N-Hydroxyalkyl Derivatives of 3*β*-Phenyltropane and 1-Methylspiro[1*H*-indoline-3,4′-piperidine]: Vesamicol Analogues with Affinity for Monoamine Transporters

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As part of our ongoing structure−activity studies of the vesicular acetylcholine transporter ligand 2-(4-phenylpiperidino)cyclohexanol (vesamicol, **1**), 22 *N*-hydroxy(phenyl)alkyl derivatives of 3*β*-phenyltropane, **6**, and 1-methylspiro[1*H*-indoline-3,4′-piperidine], **7**, were synthesized and tested for binding in vitro. Although a few compounds displayed moderately high affinity for the vesicular acetylcholine transporter, no compound was more potent than the prototypical vesicular acetylcholine transporter ligand vesamicol. However, a few derivatives of **6** displayed higher affinity for the dopamine transporter than cocaine. We conclude that modification of the piperidyl fragment of **1** will not lead to more potent vesicular acetylcholine transporter ligands.

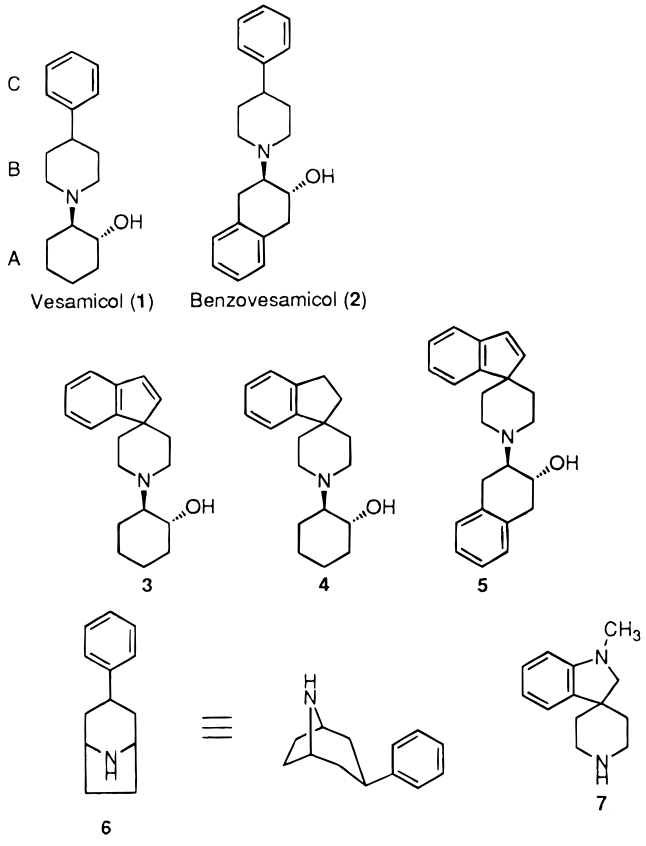
Introduction

Newly synthesized acetylcholine is actively transported from its site of synthesis in the cytosol into synaptic vesicles by a vesicular acetylcholine transporter[1−3] which is localized exclusively in cholinergic neurons.[4,5] As the synaptic vesicle is the primary vehicle for impulse-mediated release of acetylcholine, vesicular transport of acetylcholine constitutes an important component of neurotransmission at the cholinergic synapse. Consequently, vesicular acetylcholine transporter ligands will affect acetylcholine storage and related aspects of cholinergic function.

The tertiary amino alcohol 2-(4-phenylpiperidinyl)-cyclohexanol (vesamicol, AH5183, **1**; Chart 1) binds to the vesicular acetylcholine transporter with moderately high affinity.[6,7] In laboratory animals, **1** causes respiratory paralysis, convulsions, and death.[8,9] The pharmacological actions of this molecule have been attributed to inhibition of acetylcholine transport into synaptic vesicles and the subsequent quantal release of acetylcholine.[1−3] However, vesamicol also displays α-adrenoceptor activity[10] and high affinity for *σ*1 and *σ*2 receptors.[11] Consequently, a number of efforts have been launched to develop more selective ligands for the vesicular acetylcholine transporter.

Previously,[6,12,13] we and others have shown through the synthesis of analogues such as **3**−**5** that the conformational restriction of selected fragments of **1** can yield vesicular acetylcholine transporter ligands of comparable or higher potency than vesamicol. In the present communication, we report our attempts to extend the conformational restriction strategy by replacing the 4-phenylpiperidyl fragment of **1** with the 3*β*-phenyltropanyl fragment **6**. We also report our attempts to develop a new series of vesicular acetylcholine transporter ligands from the spirobase **7**.

Chart 1



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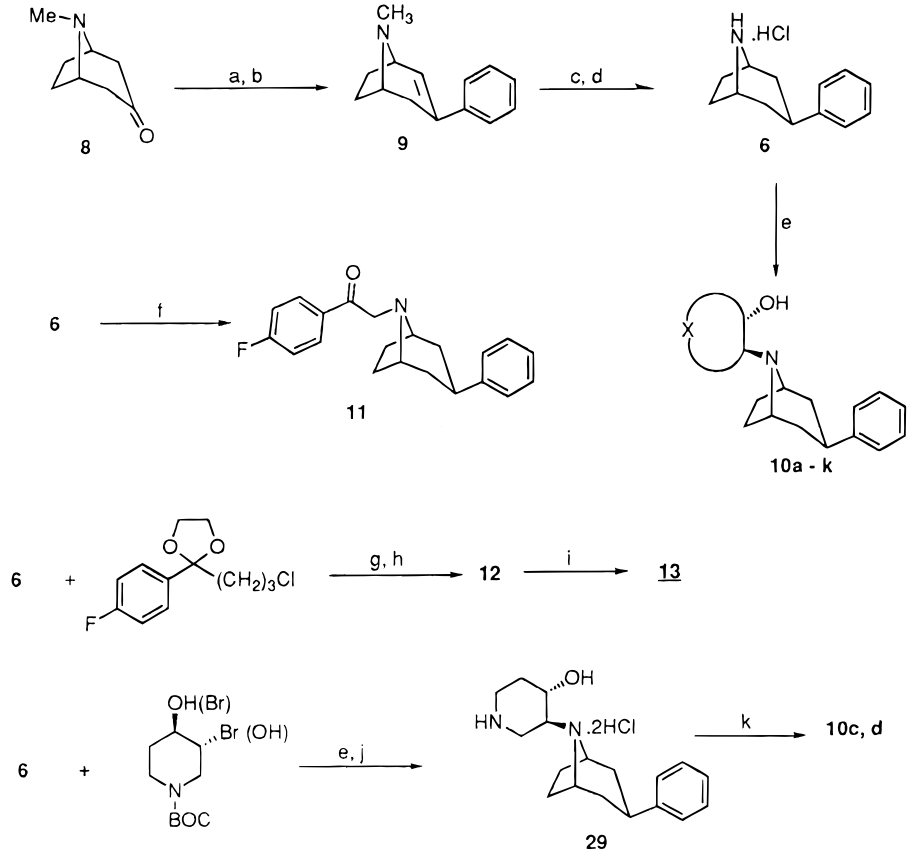
Scheme 1. Synthesis of *N*-Hydroxyalkyl/aryltropanes[a]



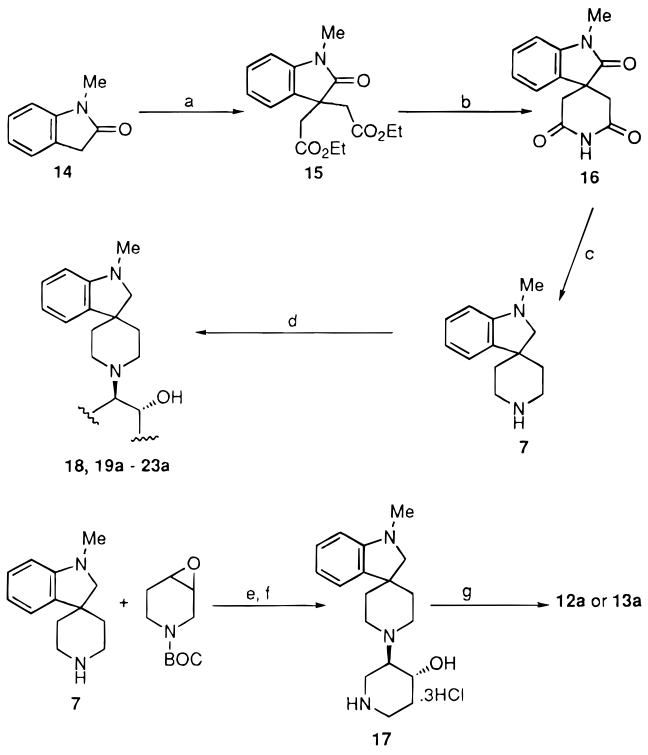
[a] (a) Ethyl bromoacetate, NaH, THF, rt; (b) Na, urea, EtOH, reflux; (c) $LiAlH_4$, THF, reflux; (d) substituted epoxide, EtOH, reflux; (e) K_2CO_3, EtOH, reflux; (f) HCl(g), EtOAc, 0 °C; (g) 4-fluoro- or 3-iodobenzyl bromide, K_2CO_3, DMF, rt.

