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CHEMICAL MODIFICATION OF RING C OF HIMBACINE: DISCOVERY OF A PHARMACOPHORIC ELEMENT FOR M_2-SELECTIVITY

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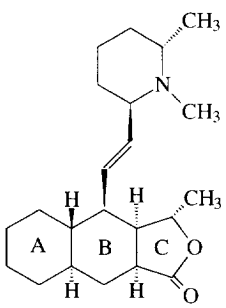
Abstract: Removal of the carbonyl oxygen of himbacine furnishes a molecule which binds with 20-fold and 4-fold lower affinity at the M_2 and M_1 receptor sites, respectively. Thus, the carbonyl oxygen constitutes an important pharmacophoric element responsible for some of the M_2 selectivity of himbacine. Other modifications of the C-ring of the tricyclic portion of himbacine were examined and tested for potency at M_1 and M_2 sites, and the data confirm that M_2 selectivity is optimal with a closed heterocyclic ring and with the presence of a proximal carbonyl oxygen.

It is well-known that basal forebrain cholinergic neurons degenerate in Alzheimer's and Parkinson's Disease.[1,2] One therapeutic approach to the treatment of these disorders is based on increasing the amount of acetylcholine released from residual cholinergic terminals that may remain functional. Blockade of the presynaptic inhibitory receptor, postulated to be either the M_2 or a pharmacologically similar M_4 receptor[3], could cause an increase in acetylcholine levels in brain areas such as the cortex and hippocampus.[1] In support of this hypothesis, it has been shown that a muscarinic antagonist with some M_2-selectivity is capable of increasing cognitive ability in an age-impaired animal model.[4] Clearly, a highly selective and potent M_2 antagonist would be of biological and therapeutic interest.

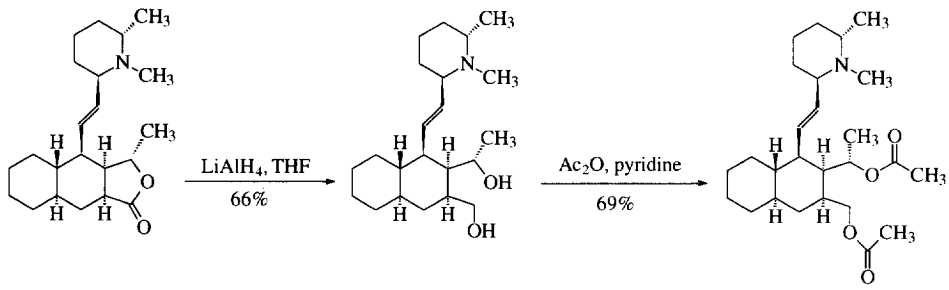


1
himbacine

Our efforts have focused on the M_2-selective antagonist himbacine.[5] In particular, our chemical and biological studies have been concerned with determining the structural elements of this molecule which are responsible for M_2-selectivity.[6,7] Previously we have described a series of analogues in which the tricycle has been modified; these compounds were found to have a high affinity for both M_1 and M_2 receptor subtypes, but unfortunately lacked selectivity.[7] Other work by Taylor *et al.* has shown that the alkene double bond of himbacine is necessary for potency; this group has also postulated that the methyl group attached to the 6-position of the piperidine ring plays a critical role for M_2-receptor selectivity.[8] Herein we describe modifications of the C-ring of the tricycle of himbacine, and the importance of the carbonyl oxygen in both the binding and selectivity at the M_2 receptor.

The C-ring of himbacine's tricycle was opened by complete reduction to the diol **2**[9] through treatment with $LiAlH_4$ (Scheme 1).[10] This diol was next converted to the diacetate **3** using standard conditions to furnish a more hydrophobic derivative for binding studies (Scheme 1).[11]

