Nonsymmetrical Bipiperidyls as Inhibitors of Vesicular Acetylcholine Storage

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Introduction of a nitrogen atom into the cyclohexane ring of 2-(4-phenylpiperidinyl)cyclohexanol (vesamicol, AH5183) yielded two positional isomers, 5-azavesamicol (**5**, prezamicol) and 4-azavesamicol (**6**, trozamicol). As inhibitors of vesicular acetylcholine transport, **5** and **6** were found to be 147 and 85 times less potent than vesamicol. N-Benzoylation of **5** (to yield **9a**) increased the potency 3-fold. In contrast, **10a**, a compound derived from N-benzoylation of **6**, was 50 times more potent than the latter and almost equipotent with vesamicol, thereby suggesting a preference for the 4-azavesamicol series. Although (−)-vesamicol is more potent than its dextrorotary isomer, (+)-**10a** was found to be 3 times more potent than (−)-**10a**, suggesting a reversal of the sign of rotation in the azavesamicol series. Reduction of **9a** and **10a** (to yield the corresponding *N*-benzyl derivatives **11a** and **12a**) increased potency 20- and 2-fold, respectively, indicating a preference for a basic nitrogen. The reaction of **5** or **6** with substituted benzyl halides yielded several potent inhibitors of vesicular acetylcholine transport, including *N*-(*p*-fluorobenzyl)trozamicol, **12d**, which is twice as potent as vesamicol. Thus the introduction of a nitrogen atom into the cyclohexane ring of vesamicol provides opportunities for developing a new class of anticholinergic agents.

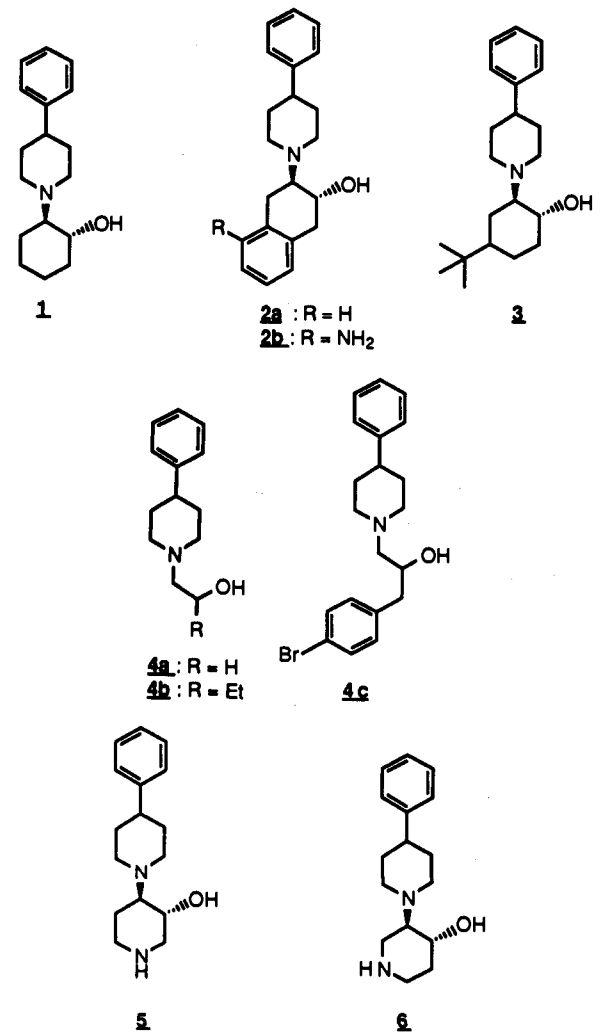
Introduction

The vesamicol receptor, a cytoplasmically-oriented site on the cholinergic synaptic vesicle, is associated with the vesicular transporter of acetylcholine (for review, see ref 1).. The location of this receptor provides opportunities for the investigation of presynaptic cholinergic phenomena associated with the release and/or storage of acetylcholine. The study of presynaptic cholinergic phenomena may be relevant to issues such as the quantum theory of neurotransmitter release and the regulation of ACh production. In addition, measurement of central presynaptic cholinergic function in the living human brain may provide valuable information for the clinical evaluation of neurodegenerative disorders (e.g., Alzheimer's disease) which are characterized by reduced cholinergic reserve.[2,3] The progress of such studies would undoubtedly be facilitated by the availability of potent, selective radiolabeled and/or fluorescent probes. The present study describes one attempt to develop easily functionalized ligands which may serve as precursors for such probes.