Chemistry

The target compounds were synthesized as outlined in Schemes 1 and 2. For the first series of target compounds, 3β-phenyltropane **6** was synthesized from 3-phenyltropidine as described.[14] The reaction of **6** with various epoxides provided the corresponding amino alcohols **10a**−**k** (Chart 2) in low to moderate yields. In contrast to 4-phenylpiperidine, the reaction of **6** with these epoxides was generally sluggish, requiring prolonged heating. For the piperidinols **10c**,**d**, 1,2,3,6-tetrahydropyridine was converted to the BOC-protected derivative as previously described,[16] and the latter was reacted with mCPBA to generate the requisite epoxide. As reported earlier,[16] the presence of the *tert*-butoxycarbonyl protecting group favors nucleophilic attack at the C4 position of the epoxide, resulting in a predominance of the desired regioisomer.

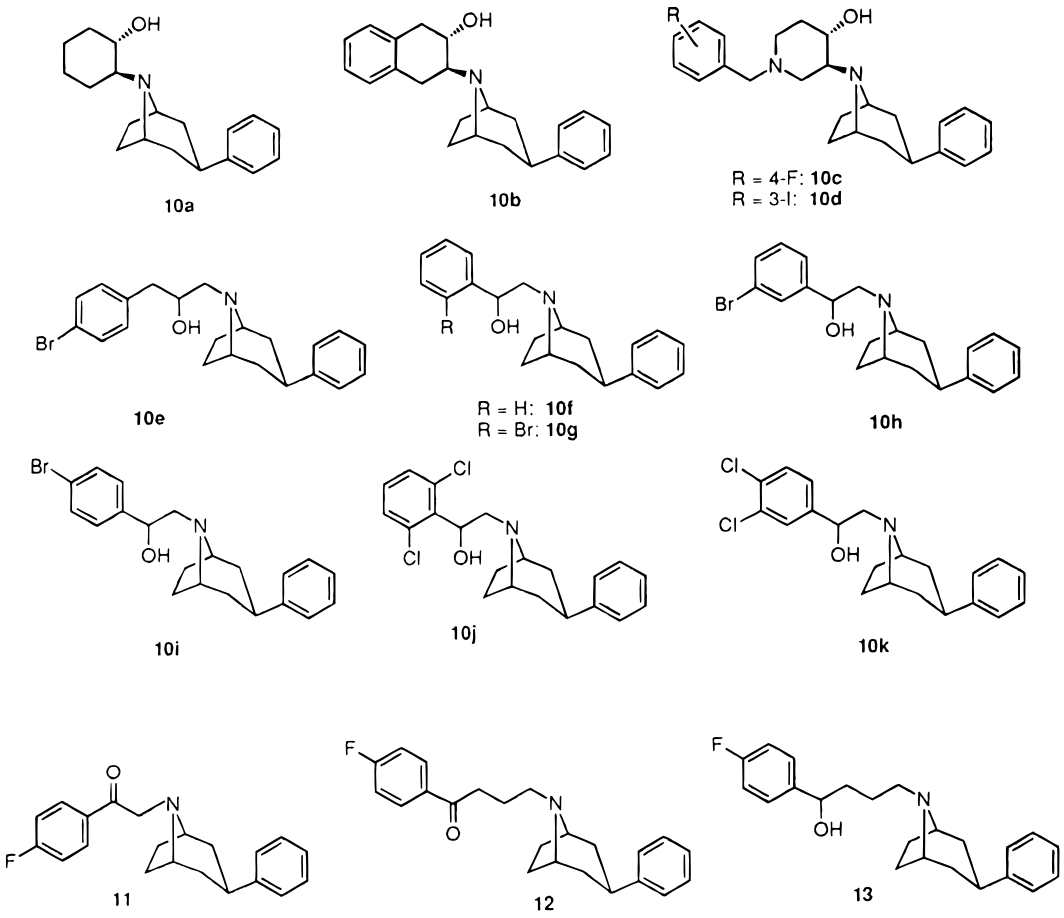
For the second series of compounds, 1-methylspiro-[indoline-1,4′-piperidine] dihydrochloride **7** was prepared as elaborated in Scheme 2. The reaction of 1-methyl-2-oxindole with a large excess of ethyl bromoacetate provided a 2:1 mixture of the di- and monoalkylated products from which **15** was separated by HPLC in 28% yield. Base-catalyzed reaction of the latter with urea provided the imide **16** which was subsequently reduced with $LiAlH_4$ to yield **7** in an overall yield of 7%. The target amino alcohols were subsequently obtained by reaction of **7** with the corresponding epoxides. Compounds **20a** and **21a** (Chart 3) were obtained as described for **10c**,**d**, respectively. Yields were typically low, even with long reaction times, suggesting that both the tropane **6** and the spirobase **7**

Scheme 2. Synthesis of Spiro[indoline-1,4′-piperidine] Analogues[a]



[a] (a) PhLi, ether; (b) 48% HBr, rt; (c) i. vinyl chloroformate, ii. 2 N HCl, EtOH, reflux; (d) 10% Pd−C/H_2, EtOH; (e) epoxide, EtOH, reflux; (f) $NaHCO_3$, aq EtOH, reflux; (g) KI,CH_3CN, Et_3N, reflux; (h) 3 N HCl, EtOH, reflux; (i) $NaBH_4$, EtOH; (j) HCl(g), EtOAc, 0 °C; (k) K_2CO_3, EtOH, reflux.

Chart 2



are significantly less nucleophilic than 4-phenylpiperidine under these conditions. Reaction of **7** or 2,3-dihydrospiro[indene-1,4′-piperidine] with the relevant epoxides provided compounds **22a,b** and **23a,b** in low to moderate yield. These two spirobases were also used to synthesize **24a**,**b** using the procedure outlined for **13** (Scheme 1).

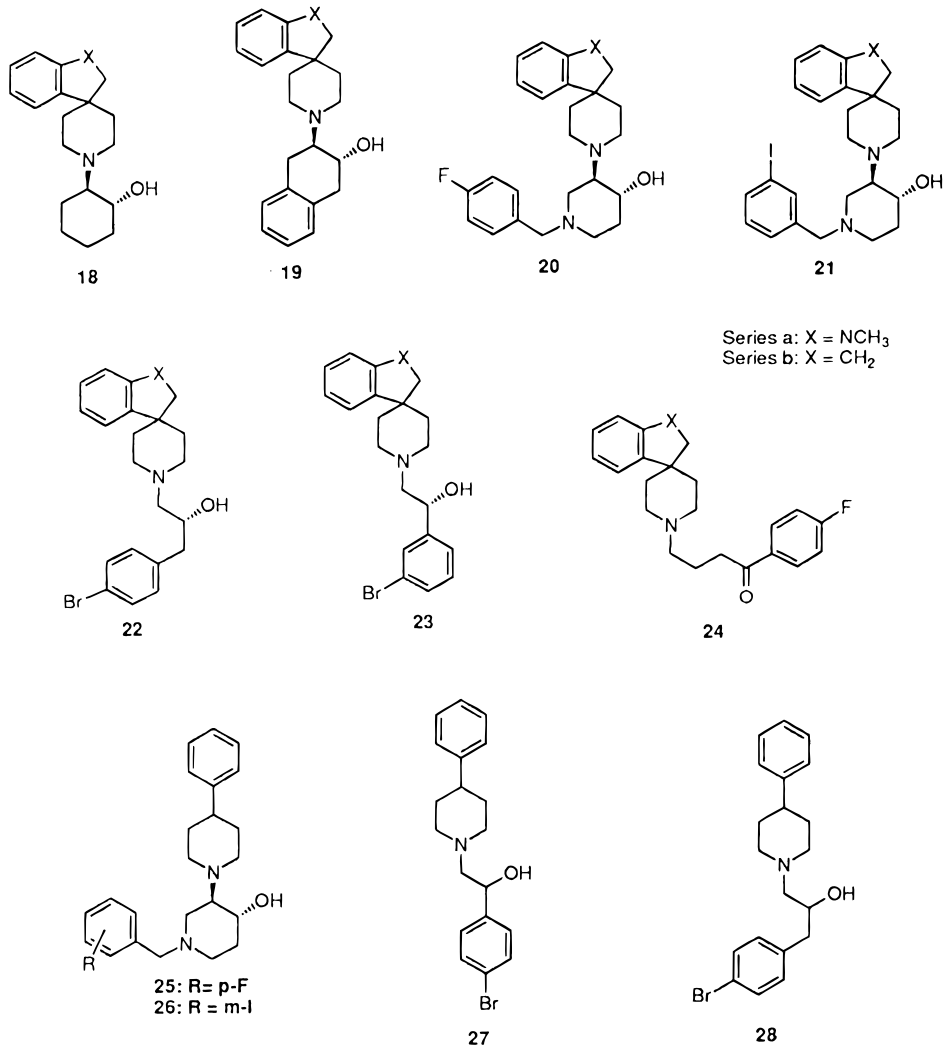
Results and Discussion

For the purpose of this discussion, the structure of vesamicol has been divided into three major fragments: A, B, and C (Chart 1). Although many vesamicol analogues have been synthesized in an effort to expand our understanding of the structure−activity relationships for binding to the vesicular acetylcholine transporter, the vast majority contains an unmodified B fragment, and the rest contains only single-point modifications of this fragment. Consequently, little is known about the interaction between this region of the molecule and the vesicular acetylcholine transporter. In previous studies,[6,12,13] we and others utilized a strategy of conformational restriction (in fragment A and/or C) to develop selective high-affinity ligands for the vesicular acetylcholine transporter. To test the limits of this strategy, we chose to extend our studies to fragment B.

