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In vitro studies on a class of quinoline containing histamine H_3 antagonists

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ABSTRACT

A series of quinoline containing histamine H_3 antagonists is reported herein. These analogs were synthesized via the Friedlander quinoline synthesis between an aminoaldehyde intermediate and a methyl ketone allowing for a wide diversity of substituents at the 2-position of the quinoline ring.

Presently, there are four known histamine receptors H_1, H_2, H_3 and H_4.[1–4] Drugs targeting H_1 and H_2 receptors have found clinical utilities in treating allergic rhinitis and reducing gastric acid secretion, respectively. The hope for novel clinical use targeting conditions such as ADHD (H_3) and asthma (H_4) has prompted intense efforts in the pharmaceutical industry to develop antagonists for histamine H_3 and H_4 receptors. The histamine H_3 receptor was first reported and characterized more than 20 years ago.[5] It is abundantly localized in CNS neurons, with lesser distribution in specific peripheral areas, predominantly neuronal.[6] On histaminergic neurons, the H_3 receptor acts as a presynaptic autoreceptor that regulates the release and synthesis of histamine; when localized on non-histamine neurons, it modulates the release of other neurotransmitters, such as acetylcholine, dopamine, noradrenaline and serotonin.[7–11] Preclinical studies of the histaminergic system have indicated the potential for H_3 antagonists as therapeutic agents for disorders involving attention, sleep, and cognition.[10,11]

Synthetic analogs of the natural ligand histamine were the first H_3 antagonists. These imidazole-based H_3 antagonists (1–3) (Fig. 1) were used to characterize the in vivo and in vitro pharmacology of the H_3 receptor.[12] The known liability of CYP inhibition induced by the imidazole moiety and the lower CNS penetration encouraged the subsequent design of non-imidazole H_3 antagonists.[13,14] In recent years, there have been reports of a number of structurally diverse non-imidazole H_3 antagonist series. Examples include prominent compounds such **4**,[15] **5**[16] and **6**.[17] ABT-239 and other benzofurans were found to be particularly potent and effective in a number of behavioral assays.[15,18,19]

Although ABT-239 has become a reference antagonist for preclinical behavioral studies, it has the liability of significant off-target activity at the hERG channel.[19] Thus, we were interested in other structural series beyond benzofurans targeting new series with equal or better affinity and good ADMET properties. Previous studies on the alkyl linker and the amine moiety of the benzofuran series uncovered that the ethyl-linked (*R*)-2-methylpyrrolidine group provided high affinity for the H_3 receptor.[16,20] During our investigation into modifications of the benzofuran, a series of naphthalenes[20] was discovered that provided good in vitro affinity for the H_3 receptor. Like **4**, members of the new naphthalene series were active in animal models of cognition. Beyond the 4-cyanophenyl group on the naphthalene and benzofuran cores, other active groups such as heterocycles could be prepared through cross-coupling reactions such as the Suzuki and Ullman reactions.

We report here a series of quinolines (general structure **13**, Scheme 1).[21] An additional benefit of this series was the ability to extensively probe the SAR of the series, due to the especially facile Friedlander condensation chemistry to install the R moiety on **13**. This reaction (step f in Scheme 1) enables a very wide variety of R group analogs to be readily generated.

The quinolines reported here were prepared through the Friedlander quinoline condensation[22] as the key step. As such, reaction of the *ortho*-aminoaldehyde **12** with methyl ketones (Scheme 1) was very efficient. Because a large variety of methyl ketones was available commercially and from internal compound collections, the chemistry allowed for the synthesis of a wide variety of derivatives not easily available via the cross-coupling chemistry used for the benzofurans.

