6β-Acetoxynortropane: A Potent Muscarinic Agonist with Apparent Selectivity toward M_2-Receptors

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A series of tropane derivatives, related in structure to baogongteng A (**1**), an alkaloid from a Chinese herb, were synthesized. 6β-Acetoxynortropane (**5**) had weak affinity (K_i 22 μM) for central (M_1) muscarinic receptors in a [^{3}H]quinuclidinyl benzilate binding assay but had extremely high affinity (K_i 2.6 nM) and selectivity for M_2-muscarinic receptors expressed in CHO cells. It had 13-fold lower affinity for M_4-receptors, 260-fold lower affinity for M_3-receptors, and 8200-fold lower affinity for M_1-receptors expressed in CHO cells. The 6β-carbomethoxy analogue (**14**) of baogongteng A had only weak affinity for M_2-muscarinic receptors, as did 6β-carbomethoxynortropane (**13**) and 6β-acetoxytropane (**4**). In transfected CHO cells, 6β-acetoxynortropane (**5**) was an agonist at M_2-receptors, based on a GTP-elicited decrease in affinity, and a full agonist with an IC_{50} of 11 nM at M_4-receptors, based on inhibition of cyclic AMP accumulation, while being a full agonist at M_1-receptors with an EC_{50} of 23 nM and a partial agonist at M_3-receptors with an EC_{50} of 3.6 nM, based in both cases on stimulation of phosphoinositide breakdown. All of the 16 tropane derivatives had weak affinities for central $\alpha_4\beta_2$-nicotinic receptors with 6β-carbomethoxynortropane (**13**) having the highest affinity, which was still 150-fold less than that of nicotine. 6β-Acetoxynortropane (**5**) represents a potent muscarinic agonist with apparent selectivity toward M_2-receptors.

Agents that enhance central cholinergic function have been the object of intensive research in recent years because of deficits in such function in Alzheimer's disease. One approach has focused on subtype-selective muscarinic agonists and/or antagonists as research tools or potential therapeutics.[1,2] At present, five major subtypes of muscarinic receptors are recognized. All are G-protein-coupled receptors with the M_1-, M_3-, and M_5-muscarinic receptors coupling through G_q-proteins to stimulate phospholipase C, while the M_2- and M_4-receptors couple through G_i-proteins to inhibit adenylyl cyclase.[3] Ion channels can also be modulated by muscarinic receptors.[4] Mixed populations of receptors occur in different brain regions and tissues complicating any pharmacological search for selective muscarinic agents.[3,5] However, cells transfected with specific muscarinic receptors do provide homogeneous subtypes.[6]

Many tropane alkaloids, such as atropine and scopolamine, are potent muscarinic receptor antagonists, but baogongteng A (**1**) (Chart 1), a nortropane alkaloid isolated from the Chinese herb *Erycibei obtusifola* Benth,[7] has been reported to have agonist activity at muscarinic receptors.[8] In addition, a synthetic analogue, 6β-acetoxynortropane (**5**), was concluded to be a selective M_2-muscarinic receptor agonist, based on binding affinity and functional assays in brain, heart, ileum smooth muscle, and iris.[8] We now report that, from a series of 16 tropane derivatives, 6β-acetoxynortropane (**5**), based on binding assays, is the most active and most selective agonist for M_2-muscarinic receptors. It also has high affinity for M_4-muscarinic receptors but has only weak affinity for M_1- and M_3-muscarinic receptors and for $\alpha_4\beta_2$-nicotinic receptors. In functional assays, **5** is a full agonist at M_4- and M_1-muscarinic receptors but only a partial agonist at M_3-muscarinic receptors. Alterations in the structure of **5** result in marked reductions or loss of activity.

Chemistry

Compounds **3**−**5** have been prepared from 6β-acetoxytropinone (**2**) as shown in Scheme 1A with an overall yield of 27%.[9] An alternate synthetic route from commercially available 6β-hydroxytropinone (**19**) (Aldrich Chemical Co., Milwaukee, WI) was developed, as shown in Scheme 1B. Wolff−Kischner decarbonylation of **19** yielded 6β-tropanol (**20**).[10] Acetylation of **20** gave **4**, which was demethylated to give the nortropane **5**. The overall yield of **5** in this alternate route is about 40%.

Compounds **6**−**9** were synthesized as shown in Scheme 2. The 1,3-dipolar cycloaddition of 1-benzyl-3-oxidopyridinium and phenyl vinyl sulfone gave exclusively 6β-isomer (**21**, 78%) as the adduct.[11,12] Catalytic hydrogenation of **21** afforded the ketone (**22**, 97%), which was reduced with $NaBH_4$ to give 2β-ol (**23**, 59%) and 2α-ol (**24**, 41%). The phenylsulfonyl group of **23** and **24** was reductively cleaved using sodium amalgam to give **25** (62%) and **26** (42%), respectively. Acetylation of **23**−**26** gave acetates **27**−**30**, which were debenzylated by catalytic hydrogenation to give nortropanes **6**−**9**.

