	*i*-Pr	4-F	12 ± 4	
12c	*c*-Bu	*c*-Pr	4-F	13 ± 1	
12d	*c*-Bu	*c*-Bu	4-F	17 ± 3	
12e	*c*-Bu	Ac	4-F	1.5 ± 1.0	9.87
12f	*c*-Bu	SO_2Me	4-F	1.2 ± 0.2	
12g	H	Ac	3-F	1970 ± 560	
12h	Me	Ac	3-F	455 ± 45	
12i	*i*-Pr	Ac	3-F	51 ± 9.4	
12j	*i*-Bu	Ac	3-F	13 ± 4.2	
12k	*c*-Bu	Ac	3-F	1.6 ± 1.4	9.50 ± 0.16
12l	*c*-Bu	EtO_2C	3-F	16 ± 7	
12m	*c*-Bu	C(O)*c*-Pr	3-F	7.5 ± 0.8	
12n	*c*-Bu	CH_2*c*-Pr	3-F	7.0 ± 0.2	
12o	c-Pent	Ac	3-F	2.2 ± 0.8	
12p	THP	Ac	3-F	69 ± 7.5	
12q	Bn	Ac	3-F	150 ± 15	

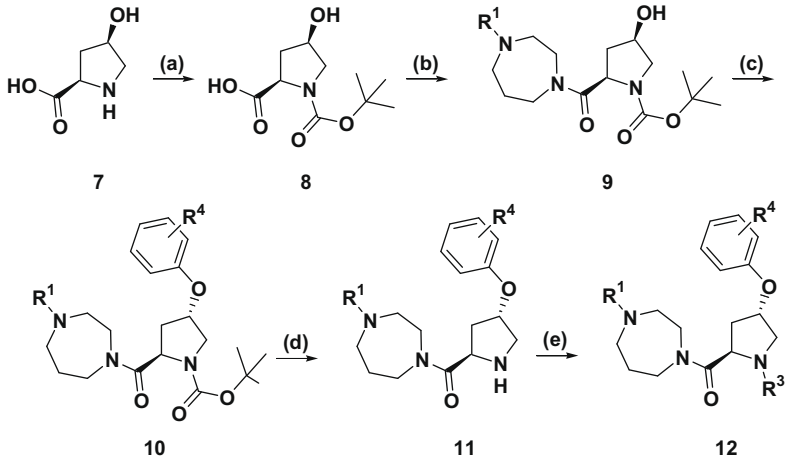
[a] K_i's are the mean of at least three experiments in triplicate. K_i ± s.d. is reported.
[b] The result of a single or duplicate experiment, in triplicate, unless s.d. is shown, then it is the mean of three experiments.



The carbon-linked analogs **20** and **21** were easily prepared as detailed in Scheme 3 from the commercially available acids **22** and **23** using an amino acid coupling reaction followed by removal of the Boc protecting group.

Tables 1–4 detail the human histamine H_3 receptor binding and functional data for representative compounds that were prepared in this series.[11] First, it was determined that the cyclobutyl diazepane amide provided sufficient affinity for the histamine H_3 receptor with an unsubstituted phenoxy substituent (i.e., **11a**, Table 1) and that the addition of small substituents (R^4) on the phenoxy ring were tolerated (**11b**–**q**) regardless of their electronic contribution. Likewise, the heteroaryloxy substituents of **19a**–**g** provided acceptable affinity for the human H_3 receptor. Replacement of the cyclobutyl ring of the diazepane with other small branched alkyls was generally well tolerated (i.e., **11o**–**q**, **12j,** and **12o**), however a hydrogen (**12g**), methyl (**12h**), tetrahydropyran, (**12p**) or benzyl substituent (**12q**) resulted in a significant loss of histamine H_3 binding affinity.