As shown in Scheme 1,[23] 4-nitrophenethyl bromide **7** was displaced with (*R*)-2-methylpyrrolidine[24] in DMF to afford compound

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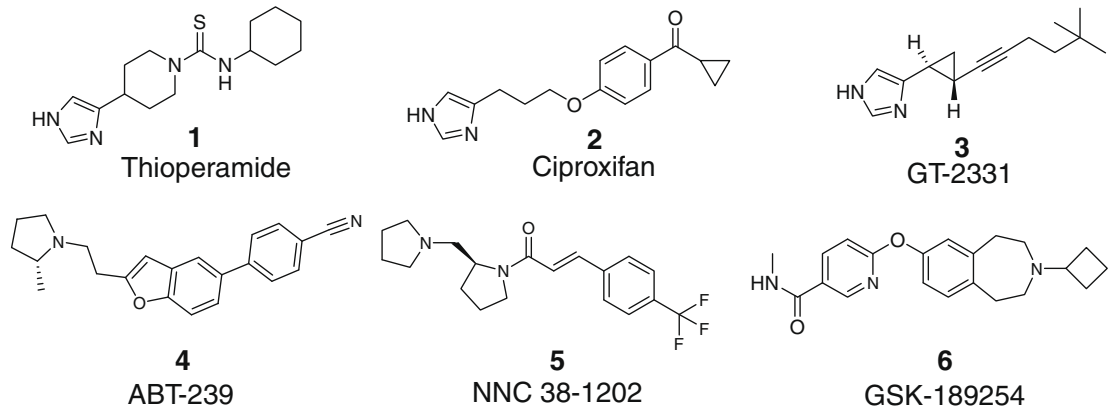
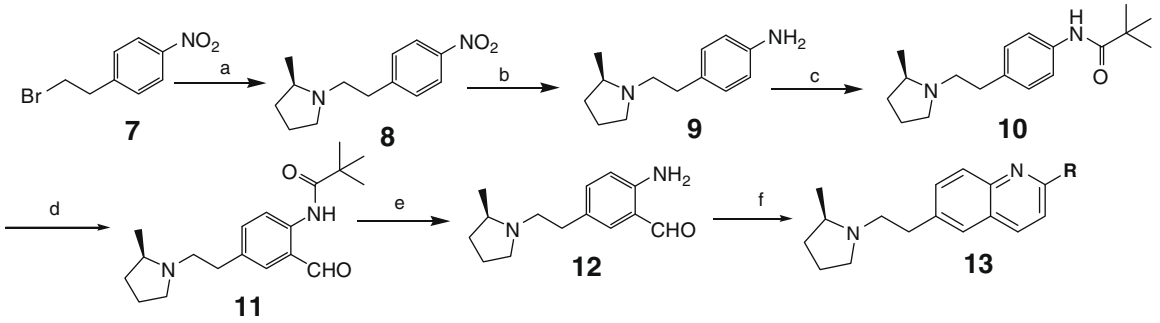


Figure 1. Structures of some H_3 antagonists.



Scheme 1. Reagents and conditions: (a) (*R*)-2-methylpyrrolidine tartrate, K_2CO_3/DMF, 65 °C, 24 h, 100%; (b) H_2, 10% Pd/C, 45 psi, 45 min, quantitative; (c) pivaloyl chloride, DCM, TEA, rt, 95%; (d) 3 equiv *n*-BuLi, 3 equiv TMEDA, ether, 0 °C, 30 min, then 6 equiv DMF, 0 °C to rt; (e) >50 mL 2 M HCl/g of **11**, reflux 45 min 59% from aniline; (f) RC(O)Me, NaOH, EtOH, reflux, 40–70%.

8, which was subsequently hydrogenated to aniline **9**. Protection of amino group as the pivaloyl amide **10** facilitated *ortho* directed formylation in the next step to provide the protected *ortho*-aminoaldehyde **11**. Deprotection of the pivaloyl amide was best carried out dilute in the hot aqueous acid to give moderate yields of the *ortho*-aminoaldehyde **12**; reactions run more concentrated gave lower yield due to the formation of a cyclotrimer of **12** through self condensation. Friedlander condensation yields varied, but were widely successful with a variety of substrates.

