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SHORT COMMUNICATION

In search of potent histamine-3 receptor antagonists

Sankar Chatterjee

Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380-4245, USA

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KEYWORDS

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Antagonist;
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Abstract Starting with modafinil, an alertness and wake-promoting agent but inactive in histamine 3 receptor (H_3R) binding assay, a series of potent H_3 receptor antagonists were developed.

1. Introduction

Due to the beneficial roles played by histamine 3 receptor (H_3R) in alertness, wake-promotion and cognition in central nervous system (CNS), the research in this field has become a subject of intense interest in recent years (Singh et al., 2013). Thus the development of potent H_3R antagonists has emerged as an attractive target for the treatment of various CNS disorders including narcolepsy, ADHD, and cognitive disorders either as a primary indication or associated with another disease state (Berlin et al., 2011; Vohora and Bhowmik, 2012; Celanire et al., 2005). Our interest in H_3R antagonists originated from modafinil (compound **1**, Fig. 1) a novel alertness and wake-promoting agent whose mechanism of action at the molecular level remains elusive to date (Saper and Scammell, 2004). Modafinil does not display any significant *in vitro* binding affinity for the H_3 receptor. However, it has been reported that in anesthetized rats, modafinil increased extracellular histamine concentrations (Ishizuka et al., 2003). Thus while designing a series of H_3R antagonists, we sought to incorporate some of modafinil's structural themes, especially its lipophilic bis-aryl moiety attached to a polar (i.e. sulfinyl) moiety. In a previous report, we disclosed results from our initial effort (Dunn et al., 2014). In this *Communication*, we offer a brief summary from our additional effort.

2. Chemistry

Scheme 1 depicts the synthesis of target compounds **9–10**. Pyridine derivative **2** was coupled with the boronic acid **3** under Suzuki condition to generate compound **4**. Separately, enantiomerically pure prolinols (compounds **5–6**) underwent reductive amination reactions with cyclobutanone to generate amino alcohols **7–8**. O-alkylation of compounds **7–8** with compound **4** generated compounds **9** and **10**, respectively.

In Scheme 2**,** the chloro group of compound **2** was displaced by the alkoxides generated from cyclic alkanols **11–13** to produce a series of compounds of general structure **14**. Separately, phenol derivative **15** underwent a Mitsunobu reaction with compound **13** to produce compound of general structure **16**. Compounds **14** and **16** underwent separate Suzuki reactions with 4-(methylsulfonyl)benzeneboronic acid to generate compounds of general structure **17** that were *N*-deprotected to produce free amines of general structure **18.** Reductive amination of compounds **18** with a set of cyclic ketones produced target compounds **19–25**.

E-mail address: Sankar.Chatterjee24@gmail.com
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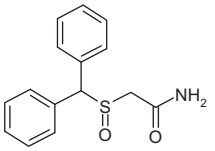

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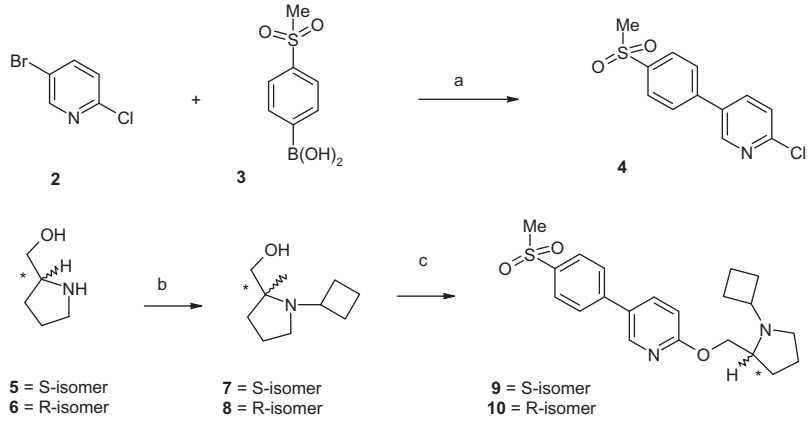
Figure 1 Chemical structure of compound **1**.

3. Biology

Following a literature procedure, binding properties of the target compounds were assessed against recombinant human H_3 (hH_3) and rat H_3 (rH_3) receptors by displacement of [^{3}H]-N-α-methylhistamine and reported as K_i values from an average of three experiments, done in duplicate (US Patent, 2012), while in triplicate for the potent analogs. The results are displayed in Table 1. The table also displays the result for a reference compound Pitolisant that entered into clinical trials (Schwartz, 2011).