Compounds **10**−**17** were synthesized as shown in Scheme 3. The 1,3-dipolar cycloaddition of 1-benzyl-3-oxidopyridinium and methyl acrylate gave a mixture of the 6β-isomer (**31**) and the 6α-isomer (2:1 ratio estimated from NMR), which could not be separated by

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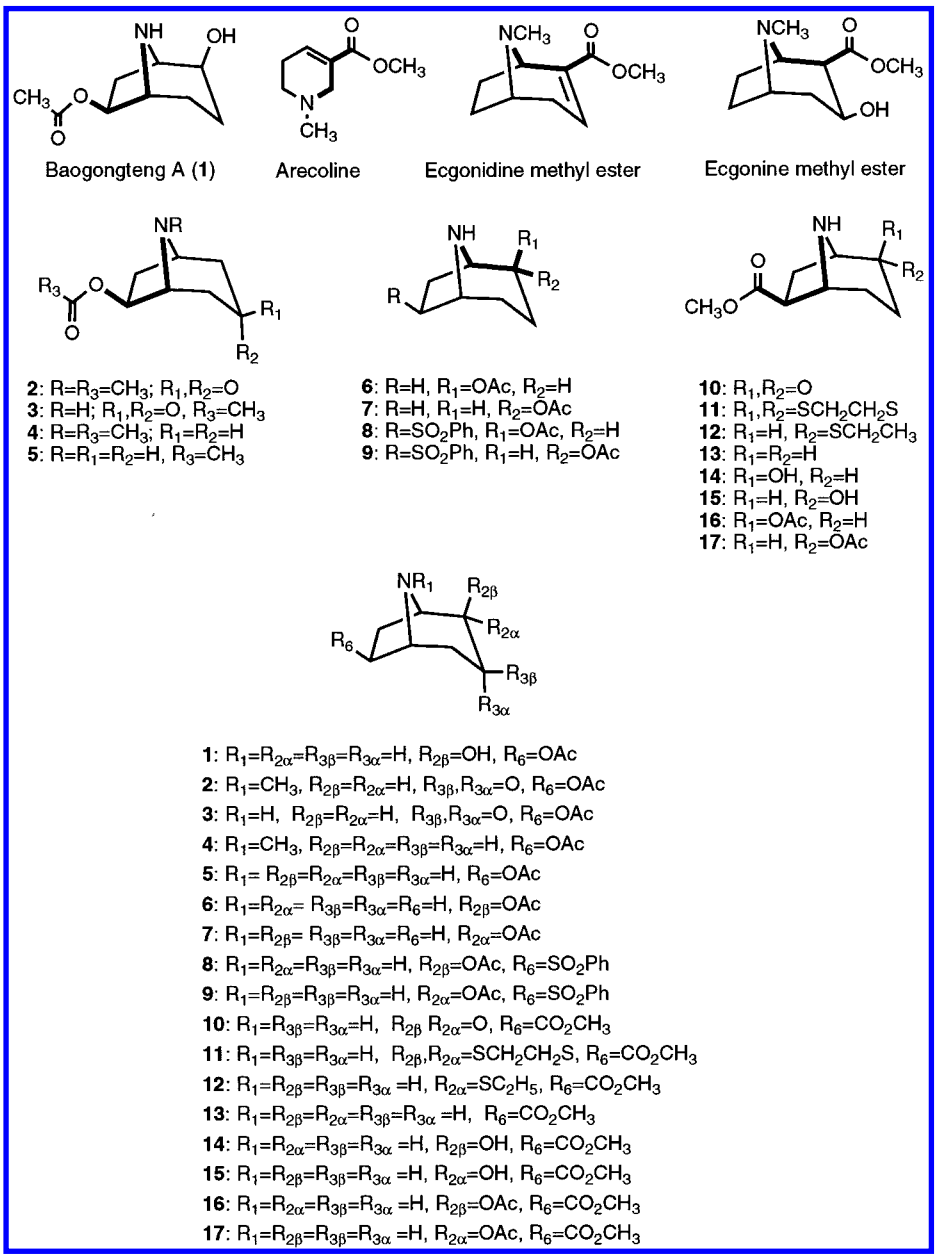
Chart 1



TLC. However, pure **31** could be crystallized from the mixture in 47% yield. Catalytic hydrogenation of **31** gave a mixture of **10** (49%) and **32** (46%). Decarbonylation of **10** was carried out by converting it to the dithioketal (**11**, 90%), followed by catalytic hydrogenation in the presence of Raney nickel to afford a mixture of **12** (12%) and **13** (14%). Reduction of **32** with $NaBH_4$ gave 2β-ol (**33**, 36%) and 2α-ol (**34**, 44%), which were converted to acetates **35** and **36**. Nortropanes **14**−**17** were obtained from **33**−**36** by catalytic hydrogenation.