Methyl ketone condensation partners containing groups sensitive to alkoxide were labile in the Friedlander reaction. For example, the nitrile-containing ketones used to generate compounds **35** and **43** provided a mixture of desired products (**35** and **43**) and side products (compounds **36** and **44**, respectively). In addition, the ketone intermediate used to generate compound **70** was a chloropyrazine, the chlorine atom of which was displaced by ethoxide during the Friedlander reaction.

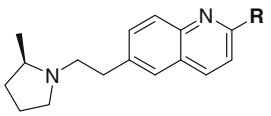
A wide variety of analogs with aryl and heteroaryl substituents on the 2-position of the quinoline were synthesized. The in vitro binding potencies for these analogs at the rat and the human H_3 receptor as well as the hERG channel are displayed in Table 1. Compounds in this series were shown to be inverse agonists in the GTPγS binding assay.[25] The 4-cyanophenyl analog **14** had similar H_3R affinity to that of the benzofuran, ABT-239, justifying the belief that the quinoline core could support high in vitro affinity. A survey of the 2-position revealed that alkyl substitution (**16**–**18**) decreased affinity relative to 4-cyano phenyl. The size of the alkyl group had no difference in terms of the H_3 affinities.

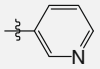
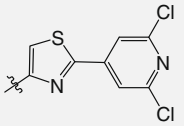
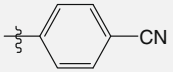
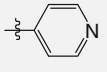
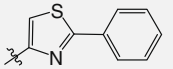
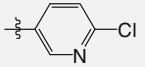
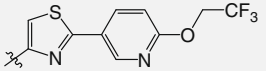
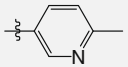
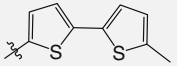
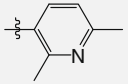
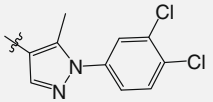
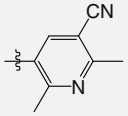
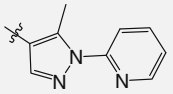
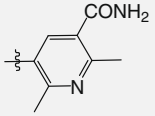
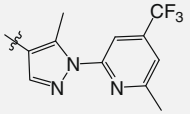
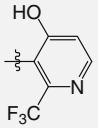
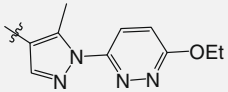
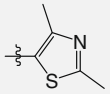
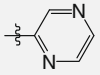
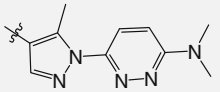
In general, the monocyclic aryl and heteroaryl substitutions (compounds **19**–**48**, **51**) had high affinity at both rat and human H_3Rs. Except for one compound (e.g., **45**), single ring substituted quinoline compounds (**19**–**48**) possessed H_3 binding potencies of less than 5 nM human and 20 nM rat. Also interesting was the almost 10-fold decrease in both rat and human H_3 affinities for dihydrothiazole **20**, a non-aromatic ring, compared to thiazole **19**. The 4-methylpiperidine analog, compound **51**,[26] was the most potent compound of the series, having an affinity of 50 pM for the hH_3R and 80 pM at the rH_3R.

Analogs **49**, **50** and **52**–**76** were made, with the aryl or heteroaryl derivatives substituted with an additional ring. Most of these second rings were at the distal position relative to the quinoline. These additional ring substitutions were well tolerated or even improved beyond most of the analogs of benzofuran series, with potencies in the subnanomolar range, with exceptions being compounds **63**–**66** and **73**. These compounds indicate that increased lipophilicity on the more distal ring may result in some decrease in binding affinity for the H_3 receptor. Three analogs, compounds **74**–**76**, possessed a phenyl ring connected to the "*ortho*" position of the middle ring. These analogs maintained high in vitro potencies for the H_3 receptor. One interesting SAR finding was the differences in potencies between compounds **73** and **74**, wherein the distal substituted **73** is over 50-fold less potent than the corresponding "*ortho*" substituted **74**. The fused analogs, compounds **77**–**87**, were highly potent as well. In general, in vitro potencies were very well maintained in spite of the different groups and orientations.