In our first attempt, we replaced the piperidyl fragment B with a tropanyl moiety. Formally, tropane may be regarded as *2,6-ethanopiperidine*. Since the ethylene bridge prevents the interconversion of piperidine conformers, the tropanyl fragment may be regarded as a conformationally restricted piperidyl residue. In a parallel effort, we also synthesized several derivatives of 1-methylspiro[1*H*-indoline-3,4′-piperidine] (**7**) as conformationally restricted vesamicol analogues. The latter study is an extension of our previous investigation[13] of spirovesamicol analogues such **3**−**5**. The target compounds were tested for binding to the vesicular acetylcholine transporter of *Torpedo* synaptic vesicles. Binding to σ receptors was also evaluated because vesamicol and some of its analogues display moderate to high affinity for these sites.[11] The compounds were also tested for binding to monoamine transporters because the 3β-phenyltropanyl moiety is found in many inhibitors of monoamine reuptake.[17−20] Finally, the affinity of these compounds for dopamine D2 receptors was evaluated because of behavioral effects in rodents (data not shown). Since the reference ligand [^{125}I]-NCQ298 does not discriminate between dopamine D2 and D3 receptors, the data obtained with this radioligand reflect binding to both dopamine receptor subtypes.

Although many of the tropane analogues displayed moderate affinity for the vesicular acetylcholine transporter, none of the new compounds was more potent than vesamicol. In fact, compared to the corresponding piperidyl analogues, most of the new compounds fared rather poorly. Thus, while compound **10a** displayed 4-fold lower affinity than vesamicol, **10b**−**d** were at least 2 orders of magnitude less potent than benzovesamicol **(2)**, **25**, and **26**, respectively (Table 1). These disparities persisted even when the cyclohexyl moiety was replaced with a hydroxyethyl group (**27** vs **10f**−**k**) but disappeared when the *N*-alkyl substituent became more flexible (**28** vs **10e**). Taken together, the foregoing

Chart 3



suggests that the two-carbon bridge (which converts the piperidyl into a tropanyl fragment) is profoundly detrimental to the ligand−receptor interaction. As the two-carbon bridge increases the steric volume of fragment B, one plausible explanation for the adverse effect of this bridge may be that fragment B fits into a narrow pocket within the binding site.

Although spirovesamicols such as **3**−**5** display comparable or higher affinity for the vesicular acetylcholine transporter than vesamicol, all analogues of **7** were significantly less potent than vesamicol. Moreover, the new compounds generally displayed 1−2 orders of magnitude lower potency than the corresponding carbon analogues (compare **18a** vs **4**, **19a** vs **19b**, **20a** vs **20b**, and **21a** vs **21b**). While the disparity between the two series of compounds was obscured by the absence of a cyclohexyl group (compare **22a** vs **22b** and **23a** vs **23b**), it is clear that **7** is not a suitable replacement for the A−B fragment in vesamicol.

All compounds tested showed moderate to weak affinity for both σ1 and σ2 receptors. In addition, several of the analogues (**10e**,**g**,**h**,**j**,**k**, **22a**, and **23b**) displayed 6−20-fold higher affinity for σ2 receptors than σ1 sites. The moderate affinity of these compounds is consistent with previous findings[11] and the view that the 4-phenylpiperidyl fragment constitutes an important recognition element for the σ receptor.[21−23]

While some of the compounds caused a reduction in locomotor activity in animals, none of the hydroxylalkyl derivatives displayed even moderate affinity for dopamine D2/D3 receptors. However, moderate to high affinity was observed with the butyrophenones **12** and **24a**,**b**. The latter observation is not particularly surprising, given the striking similarity between these compounds and the potent dopamine antagonists spiperone and haloperidol.[24] The latter also displays high affinity for σ receptors.[21]

Among the phenyltropanes, all analogues containing a cyclic B fragment (cyclohexyl or piperidyl) displayed poor affinity for the dopamine transporter (DAT). In contrast, all analogues lacking the cylohexyl group displayed moderate to high affinity for the DAT and serotonin transporter (SERT) and higher affinity for the DAT than (−)-cocaine. With the respect to N-substitution, the moderate to high affinity of these compounds for the DAT is consistent with the previously[25] observed bulk tolerance of the DAT around the nitrogen atom of cocaine. However, the absence of a C2 substituent requires further comment.

Previous studies of 3β-aryl-2β-carbomethoxytropanes (the WIN series of cocaine analogues) clearly demonstrate that replacement of the 2β-carbomethoxy group with a halovinyl[17] or propionyl[18] moiety does not adversely affect affinity for the DAT. While the present

Table 1. Relative Affinities ($K_i \pm$ SEM, nM) of Vesamicol Analogues at Selected Receptors

compd	VR	σ1	σ2	D2/D3	DAT	SERT
10a	8.5 ± 2.7	380 ± 36	118 ± 18	>38000	2813 ± 316	3422 ± 286
10b	8.5 ± 0.94	175 ± 21.4	175 ± 12.4	3385 ± 429	1066 ± 131	1673 ± 221
10c	325 ± 49	240.3 ± 44.9	260.1 ± 4.2	8795 ± 2339	4183 ± 500	9201 ± 2313
10d	14.5 ± 3.1	194.5 ± 32.2	275.1 ± 21.6	1967 ± 357	1069 ± 143	4434 ± 178
10e	10.3 ± 4.4	30.89 ± 6.72	4.95 ± 0.34	1490 ± 399	306 ± 20	234 ± 20
10f	474 ± 150	40.4 ± 11.3	32.7 ± 9.3		41.55 ± 7.1	
10 g	130 ± 30	95.69 ± 11.40	7.71 ± 1.12		67.25 ± 24.2	
10h	298 ± 106	117 ± 24.2	9.87 ± 0.03	2658 ± 1051	23.6 ± 3.2	27 ± 3
10i	144 ± 43	17.49 ± 1.94	11.28 ± 1.85	5092 ± 2062	73.8 ± 11	30.05 ± 0.75
10j	411 ± 170	302.4 ± 51.7	15.87 ± 3.74		42.05 ± 2.0	
10k	60 ± 8.3	249.3 ± 38.2	18.8 ± 2.6		23.65 ± 3.1	
11	3400 ± 3000	280.75 ± 15.29	73.33 ± 8.52		221.7 ± 34.2	
12	594 ± 94	34.10 ± 9.49	15.36 ± 1.87	16.3 ± 6.5	129 ± 6.7	88.55 ± 8.77
13	380 ± 115				463.6 ± 77.2	
18a	74 ± 9.9	286.4 ± 35.1	357.3 ± 23.7	>1000		
19a	77 ± 9.5			>1000		
20a	33 ± 4.8			>1000		
21a	17 ± 2.2	121.8 ± 14.8	139.2	>1000		
22a	420 ± 39	191.0 ± 9.2	22.9 ± 3.1	>1000		
23a	182 ± 32	517.7 ± 46.9	99.2 ± 14.2	>1000		
24a	570 ± 61	110.4 ± 29.6	60.8	56.5 ± 24.5		
4	7.60 ± 0.99[a]	21 ± 4	30 ± 8	>1000		
19b	0.36 ± 0.15[a]			>1000		
20b	0.36 ± 0.10[a]	25 ± 1	67 ± 7	>1000		
21b	0.67 ± 0.19[a]	283 ± 20	224 ± 49	>1000		
22b	86 ± 14	8.5 ± 0.9	8.8 ± 3.6	>1000		
23b	600 ± 49	106.8 ± 22.5	12.7 ± 1.6	>1000		
24b	85 ± 17	1.8 ± 0.1	3.3 ± 0.2	25.3 ± 5.4		
25	0.44 ± 0.11[b]					
26	0.13 ± 0.03[b]					
27	30 ± 5[c]					
28	170 ± 20[c]					
(±)-vesamicol	2.0 ± 1.0[d]	26 ± 8	34 ± 2			
benzovesamicol	0.055 ± 0.01[d]					

[a] Reference 13. [b] Reference 11. [c] Reference 15. [d] Reference 6. K_D values for (−)-cocaine, haloperidol, [^{125}I]IPT (DAT), [^{125}I]IPT (SERT), and [^{125}I]NCQ298 (D2/D3) are 373 ± 150, 1.21 ± 0.2, 0.25, 1.2, and 0.05 nM, respectively.

study would appear to go even further by suggesting that a C2 substituent is not required for binding to the DAT, the situation appears to be significantly more complex.

