Bioorganic & Medicinal Chemistry Letters, Vol.3, No.6, pp. 1247-1252, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

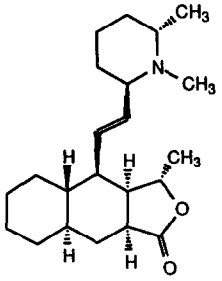
SIMPLIFIED ANALOGS OF HIMBACINE DISPLAYING POTENT BINDING AFFINITY FOR MUSCARINIC RECEPTORS

Michael J. Malaska[a], Abdul H. Fauq[a], Alan P. Kozikowski[a*], Patricia J. Aagaard[b], and Michael McKinney[b],
[a]Neurochemistry and [b]Neuropharmacology Research, Mayo Foundation for Medical Education and Research, 4500 San Pablo Road, Jacksonville, Florida 32224

(*Received in USA* 8 *March* 1993)

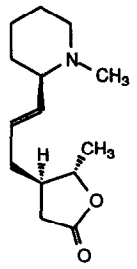
Abstract: When the tricyclic moiety of himbacine is replaced with a dihydroanthracene nucleus, an increase in affinity at both M_1 and M_2 receptor subtypes is observed. Other modifications to the himbacine skeleton were examined and the new structures tested for potency and selectivity at M_1 and M_2 sites.

The naturally occurring alkaloid himbacine **1** has been shown to bind preferentially to the M_2 and M_4 muscarinic receptors, as compared to the M_1 and M_3 receptor subtypes.[1, 2] Antagonists at the M_2 and M_4 receptors (putative presynaptic inhibitory sites[3]) could result in elevated levels of acetylcholine released during neurotransmission.[4] Such agents could find potential use as therapeutics for the treatment of symptoms resulting from Alzheimer's and Parkinson's disease, where levels of presynaptic cholinergic terminals were found to be significantly reduced.[4, 5] In order to dissect the structural elements necessary for potency and selectivity at these receptors, simplified analogs of himbacine were constructed and submitted for binding studies at both M_1 and M_2 receptor subtypes.



1
himbacine

K_d (M_1) = 168 ± 22 nM
K_d (M_2) = 9.05 ± 0.48 nM



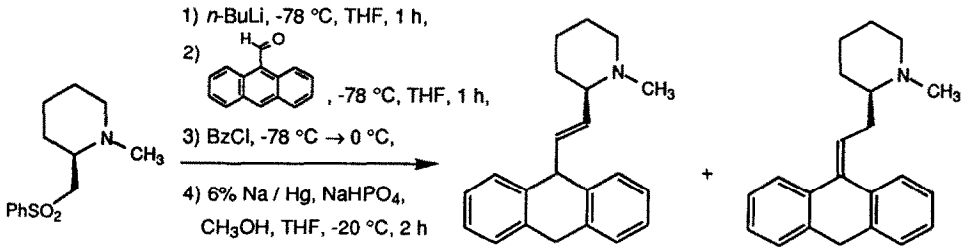
2

K_d (M_1) = 9.7 μM
K_d (M_2) = 1.21 μM

Previous work has shown that when the decalin ring was removed from the tricycle of himbacine, as in **2**, a large loss in potency was noted, as well as a decrease in selectivity.[6] It was postulated that a tricyclic substituent would better mimic himbacine's hydrophobic southern portion. Initially, a 9,10-dihydroanthracene substituent was used as a simplified replacement for

the himbacine tricyclic system. Thus, the previously described sulfone **3a**[6] underwent modified Julia coupling[7] with 9-anthraldehyde to generate **4a** and the double-bond isomerized product **5a** (Scheme 1).[8] Under the coupling conditions the central ring of the tricycle was reduced to the dihydroanthracene ring system. The *S*-enantiomers **4b** and **5b** were constructed starting from the sulfone with the opposite configuration (**3b**)[9] and the racemic versions of these two compounds (**4** and **5**) were similarly constructed from **3**. Rearomatization of the central ring of **4** was performed using DDQ in CH_2Cl_2 to furnish anthracene **6** (Scheme 2).

