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NEW ACETYLENE BASED HISTAMINE H_3 RECEPTOR ANTAGONISTS DERIVED FROM THE MARINE NATURAL PRODUCT VERONGAMINE

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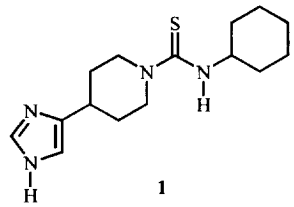
Abstract: New histamine H_3 receptor antagonists were developed using an acetylene moiety as a replacement for the amide-oxime functionality of verongamine **5**. Optimization of receptor binding was performed by following aliphatic Topliss tree guidelines. These new H_3 ligands demonstrate excellent blood-brain barrier penetration.

Since the disclosure of thioperamide **1** (K_i = 4.3 nM)[1] as a potent and selective histamine H_3 receptor antagonist, a number of 4(5)-substituted imidazole derivatives have been prepared and evaluated for H_3 receptor affinity. Most of the efforts directed towards the design of novel and potent H_3 antagonists have been comprehensively summarized by Leurs et al.[2] and Stark et al.,[3] and most recently by Phillips et al.[4] A few of the more prominent H_3 receptor antagonists that have been developed are: clobenpropit **2** (K_i = 0.18 nM),[5] GR 175737 **3** (K_i = 7 nM),[6] and GT-2016 **4** (K_i = 40 nM).[7]



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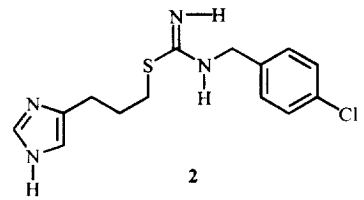

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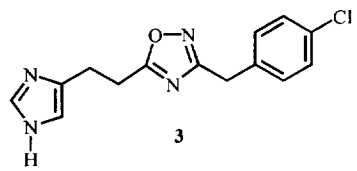

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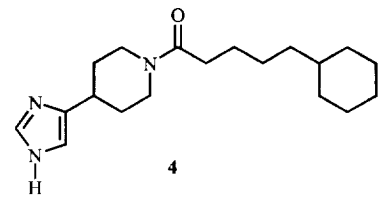

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Studies directed towards the design and development of new and potent H_3 receptor antagonists have revealed the following three structural features as being essential for good binding affinity: an imidazole head group, a spacer, and a hydrophobic tail. A variety of polar spacer groups such as amide, thioamide, guanidine, urea, thiourea, ester, carbamate, and thiocarbamate have been investigated for synthesizing potent H_3 antagonists.[2,3,8]

The efforts of design and synthesis of new histamine H_3 receptor antagonists in our group have been focused on using verongamine **5** as a structural template. Compound **5** is the only natural product that has been reported as an histamine H_3 receptor antagonist (IC_{50} = 0.5 μM).[9] Analysis of its structural features, reveal that verongamine contains an imidazole head group, a polar and planar amide-oxime spacer, and an aromatic

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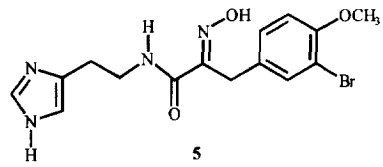
hydrophobic tail. Since our goal was to prepare new H_3 ligands with improved blood-brain barrier penetration, we replaced the polar and planar amide-oxime spacer of verongamine with a nonpolar and linear moiety as exemplified by the acetylenic congener **6**.



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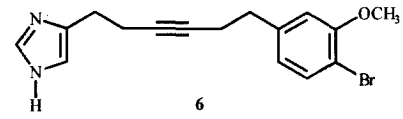

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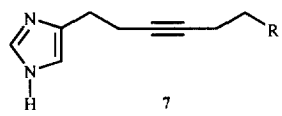
We were encouraged to pursue this approach from our molecular modeling studies that evaluated energy minimized conformations of **5** with those of the intended ligand **6**. Figure 1 shows good overlay of the energy minimized conformations of **5** and **6**.[10]

Figure 1

Although, molecular modeling comparison was performed using a substituted aromatic hydrophobic tail, we optimized binding affinity and blood-brain barrier penetration of acetylene containing H_3 ligands by following a Topliss operational scheme for aliphatic side chain substitution.[11] This strategy was based on SAR information obtained from our previous work using verongamine as a template.[12] We describe, herein, the synthesis and evaluation of H_3 receptor ligands having the general structure **7** using the acetylene derivative **8** as the base compound.