In addition to the in vitro H_3R affinities, the new compounds were also tested for interaction with hERG (Human Ether-a-go-go Related Gene) channel in a competition binding assay ([^{3}H]-dofetilide used as the radioligand).[27] This voltage-gated potassium channel has been associated with drug-induced long QT syndrome.[28] Reducing or eliminating hERG binding at an early stage

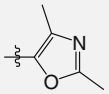
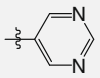
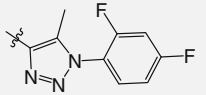
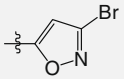
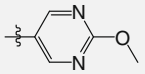
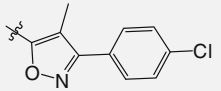
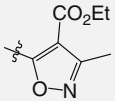
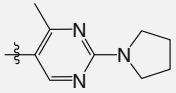
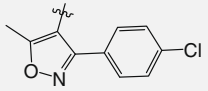
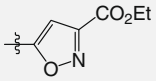
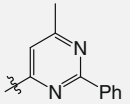
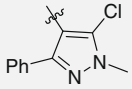
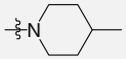
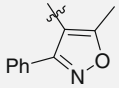
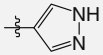
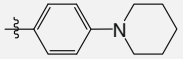
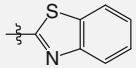
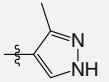
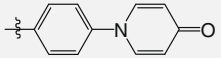
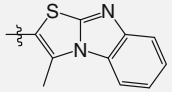
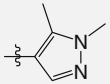
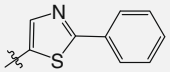
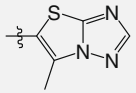
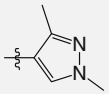
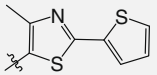
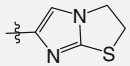
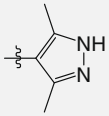
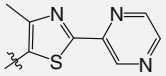
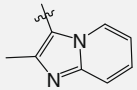
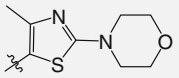
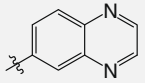
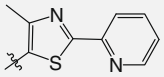
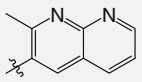
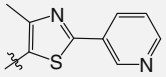
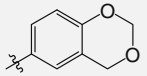
Table 1
Binding data (K_i) of quinolines at human H_3R, rat H_3R and hERG channel. Selectivity (sel. × 1000) for activity at human H_3R over hERG shown



Compd	R	H_3[a,b] (nM)		hERG[b,c]		Compd	R	H_3[a,b] (nM)		hERG[b,c]		Compd	R	H_3[a,b] (nM)		hERG[b,c]	
		Hum	Rat	μM	Sel.			Hum	Rat	μM	Sel.			Hum	Rat	μM	Sel.
4	ABT-239	0.45	1.4	0.40	1.1	**38**		0.11	0.50	3.0	27	**63**		28	55	5.1	0.2
14		0.69	3.9	0.78	1	**39**		0.22	1.9	6.9	31	**64**		6.8	31	0.4	0.06
15	H	7.4	41	10	1	**40**		0.25	0.48	0.87	4	**65**		25	58	0.38	0.02
16	Me	12	65	54	5	**41**		0.15	0.45	6.6	44	**66**		21	71	0.5	0.02
17		7.4	47	11	2	**42**		0.36	2.2	5.0	14	**67**		0.74	2.1	0.15	0.2
18	t-Bu	11	51	1.7	0.2	**43**		0.28	2.0	2.1	8	**68**		0.26	0.91	5.0	19
19		0.44	1.6	2.9	7	**44**		0.98	5.5	10	10	**69**		1.1	5.9	4.2	4
20		3.8	17	6.3	2	**45**		230	1000	10	0.04	**70**		0.19	0.49	2.6	14
21		0.19	1.4	5.1	27	**46**		0.11	0.51	6.2	56	**71**		0.19	1.1	4.5	24