The prototypical vesamicol receptor ligand 2-(4-phenylpiperidinyl)cyclohexanol (**1**, vesamicol, AH5183; Chart I) exhibits nanomolar affinity for this receptor.[4,5] However, vesamicol also exhibits significant α-adrenoceptor activity,[6,7] an attribute which limits its usefulness in the study of presynaptic cholinergic function. In an earlier structure–activity study,[8] the vesamicol analogs **2a**, **2b**, and **3** were identified as potent ligands for this receptor. In addition to providing new insights into the topography of the vesamicol receptor, these novel analogs also represent lead structures for developing a new generation of potent and selective ligands for this receptor.

Although **2a**, **2b**, and **3** emerged as potent vesamicol receptor ligands, subsequent development of potent radiolabeled and/or fluorescent probes for this receptor has relied almost exclusively on the easily functionalized analog **2b**.[9–12] The apparent neglect of **3** can be largely attributed

Chart I. Vesamicol Analogs



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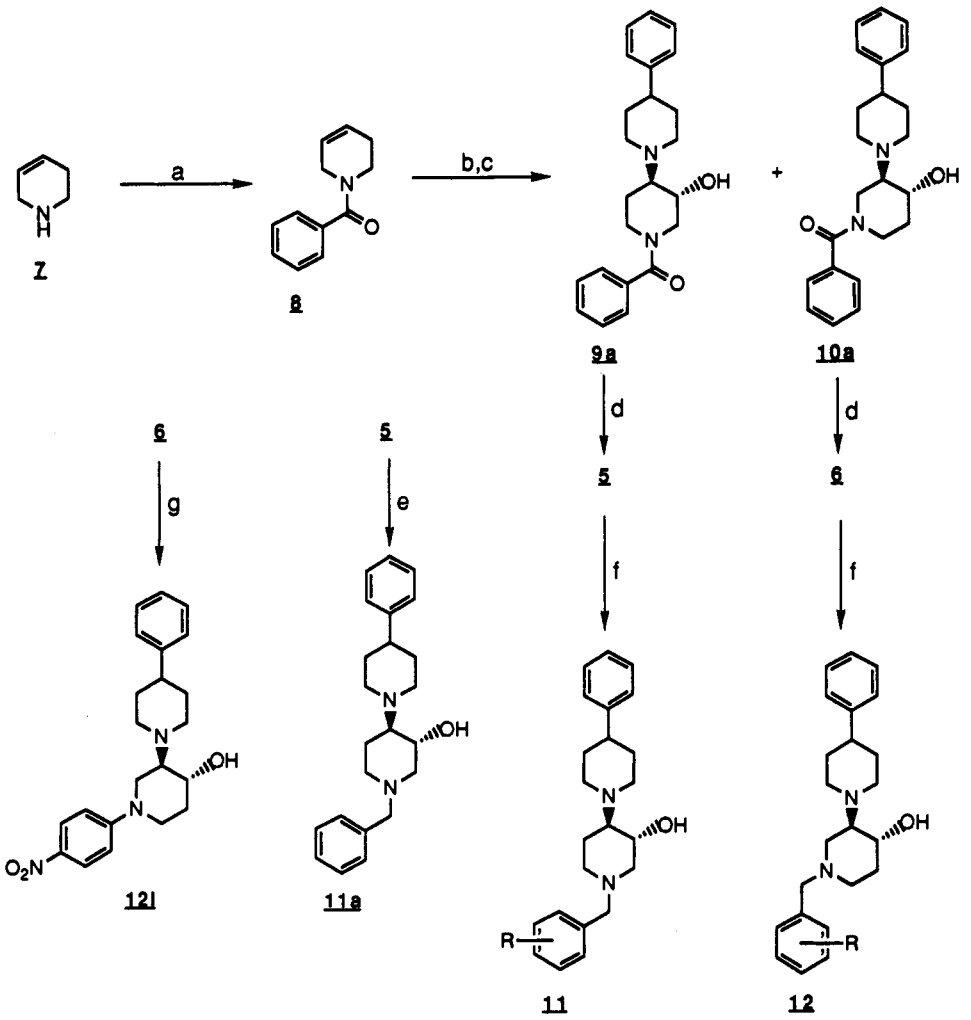
Scheme I. Synthesis of Azavesamicols