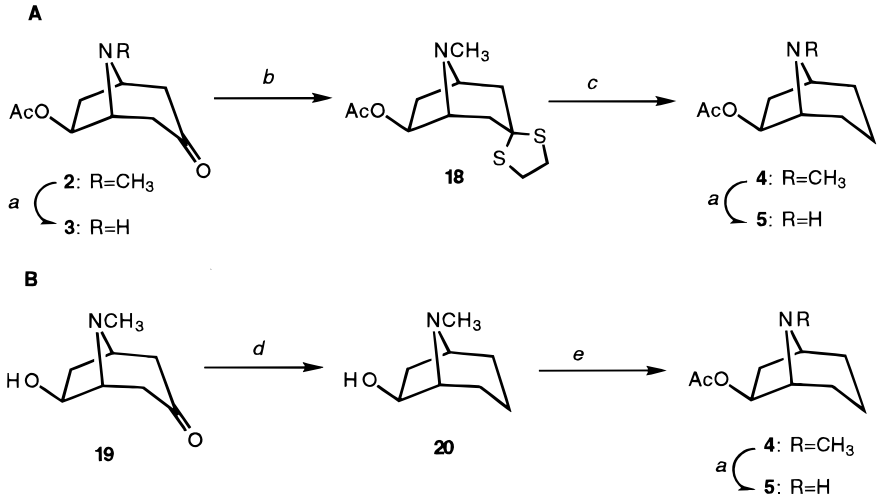
The synthesis of baogongteng A (**1**) has been described.[13]

Results and Discussion

The tropane and nortropane derivatives were rather weak or inactive versus binding of [^{3}H]quinuclidinyl benzilate to muscarinic (mainly M_1) receptors of rat cerebral cortical membranes (Table 1). Muscarinic agonists, such as muscarine and methacholine, were also very weak (Table 1 and data not shown). It should be noted that agonists do show relatively low affinities for M_1-muscarinic receptors, when assayed against an antagonist radioligand, which labels mainly a receptor state with low affinity for agonists (see ref 14). Such tropanes and nortropanes did show much higher affinities when assayed against an agonist radioligand (Table 2). The most potent compound (**5**) of the present series was about 20-fold more potent than muscarine and carbamylcholine in inhibiting agonist binding to central muscarinic receptors. The alkaloids arecoline, ecgonine methyl ester, and ecgonidine methyl ester were either weak or inactive at the brain receptors (Table 1).

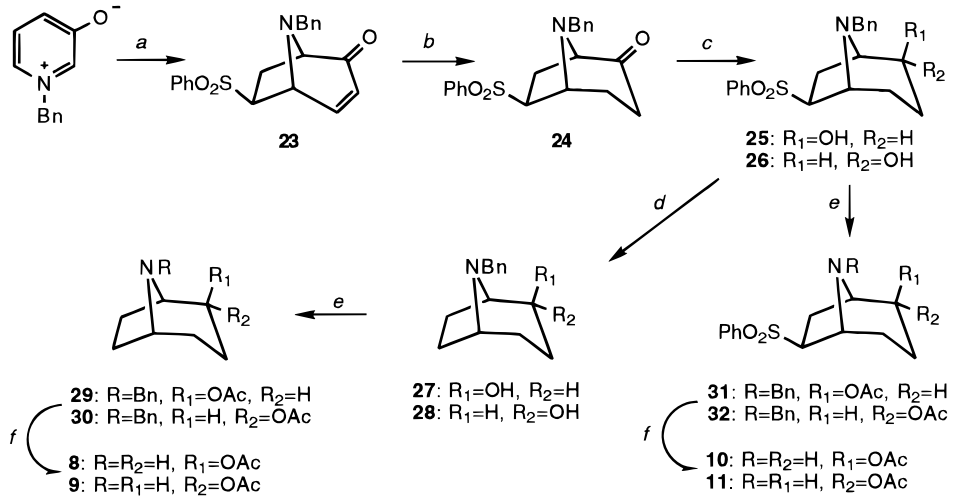
The tropane and nortropane derivatives were also very weak or inactive versus binding of [^{3}H]nicotine to the nicotinic ($\alpha_4\beta_2$) receptors of rat cerebral cortical membranes (Table 1). The most potent compound (**13**) had 150-fold lower affinity than nicotine. Replacement of the 6β-carbomethoxy group of **13** with a 6β-acetoxy group, to yield **5**, resulted in a 20-fold lower affinity. The *N*-methyl derivative **4** had somewhat higher affinity

Scheme 1[a]