(*continued on next page*)

Table 1 (*continued*)

Compd	R	H_3[a,b] (nM)		hERG[b,c]		Compd	R	H_3[a,b] (nM)		hERG[b,c]		Compd	R	H_3[a,b] (nM)		hERG[b,c]	
		Hum	Rat	μM	Sel.			Hum	Rat	μM	Sel.			Hum	Rat	μM	Sel.
22		0.56	1.5	10	18	**47**		0.19	0.66	10	53	**72**		0.29	0.35	0.72	3
23		1.8	5.5	0.22	0.1	**48**		0.23	1.0	5.6	24	**73**		7.8	36	0.44	0.06
24		0.71	5.4	1.5	2	**49**		0.29	0.72	2.6	9	**74**		0.16	0.40	0.40	3
25		3.5	16	4.2	1	**50**		1.7	1.4	0.03	0.02	**75**		0.41	0.59	1.5	4
26		3.2	14	10	3	**51**		0.05	0.08	5.6	112	**76**		0.10	0.31	1.6	16
27		0.89	5.9	10	11	**52**		1.0	2.3	0.69	0.7	**77**		2.9	8.5	0.48	0.2
28		0.2	1.5	4.4	22	**53**		0.12	0.3	5.0	42	**78**		0.36	0.78	0.43	1
29		0.16	0.68	10	63	**54**		1.1	6.8	0.34	0.3	**79**		0.10	0.25	0.71	7
30		0.45	1.6	6.2	14	**55**		0.95	4.5	0.93	1	**80**		0.85	4.9	10	12
31		0.93	5.4	10	11	**56**		0.35	1.4	7.9	23	**81**		0.12	0.50	2	17
32		5.1	20	2.1	0.4	**57**		0.56	2.9	5.1	9	**82**		0.37	1.7	2.1	6
33		2.5	6.9	1.2	0.5	**58**		0.34	2.3	2.8	8	**83**		0.34	2.0	10	29
34		1.0	3.6	0.62	0.6	**59**		0.35	1.9	1.5	4	**84**		0.40	2.3	0.91	2

of drug discovery has become a primary task of current drug discovery. The large number of new compounds made in this quinoline series allowed us to assess hERG and H_3 affinities for a wide range of substituents on the quinoline core, and to find compounds that were highly selective over hERG, while maintaining potent in vitro activity at the H_3R. From Table 1, general trends can be seen. In the monocyclic derivatives, analogs such as bromoisoxazole **23**, furan **33**, and thiophenes, **34** and **35**, had unacceptable high hERG affinities. Whereas, analogs with pyrazoles (**26**–**31**), pyridines (**37**–**39**, **41** and **42**) and pyrimidines (**47** and **48**) bound much less potently to the hERG channel, and thus possess a more favorable selectivity for H_3 over hERG. In the case of groups substituted with an additional ring, the more distal ring seems to play a bigger influence on hERG binding. Although the analogs allowing a direct comparison were not available, general trends show that replacement of the phenyl of compound **54** with groups such as pyrazine (**56**) and pyridine (**58**–**60**) result in a decrease in the hERG binding. The high selectivity of this series over the hERG site was further evaluated by examining analogs in a Purkinje fiber assay.[29] Compounds **30** and **86** displayed no adverse advents at high plasma concentration, 250 ng/mL and 200 ng/mL, respectively.

A selection of compounds (**30**, **49**, **68**, **70**, **83** and **86**) was chosen for further PK and in vivo profiling.[21] Compounds **30**, **49**, **68**, **70** and **86** displayed good PK properties, blood–brain barrier penetration and were found to be efficacious in a social recognition memory test in adult rats. In addition, compounds **30** and **86** were found to be efficacious at 0.1 and 0.3 mg/kg, respectively, in the 5-trial inhibitory avoidance model, a primary model to assess the animal learning ability as well as impulsive behaviors.