Scheme 1



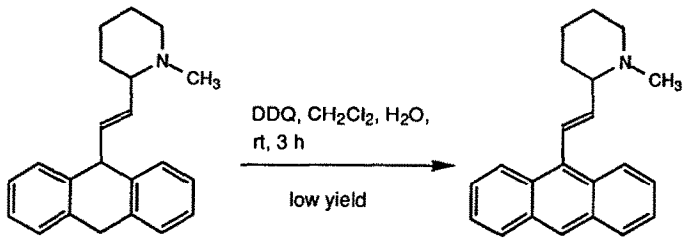
3a (*R*-enantiomer)
3b (*S*-enantiomer)
3 (racemic)

4a (*R*)
4b (*S*)
4 (racemic)

5a (*R*)
5b (*S*)
5 (racemic)

cpd.	K_d (M_1)	K_d (M_2)
4a (*R*)	1.53 ± 0.234 nM	3.17 ± 0.694 nM
4b (*S*)	4.92 ±1.4 nM	21.9 ± 2.67 nM
4 (racemic)	1.75 ± 1 nM	20.5 ± 0.758 nM
5a (*R*)	11.6 ± 1.25 nM	14.5 ± 0.478 nM
5b (*S*)	24.2 ± 6.6 nM	35.0 ± 2.62 nM
5 (racemic)	2.7 nM	3.0 nM

Scheme 2

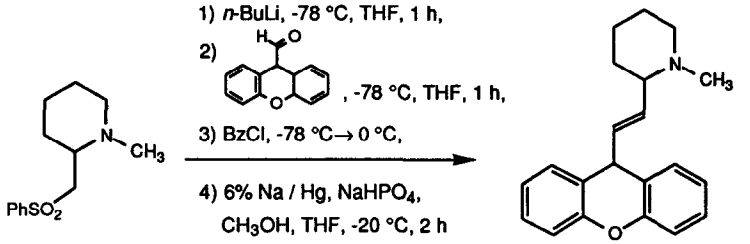


4 **6**

K_d (M_1) = 679 ± 189 nM
K_d (M_2) = 635 ± 59 nM

The xanthine ring was also examined as a tricyclic substituent (Scheme 3). As for **5**, Julia coupling of 9-xanthine carboxaldehyde[10] with racemic sulfone **3** furnished **6** following reduction. This result is surprising considering the known acidity of the α-proton of 9-xanthine carboxaldehyde.[10] It is also worthy to note that isomerization of the double bond did not occur during the reductive cleavage step of the coupling reaction, suggesting that the isomerization takes place during reduction of the central ring.

Scheme 3



3 **7** (11%)