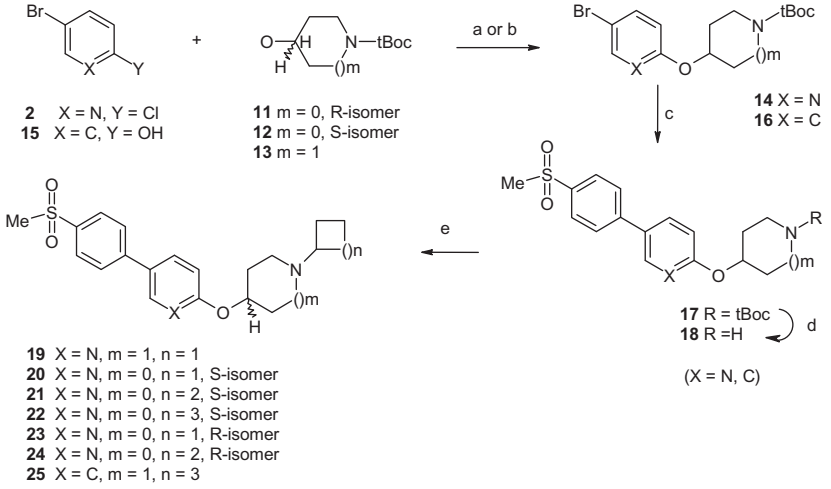
4. Discussion

As mentioned previously, compound **1** displayed no hint of any *in vitro* binding affinity (even at higher concentrations) for either H_3 receptor. Thus, our exploration began by re-orienting the aromatic region into a para-linked bi-aryl system, especially an aryl-heteroaryl system as the central lipophilic region. While a polar sulfonyl group was appended to the aryl ring, the heteroaryl moiety was attached via a methyleneoxy moiety, to a terminal cyclic amine moiety to impart some structural rigidity in that part of the molecule. Initially, structural fragments derived from (*S*)- and (*R*)- prolinol were



5 = S-isomer
6 = R-isomer
7 = S-isomer
8 = R-isomer
9 = S-isomer
10 = R-isomer

Scheme 1 Reagents and conditions: (a) 4-($Pd(PPh_3)_4$, 2 M aq. Na_2CO_3, toluene, EtOH, 95–100 °C, 3 h, 70%; (b) (i) cyclobutanone, gl. AcOH (catalytic), CH_2Cl_2, 0 °C, 20–30 min; (ii) sodium acetoxyborohydride, 0 °C to room temperature, 4–6 h, 60–65%; (c) (i) NaH, DMF, 0 °C to room temperature, 30 min; (ii) compound **4**, 100 °C, 2 h, 40–45%.



2 X = N, Y = Cl
15 X = C, Y = OH
11 m = 0, R-isomer
12 m = 0, S-isomer
13 m = 1
14 X = N
16 X = C
17 R = tBoc
18 R =H
(X = N, C)
19 X = N, m = 1, n = 1
20 X = N, m = 0, n = 1, S-isomer
21 X = N, m = 0, n = 2, S-isomer
22 X = N, m = 0, n = 3, S-isomer
23 X = N, m = 0, n = 1, R-isomer
24 X = N, m = 0, n = 2, R-isomer
25 X = C, m = 1, n = 3

Scheme 2 Reagents and conditions: for compound **14**: (a) (i) compounds **11**–**13**, 60%NaH, NMP, 0 °C to room temperature; (ii) compound **2**, 90 °C, overnight, 50–60%; for compound **16**: (b) PPh_3, 40% DEAD in toluene, THF, 0 °C to room temperature, overnight, 60%; (c) 4-(methylsulfonyl)benzeneboronic acid, 4-($Pd(PPh_3)_4$, 2 M aq. Na_2CO_3, toluene, EtOH, 95–100 °C, 6–8 h, 50–60% (d) 4 N HCl in dioxane, room temperature, 90–95%; (e) (i) cyclic ketone, gl. AcOH (catalytic), CH_2Cl_2, 0 °C, 30 min; (ii) sodium acetoxyborohydride, 0 °C to room temperature, 4–6 h, 50–60%.

Table 1 Biological data for the target compounds.

Compound	hH_3 K_i (nM)[a]	rH_3 K_i (nM)[a]
1	[b]	[b]
9	>1000	>1000
10	>1000	>1000
19[c]	7 ± 2	18 ± 3
20[c]	19 ± 3	62 ± 4
21[c]	19 ± 4	63 ± 4
22[c]	22 ± 2	20 ± 3
23	234	>1000
24	115	>1000
25	82	276
26	>1000	>1000
Pitolisant[d]	0.3–1.0	17

[a] $n = 2$.
[b] not active.
[c] $n = 3$.
[d] reference compound (data from Schwartz, J-C, 2011).