to the relative ease of functionalization of the pseudo-irreversible ligand **2b**. However, the potency of **3**, a nonfused 4-substituted vesamicol analog, suggested that the development of novel high-affinity vesamicol receptor ligands could be accomplished without extensive modification of the parent vesamicol nucleus. In an earlier study, Rogers et al.,[8] noted that the vicinal amino alcohol functionality was essential for vesamicol-like anticholinergic activity. Furthermore, these authors showed that benzo-fused analogs like **2a** and **2b** were potent inhibitors of vesicular ACh transport. In contrast, those analogs which lacked a cyclohexyl moiety (e.g. **4a,b**) were found to exhibit substantially diminished activity. It was significant, however, that the addition of a small lipophilic group to **4a**, to yield **4b**, also resulted in a 30-fold increase in activity.[8] This observation clearly suggested that further increases in potency could be achieved, even in the absence of the cyclohexyl moiety, by increasing the substituent size. In a subsequent study,[13] we demonstrated that potent non-cyclohexyl-containing vesamicol analogs could indeed be obtained by attaching arylethyl substituents onto **4a**. For example, compound **4c** was found to be 59 times more potent than **4a**.[13] The large improvement in activity clearly demonstrates the importance of nonbonded hydrophobic interactions at the vesamicol receptor site. At the same time, these improvements support the view that the cyclohexyl group is not essential for high-affinity binding to this receptor site.

In light of these earlier findings, we postulated that a new generation of potent conformationally restrained vesamicol-like anticholinergics could be developed by substituting the cyclohexyl group with a heterocycle of similar size, the piperidine ring. Since the nitrogen atom of this ring can be easily functionalized, the new parent structure would be a useful intermediate for developing anticholinergic drugs and fluorescent, radiolabeled, or photoaffinity probes for studying presynaptic cholinergic function. Finally, the nitrogen of this new piperidine ring could be introduced at various orientations relative to the amino alcohol functionality to optimize biological activity. To test this hypothesis, we proposed the novel compounds **5** (5-azavesamicol) and **6** (4-azavesamicol). In the regioisomers **5** and **6**, the orientation of the new ring nitrogen relative to the hydroxyl group is 1,3 and 1,4, respectively. For simplicity, the compounds **5** and **6** have been named prezamicol and trozamicol, respectively.

Chemistry

Equal amounts of the key intermediates **9a** and **10a** were obtained from the starting material **7**, in a combined yield of 50% (Scheme I). The regioisomers were easily separated by HPLC, and the structure of the less mobile regioisomer was determined by X-ray crystallography to be **10a**. The racemate (*dl*)-**10a** was resolved chromatographically to yield (+)-**10a** and (−)-**10a**, respectively. The enantiomeric purity was determined by HPLC (Chiralcel OD) to be greater than 99%. Acid-catalyzed hydrolysis

Table I. Characteristics of Azavesamicols

compd	R	formula[a]	procedure	% yield	mp (°C)
5		$C_{16}H_{24}N_2O\cdot 2HCl$[b]	B	86	279–282
6		$C_{16}H_{24}N_2O\cdot 2HCl$[c]	B	75	205–206
9a	H	$C_{23}H_{28}N_2O_2\cdot HCl$[d]	A	25	220–223
10a	H	$C_{23}H_{28}N_2O_2CHCl$[e]	A	25	248–251
10b	*o*-I	$C_{23}H_{27}IN_2O_2\cdot HCl$[f]	E	61	263–264
10c	*m*-I	$C_{23}H_{27}IN_2O_2\cdot HCl$	E	56	266–269
10d	*p*-I	$C_{23}H_{27}IN_2O_2\cdot HCl$[g]	E	47	248–251
11a	H	$C_{23}H_{30}N_2O\cdot 2HCl$	C	71	288–290
11b	*o*-Br	$C_{23}H_{29}BrN_2O\cdot 2HCl$	D	70	270–273
11c	*m*-Br	$C_{23}H_{29}BrN_2O\cdot 2HCl$	D	70	282–286
11d	*p*-Br	$C_{23}H_{29}BrN_2O\cdot 2HCl$	D	60	275–278
11e	I	$C_{23}H_{29}IN_2O\cdot 2HCl$	D	44	230 (sinters)
11f		$C_{22}H_{27}N_3O_3$[h]	F	39	230–232
12a	H	$C_{23}H_{30}N_2O\cdot 2HCl$[i]	D	53	235–236
12b	*o*-F	$C_{23}H_{29}FN_2O\cdot 2HCl$[j]	D	65	225–227
12c	*m*-F	$C_{23}H_{29}FN_2O\cdot 2HCl$[k]	D	73	233–235
12d	*p*-F	$C_{23}H_{29}FN_2O\cdot 2HCl$[l]	D	74	236–238
12e	*o*-Br	$C_{23}H_{29}BrN_2O\cdot 2HCl$[m]	D	68	232–234
12f	*m*-Br	$C_{23}H_{29}BrN_2O\cdot 2HCl$	D	63	220–223
12g	*p*-Br	$C_{23}H_{29}BrN_2O$[n]	D	56	159–161
12h	I	$C_{23}H_{29}IN_2O\cdot 2HCl$[o]	D	68	236–240
12i	CH_3	$C_{24}H_{32}N_2O\cdot 2HCl$[p]	D	73	233–235
12j	CH_3	$C_{24}H_{32}N_2O\cdot 2HCl$[q]	D	58	220–224
12k	CH_3	$C_{24}H_{32}N_2O\cdot 2HCl$	D	48	218–221
12l		$C_{22}H_{27}N_3O_3\cdot HCl$	F	49	247–250