Removal of the 2β-carbomethoxy group of (−)-cocaine results in a 50-fold reduction in affinity for the DAT.[26] On the other hand, both 3α-(diphenylmethoxy)tropanes[27,28] and 3α-(diphenylmethoxy)-2β-carbomethoxytropanes[29] display high affinity for the DAT. Taken together, the foregoing suggests that the requirement for a C2 substituent is partly governed by the C3 substituent. Previous investigators[29] appear to have arrived at a similar conclusion.

In summary, 3β-phenyltropanyl derivatives of vesamicol exhibit lower affinity for the vesicular acetylcholine transporter than the parent compound. Consequently, the introduction of a two-carbon bridge across the C2 and C6 positions of the piperidyl moiety of vesamicol is not a suitable strategy for enhancing affinity for the vesicular acetylcholine transporter or increasing selectivity for the vesicular acetylcholine transporter relative to σ receptors. The introduction of an aminomethyl bridge into the C fragment of vesamicol (to yield analogues of **7**) is also unsuitable for similar reasons. Since the results obtained with the tropanes suggest that fragment B binds to a narrow pocket within the binding site, we suggest that further modification of this fragment should be limited to single-point substitution.

Experimental Section

General. Synthetic intermediates were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Lancaster Synthesis (Windham, MA) and used as received. Tetrahydrofuran (THF) was distilled from sodium hydride immediately prior to use. All other reagents and solvents were purchased as reagent grade and used without further purification.

All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Yields were not optimized. Melting points were determined on a Haake-Buchler or Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 200-MHz IBM-Brucker spectrometer or a 300-MHz GE spectrometer. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. With this condition, the chemical shifts (in ppm) of residual solvents are observed at 7.26 ($CHCl_3$) and 4.78 (CD_3OH). The following abbreviations are used to describe peak patterns wherever appropriate: b = broad, d = doublet, t = triplet, q = quartet, m = multiplet. Preparative chromatography was performed on a Harrison Research Chromatotron using Merck 60 PF254 silica gel or a preparative HPLC system (Rainin Instrument Co.) using a 41.1-mm i.d. Dynamax silica gel column (delivering solvent at 80 mL/min). Analytical TLC was carried out on Analtech GHLF silica gel glass plates, and visualization was aided by UV and/or methanolic iodine.

Procedure A. Representative Procedure for the Synthesis of Epoxides. 3-Chloroperbenzoic acid (3.71 g of 57% mCPBA, 9.8 mmol) was added portionwise over a 15-min period to a cold (ice bath) stirring solution of 4-bromostyrene (1.5 g, 8.2 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was gradually warmed to room temperature and allowed to stir for 15 h, at which time it was diluted with CCl_4 (50 mL), and the precipitated 3-chlorobenzoic acid was removed by suction filtration. The filtrate was washed with a 1:1 mixture of 5% aqueous $NaHCO_3$ and 5% aqueous $NaHSO_3$ (3 × 25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the epoxide as a colorless liquid (1.48 g, 91%).

Procedure B. Synthesis of Vicinal Amino Alcohols. *trans*-2-(3β-Phenyltropan-8-yl)cyclohexanol (10a). A mix-

ture of 3β-phenyltropane hydrochloride (0.57 g, 2.55 mmol) and cyclohexene oxide (0.32 g, 3.31 mmol) was refluxed in EtOH (15 mL) and Et_3N (1 mL) for 5 days. (Subsequent reactions were only refluxed for 20 h.) Solvent was evaporated under reduced pressure, and the residue was purified by radial flow chromatography [hexane(9)−acetone(1)] to yield 0.3 g (37%) of free base. The corresponding hydrochloride was obtained by bubbling dry HCl(g) through a cold solution of the free base in MeOH followed by concentration of the resulting acidic solution and subsequent recrystallization from *i*-PrOH−Et_2O: mp 234−236 °C; ^{1}H NMR ($CDCl_3$) δ 1.17−1.73 (m, 8H, cyclohexyl), 2.12−3.78 (m, 13H, cyclohexyl methine Hs, tropanyl Hs), 4.67 (s, 1H, OH), 7.15−7.29 (m, 5H, phenyl). Anal. ($C_{19}H_{27}NO\cdot HCl\cdot H_2O$) C, H, N.

***trans*-2-Hydroxy-3-(3β-phenyltropan-8-yl)-1,2,3,4-tetrahydronaphthalene hydrochloride (10b):** procedure B, yield 8%; mp 228−231 °C (*i*-PrOH−Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.61−1.91 (m, 4H, tropanyl), 2.41−3.72 (m, 15H, tropanyl, tetrahydronaphthyl Hs), 4.47 (m, 1H, N-CH-), 4.89 (bs, 1H, OH), 6.10−7.31 (m, 9H, phenyl). Anal. ($C_{23}H_{27}NO\cdot HCl\cdot 0.5\ H_2O$) C, H, N.

1-(4-Bromophenyl)-3-(3β-phenyltropan-8-yl)propan-2-ol hydrochloride (10e): procedure B, yield 26%; mp 221−223 °C; ^{1}H NMR ($CHCl_3$) δ 1.52−2.46 (m, 12H), 2.72−3.29 (m, 3H), 3.51−3.78 (m, 1H, C*H*-OH), 4.38 (bs, 1H, O*H*), 7.16 (d, 2H, 4-Br Ar-*H*), 7.25−7.37 (m, 5H, Ar-*H*), 7.42 (d, 2H, 4-Br Ar-*H*). Anal. ($C_{22}H_{26}NOBr\cdot HCl\cdot {}^1/_4H_2O$) C, H, N.

1-Phenyl-2-(3β-phenyltropan-8-yl)ethanol hydrochloride (10f): procedure B, yield 0.26 g (45%); mp 179−182 °C; ^{1}H NMR ($CDCl_3$) δ 1.54−2.65 (m, 9H), 3.11−3.60 (m, 4H), 4.66 (dd, 1H, C*H*-OH), 4.77 (bs, 1H, O*H*), 7.18−7.39 (m, 10H, Ar-*H*). Anal. ($C_{21}H_{26}NOCl\cdot 0.25\ H_2O$) C, H, N.

1-(2-Bromophenyl)-2-(3β-phenyltropan-8-yl)ethanol hydrochloride (10 g): procedure B: yield 49%; mp 184−188 °C; ^{1}H NMR ($CDCl_3$) δ 1.51−2.53 (m, 8H), 2.81 (dd, 2H, bridge CH), 3.03−3.19 (m, 2H), 3.58 (t, 1H), 4.72 (bs, 1H, O*H*), 5.01 (dd, 1H, C*H*-OH), 7.12−7.70 (m, 9H, Ar-*H*). Anal. ($C_{21}H_{24}NOBr\cdot HCl\cdot {}^1/_4H_2O$) C, H, N.

1-(3-Bromophenyl)-2-(3β-phenyltropan-8-yl)ethanol hydrochloride (10h): procedure B, yield 27%; mp 171−173 °C; ^{1}H NMR ($CDCl_3$) δ 1.54−2.71 (m, 8H), 3.06−3.57 (m, 4H), 4.58 (dd, 2H, C*H*-OH), 4.81 (bs, 1H, O*H*), 7.18−7.56 (m, 9H, Ar-*H*). Anal. ($C_{21}H_{25}NOBrCl\cdot 0.5H_2O$) C, H, N.

1-(4-Bromophenyl)-2-(3β-phenyltropan-8-yl)ethanol hydrochloride (10i): procedure B, yield 35%; mp (MeOH−Et_2O) 200−202 °C; ^{1}H NMR (free base) ($CDCl_3$) δ 1.49−2.63 (m, 8H), 3.05−3.55 (m, 4H), 4.58 (dd, 2H, C*H*-OH), 4.81 (bs, 1H, O*H*), 7.16−7.35 (m, 7H, Ar-*H*), 7.49 (d, 2H, 4-Br Ar-*H*). Anal. ($C_{21}H_{25}NOBrCl$) C, H, N.