utilized to generate compounds **9** and **10**, respectively. The lack of reasonable activity of both compounds **9** and **10** (Table 1) indicated that this initial maneuver was not productive. It was postulated that the basic amine moiety in either compound probably was unable to interact with a critical aspartate residue on the receptor, essential for a productive binding to H_3R. Subsequently, the decision was made to incorporate the exocyclic methylene group of either compound as a part of a cyclic moiety, thus generating achiral 2-pyridyl-4-oxypiperidnyl- derivative **19** that imparted immediate potency in both assays (K_i of 7 nM and 18 nM, respectively). To expand the scope of the series, the 4-oxypiperidinyl group in compound **19** was subsequently replaced by chiral 3-oxypyrrolidinyl systems. In the (*S*)-series of ligands, variation of the size ($n = 4 - 5$) of the cycloalkyl groups attached to the basic nitrogen generated compounds **20**–**22** that displayed similar affinity in the hH_3 binding assay, but they differed ca. 3-fold in rH_3 binding assay (K_i 19–22 nm vs. 62–63 nM). From the (*R*)-series, the ligands appeared to possess at least one order of magnitude lower affinity than their corresponding counterpart from (*S*)-series in the hH3 assay (cf. affinities of compound **23** vs. compound **20**, and compound **24** vs. compound **21**, respectively). Compounds **23**–**24** appeared to be significantly less active in the rH_3 assay also. The reason for this difference in binding activity of compounds **23**–**24** in two assays is not currently understood. Thus, the chirality of the 3-oxypyrrolidinyl moiety played a role in the binding activity of this class of ligand. Subsequently, the pyridyl group of compound **22** was replaced by a phenyl group generating compound **25** that appeared to have somewhat lower affinity than the parent compound. Finally, the replacement of the entire methylsulfonylphenyl moiety of this class of ligands with a smaller lipophilic group (e.g. bromine as in compound **26**, Fig. 2) resulted in significant loss of affinity (cf. compound **26** vs. compound **25**) indicating importance of the methylsulfonylphenyl group in the binding affinity of this class of ligands. Representative compounds **19** and **21** were advanced to pharmacokinetic studies in rats. Both compounds were detected in the brain in reasonable level post 1 h ip administration (dose 10 mg/kg). Additional profiling is continuing with several members of the series. After completion of this research, a report of a set of bis-phenyl derivatives appeared in the literature without mentioning any aryl-heteroaryl combination from this current series (Semple et al., 2012).

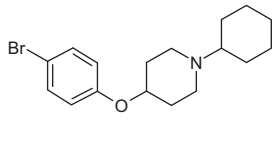


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Figure 2 Chemical structure of compound **26**.

5. Conclusions

In this *Communication*, utilizing modafinil as a launching pad, a series of para phenyl-pyridyl aromatic system compounds that bind potently to both mouse and human recombinant histamine-3 receptors were developed. In PK experiments, representative compounds **19** and **21** displayed brain permeability post 1 h ip administrations.

Acknowledgment

Author wishes to acknowledge the scientific support provided by the members of the biology team.

Appendix

Rat H_3 assay

Cell line development and membrane preparation

The rat H_3 receptor cDNA was PCR amplified from reverse-transcribed RNA pooled from rat thalamus, hypothalamus, striatum and prefrontal cortex with a sequence corresponding to bp #338–1672 of Genbank file #NM_05306, encoding the entire 445-amino acid rat histamine H_3 receptor. This was engineered into the pIRES-neo3 mammalian expression vector, which was stably transfected into the CHO-A3 cell line (Euroscreen, Belgium), followed by clonal selection by limiting dilution. Cells were harvested and cell pellets were frozen (−80 °C). Cell pellets were re-suspended in 5 mM Tris–HCl, pH 7.5 with 5 nM EDTA and a cocktail of protease inhibitors (Complete Protease Inhibitor Tablets, Roche Diagnostics). Cells were disrupted using a polytron cell homogenizer and the suspension was centrifuged at 1000×*g* for 10 min at 4 °C. The pellet was discarded and the supernatant was centrifuged at 40,000×*g* for 30 min at 4 °C. The membrane pellet was washed in membrane buffer containing 50 mM Tris–HCl, pH 7.5 with 0.6 mM EDTA, 5 mM $MgCl_2$ and protease inhibitors, re-centrifuged as above and the final pellet re-suspended in membrane buffer plus 250 mM sucrose and frozen at −80 °C.

Radioligand binding

Membranes were re-suspended in 50 mM Tris HCl (pH 7.4), 5 mM $MgCl_2$ and 0.1% BSA. The membrane suspensions (10 μg protein per well) were incubated in a 96 well microtiter plate with [^{3}H]-N-alpha-methylhistamine (approx. 1 nM final concentration), test compounds at various concentrations (0.01 nM–30 μM) and scintillation proximity beads (Perkin

Elmer, FlashBlue GPCR Scintillating Beads) in a final volume of 80 µl for 4 h at room temperature, shielded from light. Non-specific binding was determined in the presence of 10 µM clobenpropit. Radioligand bound to receptor, and therefore in proximity to the scintillation beads, was measured using a Microbeta scintillation counter.

Human H_3 assay

Cell line development and membrane preparation

CHO cells stably expressing the human H_3 receptor (GenBank: NM_007232) were harvested and cell pellets were frozen (−80 °C). Cell pellets were re-suspended in 5 mM Tris–HCl, pH 7.5 with 5 nM EDTA and a cocktail of protease inhibitors (Complete Protease Inhibitor Tablets, Roche Diagnostics). Cells were disrupted using a polytron cell homogenizer and the suspension was centrifuged at 1000×*g* for 10 min at 4 °C. The pellet was discarded and the supernatant was centrifuged at 40,000×*g* for 30 min at 4 °C. This membrane pellet was washed in membrane buffer containing 50 mM Tris–HCl, pH 7.5 with 0.6 mM EDTA, 5 mM $MgCl_2$ and protease inhibitors, re-centrifuged as above and the final pellet re-suspended in membrane buffer plus 250 mM sucrose and frozen at −80 °C.

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