K_d (M_1) = 3.35 ± 0.405 nM

K_d (M_2) = 25.4 ± 7.21 nM

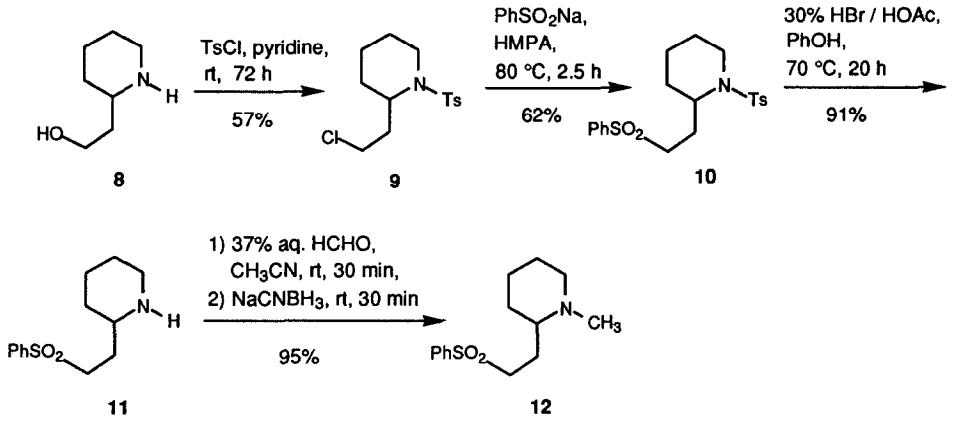
Next, the chain was extended by one methylene subunit to examine the effect of the tether flexibility on receptor selectivity. It was postulated by Eberlein et al.[11] that the orientation of the nitrogen head group in relation to the tricycle is of major importance for M_2 / M_1 selectivity. Adjustment of the chain length would therefore allow more conformational mobility between the two components. The homologated sulfone **12** was constructed in a similar fashion as for the synthesis of **3** (Scheme 4). Tosylation of the commercially available piperidine-2-ethanol (**8**) furnished the corresponding N-tosylated chloride **9** instead of the expected bis[tosylate]. This was of no concern, since nucleophilic displacement with benzene sulfinate anion in HMPA generated **10**. Next, compound **10** was N-deprotected with 30% HBr / acetic acid to afford **11**, which after reductive amination gave **12**. The Julia coupling of **12** with 9-anthraldehyde proceeded as before to give **13** together with its isomerization product **14** (Scheme 5).

The spectral data for the himbacine analogs were fully consistent with the depicted structures.[12] For affinity studies, the rat brainstem was used as a source of M_2 receptors[13] while CHO-K1 cells were transfected with hm1 receptor sequences to provide a source of M_1 receptors. Binding studies for these compounds were carried out using the displacement of radiolabelled [^{3}H]QNB to determine affinity data and the calculated K_d's determined as shown.

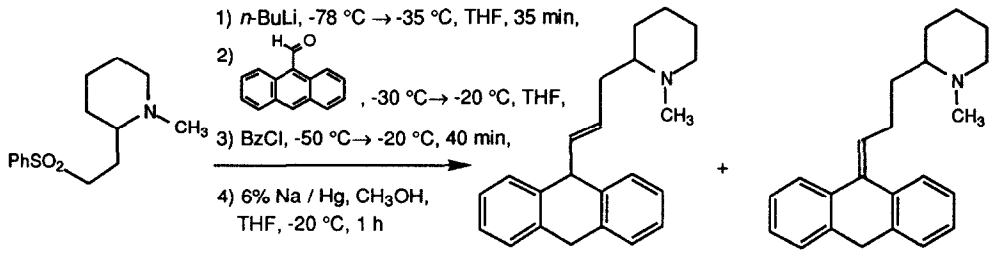
All of the compounds synthesized, with the exception of aromatic tricycle **6**, were highly potent at both M_1 and M_2 sites. For **6**, aromatization of the central ring, whether by affecting geometry, electronics, or both, caused a 30-fold loss in potency at the M_2 receptor and a 400-fold loss in potency at the M_1 receptor. In comparison, dihydroanthracene **4a** had an even higher affinity than himbacine at the M_2 receptor, and was almost as potent as DIBA[14], the most potent M_2-selective antagonist described to date. An examination of the table of Scheme 1 suggests that

the chiral center on the piperidine ring may play a role in binding at the M_2 subtype, since binding at the M_1 receptor was virtually identical among **4a**, **4b**, and **4** (the racemate), while slightly different at the M_2 receptor. In general, those analogs containing a trans double bond (again with the exception of compound **6**) showed preferential binding to the M_1 receptor, while those compounds derived from the double bond rearrangement (**5a**, **5b**, **5** and **14**) displayed nonselective binding and slightly lessened affinity when compared to their isomers.

Scheme 4



Scheme 5



K_d (M_1) = 5.23 ± 1.6 nM K_d (M_1) = 4.99 ± 1 nM

K_d (M_2) = 22.6 ± 1.4 nM K_d (M_2) = 7.64 ± 1.5 nM

Comparison of the binding characteristics of **5** and **7** reveals that replacement of the methylene group in the dihydroanthracene ring with an oxygen atom has little effect on potency or selectivity at either receptor subtype. Finally, it should be noted that when an additional methylene is inserted into the tether between the two ring components as in **13**, that there is no appreciable effect on binding relative to compound **4**.