We also explored additional functionalization of the pyrrolidine nitrogen (R^3). Compounds **12a**–**g** (Table 1) demonstrate that small substituents on the nitrogen are preferred and that the acetamide (**12e**, **12h**–**k**) may be slightly better than the unsubstituted analogs



Scheme 1. Synthesis of compounds **11** and **12** from *cis*-4-hydroxy-pyrrolidine-2-carboxylic acid. Reagents and conditions: (a) $(Boc)_2O$, NaOH, H_2O, Dioxane (84%); (b) amine, EDCI, Et_3N, DMF (15–30%); (c) phenol, PPh_3, DEAD, THF (15–40%); (d) TFA, DCM (74–98%); (e) RC(O)Cl or RSO_2Cl with Et_3N in THF or reductive amination.

Table 2
Binding and functional data for the human H_3 receptor for compounds **13**, **14**, and **18**

Compds	R^3	R^4	Human H_3 K_i[a] (nM)	Human H_3 pA_2[b]
13a	H	4-CN	184 ± 65	
13b	Ac	4-SMe	50 ± 13	7.68
13c	Ac	3-F	717 ± 85	
13d	*c*-Pr	4-F	55 ± 11	
14a	H	3-F	37 ± 4	
14b	H	4-CN	45 ± 1	7.92
14c	H	4-SMe	143 ± 24	
14d	Ac	3-F	600 ± 230	
18a	H	3-F	54 ± 33	
18b	H	4-CN	89 ± 62	
18c	Ac	3-F	840 ± 440	

[a] K_i's are the mean of at least three experiments in triplicate. K_i ± s.d. is reported.
[b] The result of a single experiment, in triplicate, unless s.d. is shown, then it is the mean of three experiments.

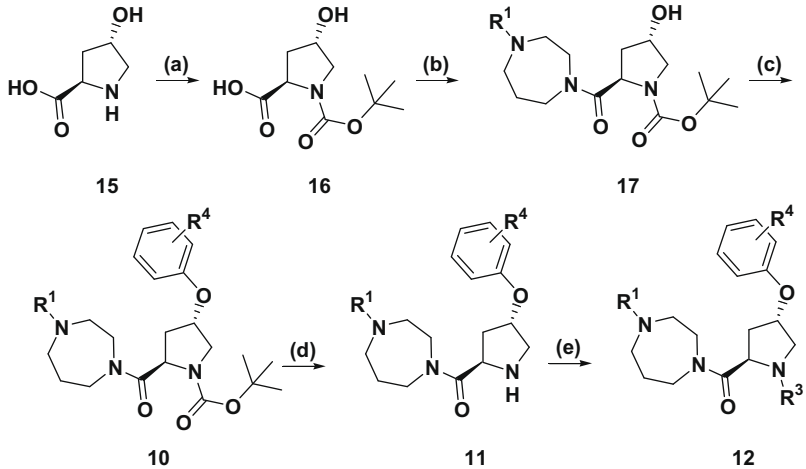
11. Compounds **13**, **14** and **18** (Table 2) reveal that the 2*S*,4*R* stereochemistry on the pyrrolidine ring (i.e., compounds **11** and **12**) is preferred for histamine H_3 affinity.

In an attempt to alter the metabolism that was observed with some aryloxy compounds, a few heteroaryloxy and carbon-linked compounds were also prepared. Compounds **19** (Table 3) demonstrate that thienyl and pyridyl substituents are tolerated as replacements for the phenoxy ring in compounds **12** and finally, the carbon-linked compounds **20a–d** and **21a–c** (Table 4) have somewhat lower affinity for the H_3 receptor and were not pursued further as they appeared to be inferior to the 4-aryloxy compounds **11** and **12**.