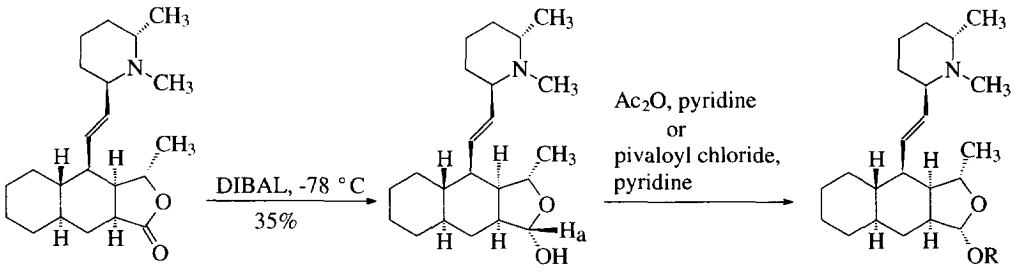
Scheme 1



1 himbacine **2** **3**

The skeletal framework of himbacine was preserved by carrying out a partial reduction of himbacine to the lactol **4**.[12] This was accomplished by treating himbacine (**1**) with diisobutylaluminum hydride in CH_2Cl_2 at -78 °C (Scheme 2). It is interesting to note that only one lactol diastereoisomer was isolated from the reaction. The hydroxyl group of the hemiacetal is postulated to be oriented as depicted based upon the lack of any discernible coupling of the hemiacetal proton (H_a) of the lactol with its neighboring ring junction proton (H_b). The lactol was reacted with acetic anhydride in pyridine to furnish the acetate **5** in 71% yield. Similarly, reaction of **4** with pivaloyl chloride in pyridine generated the pivaloyl acetal **6** in a 36% yield.

Scheme 2



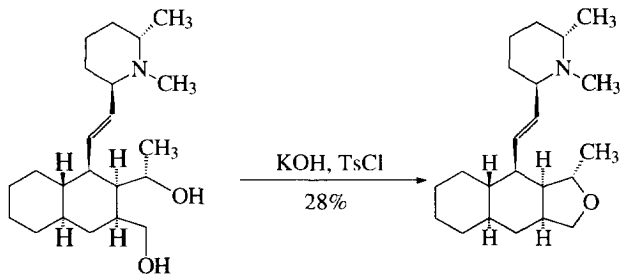
1
himbacine

4

5 (R = $COCH_3$)
6 (R = $COC(CH_3)_3$)

Finally, the carbonyl oxygen of himbacine was removed entirely to furnish the tetrahydrofuran **7** (Scheme 3).[13] This was accomplished through intramolecular cyclization of the diol **2** using powdered KOH and TsCl.

Scheme 3



2

7

The spectral data for the himbacine analogs were fully consistent with the depicted structures. [10-15] Our initial biological screens of the compounds compared their potencies at M_1 and M_2 receptors, as these two receptors are thought to play major signal transduction roles, post-synaptically and pre-synaptically, respectively. Relative M_2-selectivity would be considered therapeutically desirable.[1] For our screens of these compounds, the rat brainstem was used as a convenient source of M_2 receptors while CHO-K1 cells transfected with hm_1 human muscarinic receptor sequence were used as a source of M_1 receptors. The latter tissue was used for the M_1 assay, as native M_1 receptors always occur in mixtures with other muscarinic receptors, making determination of true M_1 potencies problematic. Binding studies for these compounds were carried out using the displacement

of radiolabeled [^{3}H]quinuclidinyl benzilate ([^{3}H])QNB) to determine receptor binding potencies, and the K_d values were calculated assuming competitive interactions. These data are summarized in Table 1.

Table 1. Binding Affinity of Himbacine and C-Ring Modified Himbacine Analogs

Compound	K_d (M_1)	K_d (M_2)	M_2 Selectivity
1 (himbacine)	148 ± 5 nM	10 ± 0.3 nM	14.8
2 (diol)	494 ± 8 nM	293 ± 7 nM	1.7
3 (diacetate)	604.7 ± 12.5 nM	248.5 ± 6 nM	2.4
4 (lactol)	423 ± 13 nM	96 ± 4 nM	4.4
5 (lactolacetate)	866 nM	89 nM	9.7
6 (lactolpivalate)	2520 nM	464 nM	5.4
7 (tetrahydrofuran)	167 ± 69 nM	353 ± 55 nM	0.5

The ring-opened diol **2** bound to M_1 and M_2 receptors with 3- and 29-fold less affinity, respectively, than himbacine, a nearly complete loss of M_2-selectivity. While removal of the C-ring could be an important factor in the loss of binding activity, it could also be speculated that the receptor pocket is not sterically capable of accomodating the two hydrophilic alcohol groups. The affinity data for **3**, in which the two hydroxyl groups are "capped off" as acetate esters, however, reveal no improved binding at both M_1 and M_2 receptor subtypes.

Four of the compounds examined contained an intact tricyclic framework (**4**, **5**, **6**, and **7**); these compounds demonstrated decreased affinity at M_1 and M_2 sites when compared with himbacine. However, when compared with diol **2**, the lactol **4** has approximately the same binding affinity at the M_1 receptor, while it binds slightly (~2.5-fold) better at the M_2 receptor than does diol **2**. It appears that an intact C-ring framework alone is not sufficient to ensure M_2 selectivity and potency. Compounds **5** and **6** have derivatized hydroxyls, but interestingly the added bulk in compound **5** does not further decrease M_2 potency relative to **4**; compound **5** retains most of the M_2 selectivity of himbacine itself. As observable in compound **6**, the pivaloyl moiety is too large to maintain the potency at either M_1 or M_2, and only modest M_2 selectivity remains. Additionally, as observable by the loss of M_2 potency and selectivity in compound **3**, a lactone C-ring is essential. Thus, a carbonyl oxygen in or around the heterocyclic ring of the himbacine template appears necessary for M_2 selectivity.