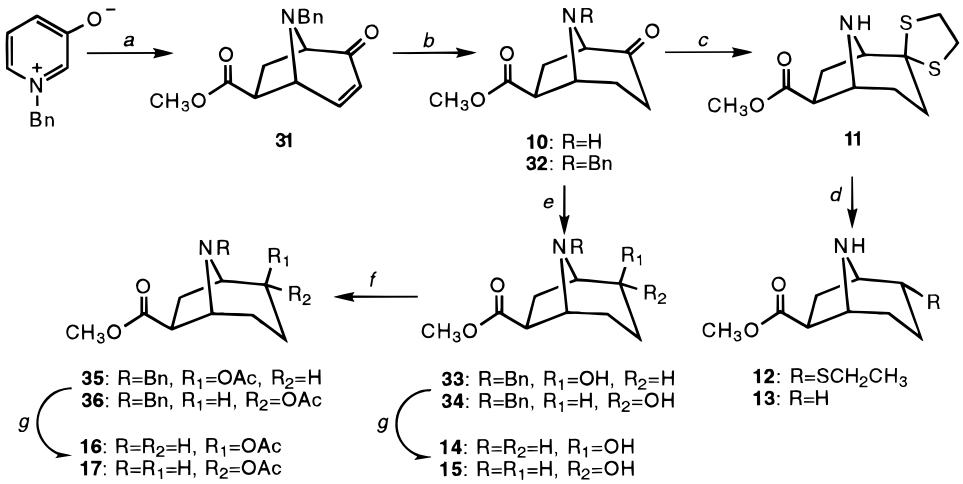
[a] (a) (i) $ClCO_2CH_2CCl_3$, (ii) Zn/AcOH; (b) $BF_3 \cdot Et_2O/(CH_2SH)_2$; (c) H_2/Raney-Ni/THF; (d) (i) $NH_2NH_2 \cdot H_2O$, (ii) KOH; (e) Ac_2O/Py.

Scheme 2[a]



[a] (a) $CH_2{=}CHSO_2Ph/Et_3N$; (b) H_2/10% Pd−C/EtOH; (c) $NaBH_4$/EtOH; (d) 6% Na−Hg/MeOH−THF/Na_2HPO_4; (e) Ac_2O/Py; (f) $H_2/Pd(OH)_2$−C/THF.

Scheme 3[a]

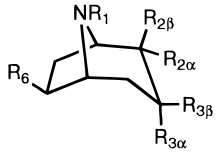


[a] (a) $CH_2{=}CHCO_2CH_3/Et_3N$; (b) H_2/10% Pd−C/EtOH; (c) $BF_3 \cdot Et_2O/(CH_2SH)_2$; (d) H_2/Raney-Ni/THF; (e) $NaBH_4$/EtOH; (f) Ac_2O/Py; (g) $H_2/Pd(OH)_2$−C/THF.

than **5**. The 2β-hydroxy group of **14** was well-tolerated, while the 2α-hydroxy of **15** and other 2-substituents in analogues of **13** were not tolerated. The 3-keto function in **2** and **3** was also not tolerated (compare to **4** and **5**). Because of the low affinities of the compounds, there was no further investigation of nicotinic systems.

Further investigations of muscarinic systems were conducted with CHO cells transfected with M_1-, M_2-,

Table 1. Affinities of Tropanes and Nortropanes for Nicotinic and Muscarinic Receptors in Rat Cerebral Cortical Membranes[a]



						K_i (μM) or % inhibition of binding	
compd	R_1	$R_{2\beta}$	$R_{2\alpha}$	R_6	$R_{3\alpha\beta}$	nicotinic ($\alpha_4\beta_2$)	muscarinic (M_1)
2	CH_3	H	H	OAc	O	5% (100 μM)	0% (100 μM)
3	H	H	H	OAc	O	13% (100 μM)	11% (100 μM)
4	CH_3	H	H	OAc	H, H	1.5 ± 0.3	280 ± 33
5	H	H	H	OAc	H, H	3.0 ± 0.3	22 ± 3
6	H	OAc	H	H	H, H	32 ± 10	18% (100 μM)
7	H	H	OAc	H	H, H	24 ± 4	6% (100 μM)
8	H	OAc	H	SO_2Ph	H, H	16% (100 μM)	0% (100 μM)
9	H	H	OAc	SO_2Ph	H, H	10% (100 μM)	6% (100 μM)
10	H	O		CO_2CH_3	H, H	15% (100 μM)	0% (100 μM)
11	H	$-SCH_2CH_2S-$		CO_2CH_3	H, H	36% (100 μM)	3% (100 μM)
12	H	H	SC_2H_5	CO_2CH_3	H, H	50% (100 μM)	7% (100 μM)
13	H	H	H	CO_2CH_3	H, H	0.15 ± 0.03	9% (100 μM)
14	H	OH	H	CO_2CH_3	H, H	4.0 ± 0.2	6% (100 μM)
15	H	H	OH	CO_2CH_3	H, H	64 ± 6	3% (100 μM)
16	H	OAc	H	CO_2CH_3	H, H	13% (100 μM)	1% (100 μM)
17	H	H	OAc	CO_2CH_3	H, H	19% (100 μM)	1% (100 μM)
nicotine						0.0010 ± 0.0001	
muscarine							3% (100 μM)
arecoline						0.27 ± 0.04	43% (100 μM)
ecgonidine methyl ester	CH_3	CO_2CH_3	2,3-ene	H	H	8.9 ± 1.6	25 ± 2
ecgonine methyl ester	CH_3	CO_2CH_3	H	H	H, OH	11% (100 μM)	27% (100 μM)

[a] Values are means ± SEM (n = 4) for inhibition of binding of [^{3}H]nicotine to nicotinic receptors or for inhibition of binding of [^{3}H]quinuclidinyl benzilate to muscarinic receptors in rat cerebral cortical membranes. Either K_i values or % inhibition at the highest concentration tested is reported. The K_d for [^{3}H]quinuclidinyl benzilate was 0.26 nM and the B_{max} 2500 fmol/mg of protein. The K_d for [^{3}H]nicotine was 1.0 nM and the B_{max} 90 fmol/mg of protein.