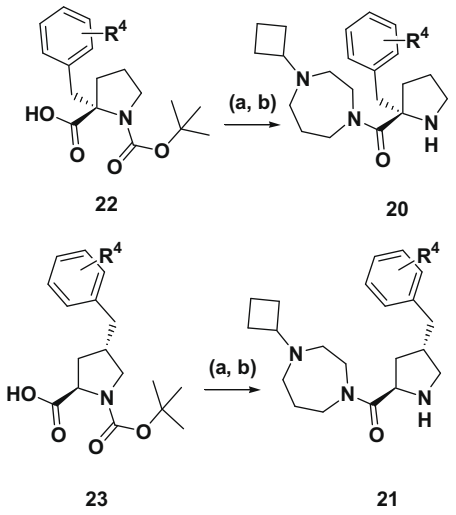
All of the compounds tested for functional antagonism at the human histamine H_3 receptor were found to be potent antagonists (pA_2's 7.7–9.9, Tables 1–3).[11]

Based on the in vitro data presented in Tables 1–4, and balancing in vitro microsomal data, hERG data and binding affinities, several compounds were chosen for further screening. Prior to any in vivo work, we typically verified in vitro functional antagonism at the rat H_3 receptor (i.e., rat pA_2), and measured hERG affinity.

Table 5 shows the data for the compounds that were tested. The compounds tested are all antagonists at the rat H_3 receptor and typically were slightly less potent at the rat H_3 receptor than at the human H_3 receptor. Table 2 also shows hERG affinity for these compounds. Compounds **11l**, **11o**, and **11p** have some affinity for the hERG channel in this assay. Following up on these initial findings, **11j**, **11k**, and **12k** were screened in a hERG patch clamp assay. The compounds had IC_{50}'s of 3.0 μM, 7.8 μM, and 6 μM,



Scheme 2. Synthesis of compounds **11** and **12** from *trans*-4-hydroxy-pyrrolidine-2-carboxylic acid. Reagents and conditions: (a) $(Boc)_2O$; (b) amine, EDCI, Et_3N, DMF; (c) ArI, CuI, Cs_2CO_3, 3,4,7,8-tetramethyl-1,10-phenanthroline, $PhCH_3$, 100 °C (26–50%); (d) TFA, DCM; (e) RC(O)Cl or RSO_2Cl with Et_3N in THF or reductive amination.



Scheme 3. Synthesis of the 2- and 4-benzyl-pyrrolidine-2-carboxylic acid amides **20** and **21**. Reagents and conditions: (a) cyclobutyldiazepane–HCl, EDCI, Et_3N, DMF; (b) TFA, DCM (47–93% over two steps).

Table 3
Binding and functional data for the human H_3 receptor for compounds **19**

Compds	R^3	Het	Human H_3 K_i[a] (nM)	Human H_3 pA_2[b]
19a	H	3-Thienyl	5.3 ± 0.6	8.65
19b	H	2-Pyridyl	4.6 ± 0.5	
19c	H	3-Pyridyl	2.6 ± 0.2	
19d	Ac	2-Thienyl	3.7 ± 0.4	
19e	Ac	3-Thienyl	2.7 ± 0.3	9.28
19f	Ac	2-Pyridyl	5.4 ± 0.3	
19g	Ac	3-Pyridyl	6.0 ± 0.4	

[a] K_i's are the mean of at least three experiments in triplicate. K_i ± s.d. is reported.
[b] The result of a single experiment, in triplicate, unless s.d. is shown, then it is the mean of three experiments.

Table 4
Binding and functional data for the human H_3 receptor for compounds **20** and **21**

Compds	R^4	Human H_3 K_i[a] (nM)
20a	4-F	16 ± 8.7
20b	3-F	44 ± 12
20c	4-CF_3	200 ± 73
20d	H	11 (n = 1)
21a	H	260 ± 150
21b	3-F	350 (n = 2)
21c	4-F	300 ± 230

[a] Except where indicated K_i's are the mean of at least three experiments in triplicate. K_i ± s.d. is reported.

respectively, which is in acceptable agreement with the binding data shown on Table 5.