[a] All elemental analyses (C, H, N) are within ±0.4% of theoretical values unless otherwise specified. [b] C: calcd, 57.66; found, 55.20. [c] C: calcd, 57.66; found 54.99. [d] C: calcd, 68.90; found, 67.68. [e] C: calcd, 68.90; found, 68.04. [f] C: calcd, 52.44; found, 51.96. [g] C: calcd, 52.44; found, 51.53. [h] C: calcd, 69.27; found, 68.43. [i] C: calcd, 65.24; found, 64.26. [j] C: calcd, 62.58; found, 60.98. [k] C: calcd, 62.58; found, 61.09. [l] C: calcd, 62.58; found, 62.08. [m] C: calcd, 55.00; found, 54.42. [n] C: calcd, 64.33; found, 63.76. [o] C: calcd, 65.90; found, 64.22. [p] C: calcd, 65.90; found, 64.62. [q] C: calcd, 65.90; found, 64.82.

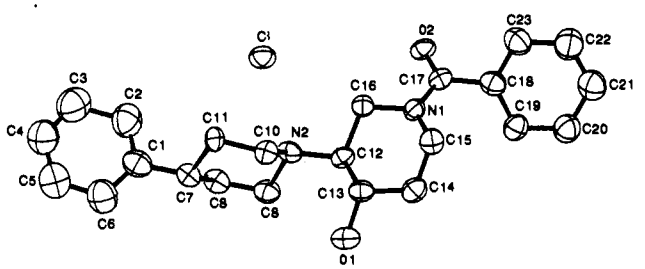


Figure 1. X-ray crystal structure of the hydrochloride of 10a showing heavy atoms only.

of 9a and 10a yielded 5 (86%) and 6 (86%), respectively. These two intermediates were subsequently derivatized by N-alkylation or N-acylation to yield the target compounds in acceptable yields (Table I).

X-ray Crystallography

The structure of 10a was unequivocally determined by X-ray diffraction of the corresponding hydrochloride (Figure 1). Both piperidine rings were found to contain equatorial substituents. As reported earlier for (−)-vesamicol,[8] the benzene ring and newly introduced piperidyl moiety are nearly coplanar. Each of these moieties is in turn almost perpendicular to the piperidyl moiety that separates them.

Results and Discussion

As evident in the in vitro binding data (Table II), replacement of the cyclohexyl moiety with the piperidyl group results in a complete loss of activity (compare 1 vs 5 and 6). Although both 5 and 6 are inactive, N-alkylation and/or N-acylation of these parent compounds provides analogs of differing potency. While the benzoyl analog 9a is essentially inactive, the corresponding regioisomer 10a

Table II. Inhibitory Potency of Azavesamicols[a]

compd	R[e]	IC_{50} (nM)[c]	compd	R[e]	IC_{50} (nM)[c]
(*dl*)-vesamicol[b]		34 ± 6	11d	*p*-Br	310 ± 130
(*dl*)-4c[d]		170 ± 20	12a	H	30 ± 7
(−)-4c[d]		36 ± 5	12b	*m*-F	42 ± 11
(*dl*)-5		5000 ± 700	12c	*o*-F	31 ± 6
(*dl*)-6		2900 ± 400	12d	*p*-F	14 ± 3
(*dl*)-9a		1900 ± 600	12e	*o*-Br	22 ± 6
(+)-10a		34 ± 7	12f	*m*-Br	21 ± 8
(−)-10a		100 ± 30	12g	*p*-Br	25 ± 10
10b	*o*-I	250 ± 60	12h	*m*-I	26 ± 11
10c	*m*-I	39 ± 7	12i	*o*-CH_3	25 ± 9
10d	*p*-I	22 ± 4	12j	*m*-CH_3	22 ± 6
11a	H	83 ± 7	12k	*p*-CH_3	24 ± 9
11b	*o*-Br	55 ± 8	12l		290 ± 30