Further support for this argument is evidenced by the poor binding of compound **7**. This compound differs from himbacine only in that the carbonyl oxygen is removed, thus the tricycle of **7** resembles himbacine sterically, but differs in its electrostatic field. The binding data for this molecule show that this compound has much less

affinity than himbacine for the M_2 receptor, and nearly identical affinity for the M_1 receptor. Thus, removal of the carbonyl oxygen affects only the binding to the M_2 receptor.

These results indicate that the carbonyl oxygen of the C-ring and a closed C-ring of himbacine are necessary for high affinity and selectivity at the M_2-receptor subtype. This constitutes the discovery of a new pharmacophoric element present in himbacine.

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References and Notes:

1. McKinney, M.; Coyle, J. T. Mayo Clinic Proceedings **1991**, *66*, 1225–1237.
2. Iversen, L. L. *Trends in Pharmacol. Sci.,* Suppl. **1986**, 44–45.
3. McKinney, M.; Miller, J. H.; Aagaard, P. J.; *J. Pharmacol. Exp. Ther.* **1993**, *264*, 74–78.
4. Doods, H. N.; Quirion, R.; Mihm, G.; Engel, W.; Rudolf, K.; Entzeroth,, M.; Schiavi, G. B.; Ladinsky, H.; Bechtel, W. D.; Ensinger, H. A.; Mendla, K. D.; Eberlein, W. *Life Sciences* **1993**, *52*, 497–503.
5. Miller, J. H.; Aagaard, P. J.; Gibson, V. A.; McKinney, M. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 663–667.
6. Kozikowski, A. P.; Fauq, A. H.; Miller, J. H.; McKinney, M. *BioMed. Chem. Lett.* **1992**, *2*, 797–802.
7. Malaska, M. J.; Fauq, A. H.; Kozikowski, A. P.; Aagaard, P. J.; McKinney, M.; *BioMed. Chem. Lett.* **1993**, *3*, 1247–1252.
8. Darroch, S. A.; Taylor, W. C.; Choo, L. K.; Mitchelson, F. *Eur. J. Pharm.* **1990**, *m182*, 131–136.
9. Pinhey, J. T.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1961**, *14*, 106–134.
10. Procedure and analytical data for himbacine diol (**2**)[9]: To a stirred solution of himbacine (**1**) (10 mg, 28.0 μmol) was added lithium aluminum hydride (0.2 mL of a 1 *M* solution in THF, 0.2 mmol). After 3 h at room temperature, the reaction mixture was diluted with saturated aqueous Rochelle salt (5 mL) and H_2O (5 mL), extracted with CH_2Cl_2 (2 x 10 mL), dried over $MgSO_4$,and concentrated by rotary evaporation to furnish **1**[9] (6.5 mg, 66%) as a clear oil: IR (neat) 3304, 2926, 2852, 1447, 1371, 1259, 1070 cm^{-1}; MS (70 eV) *m/z* 331 (M-H_2O, 10), 316 (86), 138 (32), 112 (100); ^1H NMR (300 MHz, $CDCl_3$) δ 5.62 (dd, *J* = 15.1, 9.0 Hz, 1 H), 5.50 (dd, *J* = 15.1, 8.6 Hz, 1 H), 4.09 (m, 1 H), 3.66 (d, *J* = 5.8 Hz, 2 H), 3.07 (m, 1 H), 2.83 (m, 1 H), 2.21 (s, 3 H), 2.0–1.85 (m, 4 H), 1.75–1.64 (m, 5 H), 1.62–1.30 (m, 7 H), 1.28 (d, *J* = 6.0 Hz, 3 H), 1.22 (m, 2 H), 1.10–0.9 (m, 3 H), 1.01 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.09, 132.32, 66.73, 66.31, 61.57, 53.59, 41.52, 41.16, 34.47, 33.46, 33.23, 33.00, 32.37, 27.29, 27.04, 19.49; $[\alpha]_D$ + 45.0 (*c* = 2.3 in $CHCl_3$)
11. Procedure and analytical data for himbacine diol diacetate (**3**): A mixture of himbacine diol (**2**) (11.8 mg, 33.7 μmol), 4-(dimethylamino)pyridine (DMAP, 0.8 mg, 7.0 μmol), acetic anhydride (63.6 μL, 0.674 mmol), and pyridine (5 mL) was stirred at room temperature. After 24 h, the volatiles were removed in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with $CHCl_3$. The combined organic phases were dried over Na_2SO_4, concentrated in vacuo, and subjected to silica gel chromatography (MeOH/EtOAc) to afford **3** (10.1 mg, 69%) as a clear oil: IR (neat) 2963, 2928, 1740, 1447, 1371, 1242, 1034 cm^{-1}; MS (70 eV) *m/z* 418 (M - CH_3, 2.3), 165 (6.4), 138 (72), 125 (35), 112 (100); ^1H NMR (300 MHz, $CDCl_3$) δ 5.50 (m, 2 H), 5.22 (m, 1 H), 4.20 (dd, *J* = 10.8, 6.2 Hz, 1 H), 4.08 (dd, *J* = 10.7, 7.9 Hz, 1 H), 3.00 (m, 1 H), 2.81 (m, 1 H), 2.18 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.19–1.4 (m, 20 H), 1.17 (d, *J* = 6.6 Hz, 3 H), 0.97 (d, *J* = 6.5 Hz, 3 H), 0.65 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.23, 170.12, 134.66, 132.92, 69.69, 68.24, 61.32, 53.40, 51.42, 47.55, 43.00, 41.74, 41.13, 41.08, 34.04, 33.30, 32.96, 32.83, 31.73, 26.69, 26.44, 23.34, 21.76, 21.09, 19.08,