Our strategy was next designed to identify compounds with acceptable brain exposures and clearance after oral administration. In order to determine whether a compound was likely to be of further interest, the compound was dosed po in rats and then plasma and brain concentrations, as well as ex vivo receptor occupancy, were measured at 1 and 3 h post dose.[11] The results of these studies are also shown in Table 5. Most of the compounds showed very high receptor occupancy and fairly high plasma and brain exposures.

Balancing the in vitro data and receptor occupancy/exposure data compound **12k** was chosen for further studies. This choice

Table 5
Functional data for the rat H_3 receptor, hERG affinity, and rat in vivo data for select compounds **11** and **12**

Compds	Rat H_3 pA_2[a]	hERG IC_{50}[b]	R.O.[c] (%)	Plasma/Brain concn[d]
11j	8.09	>10	95/96	3.88/3.70
11k	7.93	>10	95/96	3.71/3.40
11l	8.30	2.0	99/100	6.55/5.28
11m	8.59	7.2	97/100	9.94/8.16
11n	7.90	>10	94/97	3.93/3.76
11o	8.21	4.6	100/97	0.56/0.76
11p	8.51	2.9	96/98	0.38/0.40
12e	8.64	>10	67/46	0.77/0.12
12k	8.22 ± 0.21	>10	84/56	0.72/0.43

[a] The result of a single or duplicate experiment, in triplicate, unless s.d. is shown, then it is the mean of three experiments.
[b] The result, in μM, of a single experiment measuring the displacement of labeled dofetilide from the hERG channel.
[c] Receptor occupancy (R.O.) at 1 h/3 h after a 10 mpk po dose.
[d] Plasma/Brain concentrations (μM) at 1 h after a 10 mpk po dose.

was primarily based on the fact that **12k** showed robust receptor occupancy which appeared to be clearing over time therefore the compound was not likely to have a long tissue half-life. Additional pre-clinical pharmacokinetic data in rat, dog, and mouse for **12k** are shown on Table 6.

Compound **12k** on Table 6 has relatively high clearance, a relatively short half-life and a moderately high V_{ss} in all species tested. Bioavailability was good (⩾30%) in rat and dog, but rather low in mouse.

In a commercial panel of 50 receptor, ion channel and transporter assays (CEREP, www.cerep.com) **12k** did not have any significant affinity (at 1 μM) for any of the targets that were

Table 6
Pre-clinical pharmacokinetic data for compound **12k**[a]

Species	Cl (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	% F
Rat	66	3.0	0.81	30
Dog	31	2.1	0.87	48
Mouse	76	2.8	0.51	5

[a] For rat and mouse, the compound was dosed 1 mg/kg iv and 10 mg/kg po, for dog the doses were 1 mg/kg iv and 5 mg/kg po.

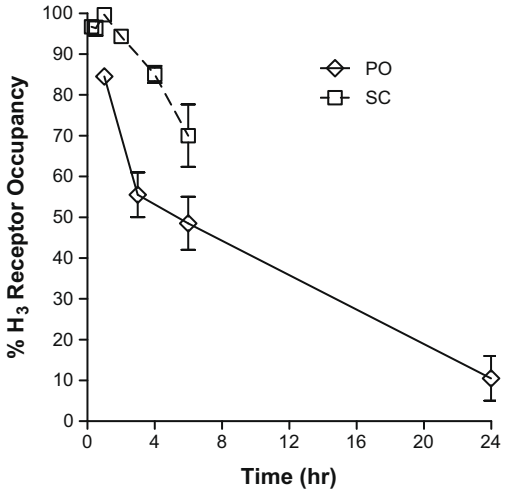


Figure 3. Ex vivo H_3 receptor occupancy with compound **12k** in rat striatum: time dependency after oral or subcutaneous administration (10 mg/kg). Results are expressed as average percentage receptor occupancy versus vehicle treated rats ± SEM. (n = 3).

