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Bioorganic & Medicinal Chemistry Letters 18 (2008) 1490–1494
Bioorganic & Medicinal Chemistry Letters

A new family of H_3 receptor antagonists based on the natural product Conessine

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Received 20 November 2007; revised 17 December 2007; accepted 21 December 2007
Available online 25 December 2007

Abstract—A new family of Histamine H_3 receptor antagonists (**5a**–**t**) has been prepared based on the structure of the natural product Conessine, a known H_3 antagonist. Several members of the new series are highly potent and selective binders of rat and human H_3 receptors and display inverse agonism at the human H_3 receptor. Compound **5n** exhibited promising rat pharmacokinetic properties and demonstrated functional antagonism of the H_3 receptor in an in-vivo pharmacological model.

The histamine H_3 receptor is one of four distinct G-protein coupled histamine receptors known as H_1, H_2, H_3, and H_4.[1] Histamine H_1 and H_2 receptors are validated drug targets and involved in allergic reactions (H_1 antagonists) and gastric acid secretion (H_2 antagonists). The more recently discovered H_4 receptor is found in immune cells and is being investigated as a target for inflammatory disorders. H_3 receptors are expressed primarily in the central nervous system (CNS) where they are located presynaptically, and function as constitutively active regulators of the synthesis and release of histamine and several other neurotransmitters.[2] H_3 antagonists have attracted interest recently as potential treatments for CNS disorders relating to feeding, wakefulness, cognition, and pain.[3] Many of the early H_3 antagonists contained an imidazole group similar to the natural ligand histamine. The potential for imidazole-containing ligands to inhibit CYP-450 enzymes[4] and the low receptor selectivity exhibited by some early H_3 antagonists[5] encouraged the development of second generation H_3 antagonists which lacked this group. Such 'nonimidazole' H_3 antagonists are now at the forefront of H_3 drug development and several compounds in this class have recently entered human clinical trials.[6]

We were interested in developing novel H_3 antagonists/inverse agonists as wake promoting agents, and were intrigued when a high throughput screen of our compound collection identified the steroidal alkaloid natural product Conessine (**1**) as a fairly potent H_3 antagonist.[7] Compound **1** was found to inhibit [^{3}H] *N*-methyl histamine binding in a rat cortex membrane assay with a K_i of 66 nM. We hypothesized that the relative positioning of the two amine functionalities of **1** was crucial to its biological activity, and sought to construct novel H_3 antagonists by designing a series of compounds that would orient two amines in a manner similar to that of **1**. This approach was further supported by several reports of diamine-based H_3 antagonists (e.g., **2**,[8] **3**,[9] and **4**,[10] Fig. 1) that appeared during the course of our work. As outlined in Figure 2, the initial target compounds consisted of a series of diamines **5** in which the structure of **1** was simplified by aromatization of the central six-membered ring,

Keywords: Histamine H_3 receptor antagonist; Histamine H_3 receptor inverse agonist; Conessine.
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doi:10.1016/j.bmcl.2007.12.059

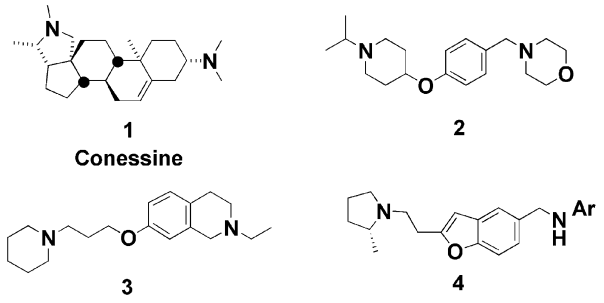


Figure 1. Structure of Conessine and representative diamine H_3 antagonists.

removal of several carbon atoms, and the introduction of a double bond in the remaining bicyclic portion of the scaffold.

The initial target compounds were prepared using the methods shown in Scheme 1. Commercially available azomethine ylide precursor **6** was treated with cyclopentenone **7** to give bicyclic ketone **8** via 3 + 2 cycloaddition. Reaction of **8** with the alkyllithium reagent derived from silyl-ether **9** provided the alcohol **10**, which was dehydrated and deprotected with HCl then activated with methanesulfonyl chloride to give mesylate derivative **11**. Reaction of **11** with various secondary amines under basic conditions provided compounds **5a**–**m**, which enabled exploration of phenethylamine substituent effects on binding.