Table 2. Affinities of Certain Tropanes and nortropanes versus Agonist and Antagonist Binding to Muscarinic (M_1) Receptors in Rat Cerebral Cortical Membranes.[a]

	K_i (μM) or % inhibition of binding versus	
compd	[^{3}H]oxotremorine M	[^{3}H]quinuclidinyl benzilate
4	2.6 ± 0.1	280 ± 33
5	0.0056 ± 0.0005	22 ± 3
13	0.26 ± 0.02	9% (100 μM)
14	18 ± 1	6% (100 μM)
muscarine	0.027 ± 0.002	3% (100 μM)
carbamylcholine	0.031 ± 0.01	5% (100 μM)

[a] Values are means ± SEM (n = 4) for inhibition of binding of the agonist [^{3}H]oxotremorine M and of the antagonist [^{3}H]quinuclidinyl benzilate to muscarinic receptors in rat cerebral cortical membranes. Either K_i values or percent inhibition at the highest concentration tested is reported. The K_d for [^{3}H]oxotremorine M was 0.75 nM, and the K_d for [^{3}H]quinuclidinyl benzilate was 0.26 nM.

M_3-, or M_4-muscarinic receptors.[6] Compound **5** had an affinity for M_1-receptors in CHO cell membranes (Table 3) identical to its affinity for muscarinic receptors in cerebral cortical membranes (Table 1), consonant with M_1-receptors being the major muscarinic receptor in the brain membranes. Compound **5** had a 30-fold higher affinity for M_3-receptors than for M_1-receptors and was manyfold more potent at M_3-receptors than the muscarinic agonists muscarine or methacholine (Table 2). Compound **5** proved to be highly selective for the M_2- and M_4-receptors that are inhibitory to adenylyl cyclase via G_i-proteins. The highest affinity was for M_2-receptors, where compound **5** had an affinity (K_i value of 2.6 nM) 30-fold greater than that of muscarine. Compound **5** had a K_i value for M_4-receptors of 33 nM and was 35-fold more potent at M_4-receptors than muscarine. The *N*-methyl derivative **4** had manyfold lower affinity than compound **5** at M_1-, M_2-, M_3-, and M_4-receptors (Table 3). The lack of tolerance for the *N*-methyl substituent was particularly striking at the M_2-receptor where compound **4** had 1000-fold lower affinity than **5**. A similar large reduction in affinity for compound **5** compared to compound **4** was seen when assayed versus agonist binding to muscarinic (M_1) receptors in brain membranes (Table 2). Replacement of the 6β-acetoxy group of **5** with a 6β-carbomethoxy group in **13** resulted in a very marked reduction in affinity at all muscarinic receptors.

The K_i value of **5** for inhibition of binding of [^{3}H]-quinuclidinyl benzilate to M_2-receptors was markedly increased in the presence of GTP, indicative of agonist-like properties. The IC_{50} values for **5**, muscarine, methacholine, and carbamylcholine in the absence and presence of GTP are listed in Table 4. GTP increased the K_i value for compound **5** and muscarine to a similar extent, while causing greater shifts for methacholine and carbamylcholine.

Functional assays for inhibition of adenylate cyclase in membranes of cells transfected with M_2-receptors proved difficult, perhaps due to the low density of M_2-receptors expressed (see legend of Table 3). Inhibition of adenylate cyclase by compound **5** and muscarinic agonists was examined in the CHO cells transfected with M_4-receptors. Compound **5** was a full agonist with an IC_{50} of 11 ± 2 nM in such cells (Figure 1). It is to be expected that compound **5** would be much more potent at M_2-receptors, since it had a 15-fold higher binding affinity for M_2-receptors compared to M_4-receptors

Table 3. Affinities of Tropanes and Nortropanes for Muscarinic Receptors in Membranes from Transfected CHO Cells[a]

compd	K_i (μM) or % inhibition of binding to the muscarinic receptor			
	M_1	M_2	M_3	M_4
4	48% (30 μM)	2.7 ± 0.2	15 ± 1	8.3 ± 1.0
5	21 ± 1	0.0026 ± 0.0005	0.68 ± 0.03	0.033 ± 0.011
13	24% (300 μM)	0.48 ± 0.05	18 ± 3	7.9 ± 1.6
14	2% (300 μM)	12 ± 1	0% (100 μM)	33% (300 μM)
muscarine	15% (300 μM)	0.075 ± 0.020	28% (100 μM)	1.4 ± 0.4
arecoline	13% (100 μM)	0.040 ± 0.011	5.7 ± 0.3	2.4 ± 0.5
ecgonidine methyl ester	26% (100 μM)		28% (100 μM)	
ecgonine methyl ester	13% (100 μM)		19% (100 μM)	
methacholine	8% (300 μM)	0.059 ± 0.009	34% (100 μM)	1.6 ± 0.4
carbamylcholine	5% (300 μM)	0.020 ± 0.001	16% (300 μM)	2.6 ± 0.7