1-(2,6-Dichlorophenyl)-2-(3β-phenyltropan-8-yl)ethanol hydrochloride (10j): procedure B, yield 28%; mp 180−182 °C; ^{1}H NMR ($CDCl_3$) δ 1.48−3.40 (m, 13H), 4.38 (bs, 1H, O*H*), 5.47 (dd, 1H, C*H*-OH), 7.11−7.31 (m, 8H, Ar-H). Anal. ($C_{21}H_{24}NOCl_3\cdot 1.5H_2O$) C, H, N.

1-(3,4-Dichlorophenyl)-2-(3β-phenyltropan-8-yl)ethanol hydrochloride (10k). Sodium borohydride (2.0 g, 53 mmol) was added portionwise to a solution of 3′,4′-dichloroacetophenone (2.5 g, 13 mmol) in MeOH (30 mL). After completion of addition the mixture was stirred at room temperature for 1 h, and the solvent was removed in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and water (50 mL); the organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure to afford the desired alcohol (2.25 g, 89%) as a chromatographically homogeneous sample. The latter was combined with *p*-TsOH (0.23 g, 1.2 mmol) and benzene (50 mL), and the mixture was refluxed for 4 h with azeotropic distillation of water. The reaction mixture was subsequently cooled to room temperature, washed with 5% aqueous $NaHCO_3$ (3 × 25 mL), dried over anhydrous Na_2SO_4, and concentrated to yield 3,4-dichlorostyrene as a colorless liquid (1.5 g, 68%): ^{1}H NMR ($CDCl_3$) δ 5.4 (d, 1H, -CH=CH-*H*), 5.8 (d, 1H, -CH=C*H*-H), 6.6 (dd, 1H, -C*H*=CH_2), 7.4−7.6 (m, 3H, phenyl).

The title compound was synthesized from 3,4-dichlorostyrene as described in procedure B: yield 0.13 g (17%); mp 204−206 °C; ^{1}H NMR ($CDCl_3$) δ 1.53−2.60 (m, 10H), 3.05−3.16 (m, 2H), 3.40 (m, 1H), 4.55 (dd, 1H, C*H*-OH), 4.58 (bs, 1H, O*H*), 7.18−7.49 (m, 8H, Ar-H). Anal. ($C_{21}H_{24}NOCl_3$) C, H, N.

***trans*-4-Hydroxy-3-(3β-phenyltropan-8-yl)piperidine Dihydrochloride (29).** A mixture of 3β-phenyltropane hydrochloride (1.14 g, 6.1 mmol), 2.15 g (7.6 mmol) of the isomeric *trans*-bromohydrins 1-(*tert*-butoxycarbonyl)-3(4)-bromo-4(3)-hydroxypiperidine,[16] and potassium carbonate (1.68 g, 14.0 mmol) was refluxed in ethanol (50 mL) for 4 days. After cooling, the insoluble material was removed by filtration, and the filtrate was concentrated to yield a tan residue. The latter was passed through a short column of silica gel using 25% acetone−hexane (plus trace Et_3N). The eluent was concentrated, and the residue thus obtained was purified by HPLC (5% *i*-PrOH−hexane, plus trace Et_3N) to afford 0.24 g (10%) of the title compound as a clear syrup: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, BOC), 1.5−1.95 (m, 6H, piperidyl CH_2), 2.13−3.15 (m, 8H, piperidyl CH_2), 3.38−3.56 (m, 3H, piperidyl CH), 3.85−4.2 (bs, 3H, O*H*, C*H*-N, C*H*-OH), 7.18−7.36 (m, 5H, Ar-H).

Similar yields were obtained from subsequent runs. For removal of the butoxycarbonyl protecting group, a stirring solution of this intermediate (1.0 g, 0.25 mmol) in EtOAc was cooled in an ice bath, and dry HCl(g) was bubbled vigorously through it for 30 min. Stirring and cooling were continued for an additional 30 min, at which time the mixture was concentrated under reduced pressure. Coevaporation of the residual solvent with toluene (3 × 25 mL) and subsequent drying yielded crude **29** as a white solid (0.91 g, 99%). The latter was used without further purification.

3,4-*trans*-1-(4-Fluorobenzyl)-4-hydroxy-3-(3β-phenyltropan-8-yl)piperidine Dihydrochloride (10c). 4-Fluorobenzyl bromide (0.26 g, 1.37 mmol) was added to a mixture of **29** (0.41 g, 1.14 mmol) and anhydrous potassium carbonate (0.4 g, 2.85 mmol) in DMF (20 mL). The resulting mixture was stirred at room temperature for 18 h, diluted with methylene chloride (25 mL), and filtered. The filtrate was concentrated under reduced pressure and the tan residue purified by radial flow chromatography using a mobile phase of 15% acetone−hexane (plus trace Et_3N) to afford **10c** as a solid (0.27 g, 64%). The free base was converted to the corresponding hydrochloride with cold methanolic HCl, and recrystallization was performed in *i*-PrOH−ether: mp 183−186 °C; ^{1}H NMR ($CDCl_3$) δ 1.53−2.10 (m, 10H, piperidinyl CH_2), 2.41−2.50 (m, 3H, piperidinyl CH_2, CH), 2.70−3.1 (m, 3H, piperidinyl CH_2, CH), 3.2−3.49 (m, 5H, benzyl CH_2, piperidinyl CH, OH), 4.22 (s, 1H), 6.94−7.31 (m, 9H, Ar-H). Anal. ($C_{25}H_{33}N_2OFCl_2\cdot 0.5H_2O$) C, H, N.

3,4-*trans*-1-(3-Iodobenzyl)-4-hydroxy-3-(3β-phenyltropan-8-yl)piperidine Dihydrochloride (10d). Alkylation of **29** with 3-iodobenzyl bromide as described for **10c** above provided an 89% yield of **10d** as a pale oil. Conversion to the hydrochloride and subsequent recrystallization were performed as described above: mp 180−184 °C; ^{1}H NMR ($CDCl_3$) δ 1.55−2.10 (m, 10H, piperidinyl CH_2), 2.39−3.15 (m, 6H, piperidinyl CH_2, CH), 3.2−3.49 (m, 5H, benzyl CH_2, piperidinyl CH, OH), 4.20 (s, 1H), 7.02−7.31 (m, 7H, Ar-H), 7.57−7.65 (m, 2H, Ar-H). Anal. ($C_{25}H_{33}N_2OICl_2\cdot 0.5H_2O$) C, H, N.

8-[(4-Fluorobenzoyl)methyl]-3β-phenyltropane Hydrochloride (11). A mixture of 3β-phenyltropane (0.4 g, 2.14 mmol), 2-bromo-4′-fluoroacetophenone (0.56 g, 2.57 mmol), and $NaHCO_3$ (0.91 g, 8.6 mmol) was refluxed in 25% aqueous EtOH (40 mL) for 17 h. The reaction mixture was cooled to room temperature, diluted with water (75 mL), and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to a dark yellow residue. Purification of the crude product by radial flow chromatography [hexane(80)−acetone(20)−Et_3N(1)] afforded 0.35 g (50%) of the title compound as a pale yellow syrup: ^{1}H NMR ($CDCl_3$) δ 1.48−1.61 (m, 4H, tropane CH_2-CH_2), 2.02−2.06 (m, 2H, tropane CH_2-CH-CH_2), 2.39−2.58 (m, 2H, tropane CH_2-CH-CH_2), 3.07−3.12 (m, 1H, tropane C*H*-Ph), 3.35 (m, 2H, bridge CH), 3.72 (s, 2H, N-CH_2), 7.09−7.28 (m, 7H, Ar-H), 8.10−8.17 (m, 2H, fluorophenyl Ar-H). The hydrochloride was prepared in methanolic HCl and recrystallized from MeOH−Et_2O: mp 121−124 °C. Anal. ($C_{21}H_{23}NOClF\cdot {}^3/_4H_2O$) C, H, N.

3-(4-Fluorobenzoyl)-1-(3β-phenyltropan-8-yl)propane (12). A mixture of 4-chloro-1,1-ethylenedioxy-1-(4-

fluorophenyl)butane[30] (0.52 g, 2.14 mmol), 3β-phenyltropane (0.4 g, 2.14 mmol), KI (0.36 g, 2.14 mmol), and Et_3N (3 mL) was refluxed in MeCN (15 mL) for 22 h. After the mixture cooled to room temperature, the solvent was evaporated in vacuo and the residue reconstituted in CH_2Cl_2 (25 mL) and washed successively with 5% aqueous $NaHCO_3$ and water. The organic extract was dried (Na_2SO_4) and concentrated in vacuo to afford a pale brown residue of the alkylated ketal which was sufficently pure for the next reaction. The ketal was refluxed in 3 N HCl (10 mL) for 20 min, and the solution was concentrated under reduced pressure. The residue was treated with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (2 × 25 mL). The combined organics were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a yellow syrup which was purified by radial flow chromatography (15% acetone−hexane) to provide 0.36 g (48%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 1.38 (m, 5H), 1.85−1.99 (m, 4H), 2.27−2.42 (m, 4H), 2.87−3.10 (m, 2H), 3.26 (m, 2H), 7.09−7.31 (m, 7H, Ar-*H*), 8.01−8.08 (m, 2H, *p*-F-phenyl Ar-*H*); mp (hydrochloride) 195−198 °C. Anal. ($C_{23}H_{26}NOF\cdot HCl\cdot 0.25H_2O$) C, H, N.