Alteration of the bicyclic portion of these molecules was examined by preparing diamines **5n**–**t**, which incorporate the phenethyl pyrrolidine fragment of compound **5f**. In this case, bicyclic ketone **8** was reacted with the lithium reagent derived from 4-bromo-phenethylamine **12** to give the alcohol **13**. Deprotection of the *N*-benzyl group of **13** followed by dehydration with HCl provided diamine **14**, which was then substituted via reductive amination or arylation to give analogs **5n**–**t**.

Compounds **5a**–**t** were tested in a rat cortex *N*-[^{3}H]-methylhistamine binding assay,[11] the results of which are shown in Table 1. Several compounds in the initial series bound the receptor with high affinity, and it was apparent that the basicity and substitution patterns of both nitrogens were important for binding affinity. For example, the phenethylpyrrolidine analog **5f** (K_i = 0.3 nM) was 10-fold more potent than difluoropyrrolidine analog **5j** (2.8 nM) and 1000 times more potent than the amide **5k** (360 nM). Compound **5f** was also significantly more potent than the indoline analogs **5l** and **5m**, suggesting that small to medium sized substituents are preferred at this position. Substitution in the phenethylpyrrolidine ring also influenced receptor affinity. This is demonstrated by the six-fold increase in potency for methylpyrrolidine analog **5g** (0.05 nM) compared to unsubstituted pyrrolidine analog **5f**, and the apparent preference for the *S*-hydroxymethyl fragment in **5i** over the R-isomer in **5h**, a trend that has been observed with other H_3 antagonists.[12]

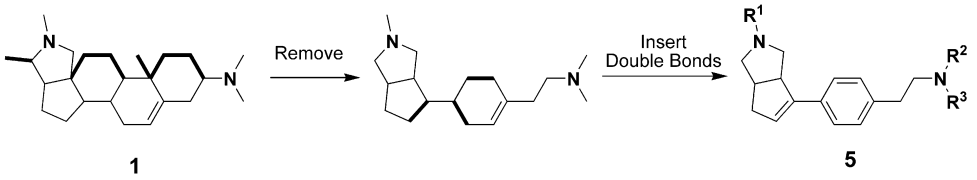
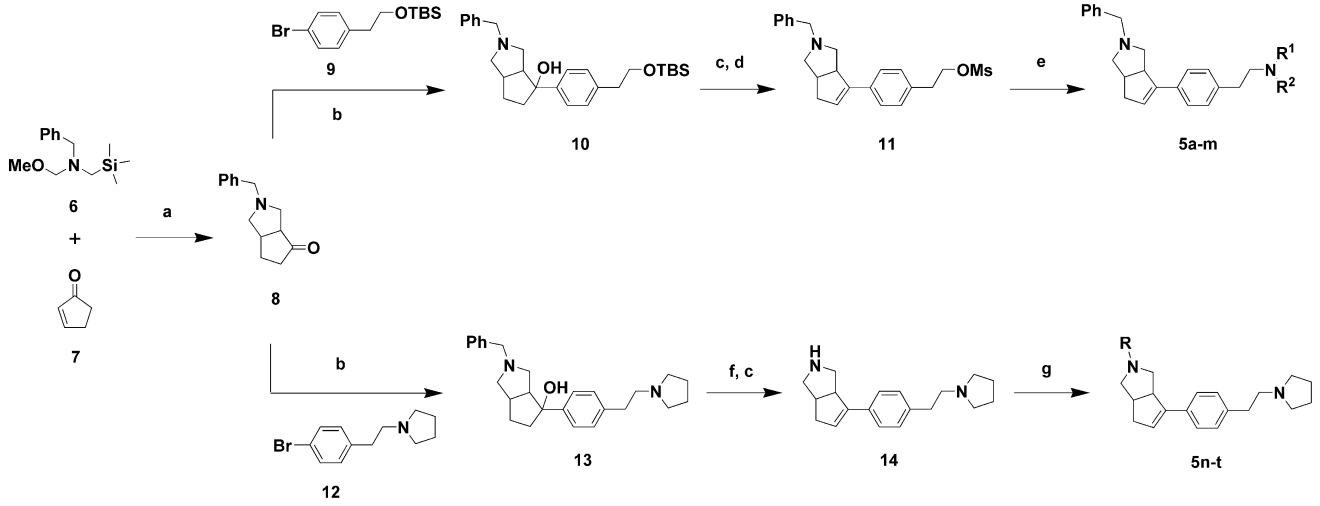
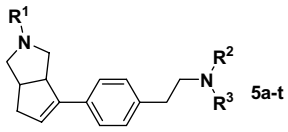


Figure 2. Design of H_3 antagonists based on Conessine.