[a] The hydrochlorides were tested using highly purified synaptic vesicles harvested from the electric organ of *T. californica*. [b] Data obtained from ref 4. [c] Values are for the inhibition of [^{3}H]vesamicol binding. IC_{50} values are quoted ±1 standard deviation. [d] Data obtained from ref 13. [e] Substituents are shown for the *N*-benzylazavesamicols only (see Scheme I). Compounds for which substituents are not shown either belong to other structural classes or are substituted with other groups (see Chart I and Scheme I).

is almost as potent as vesamicol. This observation clearly suggests that the preferred orientation of the nitrogen of the new piperidine ring relative to the hydroxyl group is 1,4 (referred to as the 1,4 series). Additionally, the dextrorotatory isomer (+)-10a was found to be more potent than the corresponding antipode, (−)-10a, indicating some degree of stereoselectivity. Substitution at the meta and para positions of the benzoyl group in 10a yielded equipotent analogs (10a vs 10b and 10c). However, substitution at the ortho position was unfavorable (10a vs 10d).

In the 1,3 series, reduction of 9a to the benzyl analog 11a increases the potency by 15–20 times. Since the benzyl group is less constrained than the benzoyl group, the observed increase in potency may be related to flexibility. Alternatively, the greater potency of 11a may be attributed to the presence of a second basic nitrogen resulting from the reduction of 9a to 11a. Substitution with bromine at the ortho position of 11a results in a slight but noticeable increase in activity (compare 11a vs 11b). However, the para-substituted analog 11c is much less potent than 11a. This observation suggests that in this series the biological activity is sensitive to the position of substituents on the pendant phenyl ring.

In the 1,4 series, reduction of 10a to 12a also results in increased potency. However, the increase is less pronounced than in the 1,3 series. The introduction of fluorine, a small electron-withdrawing atom, into the para position of the benzyl group of 12a also enhances potency (compare 12a vs 12d). However, the ortho and meta-substituted analogs 12b and 12c were less potent than the para-substituted compound 12d. Increasing the size of the electron-withdrawing substituent did not significantly alter the potency (compare 12a vs 12e,f,g). In addition the methyl-substituted compounds 12i–k were equipotent with 12a. Thus, to a first approximation, small electron-donating and electron-withdrawing substituents can be tolerated at this site. Finally, with the exception of the fluorinated analogs 12b–d, the biological activity was apparently not sensitive to the position of the substituent on the ring (compare 12e vs 12f,g and 12i vs 12j,k). This preference for lipophilic substituents may partially explain the low potency of the nitrophenyl analog 12l. Alternatively, the increased conformational restraints in this molecule may have resulted in its inability to interact effectively with the receptor.

The potency of these new compounds supports our hypothesis that the cyclohexyl group of vesamicol may be replaced with heterocycles. It is also worth noting that compounds in the 1,4 series are more potent than the acyclic compounds described earlier.[13] A superposition of one of these compounds, **4c** and **12a**, suggests that the latter can extend further into the putative hydrophobic pocket within the receptor site (data not shown). In addition, the structural dissimilarity between **12a** and **4c** suggests that the pendant phenyl groups in these two compounds can explore different regions of the receptor. These structural differences may underlie the enhancements in potency observed. Thus, by replacing the cyclohexyl group of vesamicol with the piperidine ring we have produced a new series of potent inhibitors of vesicular acetylcholine transport. Given the ease of functionalization of trozamicol, **5**, a variety of side chains may be incorporated into the latter to yield fluorescent, radiolabeled, and photoaffinity probes for studying the cholinergic system. In addition, **5** may be a useful parent molecule for developing new agents for the modulation of presynaptic cholinergic function.

Experimental Section

General Section. Synthetic intermediates were purchased from Aldrich, Inc. (Milwaukee, WI) and were used as received. Solvents were distilled immediately prior to use. Commercially available reagents were used without subsequent purification.

All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. The specific rotation was determined on an automatic polarimeter (Autopol III, Rudolph Research, Flanders, NJ). ^{1}H NMR spectra were recorded on an IBM-Brucker spectrometer at 200 MHz. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. Under these conditions, the chemical shifts (in ppm) of residual solvent in the ^{1}H NMR spectra were found to be as follows: $CHCl_3$, 7.26; DMSO, 2.56; HOD, 4.81. The following abbreviations are used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Both low- and high-resolution MS were performed on an AEI MS-30 instrument. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Unless otherwise indicated, these values are within ±0.4% of the theoretical.