[a] Values are means ± SEM (n = 3) for inhibition of binding of [^{3}H]quinuclidinyl benzilate to muscarinic receptors in membranes of CHO cells transfected with M_1-, M_2-, M_3-, or M_4-muscarinic receptors. Either K_i values or percent inhibition at the highest concentration tested is reported. The K_d and B_{max} values for [^{3}H]quinuclidinyl benzilate binding were as follows: M_1-receptor, 0.13 nM and 1800 fmol/mg of protein; M_2-receptor, 0.018 nM and 71 fmol/mg of protein; M_3-receptor, 0.071 nM and 2050 fmol/mg protein; M_4-receptor, 0.031 nM and 340 fmol/mg of protein.

Table 4. Inhibition of [^{3}H]Quinuclidinyl Benzilate Binding to M_2-Muscarinic Receptors by Nortropane **5** and Muscarinic Agonists in the Absence and Presence of GTP[a]

compd	IC_{50} (μM)		
	−GTP	+GTP	-fold shift
5	0.017 ± 0.003	0.12 ± 0.01	6
muscarine	0.49 ± 0.10	3.9 ± 0.7	8
methacholine	0.38 ± 0.06	5.2 ± 1.0	14
carbamylcholine	0.13 ± 0.01	3.1 ± 0.9	24

[a] Values are means ± SEM (n = 3) for inhibition of 0.1 nM [^{3}H]quinuclidinyl benzilate binding to M_2-receptors in membranes of transfected CHO cells in the absence or presence of 10 μM GTP.

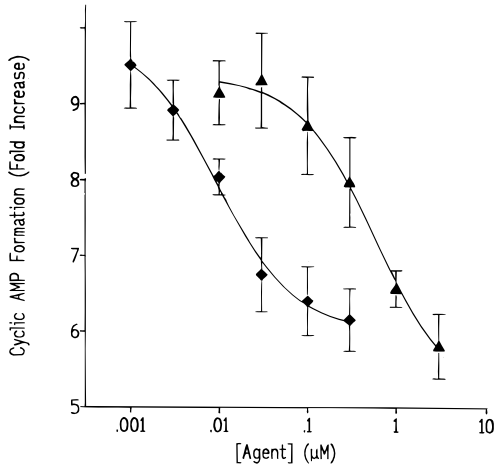


Figure 1. Inhibition of [^{3}H]cyclic AMP accumulation in CHO cells transfected with M_4-muscarinic receptors: nortropane **5** (◆) and muscarine (▲). Values are means ± SEM (n = 3). See Experimental Section for assay conditions.

(Table 3). Carbamylcholine (IC_{50} 230 ± 60 nM), muscarine (IC_{50} 470 ± 120 nM), and methacholine (IC_{50} 700 ± 440 nM) were much less active than nortropane **5** at M_4-receptors (Figure 1 and data not shown).

Functional assays for stimulation of phosphoinositide breakdown by phospholipase C were conducted with cells transfected with either M_1- or M_3-receptors. Nortropane **5** was a potent full agonist at M_1-receptors with an EC_{50} of 23 ± 2 nM (Figure 2A). Muscarine (EC_{50} 219 ± 3 nM), methacholine (EC_{50} 400 ± 40 nM), and carbamylcholine (EC_{50} 650 ± 50 nM) were much less potent but also were full agonists. In cells transfected with M_3-receptors, nortropane **5** was a potent agonist with an EC_{50} of 3.6 ± 0.3 nM (Figure 2B). Muscarine