Scheme 1. Synthesis of target compounds **5**. Reagents and conditions: (a) CH_3CN, rt, 69%; (b) *n*-BuLi, THF, −78 °C, 64–66%; (c) HCl, *i*-PrOH, 60 °C, 98%; (d) MsCl, Et_3N, CH_2Cl_2, rt, 82%; (e) HNR_1R_2, Na_2CO_3, CH_3CN, microwave, 120 °C, 25–60%; (f) NH_4CHO_2, $Pd(OH)_2$/C, MeOH, 98%; (g) i—RCHO or R_2CO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 22–88%; or ii—ArBr, $Pd(dba)_2$, *t*-BuOK, DMSO, 175 °C, 8–31%.

Table 1. Binding Affinities (K_i and pK_i) of compounds **5a**–**t** at rat H_3 receptor[a]



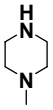
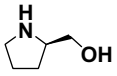
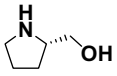
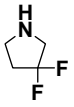
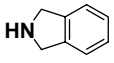
Compound	R^1	NR^2R^3	Rat H_3 K_i[b] (nM)	Rat pK_i[b]
5a	Bn		2.3	8.64 ± 0.24
5b	Bn		2.4	8.62 ± 0.29
5c	Bn		0.4	9.43 ± 0.09
5d	Bn		1.7	8.77 ± 0.27
5e	Bn		58	7.23 ± 0.24
5f	Bn		0.3	9.51 ± 0.25
5g	Bn		0.05 (**h Antag.** = **0.1**)[d]	10.23 ± 0.25
5h	Bn		0.51 (**h Antag.** = **8.5**)[d]	9.29 ± 0.28
5i	Bn		0.16 (**h Antag.** = **1.5**)[d]	9.80 ± 0.12
5j	Bn		2.8	8.56 ± 0.15
5k	Bn		310	6.88 ± 0.14
5l	Bn		NA[c]	NA[c]
5m	Bn		256	6.59 ± 0.20
5n			0.1	9.95 ± 0.09
5o			0.4 (**h Antag.** = **1.7**)[d]	9.38 ± 0.30

Table 1 (*continued*)

Compound	R^1	NR^2R^3	Rat H_3 K_i[b] (nM)	Rat pK_i[b]
5p			0.9	9.02 ± 0.04
5q			67	7.17 ± 0.39
5r			2	8.63 ± 0.19
5s			36	7.45 ± 0.06
5t			7	8.17 ± 0.19
(3a*S*, 6a*S*)-**5f**	Bn		0.75	9.12 ± 0.18
(3a*R*, 6a*R*)-**5f**	Bn		0.05 (**h Antag.** = **0.6**, **Inverse Ag.** = **2.0**)[d]	10.35 ± 0.10
(3a*R*, 6a*R*)-**5n**			0.07 (**h Antag.** = **0.9**, **Inverse Ag.** = **1.0**)[d]	10.17 ± 0.21
(3a*R*, 6a*R*)-**5o**			0.08	10.11 ± 0.44
(3a*R*, 6a*R*)-**5p**			0.29	9.53 ± 0.13

[a] Displacement of *N*-[^{3}H]-methylhistamine from rat cortex membranes.[11]
[b] Values are reported as average of $n \geqslant 3$ independent measurements for all compounds. Errors are ± log SD.
[c] NA, no activity below 5 μM.
[d] Antagonism determined by displacement of [^{3}H] R(−)-α-methylhistamine from recombinant CHO-K1 cells expressing the human H_3 receptor ($n = 1$). Inverse Agonism determined by GTPγS binding in CHO-K1 cells expressing the human H_3 receptor in the absence of agonist ($n = 1$). Testing performed at MDS Pharma Services (Taiwan).[14]

The basicity and substitution of the bicyclic nitrogen was also important in determining potency. Substitution of the bicyclic nitrogen with simple alkyl groups produced compounds **5n**–**p**, which exhibited sub-nanomolar potency similar to that seen for the *N*-benzyl derivative **5f**. In contrast, the less basic *N*-aryl analogs **5q**–**t** were significantly less potent than the *N*-alkyl analogs although some still bound with affinities in the low nM range.

Having confirmed that the new scaffold could be used to identify potent H_3 antagonists, the preferred stereochemistry of the bicyclic moiety was examined. To this end, both isomers of **5f** were prepared from the resolved isomers of ketone **8**. The more potent isomer of **5f** was identified as having the 3a*R*, 6a*R* configuration based on the stereochemistry of (3a*S*, 6a*R*)-**8**, which was assigned by X-ray crystallography.[13] The same isomer of **8** was then used to prepare the (3a*R*, 6a*R*) isomers of **5n**–**p**, which were significantly more potent than their respective racemic mixtures.