Column chromatography was performed using "Baker Analyzed" silica gel (60–200 mesh). Preparative chromatography was performed on either a Harrison Research Chromatotron using Merck 60 PF_{254} silica gel or a preparative HPLC (Rainin Instrument Co.) using a 41.1 mm i.d. Dynamax silica gel column (at a solvent delivery rate of 80 mL/min). Enantiomeric purity was determined by HPLC with a Chiralcel OD column [isopropyl alcohol (10)–hexane (89)–Et_3N (1); flow rate, 1 mL/min]. Analytical TLC was performed on Analtech glass TLC plates coated with silica gel GHLF and the plates were visualized with UV light and/or methanolic iodine. All target compounds were checked for purity by HPLC (silica gel; isopropyl alcohol (10–20)–hexanes (90–80), trace Et_3N). Representative procedures for the steps shown in Scheme I are provided below as procedures A–F.

X-ray Crystallography. Clear colorless crystals of 1-benzoyl-4-hydroxy-3-(4-phenylpiperidinyl)piperidine hydrochloride (**10a**) were obtained from ethanol by slow evaporation of solvent. Compound **10a** crystallizes in the orthorhombic space group $P2_12_12_1$ (No. 19), with a = 9.119 (6) Å, b = 7.78 (7) Å, c = 31.793 (22) Å, and V = 2255 Å^3. The density was calculated to be 1.073 g/cm^3 for Z = 4. Diffraction data were collected at room temperature, using a Siemens/Nicolet/Syntex $P2_1$ diffractometer with Mo Kα radiation up to a 2θ limit of 45.0°. The positions of the non-hydrogen atoms were obtained by direct methods.[15] Hydrogen atom positions were calculated and fixed in the subsequent less squares full matrix refinement cycles.[16] The final agreement factor was R = 5.96%, using 1161 reflections with $I \geq 3\sigma(I)$. Molecular plots of the title compound are given in Figure 1.

Procedure A. 1-Benzoyl-3-hydroxy-4-(4-phenylpiperidinyl)piperidine (9a) and 1-Benzoyl-4-hydroxy-3-(4-phenylpiperidinyl)piperidine (10a). Benzoyl chloride (6.22 g, 44.3 mmol) was added dropwise to a cold (ice bath) stirring solution containing 3.2 g (38.5 mmol) of 1,2,3,6-tetrahydropyridine in Et_3N (30 mL). Following the addition, the mixture was allowed to slowly warm up to room temperature. After 24 h, the reaction mixture was diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (85 mL). The organic extract was washed with saturated $NaHCO_3$ (50 mL), dried over Na_2SO_4, and concentrated in vacuo to yield a chromatographically homogeneous yellow liquid (quant): ^{1}H NMR ($CDCl_3$) δ 2.20 (d, 2 H), 3.46 (br s, 2 H), 3.86 (br s, 2 H), 4.20 (br s, 1 H), 5.86 (m, 1 H), 7.40 (s, 5 H). The latter was redissolved in CH_2Cl_2 (140 mL) and cooled in an ice bath. To this cold stirring solution was added *m*-CPBA (14.40 g; 50–60% pure) portionwise over 10 min. After completion of the addition, the reaction mixture was maintained a 4 °C for 1 h and allowed to slowly warm up to room temperature. Sixteen hours later, the reaction mixture was diluted with CCl_4 (100 mL) and filtered to remove precipitated 3-chlorobenzoic acid. The filtrate was washed consecutively with 5% aqueous $NaHSO_3$ (2 × 100 mL) and saturated $NaHCO_3$ (2 × 100 mL), dried over Na_2SO_4, and concentrated in vacuo to provide 6.93 g of the crude epoxide as a brown syrup. A solution of the epoxide and 5.64 g (35.0 mmol) of 4-phenylpiperidine in EtOH (100 mL) was refluxed for 15 h, cooled, and concentrated to a brown residue. The latter was dissolved in CH_2Cl_2 (150 mL) and the resulting solution was washed with H_2O (70 mL), dried over Na_2SO_4, and concentrated to a syrup which was purified by preparative HPLC [silica gel; i-PrOH (10)–hexanes (90)–Et_3N (1)] to yield two major components. The more mobile component identified as **9a** was obtained in 25% yield: The corresponding hydrochloride was recrystallized from *i*-PrOH–MeOH: mp 220–223 °C; ^{1}H NMR ($CDCl_3$) δ 1.61–1.89 (m, 6 H), 2.29 (t, 1 H), 1.46–1.53 (m, 2 H), 2.81 (m, 4 H), 2.96 (m, 1 H), 3.45 (m, 1 H), 3.83 (m, 1 H), 4.69 (m, 1 H), 4.88 (m, 1 H), 7.19–7.40 (m, 10 H). The less mobile component, obtained in 25% yield, was assigned the structure **10a**: mp (HCl) 248–251 °C; ^{1}H NMR ($CDCl_3$) δ 1.50–3.07 (m, 12 H), 3.67–3.79 (m, 4 H), 4.78 (m, 1 H), 4.92 (m, 1 H), 7.20–7.44 (m, 10 H).