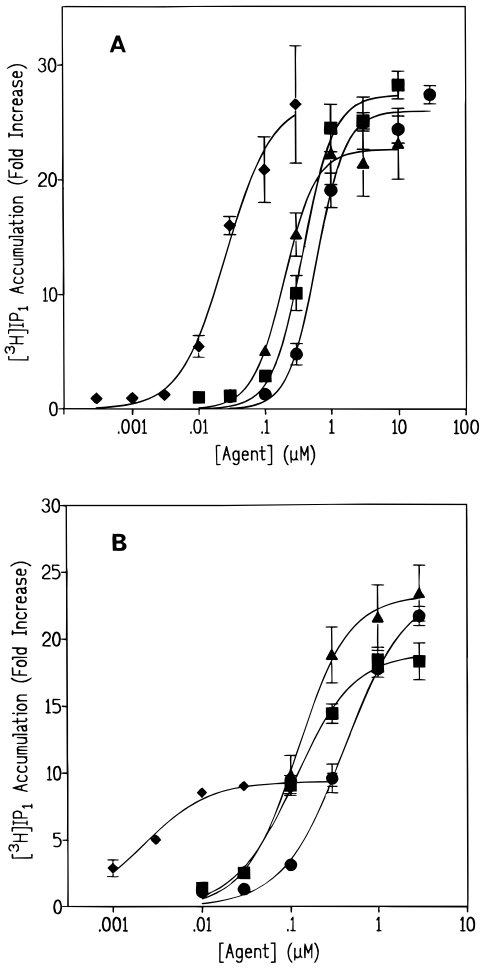


Figure 2. Stimulation of [^{3}H]inositol monophosphate (IP_1) accumulation in [^{3}H]inositol-labeled CHO cells transfected with either (A) M_1-muscarinic receptors or (B) M_3-muscarinic receptors: nortropane **5** (◆), muscarine (▲), methacholine (■), and carbamylcholine (●). Values are means ± SEM (n = 3). Error bars are in some cases smaller than the symbol. See Experimental Section for assay conditions.

(EC_{50} 130 ± 10 nM), methacholine (EC_{50} 120 ± 4 nM), and carbamylcholine (EC_{50} 480 ± 90 nM) were much weaker agonists. However, compared to muscarine, methacholine, and carbamylcholine, compound **5** was only a partial agonist (Figure 2B). The transfected cells apparently had a large excess of spare receptors, since the EC_{50} values for stimulation of phosphoinositide

breakdown were manyfold lower than the K_i values from binding experiments for all four muscarinic agonists (see Table 3).

The present results confirm and extend a prior report on muscarinic activity of (−)-6β-acetoxynortropane (6β-AN),[8] the levorotatory enantiomer of the presently described compound **5**. Yu and Sun[8] report K_i values versus [^{3}H]quinuclidinyl benzoate for (−)-6β-AN of 0.43 μM for rat cortical membranes, 0.026 μM for rat heart membranes, 2.3 μM for guinea pig ileum muscle membranes, and 3.2 μM for rabbit pupil. The value for heart (M_2-receptors) is 10-fold higher than our value for transfected M_2-receptors, while the value for rat brain is 50-fold lower than our value for rat brain. 6β-AN had potent negative inotropic and chronotropic effects on guinea pig heart and had potent activity in contracting guinea pig longitudinal smooth muscle and constricting rabbit pupils.[8] All these functional effects were blocked by atropine. The (+)-enantiomer of 6β-AN was severalfold less potent than (−)-6β-AN, as was baogongteng A.[8] In such functional assays[8] 6β-AN appeared to be a selective M_2-receptor agonist. The present results confirm that compound **5** ((±)-6β-AN) is an extremely potent muscarinic agonist with high affinity for M_2-muscarinic receptors. Compound **5** should readily pass into the central nervous system, and indeed Yu and Sun[8] did report cognitive enhancement in a three-arm maze for mice. The facile synthetic route to compound **5** makes this potent muscarinic agonist readily accessible for research on cholinergic function.

Experimental Section

General. Melting points (uncorrected) were measured with a Thomas-Hoover capillary melting point apparatus. ^{1}H NMR were recorded on a Varian XL-300 MHz spectrometer. Chemical shifts are reported as δ values (ppm) relative to Me_4Si as an internal standard. Chemical ionization (CI) mass spectral data were obtained on a Finnigan-1015D mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Unless otherwise indicated, all separations were carried out by column chromatography (Merck silica gel 60, 230−400 mesh), using the described solvents. All reactions involving nonaqueous solutions were performed under an inert atmosphere and with anhydrous solvents, unless otherwise noted. All the synthesized compounds were racemates.

8-Methyl-6β-acetoxy-8-azabicyclo[3.2.1]octane (4) (6β-Acetoxytropane). 6β-Tropanol (**20**; 523 mg, 3.71 mmol) was dissolved in $CHCl_3$ (5 mL), and pyridine (1 mL) and Ac_2O (1 mL) were added. The mixture was stirred at room temperature overnight and poured into a saturated $NaHCO_3$ solution (10 mL). The $CHCl_3$ layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 10 mL). After the removal of $CHCl_3$ under reduced pressure, the remaining oil was distilled in a vacuum (54−56 °C/1 mmHg) to give **4** (537 mg, 84%) as a colorless oil. The spectrum was identical with the published spectrum.[9]