18.99; $[\alpha]_D$ +45.0 (*c* = 2.3 in $CHCl_3$).

12. Procedure and analytical data for himbacine lactol (**4**): to a stirred solution of himbacine (10.5 mg, 30.3 μmol) in CH_2Cl_2 (2 mL) at -78 °C was added diisobutylaluminum hydride (151 μL of a 1.0 M solution in hexane, 0.151 mmol). After 3 h at -78 °C, H_2O (5 mL) and NaF (20 mg) were added and the mixture stirred for 30 min at room temperature. The mixture was extracted with CH_2Cl_2 (2 x 5 mL) and dried over $MgSO_4$, and the solvents were removed by rotary evaporation to provide **4** (3.7 mg, 35%) as a clear oil: IR (neat) 3373, 2928, 1448, 1259, 1097, 1020 cm^{-1}; MS (70 eV) *m/z* 347 (M^+, 13), 332 (67), 301 (15), 138 (28), 112 (100); ^1H NMR (300 MHz, $CDCl_3$) δ 5.58 (dd, *J* = 15.2, 8.9 Hz, 1 H), 5.09 (br s, 1 H), 5.05 (dd, *J* = 15.2, 9.9 Hz, 1 H), 4.19 (m, 1 H), 3.40 (br s, 1 H), 3.03 (m, 1 H), 2.82 (m, 1 H), 2.44 (m, 1 H), 2.24 (s, 3 H), 2.22 (m, 1 H), 1.93 (td, *J* = 10.0, 5.4 Hz, 1 H), 1.9–1.6 (m, 5 H), 1.50 (d, *J* = 6.0 Hz, 3 H), 1.6–1.48 (m, 4 H), 1.46–1.1 (m, 4 H), 1.02 (d, *J* = 6.5 Hz, 3 H), 1.05–0.60 (m, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.06, 132.71, 102.26, 75.51, 61.57, 53.51, 49.33, 47.63, 46.63, 41.49, 41.24, 40.38, 34.38, 33.59, 38.18, 31.90, 27.06, 26.75, 25.73, 19.60; $[\alpha]_D$ + 68.5 (*c* = 1.8 in $CHCl_3$).