In order to broadly assess binding affinity at the human H_3 receptor, six compounds from the new series were tested in a human H_3 receptor binding assay (Table 1).[14] In each case there was a shift toward lower potency at the human receptor that ranged from 3- to 20-fold compared to that at the rat receptor. Compounds (3a*R*, 6a*R*)-**5f** and (3a*R*, 6a*R*)-**5n** also displayed inverse agonist activity at the human receptor in the low nanomolar range when tested in an H_3 GTPγS—functional assay in the absence of agonist (Table 1) and displayed

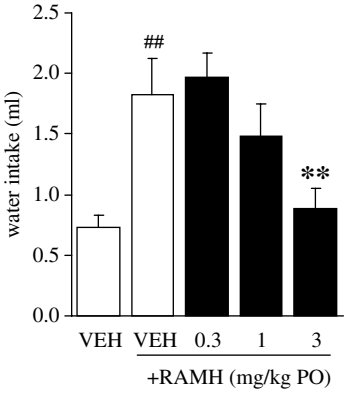


Chart 1. Inhibition of (*R*)-α-methylhistamine induced drinking after oral administration of compound (3a*R*, 6a*R*)-**5n**.

greater than 1000-fold selectivity for the H_3 receptor when screened against a panel of over 100 human GPCR's, including H_1, H_2, and H_4.

Preliminary evaluation of the rat pharmacokinetics of selected compounds from the new series was used to select a compound for in-vivo study. In general, the diamine analogs exhibited good to excellent oral bioavailability, long half-lives, and extensive tissue distribution. The pharmacokinetic profile of (3a*R*, 6a*R*)-**5n** ($F = 78\%$, $t_{1/2} = 6$ h) was considered the most promising and this compound was chosen for further testing.

Functional antagonism of the H_3 receptor in vivo was demonstrated for (3a*R*, 6a*R*)-**5n** in a rat dipsogenia model, in which an acute drinking response induced by an H_3 agonist ((*R*)-α-methylhistamine) is attenuated by pre-administration of an H_3 antagonist.[15] As shown in Chart 1, compound (3a*R*, 6a*R*)-**5n** (0.3, 1 and 3 mg/kg) or vehicle was administered orally to rats prior to the subcutaneous administration of 10 mg (*R*)-α-methylhistamine. Compound (3a*R*, 6a*R*)-**5n** was found to inhibit agonist induced drinking in a dose dependent manner with a minimum effective dose of 3 mg/kg.

In summary, we have developed a new family of H_3 antagonists based on the natural product Conessine. Several members of this family exhibit sub- to low-nanomolar potency at rat and human H_3 receptors. Preliminary in-vitro and in-vivo screening results suggest that the new compounds possess promising selectivity and pharmacokinetic profiles. Results for compound (3a*R*, 6a*R*)-**5n**, which displays in-vivo activity consistent with that of a potent, orally available H_3 antagonist, are particularly noteworthy.