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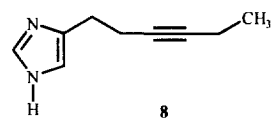

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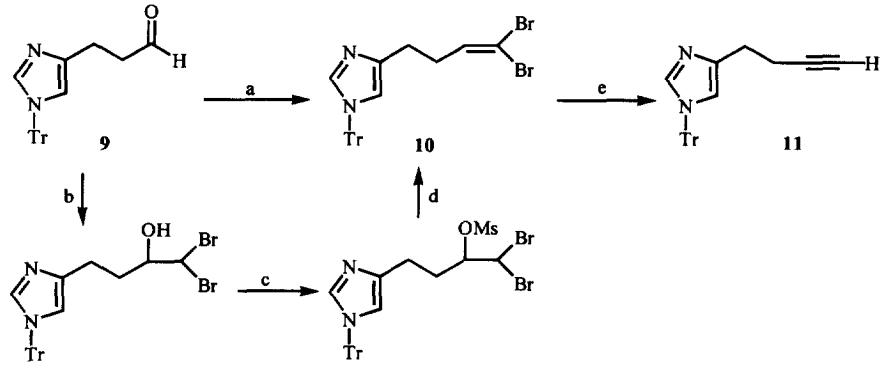
Synthesis

H_3 receptor ligands having the general formula **7** were prepared from the key intermediate 4-But-3-ynyl-1-(triphenylmethyl)imidazole **11**. This intermediate is available from aldehyde **9**[8a] following either of two synthetic pathways outlined in Scheme 1. The preparation of the vinyl dibromide **10** by the standard treatment

with triphenylphosphine carbon tetrabromide is quite effective, but isolation of **10** from triphenylphosphine is quite troublesome. The three step preparation of **10** from **9** gave comparable overall yields (70%). Treatment of vinyl dibromide **10** with an excess of *n*-BuLi gave the terminal acetylene **11** (90%). The acetylenes **8** and **12–17** were obtained by alkylation of **11** followed by deprotection of the trityl group as shown in Scheme 2.

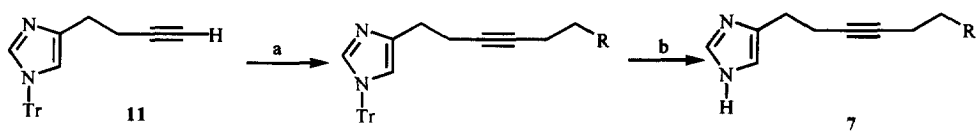
Scheme 1



Reagents: (a) PPh_3, CBr_4; (b) Dibromomethane, Lithium dicyclohexylamide, –78 °C, THF; (c) MsCl, TEA, THF, 0 °C; (d) TEA, THF, rt; (e) 2 equiv *n*-BuLi, THF, –78 °C.

Deprotonation of the terminal acetylene **11** using NaH or NaHMDS (0 °C, THF) followed by treatment with alkyl iodide gave no product even after 48 h. Reasonable alkylation yields of the lithium carbanion of **11** using excess alkyl iodide were obtained after lengthy reaction times (seven days). Better results were obtained with reactions performed using *n*-BuLi:TMEDA complex in tetrahydrofuran at 55 °C for 24–36 h.

Scheme 2

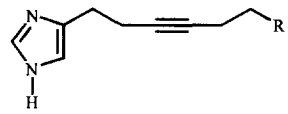


Reagents: (a) *n*-BuLi:TMEDA, R-I, 55 °C, 24 h; (b) 1 N HCl, EtOH, 90 °C, 1 h.

Results and Discussion

Histamine H_3 receptor binding data for all the compounds prepared in this study were determined using the methods outlined by Tedford et al.[7] and are shown in Table 1. The base compound **8** had H_3 receptor binding affinity of K_i = 79 nM, whereas its methyl substituted analog **12** had a K_i = 27 nM. On the premise that a + π (hydrophobic) effect would be most probable, we synthesized **13** in which the isopropyl group replaced the methyl group of **12**. Compound **13** demonstrated an improvement in binding affinity (K_i = 3.7 nM), a + π effect. Following the + π branch of the aliphatic Topliss tree, and minimizing the change in steric factors (E_s), we prepared **14** (K_i = 0.95 nM) and again observed a + π effect. To further investigate the π effect, we prepared **15** (K_i = 2.9 nM) and **16** (K_i = 3.5 nM). The drop in binding affinity observed for **15** and **16** suggested that the optimum hydrophobicity (π factor) had been obtained with the cyclopentyl substitution in **14**. In order to evaluate the steric effect, we prepared analog **17**. The bulky *tert*-butyl substitution was tolerated quite well, and **17** exhibited a K_i = 0.8 nM.