Procedure B. 3-Hydroxy-4-(4-phenylpiperidinyl)piperidine (5). A suspension of 2.0 g (5.5 mmol) of **9a** in 6 N HCl (50 mL) was refluxed overnight. The reaction was shown to be complete by TLC (silica gel; 50% acetone–hexanes) after 24 h. The reaction mixture was cooled and filtered to remove precipitated benzoic acid. The filtrate was extracted with CH_2Cl_2 (50 mL) and concentrated to a solid residue. The latter was redissolved in MeOH (25 mL), concentrated to a minimum volume, and triturated with *i*-PrOH to yield, after filtration, 1.57 g (86%) of a white solid: mp 279–282 °C; ^{1}H NMR (D_2O) δ 1.95–2.15 (m, 5 H), 2.46 (d, 1 H, J = 12 Hz), 2.88–3.02 (m, 2 H), 3.06–3.16 (t, 1 H, J = 14, 3 Hz), 3.30–3.39 (t, 1 H, J = 12, 2.4 Hz), 3.42–3.66 (m, 7 H), 4.19–4.28 (m, 1 H), 7.26–7.40 (m, 5 H). A similar procedure yielded 1.6 g (86%) of **6**: mp 205–206 °C; ^{1}H NMR (D_2O) 1.75–1.92 (m, 1 H), 1.95–2.20 (m, 4 H), 2.34 (d, 1 H), 2.91 (brs, 1 H), 3.08 (t, 1 H), 3.30–3.62 (m, 6 H), 3.71 (d, 1 H), 3.92 (d, 1 H), 4.22 (m, 1 H), 7.24–7.39 (m, 5 H).

Procedure C. 1-Benzyl-3-hydroxy-4-(4-phenylpiperidinyl)piperidine (11a). A solution of 1.08 g (2.96 mmol) of **9a** in dry THF (20 mL) was added dropwise under N_2 to a stirring suspension of $LiAlH_4$ (0.5 g, 13.2 mmol) in anhydrous THF (25 mL). Following the addition, the reaction mixture was refluxed overnight. After 22 h, the mixture was cooled to room temperature and diluted with dry THF (30 mL). The reaction was quenched by sequential dropwise addition of H_2O (0.5 mL), 15% NaOH (0.5 mL), and H_2O (1.5 mL). The resulting mixture was filtered, and the cake was washed repeatedly with THF and discarded. The filtrate was dried over Na_2SO_4 and concentrated to a chromatographically homogeneous pale yellow syrup which solidified on standing. The solid was redissolved in MeOH (15 mL). The solution was cooled in an ice bath and HCl(g) was bubbled through the solution for 5 min with concomitant

precipitation of the hydrochloride. The latter was collected by filtration, washed consecutively with a minimum volume of MeOH and cold *i*-PrOH, and dried in vacuo to yield 0.89 g (71%) of a white solid: mp 288–290 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.87–2.02 (m, 5 H), 2.70 (d, 1 H), 2.77 (m, 1 H), 2.90 (t, 1 H), 3.04 (t, 1 H), 3.20–3.45 (m, 7 H), 4.11 (m, 1 H), 7.16–7.28 (m, 5 H), 7.39–7.46 (m, 5 H).

Procedure D. 1-(4-Bromobenzyl)-4-hydroxy-3-(4-phenylpiperidinyl)piperidine (12g). A mixture of 0.5 g (1.5 mmol) of the hydrochloride of **4**, 0.41 g (1.66 mmol) of 4-bromobenzyl bromide, and 0.51 g (6.0 mmol) of $NaHCO_3$ in EtOH (13 mL) and H_2O (6 mL) was heated under reflux for 24 h. The mixture was cooled to room temperature and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to a brown syrup. The latter was redissolved in CH_2Cl_2 and passed through a short column of silica gel [eluted with CH_2Cl_2 (99)–Et_3N (1)]. The eluent was concentrated to provide 0.58 g of a golden brown syrup which was dissolved in MeOH and converted to the corresponding hydrochloride as described above. The hydrochloride was crystallized from cold MeOH and collected by filtration to provide 0.45 g (60%) of a white powder: mp 159–161 °C; ^{1}H NMR ($CDCl_3$) δ 1.57–2.20 (m, 9 H), 2.25 (t, 1 H), 2.52 (m, 2 H), 2.72 (m, 1 H), 2.82 (br s, 2 H), 3.04 (m, 2 H), 3.52 (m, 2 H), 3.73 (s, 1 H), 7.17–7.33 (m, 7 H), 7.48 (m, 2 H).