1-(4-Fluorophenyl)-3-(3β-phenyltropan-8-yl)butanol Hydrochloride (13). To a methanolic solution of **12** (0.33 g, 0.93 M) was added $NaBH_4$ (0.15 g, 3.72 mmol) portionwise at room temperature. The reaction mixture was then stirred at room temperature for an additional 15 min. The solvent was evaporated in vacuo, the white residue dissolved in CH_2Cl_2 (50 mL), and the resulting solution washed with water (2 × 25 mL). Drying of the organic extract (Na_2SO_4) followed by solvent removal under reduced pressure afforded the title compound as a pale yellow syrup (0.31 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.47−2.02 (m, 9H), 2.16−2.46 (m, 5H), 3.17−3.39 (m, 3H), 4.69−4.73 (dd, 1H, C*H*-OH), 6.96−7.39 (m, 9H, Ar-*H*), 8.51 (bs, 1H, O*H*); mp (hydrochloride) 130−134 °C. Anal. ($C_{23}H_{28}NOF\cdot HCl\cdot 0.75H_2O$) C, H, N.

3,3-Bis(carbethoxymethyl)-1-methyloxindole (15). A suspension of sodium hydride (28.2 g of a 50% dispersion in mineral oil, 1.172 mol) was washed with dry THF (20 mL) and resuspended in the same solvent (40 mL). To this stirring suspension was added a solution of *N*-methyloxindole[31] (43.0 g, 0.293 mol) in 50 mL of dry THF dropwise under nitrogen for 20 min. This was followed by the dropwise addition of a solution of ethyl bromoacetate (147.0 g, 0.88 mol) in 30 mL of THF, after which the reaction mixture was stirred at room temperature. for 1.15 h. The reaction was then quenched with 100 mL of saturated aqueous NH_4Cl, diluted with water, and extracted with CH_2Cl_2 (5 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to a syrup. The latter was applied onto a short silica gel column which was eluted with 25% acetone−hexane to afford 76.6 g of a product containing both the mono- and dialkylation reaction products (approximately 1:2) along with unreacted ethyl bromoacetate. The desired compound was separated by preparative HPLC (10% *i*-PrOH−hexane with trace Et_3N) in 28% yield (17.8 g): ^{1}H NMR ($CDCl_3$) δ 1.01 (t, 6H, $COOCH_2CH_3$), 2.88−3.11 (bq, 4H, CH_2-CO), 3.23 (s, 3H, N-CH_3), 3.84 (q, 4H, $COOCH_2$), 6.80 (d, 1H, phenyl), 6.95−7.33 (m, 3H, phenyl).

1-Methyl-2,2′,6′-trioxospiro[indoline-1,4′-piperidine] (16). The compound was synthesized according to a previously published procedure.[32] To a refluxing solution of sodium (0.5 g, 2.2 g-atom) in absolute EtOH (35 mL) was added a solution of **15** (3.0 g, 9.4 mmol) in absolute EtOH (15 mL) and urea (0.85 g, 14.1 mmol). The resulting mixture was refluxed under a N_2 atmosphere for 6.5 h. After the reaction cooled to room temperature, the volatiles were evaporated in vacuo to provide a tan solid. This was treated with 6 N HCl (25 mL), and the resulting mixture was extracted consecutively with CH_2Cl_2 (3 × 25 mL) and EtOAc (50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue thus obtained was passed through a short silica gel column using 25% acetone−hexane to give 0.62 g (27%) of a pale brown solid: mp 199−201 °C; yields of 41% were subsequently obtained upon scaleup; ^{1}H NMR ($CDCl_3$) δ 2.53 (d, 2H, CH_2), 2.94 (d, 2H, CH_2), 3.24 (s, 3H, N-CH_3), 6.90 (d, 1H, phenyl), 7.05−7.10 (m, 1H, phenyl), 7.20−7.24 (m, 1H, phenyl), 7.33−7.38 (m, 1H, phenyl). Anal. ($C_{13}H_{11}N_2O_2$) H, N; C: calcd, 63.93; found, 62.94.

1-Methylspiro[indoline-1,4′-piperidine] (7). A solution of **16** (0.60 g, 2.46 mM) in 10 mL of dry THF was added dropwise to a stirring suspension of lithium aluminum hydride (0.93 g, 24.6 mM) in dry THF (20 mL) cooled in an ice bath. At the end of the addition, the reaction mixture was warmed to room temperature, refluxed for 6 h, and cooled to room temperature. The reaction was then quenched by dropwise addition of water (1 mL), 15% NaOH (1 mL), and water (3 mL), consecutively. The resulting precipitate was removed by suction filtration, washed with EtOAc (25 mL), and discarded, while the filtrate was dried over Na_2SO_4 and concentrated under reduced pressure to yield a pale brown syrup (0.46 g, 93%): ^{1}H NMR ($CDCl_3$) δ 1.70−1.98 (m, 4H, piperidyl), 2.74 (s, 3H, N-CH_3), 2.76−2.81 (m, 2H, piperidyl), 3.07−3.23 (m, 2H, piperidyl), 3.35 (s, 2H, indoline CH_2), 4.09 (bm, 1H, N-*H*), 6.46 (d, 1H, phenyl), 6.67−6.74 (m, 1H, phenyl), 7.08−7.14 (m, 2H, phenyl).

Procedure C. Synthesis of Spiroindoline-Containing Amino Alcohols. 1′-(1-Hydroxycyclohex-2-yl)-1-methylspiro[indoline-3,4′-piperidine] Dihydrochloride (18a). A mixture of **7** (0.49 g, 2.4 mmol), cyclohexene oxide (0.35 g, 3.2 mmol), and triethylamine (5 mL) was refluxed in absolute ethanol (50 mL) for 3 days. The reaction mixture was cooled to room temperature and concentrated to a residue which was passed through a short column of silica gel [eluting with hexane(70)−acetone(30)−Et_3N(1)]. Concentration of the eluent yielded a residue which was further purified by radial flow chromatography, using a mobile phase of hexane(85)−acetone-(15)−Et_3N(1), to provide 0.40 g (55%) of the title compound. The latter was converted to the corresponding hydrochloride as elaborated above and recrystallized from *i*-PrOH−ether: mp 233−235 °C; ^{1}H NMR ($CDCl_3$) δ (free base) 1.17−3.70 (m, 23H), 4.09 (bs, 1H, O*H*), 6.45 (d, 1H, indoline Ar-*H*), 6.70 (t, 1H, indoline Ar-*H*), 7.04−7.13 (m, 2H, indoline Ar-*H*). Anal. Calcd for $C_{19}H_{28}N_2O\cdot 2HCl\cdot H_2O$: C, 58.31; H, 8.24; N, 7.15. Found C, 58.42; H, 7.02; N, 6.20.

1′-(2-Hydroxy-1,2,3,4-tetrahydronaphth-3-yl)-1-methylspiro[indoline-3,4′-piperidine] hydrochloride (19a): procedure C, purified by radial flow chromatography [acetone-(20)−hexane(80)−Et_3N(1)], yield: 20%; ^{1}H NMR ($CDCl_3$) δ 1.77−1.89 (m, 6H, piperidyl), 2.75 (s, 3H, CH_3), 2.78−3.38 (m, 9H, piperidyl, tetrahydronaphthyl, OH, indoline CH_2), 3.71 (m, 1H, CH-N), 3.93 (m, 1H, CH-OH), 6.46 (m, 1H, indoline phenyl), 6.71 (m, 1H, indoline phenyl), 7.07−7.50 (m, 6H, indoline, tetrahydronaphthyl ArH). The corresponding hydrochloride was obtained in methanolic HCl and recrystallized from MeOH−Et_2O as a dark yellow solid: mp 242−246 °C. Anal. ($C_{23}H_{30}N_2OCl_2\cdot {}^3/_4H_2O$) C, H, N.