In summary, replacement of the tricyclic component of himbacine with other ring systems affords compounds displaying higher affinity to both receptors, although preferential binding at the

M_1 receptor subtype is observed. Work is in progress to determine the components of himbacine which confer M_2 selectivity.

Acknowledgements: We are indebted to the Alzheimer's Disease Core Center for a pilot grant (08031-02P1). The work was also supported by the Mayo Foundation and by grant AGO9973 (Michael McKinney).

References and Notes

1. Darroch, S. A.; Taylor, W. C.; Choo, L. K.; Mitchelson, F. *Eur. J. Pharmacol.* **1990**, *182*, 131-136. "Structure-activity Relationships of some *Galbulimima* Alkaloids Related to Himbacine".
2. Miller, J. H.; Aagaard, P. J.; Gibson, V. A.; McKinney, M. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 663-667. "Binding and Functional Selectivity of Himbacine for Cloned and Neuronal Muscarinic Receptors".
3. McKinney, M.; Miller, J. H.; Aagaard, P. J. *J Pharmacol. Exp. Ther.* **1993**, *264*, 74-78. "Pharmacological Characterization of the Rat Hippocampal Muscarinic Autoreceptor".
4. McKinney, M.; Coyle, J. T. *Mayo Clinic Proceedings* **1991**, *66*, 1225-1237. "The Potential for Muscarinic Receptor Subtype-Specific Pharmacotherapy for Alzheimer's Disease".
5. Iversen, L. L. *Trends Pharmacol. Sci., Suppl.* **1986**, 44-45. "The Cholinergic Hypothesis of Dementia".
6. Kozikowski, A. P.; Fauq, A. H.; Miller, J. H.; McKinney, M. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 797-802. "Alzheimer's Therapy: An Approach to Novel Muscarinic Ligands Based Upon the Naturally Occurring Alkaloid Himbacine".
7. Trost, B. M.; Lynch, J.; Renaut, P.; Steinman, D. H. *J. Am. Chem. Soc.* **1986**, *108*, 284-291. "Enantiocontrolled Cycloaddition Approach to (+)-Brefeldin A".
8. For a representative coupling procedure: To a solution of **3a** (107.1 mg, 0.4227 mmol) in THF (2 mL) at -78 °C was added *n*-butyllithium (203 μL of a 2.5 *M* solution in hexanes, 0.5073 mmol) by gastight syringe. After 1 h at -78 °C a solution of 9-anthraldehyde (104.6 mg, 0.5073 mmol) in THF (1 mL) was slowly added. Stirring at -78 °C was continued for 1 h, then benzoyl chloride (90.7 μL, 0.7814 mmol) was added and the bright yellow solution allowed to warm to 0 °C. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with $CHCl_3$ (3 x 20 mL). The organic phases were dried over Na_2SO_4, concentrated by rotary evaporation and subjected to silica gel chromatography (CH_3OH : ethyl acetate) to furnish a mixture of benzoylated sulfone diastereomers as determined by ^1H NMR analysis. Further elution provided unreacted **3a** (86.2 mg, 80% recovery). The crude benzyloxy sulfones were stirred with 6% Na / Hg amalgam (1.64 g, 4.28 mmol of Na) and $NaHPO_4$ (302.9 mg, 2.139 mmol) in CH_3OH (6 mL) and THF (2 mL) at -20 °C for 2 h. The bright orange mixture was diluted with H_2O (20 mL) and extracted with ethyl acetate (3 x 25 mL). The organic phases were dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to silica gel chromatography (CH_3OH : ethyl acetate) and

the more non-polar fractions collected to provide **4a** (15.8 mg, 66% based on recovered **3a**), while further elution furnished **5a** (5.8 mg, 22% based on recovered **3a**).