References and notes

1. (a) Hill, S.; Ganellin, C.; Timmerman, H.; Schwartz, J.; Shankley, N.; Young, J.; Schunack, W.; Levi, R.; Haas, H. *Pharmacol. Rev.* **1997**, *49*, 253; (b) Hough, L. B. *Mol. Pharmacol.* **2001**, *59*, 415.
2. (a) Schwartz, J. C.; Arrang, J. M.; Garbarg, M.; Pollard, H.; Ruat, M. *Physiol. Rev.* **1991**, *71*, 1; (b) Leurs, R.; Smit, M. J.; Timmerman, H. *Pharmacol. Ther.* **1995**, *66*, 413; (c) Morisset, S. A.; Rouleau, A.; Ligneau, X.; Gbahou, F.; Tardivel-Lacombe, J.; Stark, H.; Schunack, W.; Ganellin, C. R.; Schwartz, J.-C.; Arrang, J.-M. *High Nat.* **2000**, *408*, 860; (d) Garduno-Torres, B.; Trevino, M.; Gutierrez, R.; Arias-Montano, J.-A. *Neuropharmacology* **2007**, *52*, 527; (e) Threlfell, S.; Cragg, S.; Kallo, I.; Turi, G.; Coen, C.; Greenfield, S. *J. Neurosci.* **2004**, *24*, 8704.
3. (a) Medhurst, A.; Briggs, M.; Bruton, G.; Calver, A.; Chessell, I.; Crook, B.; Davis, J.; Davis, R.; Foley, A.; Heslop, T.; Hirst, W.; Medhurst, S.; Ociepka, S.; Ray, A.; Regan, C. M.; Sargent, B.; Schogger, J.; Stean, T.; Trail, B.; Upton, N.; White, T.; Orlek, B.; Wilson, D. *Biochem. Pharmacol.* **2007**, *73*, 1182; (b) Passani, M.; Lin, J.-S.; Hancock, A.; Crochet, S.; Blandina, P. *Trends Pharmacol. Sci.* **2004**, *25*, 618; (c) Hancock, A.; Brune, M. *Expert Opin. Investig. Drugs* **2005**, *14*, 223; (d) Bernaerts, P.; Lamberty, Y.; Tirelli, E. *Behav. Brain Res.* **2004**, *154*, 211; (e) Parmentier, R.; Anaclet, C.; Guhennec, C.; Brousseau, E.; Bricout, D.; Giboulot, T.; Bozyczko-Coyne, D.; Spiegel, K.; Ohtsu, H.; Williams, M.; Lin, J. S. *Biochem. Pharmacol.* **2007**, *73*, 11571; (f) Esbenshade, T.; Fox, G.; Cowart, M. *Mol. Interv.* **2006**, *59*, 77.
4. (a) Murray, M. *Drug Metab. Rev.* **1987**, *18*, 55; (b) Yang, R.; Hey, J.; Aslanian, R.; Rizzo, C. *Pharmacology* **2002**, *66*, 128; (c) Halpert, J. R.; Guengerich, F. P.; Bend, J. R.; Correia, M. A. *Toxicol. Appl. Pharmacol.* **1994**, *125*, 163.
5. Leurs, R.; Tulp, M.; Menge, W.; Adolfs, M.; Zuiderveld, O.; Timmerman, H. *Br. J. Pharmacol.* **1995**, *116*, 2315.
6. Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. *Drug Discovery Today* **2005**, *10*, 1613.
7. Conessine's activity at the H_3 receptor has been reported by others, see: Zhao, Chen; Bennani, Y. L.; Gopalakrishnan, S.; Sun, M.; Esbenshade, T. A.; Krueger, K. M.; Miller, T. R.; Witte, D. G.; Marsh, Kennan C.; Cowart, M. D.; Hancock, A. A. Abstracts of Papers, 230th ACS National Meeting, Washington, DC, United States, Aug. 28–Sept. 1, **2005**, MEDI-104.
8. Dvorak, C.; Apodaca, R.; Barbier, A.; Berridge, C.; Wilson, S.; Boggs, J.; Xiao, W.; Lovenberg, T.; Carruthers, N. *J. Med. Chem.* **2005**, *48*, 2229.
9. Jesudason, C.; Beavers, L.; Cramer, J.; Dill, J.; Finley, D.; Lindsley, C.; Stevens, F. C.; Gadski, R.; Oldham, S.; Pickard, R.; Siedem, S.; Sindelar, D.; Singh, A.; Watsona, B.; Hipskind, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3415.
10. Sun, M.; Zhao, C.; Gfesser, G.; Thiffault, C.; Miller, T.; Marsh, K.; Wetter, J.; Curtis, M.; Faghih, R.; Esbenshade, T.; Hancock, A. A.; Cowart, M. *J. Med. Chem.* **2005**, *48*, 6482.
11. Esbenshade, T.; Fox, G.; Krueger, K.; Miller, T.; Kang, C.; Denny, L.; Witte, D.; Yao, B.; Pan, L.; Wetter, J.; Marsh, K.; Bennani, Y.; Cowart, M.; Sullivan, J.; Hancock, A. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 165.
12. Cowart, M.; Pratt, J. K.; Stewart, A. O.; Bennani, Y. L.; Esbenshade, T. A.; Hancock, A. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 689.
13. Single isomers of **8** (>99.5%ee) were isolated by diastereomeric salt formation, starting from racemic **8** and L- or D- di-*p*-toluoyl-tartaric acid, followed by repetitive recrystallization from isopropanol. Crystallographic data for (3a*S*, 6a*R*)-**8**·L-di-*p*-toluoyl-tartaric acid have been deposited with the Cambridge Crystallographic Data Centre (CCDC 670453). Optical rotation for (3a*S*, 6a*R*)-**8**·L-di-*p*-toluoyl-tartaric acid: $[\alpha]_D^{22} = -113.8$ (c 0.0016, EtOH).
14. Human H_3 binding and functional (GTPγS) assays were performed by MDS Pharma Services, Taiwan (http://www.mdsps.com).
15. Clapham, J.; Kilpatrick, G. J. *Eur. J. Pharmacol.* **1993**, *232*, 99.