Procedure E. 4-Hydroxy-1-(2-iodobenzoyl)-3-(4-phenylpiperidinyl)piperidine (10b). Dicyclohexylcarbodiimide (2.48 g, 12 mmol) was added to a solution of 2-iodobenzoic acid (2.48 g, 10.0 mmol) and N-hydroxysuccinimide (1.26 g, 11 mmol) in CH_2Cl_2 (75 mL). The reaction mixture was stirred for 20 h and filtered to remove precipitated dicyclohexylurea. The filtrate was concentrated in vacuo to a minimum volume and filtrated with hexanes. The resulting precipitate was collected by filtration, washed with hexanes, and dried to yield the activated ester as a white powder (3 g, 90%): ^{1}H NMR ($CDCl_3$) δ 2.84 (s, 4 H), 7.21 (br s, 1 H), 7.42 (s, 1 H), 8.02 (br s, 2 H). This material was used without purification. A suspension of the amino alcohol dihydrochloride **6** (0.33 g, 1.0 mmol) and the activated ester (0.34 g, 1.0 mmol) in reagent-grade acetonitrile (20 mL) and Et_3N (6 mL) was refluxed for 21 h, cooled to room temperature, and concentrated in vacuo. The residue was treated with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to a brown residue which was purified by radial-flow chromatography [silicxa gel; acetone (30%)–hexanes (70)–Et_3N (1)]. The desired fractions were concentrated to yield a pale yellow foam which was converted to the hydrochloride in methanol. The product crystallized from cold methanol to yield 0.32 (61%) of a white solid: mp 263–264 °C.

Procedure F. 4-Hydroxy-1-(4-nitrophenyl)-3-(4-phenylpiperidinyl)piperidine (12l). A solution of 0.50 g (1.50 mmol) of the hydrochloride of **6** and 0.17 g (3.16 mmol) of NaOMe in MeOH (20 mL) was stirred for 5 min and concentrated in vacuo. The resulting solid was dried in vacuo at 50 °C and redissolved in dry DMF (10 mL). Sodium carbonate (0.10 g, 0.9 mmol) and *p*-fluoronitrobenzene (0.42 g, 3.0 mmol) were then added, and the resulting solution was refluxed under nitrogen. After 15 h, heating was stopped. The reaction mixture was cooled and concentrated in vacuo. The residue was triturated with CH_2Cl_2 (30 mL) and filtered to remove insoluble material. The filtrate was concentrated in vacuo and purified by radial-flow chromatography (silica gel; CH_2Cl_2 (35)–hexane (65)]. The desired fractions were concentrated to a residue which was triturated with Et_2O–hexane, filtered, and dried to yield 0.28 g (49%) of the fluffy yellow solid: mp 163–164 °C; ^{1}H NMR ($CDCl_3$) δ 1.69–4.08 (m, 18 H), 7.02 (d, 2 H), 7.28 (m, 5 H), 8.05 (d, 2 H, J = 10 Hz); CIMS (NH_3) *m/e* (intensity) 381 (M^+, 39.64).

The free base was converted to the corresponding hydrochloride as described above and subsequently recrystallized from *i*-PrOH: mp 247–250 °C; ^{1}H NMR ($CDCl_3$) δ 1.66 (m, 2 H), 1.80–2.00 (m, 3 H), 2.19–2.24 (d, 1 H, J = 12 Hz), 2.35–2.65 (m, 3 H), 2.88–3.05 (m, 4 H), 3.14 (d, 1 H), 3.68–3.85 (m, 2 H), 3.97 (d, 1 H), 4.09 (d, 1 H, J = 18 Hz), 6.83 (d, 2 H), 7.24 (d, 3 H), 7.33 (m, 2 H), 8.12 (d, 2 H).

Biological. The compounds were evaluated in a purified preparation of cholinergic synaptic vesicles isolated from the electric organ of *Torpedo californica*. A full description of the assay is given in ref 8. The present study was performed with a trace amount of [^{3}H]vesamicol, and in the absence of ATP and acetylcholine. Each drug concentration was tested in duplicate and the data showed a variation of less than 5%. The average values were subjected to nonlinear regression analysis using MINSQ (Micro Math Scientific Software, Salt Lake City, UT). Protein determination was performed according to standard methods,[12] using a bovine serum albumin standard.

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Supplementary Material Available: X-ray crystallographic data for **10a** (6 pages). Ordering information is given on any current masthead page.

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