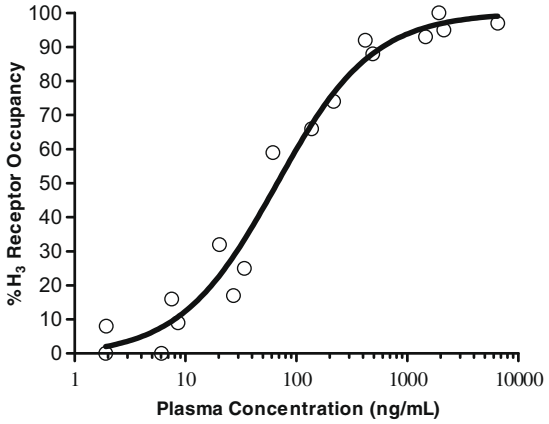


Figure 4. Ex vivo H_3 receptor occupancy with compound **12k** in rat striatum: dose dependency after oral administration. Results are presented as percentage receptor occupancy versus vehicle treated rats (each data point represents an individual animal).

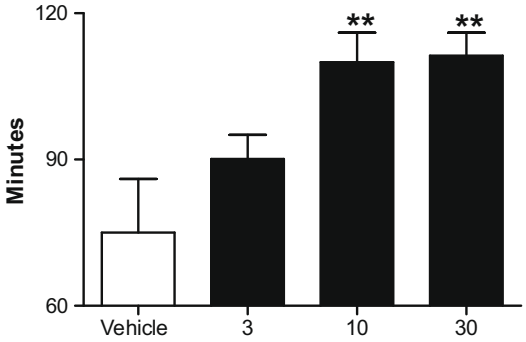


Figure 5. Dose–response effects of compound **12k** (3–30 mg/kg sc, $n = 7$ animals per dose) on total wake duration (minutes, mean ± SEM) during the first 4 h following the administration at the beginning of the light phase. $^{*}p < 0.05$, $^{**}p < 0.01$, Newman–Keuls post-hoc test following One Way ANOVA.

screened. The compound was also evaluated for the potential to be subject to efflux using a Caco-2 assay. The compound is highly permeable (A–B = 13.7 × 10^{-6} cm/s, B–A = 52.9 × 10^{-6} cm/s) and has a relatively low potential for efflux (ratio of B–A/A–B = 3.9).

More extensive ex vivo H_3 receptor occupancy experiments were then conducted in order to set doses for pharmacology experiments. Figure 3 shows receptor occupancy versus time in rat following oral and subcutaneous dosing (10 mg/kg) and Figure 4 shows a dose response for receptor occupancy versus plasma concentration.[11] Good receptor occupancy is achieved following oral dosing and the compound clears from the H_3 receptor quickly over time.

Figure 3 also shows that **12k** achieves maximal (90%) H_3 receptor occupancy in rat at a plasma concentration of ~450 ng/mL, which considering the differences in affinities for the human and rat histamine H_3 receptors (pA_2's 9.50 and 8.33, respectively) should conservatively translate to a human C_{eff} of under 45 ng/mL, assuming >90% H_3 receptor occupancy is required for efficacy.[13]

The wake promoting properties of **12k** in rat were also investigated by EEG. As detailed in Figure 5, the compound dose dependently increases total wake time after sc administration.

Finally, selective histamine H_3 antagonist is expected to modulate the levels of acetylcholine in the brain.[12] In order to verify this was the case for our compound, we used microdialysis experiments in freely moving rats (shown in Fig. 6) which clearly demonstrate a dose dependent increase in extracellular level of acetylcholine following oral administration of **12k**.

In conclusion, we have designed and prepared a series of pyrrolidines that are high affinity histamine H_3 receptor antagonists. Selected members efficiently penetrate the CNS and occupy the histamine H_3 receptor. One compound, **12k**, was extensively characterized, and was found to occupy the rat H_3 receptor, promote wake in rat and to increase the levels of extracellular acetylcholine in rat cortex. This compound has potential as a clinical candidate based on its strong pre-clinical profile.