Table 1. Histamine H_3 Receptor Binding Affinity for Compounds 8 and 12–17

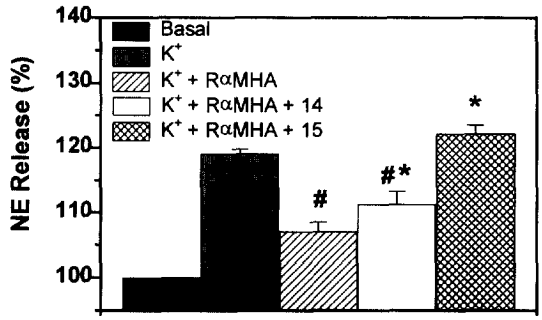


Ki = (nM)

Thioperamide	1.54 ± 0.37
R-α-methylhistamine	1.0 ± 0.12
8 R = H	79 ± 22
12 R = CH_3	27 ± 7.5
13 R = *i*-C_3H_7	3.7 ± 1.1
14 R = cyclo-C_5H_9	0.95 ± 0.3
15 R = cyclo-C_6H_{11}	2.9 ± 0.2
16 R = $(CH_2)_2Ph$	3.5 ± 1.0
17 R = *tert*-butyl	0.8 ± 0.04

The derivatives **14** and **15** were evaluated for blood-brain barrier penetration using ex vivo binding techniques.[7,13] Compounds **14** and **15** crossed the blood-brain barrier with ED_{50}s of 0.48 ± 0.14 and 0.43 ± 0.12 mg/kg, respectively. In addition, the H_3 antagonist properties of compounds **14** and **15** were evaluated in vitro for their ability to block the H_3 agonist, *R*-α-methylhistamine (RαMHA)-induced inhibition of norepinephrine (NE) release from cardiac synaptosomes.[14] RαMHA significantly inhibited K^+-induced release of NE via activation of H_3 hetereoreceptors and coincubation with either compound **14** or **15** significantly attenuated the effects of RαMHA, demonstrating H_3 antagonist activity (Figure 2).

Figure 2. Effect of Compounds 14 and 15 on RαMHA-Mediated Inhibition of NE Release from Guinea Pig Cardiac Synaptosomes



The H_3 agonist RαMHA (300 nM) was incubated alone or in the presence of the H_3 antagonists (300 nM) and K^+ (30 mM). Bars represent mean ± SEM (n = 8). Basal NE release levels were 1.45 ± 0.07 pmol/mg protein. # Indicates a significant difference from K^+-evoked NE release and * indicates a significant difference from K^+ + RαMHA evoked NE release ($p < 0.05$, ANOVA followed by post-hoc Dunnett's test).

Summary

New and potent H_3 receptor antagonist have been prepared using as a basis for design the replacement of the amide-oxime functionality of verongamine with a non polar yet planar acetylene spacer. These ligands are the first H_3 receptor antagonists that contain no heteroatom in the spacer group. Furthermore, lead optimization studies were performed rapidly and efficiently using a Topliss operational scheme for side chain modifications. Using these two principles, we have identified new H_3 receptor antagonists that have excellent binding affinity and blood-brain barrier penetration.

Selected Experimental and Analytical Data

4-But-3-ynyl-1-(triphenylmethyl)imidazole (11). *n*-BuLi (2.5 M in hexane, 50 mL, 0.125 mol) was added to a –78 °C solution of 4-(4,4-dibromobut-3-enyl)-1-(triphenylmethyl)imidazole (16.0 g, 0.031 mol) in dry tetrahydrofuran (400 mL). The reaction mixture was kept at –78 °C for 2.5 h and treated with aq satd solution of ammonium chloride. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with water and dried over anhyd sodium sulfate. Crude product was purified by flash column (ethyl acetate:hexanes, 30:80) giving **11** as solid. Yield 10 g. ^{1}H NMR (300 MHz, CD_3OD) δ 8.83 (s, 1H), 7.42 (s, 1H), 2.95 (t, J = 6.9 Hz, 2H), 2.60 (dt, J = 2.4 and 6.9 Hz, 2H), 2.36 (t, J = 2.4 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 134.9, 128.9, 117.7, 82.8, 71.6, 24.8, 18.8.

General Procedure for the alkylation of acetylene 11. *n*-BuLi (2.5 M, 1.3 equiv) was added to TMEDA (1.3 equiv) at 0 °C. The mixture was stirred at 0 °C for 30 min. and cooled to –20 °C. A solution of acetylene (1 equiv) in anhyd. tetrahydrofuran (0.1 M) was added. After 45 min at rt a solution of alkyl iodide (1.5 equiv) in anhyd. tetrahydrofuran was added. The reaction mixture was stirred at 50 °C for 24–36 h. The reaction mixture was treated with water and extracted with ethyl acetate. The organic layer was separated, washed with water, and dried over anhydrous sodium sulfate. Removal of the solvent gave crude product which was purified by flash column chromatography (eluted with ethyl acetate:hexanes (1:1) to afford alkylated products in 60-90% yield. Deprotection of the alkylated acetylenes (2 N HCl, 90 °C, 2 h) gave final products **12–17** in 90–95% yield.