13. Procedure and analytical data for himbacine lactol, acetate (**5**): a mixture of **4** (16.2 mg, 46.7 μmol), 4-(dimethylamino)pyridine (DMAP, 1.0 mg, 9.0 μmol), acetic anhydride (44 μL, 0.467 mmol), and pyridine (2 mL) was stirred at room temperature. After 24 h, the volatiles were removed in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with $CHCl_3$ (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, concentrated in vacuo, and subjected to silica gel chromatography (MeOH/EtOAc) to furnish **5** (12.8 mg, 71%) as a white crystalline solid: IR (KBr) 2974, 2932, 2855, 1735, 1441, 1375, 1244, 1125, 984, 947, 914 cm^{-1}; MS (70 eV) *m/z* 329 (M-HOAc, 22), 314 (71), 138 (27), 125 (14), 112 (100), 91 (17); ^1H NMR (300 MHz, $CDCl_3$) δ 5.78 (s, 1 H), 5.53 (dd, *J* = 15.1, 9.1 Hz, 1 H), 5.22 (dd, *J* = 15.2, 9.6 Hz, 1 H), 4.22 (m, 1 H), 3.03 (m, 1 H), 2.86 (m, 1 H), 2.54 (m, 1 H), 2.21 (m, 1 H), 2.21 (s, 3 H), 2.10 (m, 2 H), 2.00 (s, 3 H), 1.6–1.4 (m, 11 H), 1.26 (d, *J* = 6.0 Hz, 3 H), 1.20 (m, 2 H), 0.99 (d, *J* = 6.5 Hz, 3 H), 0.90 (m, 3 H), 0.70 (m, 1 H); $[\alpha]_D$ + 69.9 (*c* = 2.1 in $CHCl_3$).

14. Procedure and analytical data for himbacine lactol pivalate (**6**): a mixture of himbacine lactol (11.2 mg, 32.1 μmol), 4-(dimethylamino)pyridine (DMAP, 1.0 mg, 9.0 μmol), pivalic anhydride (39 μL, 0.32 mmol), and pyridine (2 mL) was stirred at room temperature. After 24 h, the volatiles were removed in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with $CHCl_3$ (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, concentrated in vacuo, and subjected to silica gel chromatography (MeOH/EtOAc) to afford **6** (5.1 mg, 36%) as a clear oil: IR (KBr) 2972, 2930, 2855, 1732, 1452, 1283, 1157, 1117, 912 cm^{-1}; MS (70 eV) *m/z* 329 (M-HOAc, 24), 314 (6.7), 138 (26); 112 (100), 91 (15); ^1H NMR (300 MHz, $CDCl_3$) δ 5.79 (s, 1 H), 5.53 (dd, *J* = 15.2, 9.0 Hz, 1 H), 5.21 (dd, *J* = 15.1, 9.6 Hz, 1 H), 4.22 (m, 1 H), 3.00 (m, 1 H), 2.84 (m, 1 H), 2.21 (m, 1 H), 2.21 (s, 3 H), 2.09 (m, 2 H), 1.7–1.3 (m, 14 H), 1.25 (d, *J* = 6.0 Hz, 3 H), 1.17 (s, 9 H), 0.98 (d, *J* = 6.5 Hz, 3 H), 0.90–0.80 (m, 3 H), 0.70 (m, 1 H); $[\alpha]_D$ + 56.9 (*c* = 2.5 in $CHCl_3$).

15. Procedure and analytical data for himbacine tetrahydrofuran (himbacine anhydrodiol) (**7**): to a solution of himbacine (16.8 mg, 48.1 μmol) in Et_2O at 0 °C was added *p*-toluenesulfonyl chloride (9.2 mg, 48.1 μmol) and powdered KOH (26.9 mg, 0.481 mmol). The mixture was stirred at 0 °C for 2 h, then diluted with 10% aqueous Na_2CO_3 solution (10 mL), extracted with $CHCl_3$ (4 x 10 mL), dried over Na_2SO_4, and concentrated by rotary evaporation. Purification by silica gel chromatography (MeOH/EtOAc) provided **7** (4.5 mg, 28%) as a white crystalline solid: mp 116-118 °C (lit.[9] mp 123 °C); IR (neat) 3500 (br), 2926, 2852, 1468, 1442, 1076, 984 cm^{-1}; MS (70 eV) *m/z* 331 (M^+, 8.5), 316 ($M^+ - CH_3$, 94), 138 (35), 125 (28), 112 (100); ^1H NMR (300 MHz, $CDCl_3$) δ 5.50 (dd, *J* = 15.2, 9.0 Hz, 1 H), 5.26 (dd, *J* = 15.2, 9.9 Hz, 1 H), 3.04 (m, 1 H), 2.86 (m, 1 H), 2.22 (s, 3 H), 2.11 (m, 2 H), 1.89 (m, 1 H), 1.7–1.4 (m, 14 H), 1.16 (d, *J* = 5.9 Hz, 3 H), 1.00 (m, 2 H), 0.99 (d, *J* = 6.5 Hz, 3 H), 0.9 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.79, 131.35, 73.54, 72.06, 65.49, 53.60, 52.60, 47.18, 41.30, 41.07, 41.02, 40.99, 35.08, 34.08, 33.11, 32.67, 31.74, 26.75, 26.41, 23.18, 18.93, 14.04; $[\alpha]_D$ + 37.4 (*c* = 3.6 in $CHCl_3$).

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