1′-(4-Hydroxypiperidin-3-yl)-1-methylspiro[indoline-3,4′-piperidine] Trihydrochloride (17). A mixture of **7** (1.5 g, 7.5 mmol), 1-(*tert*-butoxycarbonyl)-3,4-epoxy-1,2,3,6-tetrahydropiperidine (1.8 g, 9.0 mmol), and K_2CO_3 (1.15 g, 8.0 mmol) was refluxed in absolute EtOH (50 mL) for 2 days. At this stage another batch of epoxide (1.8 g, 9.0 mmol) was added, and heating was continued for 1.5 days. The reaction mixture was then cooled to room temperature, concentrated under reduced pressure, and passed through a short silica gel column (eluting with 25% acetone−hexane plus trace Et_3N). The above desired regioisomer was isolated as a liquid by preparative HPLC [Et_3N(1)−isopropyl alcohol(3)−hexane(97); retention time, 8.95 min]: yield 0.45 g (16%);^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, *tert*-butyl *H*), 1.77−2.67 (m, 12H), 2.78 (s, 3H, N-Me), 2.84−3.74 (m, 5H), 4.06 (bs, 2H, C*H*-OH, *OH*), 6.45 (d, 1H, Ar-*H*), 6.70 (t, 1H, Ar-*H*), 7.02−7.14 (m, 2H, Ar-*H*).

The product (0.45 g, 1.12 mmol) was dissolved in EtOAc (40 mL) and cooled in an ice bath. Hydrogen chloride gas was passed through this solution for 0.5 h, after which the reaction mixture was allowed to stir under ice bath conditions for an additional 0.5 h. Concentration of the mixture yielded a pale yellow solid which was dried to give 0.42 g (91%) of the product: ^{1}H NMR (CD_3OD) δ 1.93−2.60 (m, 8H), 3.14−4.20 (m, 16H), 7.56 (bs, 4H, Ar-*H*).

1′-[1-(4-Fluorobenzyl)-4-hydroxypiperidin-3-yl]-1-methylspiro[indoline-3,4′-piperidine] Trihydrochloride

(20a). A mixture of **17** (0.21 g, 0.51 mM) and 4-fluorobenzyl bromide (0.12 g, 0.61 mmol) was stirred in DMF (5 mL) containing anhydrous K_2CO_3 (0.36 g, 2.55 mmol) at room temperature for 18 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL), and the inorganics were filtered. The filtrate was evaporated under reduced pressure and the compound purified from the crude residue by radial flow chromatography [acetone(20)−hexane(80)−Et_3N(1)]: yield 85 mg (21%); ^{1}H NMR ($CDCl_3$) δ 1.23−2.24 (m, 11H, piperidine), (m, 13H, piperidine, N-Me, C*H*-OH, *OH*, benzyl CH_2), 6.43 (d, 1H, indoline Ar-*H*), 6.64 (t, 1H, indoline Ar-*H*), 6.99−7.25 (m, 6H, indoline, phenyl Ar-*H*). The corresponding hydrochloride was prepared in methanolic HCl and recrystallized from *i*-PrOH−Et_2O: mp 190−194 °C. Anal. ($C_{25}H_{33}N_3OF\cdot 3HCl\cdot 1.5H_2O$) C, H, N.

1′-[1-(3-Iodobenzyl)-4-hydroxypiperidin-3-yl]-1-methylspiro[indoline-3,4′-piperidine] Trihydrochloride (21a). A mixture of **17** (0.19 g, 0.47 mmol) and 3-iodobenzyl bromide (0.16 g, 0.55 mmol) was stirred in DMF (5 mL) containing anhydrous K_2CO_3 (0.33 g, 2.35 mmol) at room temperature for 18 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL), and the inorganics were removed by filtration. The filtrate was concentrated under reduced pressure and the compound purified from the crude residue by radial flow chromatography (20% acetone−hexane with trace Et_3N): yield 0.16 g (24%); mp 225−228 °C. ^{1}H NMR ($CDCl_3$) δ 1.23−2.24 (m, 11H, piperidine), (m, 13H, piperidine, N-Me, C*H*-OH, *OH*, & benzyl CH_2), 6.34 (d, 1H, indoline Ar-*H*), 6.58 (t, 1H, indoline Ar-*H*), 6.91−7.15 (m, 2H, indoline Ar−H), 7.20−7.56 (m, 4H, phenyl Ar-*H*). Anal. ($C_{25}H_{33}N_3OI.3HCl\cdot 0.75H_2O$) C, H, N.

1′-[3-(1-(3-Bromophenyl)-2-hydroxypropyl]]-1-methylspiro[indoline-3,4′-piperidine] (22a): procedure C, purified by radial flow chromatography [acetone(20)−hexane(80)−Et_3N(1)], yield 0.26 g (46%); mp (hydrochloride) 209−212 °C (MeOH−ether); ^{1}H NMR ($CDCl_3$) δ 1.20−3.56 (m, 17H), 3.99 (bm, 2H, O*H*, C*H*-OH), 6.45 (d, 1H, indoline Ar-*H*), 7.01 (t, 1H, indoline Ar-*H*), 7.09−7.45 (m, 6H, indoline, bromophenyl Ar-*H*). Anal. ($C_{22}H_{27}BrN_2O$) H; C: calcd, 63.62; found, 62.59. N: calcd, 6.74; found, 5.74.

1′-[2-(1-(3-Bromophenyl)-1-hydroxyethyl]-1-methylspiro[indoline-3,4′-piperidine] (23a): procedure C (26 h), purified by radial flow chromatography [acetone(15)−hexane(85)−Et_3N(1)], yield 27%; hydrochloride recrystallized from MeOH−Et_2O; mp 215−219 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.78 (m, 2H, piperidyl), 1.95 (m, 2H, piperidyl), 2.43−2.60 (m, 4H, piperidyl), 2.80 (s, 3H, N-CH_3), 3.22 (s, 2H, indoline CH_2), 3.60 (m, 2H, N-CH_2), 4.20 (bs, 1H, O*H*), 4.65 (dd, 1H, C*H*-OH), 6.45 (d, 1H, indoline Ar-*H*), 6.75 (m, 1H, indoline Ar-*H*), 7.10−7.60 (m, 6H, indoline Ar-*H*, styryl Ar-*H*). Anal. ($C_{21}H_{27}N_2OBrCl_2\cdot 0.25H_2O$) C, H, N.

1′-[3-(4-Fluorobenzoyl)propyl]-1-methylspiro[indoline-3,4′-piperidine] (24a). A mixture of 4-chloro-1,1-ethylenedioxy-1-(4-fluorophenyl)butane[30] (0.43 g, 1.7 mmol), **7** (0.35 g, 1.7 mmol), KI (0.12 g, 1.7 mmol), and Et_3N (3 mL) was refluxed in MeCN (10 mL) for 22 h. After the reaction mixture cooled to room temperature, the solution was taken to dryness in vacuo and the residue was reconstituted in CH_2Cl_2 (25 mL). The resulting solution was washed successively with 5% aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated in vacuo to yield the alkylated ketal as a pale brown residue. The crude ketal was refluxed in 3 N HCl (10 mL) for 30 min, and the reaction mixture was concentrated to a residue. The latter was treated with saturated aqueous $NaHCO_3$ and the resulting mixture extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extracts were dried and concentrated in vacuo to provide a tan syrup which was purified by radial flow chromatography (10% acetone−hexane, trace Et_3N) to give 0.16 g (25%) of the desired compound. The hydrochloride was prepared in methanolic HCl and recrystallized from MeOH−diethyl ether: mp 226−228 °C; ^{1}H NMR (2HCl) (CD_3OD) δ 0.64−3.45 (m, 19H), 6.17 (m, 1H, indoline Ar-H), 6.40 (m, 1H, indoline Ar-*H*), 6.8 (m, 4H, indoline, phenyl Ar-*H*), 7.74 (m, 2H, phenyl Ar-*H*). Anal. ($C_{23}H_{27}N_2OF.2HCl\cdot {}^3/_4H_2O$) C, H, N.

2,3-Dihydro-1′-[3-(4-bromophenyl)-2-hydroxypropan-1-yl]spiro[1*H*-indene-1,4′-piperidine] (22b). A mixture of 2,3-dihydrospiro[1*H*-indene-1,4′piperidine] hydrochloride[23] (0.5 g, 2 mmol), 1-(4-bromophenyl)propane 1,2-dioxide (0.6 g, 2.6 mmol), and anhydrous potassium carbonate (0.7 g, 5 mmol) was refluxed in anhydrous EtOH (50 mL) for 21 h and cooled to room temperature. Removal of the insoluble material by filtration followed by concentration of the filtrate and purification of the residue by radial flow chromatography (10% acetone−hexane with trace Et_3N) afforded 0.36 g (41%) of the desired compound as a pale brown liquid: ^{1}H NMR ($CDCl_3$) δ (free base) 1.25 (t, 2H, piperidyl), 1.96−3.97 (m, 16H), 7.13−7.35 (m, 8H, Ar-*H*). The hydrochloride was prepared with cold methanolic HCl and recrystallized from MeOH−Et_2O: mp 196−200 °C. Anal. ($C_{22}H_{26}NOBr\cdot HCl$) C, H, N.