9. **3b** was constructed by the same methodology as for **3a** using L-pipecolic acid, which was resolved following the method of Rodwell, V. W. *Meth. Enzymol.* **1971**, *17*, 174. "Pipecolic Acid".
10. Rochlin, E.; Rappoport, Z. *J. Am. Chem. Soc.* **1992**, *114*, 230-241. "Substituted Xanthenylidene Enols. The Importance of β-Ar-C=C Conjugation in the Stabilization of Aryl-Substituted Enols".
11. Eberlein, W.; Engel, W. Trummlitz, G.; Mihm, G.; Mayer, N.; Hasselbach, K. *Trends Pharmacol. Sci., Suppl.* **1988**, 92. "Conformational Requirements for M_2-Selectivity Towards the Muscarinic Receptor in a Series of Pirenzepine Analogs".
12. Spectral data for **4a** and **5a** follow: **4a**: light yellow oil; IR (neat) 3400 (br), 2932, 2853, 2778, 1667, 1479, 1452, 1026, 752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26-7.17 (m, 8 H), 5.68 (dd, *J* = 15.2, 6.7 Hz, 1 H), 5.23 (ddd, *J* = 15.2, 8.7, 0.8 Hz, 1 H), 4.55 (d, *J* = 6.7 Hz, 1 H), 3.97 (d, *J* = 18.0 Hz, 1 H), 3.84 (d, *J* = 18.0 Hz, 1 H), 2.83 (br d, *J* = 11.5 Hz, 1 H), 2.27 (m, 1 H), 2.12 (s, 3 H), 1.93 (t d, *J* = 11.3, 3.3 Hz, 1 H), 1.85 - 1.1 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.6, 136.2, 133.8, 132.7, 127.61, 127.56, 126.3 (4 C), 67.5, 56.4, 49.1, 44.4, 35.3, 33.5, 25.9, 23.9; MS (70 eV) *m/z* 303 (M^+, 48.8), 215 (23.8), 202 (16.2), 191 (18.2), 179 (25.2), 178 (46.6), 124 (83.0), 98 (100); $[\alpha]_D$ +31.9 °(*c* = 3.2, $CHCl_3$);

    5a: yellow oil; IR (neat) 3410 (br), 2931, 2854, 2779, 1597, 1473, 1450, 1034, 754 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45 (m, 2 H), 7.22 (m, 6 H), 5.94 (t, *J* = 7.3 Hz, 1 H), 3.89 (d, *J* =18.8 Hz, 1 H), 3.82 (d, *J* = 18.8 Hz, 1 H), 3.06 (m, 1 H), 2.96 (ddd, *J* = 15.2, 6.3, 3.9 Hz, 1 H), 2.77 (quintet, *J* = 8.2 Hz, 1 H), 2.52 (br s, 1 H), 2.44 (s, 3 H), 1.8 - 1.2 (m, 7 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.2, 137.7, 137.3, 134.9, 134.6, 127.42, 127.36, 127.1, 126.8, 126.6 (2 C), 125.8, 123.9, 123.8, 64.0, 55.7, 40.8, 36.4, 31.7, 28.9, 23.6, 23.0; MS (70 eV) *m/z* 203 (3.3), 202 (4.4), 99 (7.5), 98 (100), 70 (8.1); $[\alpha]_D$ +48.2 °(*c* = 1.7, $CHCl_3$).
13. McKinney, M.; Anderson, D.; Forray, C.; El-Fakahany, E. E. *J. Pharm. Exp. Ther.* **1989**, *250*, 565-572. "Characterization of the Striatal M_2 Muscarinic Receptor Mediating Inhibition of Cyclic AMP Using Selective Antagonists: A Comparison with the Brainstem M_2 Receptor".
14. Gitler, M. S.; Reba, R. C.; Cohen, V. I.; Rzeszotarski, W. J.; Baumgold, J. *Brain Res.* **1992**, *582*, 253-260. "A Novel M_2-Selective Muscarinic Antagonist: Binding Characteristics and Autoradiographic Distribution in Rat Brain".