In conclusion, a novel series of quinoline H_3 antagonists was discovered. The facile synthesis allowed a very wide variety of aromatic and heteroaromatic substitutions to be synthesized. The SAR of the analogs in this quinoline series showed very high H_3R affinity and selectivity over hERG for the majority of the compounds.

Acknowledgements

The authors thank Ms. Tracy Carr, Mr. David Witte and Mr. John Baranowski for in vitro data of some of the compounds.

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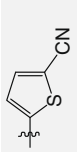
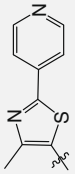
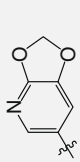
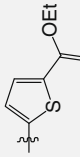
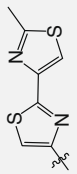
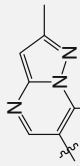
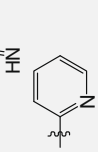
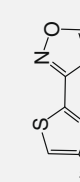
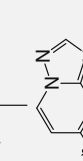
35		0.32	1.2	0.48	2	**60**		0.32	2.8	1.2	4	**85**		0.83	2.6	10	12
36		0.65	2.3	1.2	2	**61**		1.7	11	3.1	2	**86**		0.24	0.93	2.2	9
37		0.76	7.2	4.5	6	**62**		0.89	4.9	0.71	0.8	**87**		0.69	2.8	5.2	8

[a] Binding affinities (pK_i) were assessed by displacement of [^{3}H]-*N*-α-methylhistamine, using cloned human H_3 receptors and cloned rat H_3 receptors.[16] Data expressed as the K_i (nM).
[b] The values are reported as the average of $n \geqslant 3$ independent measurements for all compounds. Statistical significance was measured as the $pK_i \pm$SEM (standard error of the mean). The SEM was <0.25 for all compounds in Table 1.
[c] Binding affinities (pK_i) were assessed by displacement of [^{3}H] dofetilide from membrane preparations of HEK-293 cells heterogeneously expressing the hERG K^+ channel.[26] Data expressed as the K_i (μM).

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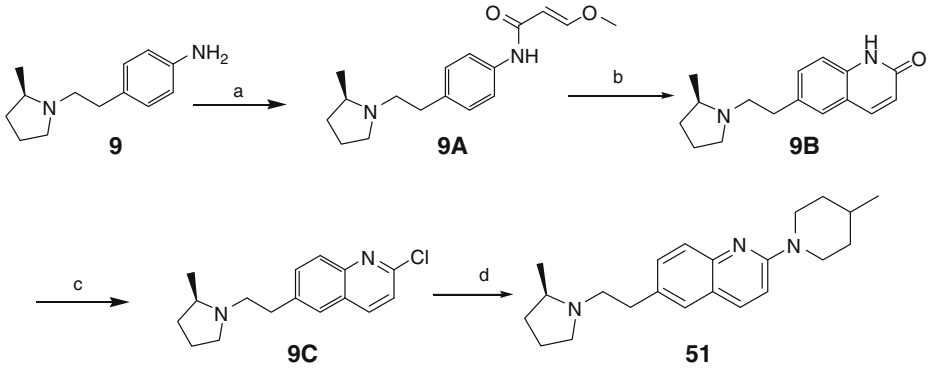

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26. Compound **51** was prepared by the following scheme:

Reagents and conditions: (a) (*E*)-3-methoxyacryloyl chloride, pyridine (1.4 equiv)/toluene, 0 °C to rt, 16 h, 70%; (b) concd H_2SO_4(10 mL/g SM), rt, 2 h, 74%; (c) $POCl_3$(8 mL/g SM), 110 °C, 2 h, 95%; (d) 4-methylpiperidine (20 equiv), sealed tube, 90 °C, 16 h, 68%.
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