2,3-Dihydro-1′-[2-(3-bromophenyl)-2-hydroxyethyl]-spiro[1*H*-indene-1,4′piperidine] hydrochloride (23b): synthesized following the procedure described for **22b**, yield 0.22 g (26%); mp 218−221 °C; ^{1}H NMR ($CDCl_3$) δ (free base) 1.25 (t, 2H, piperidyl), 1.96−3.62 (m, 14H), 7.24−7.56 (m, 8H, Ar-*H*). Anal. ($C_{21}H_{24}NOBr\cdot HCl$) C, H, N.

2,3-Dihydro-1′-[3-(4-fluorobenzoyl)propyl]spiro[1*H*-indene-1,4-′piperidine] (24b). A mixture of 2,3-dihydrospiro-[1*H*-indene-1,4′-piperidine] hydrochloride (0.25 g, 1.2 mmol) and 4-chloro-1,1-ethylenedioxy-1-(4-fluorophenyl)butane (0.31 g, 1.2 mmol) was refluxed in MeCN (25 mL) containing KI (0.2 g, 1.2 mmol) and Et_3N (2 mL) for 23 h. After the reaction mixture cooled to room temperature, the volatiles were removed under reduced presure. The residue was redissolved in CH_2Cl_2 (50 mL), and the resulting solution was washed successively with saturated aqueous $NaHCO_3$ (2 × 25 mL) and water (25 mL), dried over Na_2SO_4, and concentrated in vacuo to a yellow syrup. The latter was refluxed in 3 N HCl (10 mL) for 20 min, after which the volatiles were evaporated in vacuo. The residue was then treated with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to provide a tan residue. The title compound was isolated as a hydrochloride (0.12 g, 27%) by recrystallization from MeOH−Et_2O: mp 218−220 °C; ^{1}H NMR (CD_3OD) δ 1.79−2.20 (m, 7H), 2.93−3.70 (m, 11H), 7.19−7.28 (m, 6H, Ar-*H*), 8.06−8.13 (m, 2H, Ar-*H*). Anal. ($C_{23}H_{26}NOF.HCl\cdot 0.5\cdot H_2O$) C, H; N: calcd, 3.53; found, 4.10.

Biological Testing. All compounds were tested in the form of the corresponding hydrochlorides.

Vesicular Acetylcholine Transporter Binding. Dissociation constants of novel compounds were determined by competition against the binding of [^{3}H]vesamicol to electric organ synaptic vesicles at 22 °C, after 24 h of incubation, by the method of Rogers et al.[6]

***σ* Receptor Binding.** σ1 binding sites were labeled with the σ1- selective radioligand [^{3}H]-(+)-pentazocine (DuPont-NEN) in guinea pig brain membranes (Rockland Biological) according to published procedures.[33,34] σ-2 sites were assayed in rat liver membranes, a rich source of these sites, with [^{3}H]-DTG (DuPont-NEN) in the presence of (+)-pentazocine (100 nM).

Membrane Preparation. The crude P2 membrane fraction was prepared from frozen guinea pig brains minus cerebellum. Brains were allowed to thaw slowly on ice before homogenization. The crude P2 membrane fraction was also prepared from the livers of male Sprague−Dawley rats (175−225 g). Animals were sacrificed by decapitation and the livers removed and minced before homogenization.

Tissue homogenization was carried out at 4 °C in 10 mL/g of tissue weight 10 mM Tris-HCl/0.32 M sucrose, pH 7.4, using a Porter-Elvehjem tissue grinder. The crude homogenate was centrifuged for 10 min at 1000*g* and the supernatant saved on ice. The pellet was resuspended in 2 mL/g of tissue weight ice-cold 10 mM Tris-HCl/0.32 M sucrose, pH 7.4, by vortexing. After centrifugation at 1000*g* for 10 min, the pellet was discarded and the supernatants were combined and centrifuged at 31000*g* for 15 min. The pellet was resuspended in 3 mL/g 10 mM Tris-HCl, pH 7.4, by vortexing, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellet was resuspended by gentle homogenization to 1.53 mL/g in 10 mM Tris-HCl (pH 7.4), and aliquots were stored at −80 °C until used. The protein concentration of the suspension was determined

by the method of Bradford and generally ranged from 6 to 11 mg of protein/mL.

***σ*1 Binding Assay.** Guinea pig membranes (100 μg of protein) were incubated with 3 nM [^{3}H]-(+)-pentazocine (31.6 Ci/mmol) in 50 mM Tris-HCl, pH 8.0, at 25 °C for either 120 or 240 min. Test compounds were dissolved in ethanol and then diluted in buffer to give a total incubation volume of 0.5 mL. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl, pH 8.0, followed by rapid filtration through Whatman GF/B glass filters (presoaked in 0.5% poly(ethylenimine)) using a Brandel harvester (Gathersburg, MD). Filters were washed twice with 5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine. Liquid scintillation counting was carried out in Ecolite(+) (ICN Radiochemicals, Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%. Typical counts were 70 dpm/μg of protein for total binding, 6 dpm/μg for nonspecific binding, and 64 dpm/μg for specific binding.

***σ*2 Binding Assay.** Rat liver membranes (35 μg of protein) or guinea pig brain membranes (360 μg) were incubated with 3 nM [^{3}H]DTG (38.3 Ci/mmol) in the presence of 100 nM (+)-pentazocine to mask *σ*1 sites. Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a total incubation volume of 0.5 mL. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl, pH 8.0, followed by rapid filtration through Whatman GF/B glass filters (presoaked in 0.5% poly(ethylenimine)) using a Brandel harvester (Gaithersburg, MD). Filters were then washed twice with 0.5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 5 μM DTG. Liquid scintillation counting was carried out in Ecolite(+) (ICN Radiochemicals, Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%. Typical counts for rat liver were 297 dpm/μg of protein for total binding, 11 dpm/μg for nonspecific binding, and 286 dpm/μg for specific binding. Typical counts for guinea pig brain were 16 dpm/μg of protein for total binding, 2 dpm/μg for nonspecfic binding, and 14 dpm/μg for specific binding.

Data Analysis. The IC_{50} values for *σ* sites were determined in triplicate by nonlinear regression analysis using JMP (SAS Institute, Cary, NC), with 5−10 concentrations of each acetylcholine compound. K_i values were calculated using the Cheng−Prusoff equation[35] and represent mean values ± SEM. All assays were done in triplicate unless otherwise noted. For the radioligands, the following previously[33,34] reported K_d values were employed: [^{3}H]DTG, 17.9 nM (rat liver); [^{3}H]-(+)-pentazocine, 4.8 nM (guinea pig brain). The K_d value for [^{3}H]-DTG in guinea pig brain was determined by a Scatchard analysis to be 21.6 nM.

Dopamine D2/D3 Receptor Binding Assay. The frozen membrane preparations from *Spodoptera frugiperda* (Sf9) insect cells expressing rat dopamine D2 receptors were kindly provided by Dr. Perry Molinoff (Bristol-Myers Squibb Pharmaceutical Research Inc. Wallingford, CT). Rat striatal homogenates were prepared as described previously.[36] Competition experiments were carried out with 0.2 nM [^{125}I]NCQ298 (for Sf9/D2 receptors) or 0.05 nM [^{125}I]NCQ298 (for D2/D3 receptors in striatal homogenates) as the radioligand and 8−10 concentrations (10^{-10}−10^{-5} M) of competing drugs (serially diluted in Tris-HCl buffer containing 0.1% BSA) in these cell membranes as described previously.[37] Nonspecific binding was defined with 1 μM spiperone. Incubation was carried out at 37 °C for 30 min. The reaction was terminated by separation of bound from free radioligand by filtration through glass fiber filters (no. 25, Schleicher & Schuell, Keene, NH) presoaked with 1% poly(ethylenimine). The filters were then washed three times with 3 mL of ice-cold 20 mM Tris buffer and counted in a gamma counter (Packard 5000) with 70% efficiency. Competition experiments were analyzed using the iterative nonlinear least-squares curve-fitting program LIGAND.[38]

Dopamine and Serotonin Tranporter Binding Assay. Binding of [^{125}I]IPT to dopamine and serotonin transporters was carried out in rat striatal and cortical homogenates, respectively, as previously described.[39] Competition experiments were performed in the buffer containing 50 mM Tris-HCl, pH 7.4, and 120 mM NaCl with 0.2 M [^{125}I]IPT (for striatal homogenates) or 0.5 M [^{125}I]IPT (for cortical homogenates) and 8−10 concentrations (10^{-10}−10^{-5} M) of competing drugs. Nonspecific binding was defined in the presence of 40 μM (−)-cocaine. Competition experiments were analyzed using the iterative nonlinear least-squares curve-fitting program LIGAND.

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