4-Hex-3-ynylimidazole (8). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 6.83 (s, 1H), 2.80 (t, J = 7.2 Hz, 2H), 2.45 (m, 2H), 2.15 (m, 2H), 1.08 (t, J = 7.2 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 134.4, 118.0, 82.5, 79.0, 69.0, 26.5, 19.2, 14.2, 12.4; MS (CI) *m/e* 149(M+1).

4-Heptyl-3-ynylimidazole (12). R_f 0.4 (EtOAC:MeOH, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.33 (brs, 1H), 7.55 (s, 1H), 6.83 (s, 1H), 2.79 (t, J = 7.2 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 2.10 (t, J = 7.2 Hz, 2H), 1.46 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 134.4, 118.0, 81.0, 79.7, 68.9, 26.6, 22.4, 20.7, 19.2, 13.4; MS (CI) *m/e* 163(M+1).

4-(7-Methyloct-3-ynyl)imidazole (13). R_f 0.3 (EtOAc:MeOH, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1H), 6.82 (s, 1H), 5.96 (brs, 1H), 2.78 (t, J = 7.2 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.12 (t, J = 7.5 Hz, 2H), 1.37 (m, 1H), 0.85 (d, J = 6.9 Hz, 6H); MS (CI) *m/e* 191(M+1).

4-(6-Cyclopentylhex-3-ynyl)imidazole (14). R_f 0.4 (EtOAC:MeOH, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 6.84 (s, 1H), 2.79 (t, J = 7.2 Hz, 2H), 2.46 (t, J = 7.2 Hz, 2H), 2.15 (t, J = 7.2 Hz, 2H), 1.0–1.80 (m, 11H); ^{13}C NMR (300 MHz, CD_3OD) δ 134.8, 117.6, 83.2, 78.5, 40.6, 36.6, 33.4, 26.2, 25.5, 19.2, 18.6; MS (CI CH_4) *m/e* 217(M+1).

4-(6-Cyclohexylhex-3-ynyl)imidazole (15). R_f 0.4 (EtOAC:MeOH, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 6.82 (s, 1H), 2.78 (t, 2H, J = 7.1 Hz), 2.45 (m, 2H), 2.10 (m, 2H), 1.64 (m, 5H), 1.34 (t, 2H, J = 7.1 Hz), 1.19 (m, 4H), 0.84 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 134.4, 118.2, 100.0, 84.4, 79.4, 36.6, 36.5, 32.8, 26.5, 26.4, 26.1, 19.1, 16.1; MS (CI) *m/e* 231(M+1).

4-(8-Phenyloct-3-ynyl)imidazole (16). R_f 0.5 (EtOAC:MeOH, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.42 (m, 2H), 7.23 (m, 3H), 6.94 (s, 1H), 2.94 (t, J = 6.9 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H), 2.54 (m, 2H), 2.36 (m, 2H), 2.1 (m, 2H), 1.8 (m, 2H); MS (CI CH_4) *m/e* 253(M+1).

4-(7,7-Dimethyloct-3-ynyl)imidazole (17). R_f 0.5 (EtOAC:MeOH, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 6.86 (s, 1H), 2.85 (t, J = 6.9 Hz, 2H), 2.76 (t, J = 7.2, 2H), 2.07 (m, 2H), 1.46 (m, 2H), 1.02 (s, 9H); MS (CI CH4) *m/e* 205(M+1).

Histamine H_3 Receptor Binding Assay: Histamine H_3 receptor affinity was determined in rat cortical membranes using the H_3 agonist ligand, [^{3}H]-Nα-methylhistamine (78.9 Ci/mmol, DuPont NEN Research Products, Boston, MA) according to reference 6. The binding assay was carried out in polypropylene tubes in a total volume of 0.4 mL of 50 mM Na^+ phosphate buffer (pH 7.4), containing 150–200 mg of tissue protein and 0.8–1.2 nM [^{3}H]-Nα-methylhistamine. Nonspecific binding (NSB) was accounted for by the inclusion of thioperamide (10 μM). The samples were incubated for 40 min at 25 °C. Samples were filtered through glass fiber strips, pre-washed with 0.3% polyethyleneimine, using a Brandell cell harvester. The filters were rapidly washed three times with 4 mL of 25 mM Tris buffer containing 145 mM NaCl (pH 7.4, 4 °C). Competition experiments were analyzed and K_i's were determined using the equation $K_i = IC_{50}/(1 + ([Ligand]/[K_d])$.

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