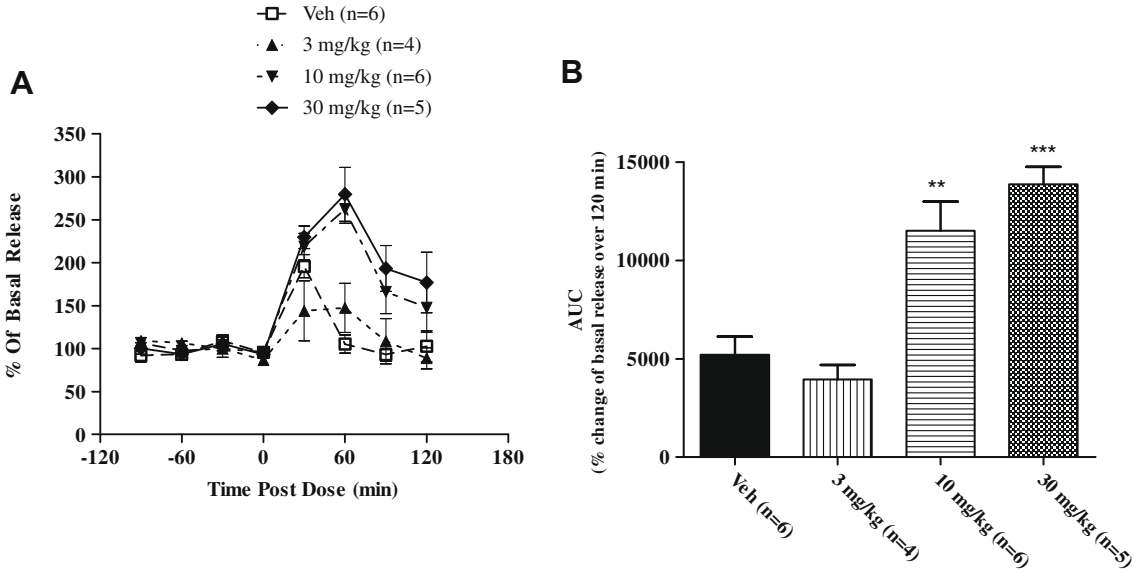


Figure 6. (A) Effects of compound **12k** (3, 10, 30 mg/kg po) on extracellular ACh levels from rat prefrontal cortex. (B) Area under the curve values of the data presented in panel A. All rats were administered the drug after measuring stable baseline. Data represents the means ± SEM, $n = 4$–6. $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle Dunnett's multiple comparison post-hoc following One Way ANOVA.[12]

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9. Additional synthetic details and compound characterization are described in Carruthers, N. I.; Letavic, M. A.; Ly, K. S.; Mani, N. S.; Pippel, D. J.; Shah, C. R.; Soyode-Johnson, A.; Stocking, E. M.; Young, L. PCT Int. Appl. (2009), WO 2009143153 A1.
10. The use of resin bound PPh_3 gave incomplete inversion in the Mitsunobu reaction. Compounds **13** and **14** were separated by reverse phase HPLC (0–99% MeCN in 20 mM NH_4OH) after *N*-Boc deprotection. Typical yields are shown on the schemes.
11. The affinity of test compounds for the human recombinant H_3 receptor was determined as described in Ref. 6. Functional data (i.e., pA_2 data) and all rat in vivo data was determined as described in Barbier, A. J.; Berridge, C.; Dugovic, C.; Laposky, A. D.; Wilson, S. J.; Boggs, J.; Aluisio, L.; Lord, B.; Mazur, C.; Pudiak, C. M.; Langlois, X.; Xiao, W.; Apodaca, R.; Carruthers, N. I.; Lovenberg, T. W. *Br. J. Pharmacol.* **2004**, *143*, 649.
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13. The relative binding affinities in rat and human could also be used to estimate human C_{eff}. The rat K_i for **12k** is 27 nM, therefore the ratio of binding affinities would suggest a human C_{eff} of $\sim$30 nM.