8-Benzyl-6β-(phenylsulfonyl)-8-azabicyclo[3.2.1]oct-3-en-2-one (21). A mixture of 1-benzyl-3-oxidopyridinium chloride (6.45 g, 29 mmol), phenyl vinyl sulfone (4.89 g, 29 mmol), Et_3N (6 mL), and hydroquinone (60 mg) in THF (60 mL) was refluxed with stirring overnight, cooled to room temperature, and filtered. The solvent was evaporated in a vacuum, and the residue was recrystallized from EtOAc to give **21** (7.94 g, 78%) as yellow crystals: mp 146−148 °C; CI-MS *m*/*z* 354 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.89−7.06 (10H, m, 2Ph), 6.92 (1H, dd, J = 9.8, 5.0 Hz, 4-CH), 6.14 (H, d, J = 9.8 Hz, 3-CH), 4.18 (1H, d, J = 5.0 Hz, 5-CH), 3.79 (1H, d, J = 13.0 Hz, PhCH), 3.68 (1H, d, J = 13.4 Hz, PhCH), 3.60 (1H, dd, J = 9.8, 3.9 Hz, 6α-CH), 3.60 (1H, d, J = 7.6, 1-CH), 2.82−2.77 (1H, m, 7β-CH), 2.01 (1H, dd, J = 14.3, 9.3 Hz, 7α-H). Anal. ($C_{20}H_{19}NO_3S$) C, H, N.

8-Benzyl-6β-(phenylsulfonyl)-8-azabicyclo[3.2.1]octan-2-one (22). Compound **21** (707 mg, 2.0 mmol) was dissolved in EtOH (10 mL), and 10% Pd−C (80 mg) was added. The mixture was stirred at room temperature under H_2 and monitored by TLC until all the starting material disappeared. The catalyst was removed by filtration, and the solvent was evaporated in a vacuum to give **22** (692 mg, 97%) as colorless crystals. An analytical sample was obtained by recrystallization from EtOH: mp 125−128 °C; CI-MS *m*/*z* 356 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.93−7.17 (10H, m, 2Ph), 3.88 (1H, br s, 5-CH), 3.84 (1H, d, J = 13.8 Hz, PhCH), 3.71 (1H, d, J = 13.5 Hz, PhCH), 3.66 (1H, dd, J = 9.2, 6.7 Hz, 6α-CH), 3.43 (1H, d, J = 6.9, 1-CH), 2.66 (1H, m, 7β-CH), 2.45−2.26 (3H, m, 4β-H, 3-CH_2), 2.11 (1H, dd, J = 14.3, 9.3 Hz, 7α-CH), 1.82−1.77 (1H, m, 4α-CH). Anal. ($C_{20}H_{21}NO_3S$) C, H, N.

8-Benzyl-6β-(phenylsulfonyl)-8-azabicyclo[3.2.1]octan-2β- and -2α-ols (23 and 24). Compound **22** (1.06 g, 3.0 mmol) was dissolved in EtOH (50 mL), and $NaBH_4$ (120 mg, 3.0 mmol) was added. The mixture was stirred at room temperature for 1 h. The solvent was evaporated in a vacuum. The residue was added to H_2O (50 mL) and extracted with Et_2O (2 × 50 mL). After the removal of Et_2O in a vacuum, the residue was chromatographed (CH_2Cl_2/MeOH = 40/1) to give first **23** (636 mg, 59%) as colorless crystals and then **24** (444 mg, 41%) as colorless crystals. Analytical samples were obtained by recrystallization from EtOAc. **23**: mp 126−128 °C; CI-MS *m*/*z* 358 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.95−7.29 (10H, m, 2Ph), 4.07 (1H, d, J = 13.4 Hz, PhCH), 3.73 (1H, d, J = 13.4 Hz, PhCH), 3.85 (1H, brs, 2α-CH), 3.60−3.41 (3H, m, 1,5,6α-CH), 2.61 (1H, p, 7β-CH), 2.05−1.95 (1H, m, 2β-OH), 1.84 (1H, dd, J = 14.1, 9.1 Hz, 7α-CH), 1.67−1.31 (4H, m, 3,4-CH_2). Anal. ($C_{20}H_{23}NO_3S$) C, H, N. **24**: mp 111−113 °C; CI-MS *m*/*z* 358 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.92−7.20 (10H, m, 2Ph), 4.11−3.49 (6H, m, $PhCH_2$, 1,2β-, 5,6α-CH), 2.40 (1H, m, 7β-CH), 2.14−2.02 (1H, m, 2α-OH), 2.10 (1H, dd, J = 14.2, 9.2 Hz, 7α-CH), 1.78−1.23 (4H, m, 3,4-CH_2). Anal. ($C_{20}H_{23}NO_3S$) C, H, N.

8-Benzyl-8-azabicyclo[3.2.1]octan-2β-ol (25). Compound **23** (357 mg, 1.0 mmol) was dissolved in MeOH (5 mL) and THF (10 mL), and Na_2HPO_4 (681 mg, 5 mmol) and 6% Na−Hg (2.30 g) were added. The mixture was refluxed with stirring for 24 h, cooled to room temperature, poured into H_2O (40 mL), and extracted with EtOAc (2 × 30 mL). After removal of AcOEt in a vacuum, the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give **25** (154 mg, 62%) as a colorless oil: CI-MS *m*/*z* 218 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.40−7.21 (5H, m, Ph), 4.01 (1H, d, J = 13.7 Hz, PhCH), 3.70 (1H, d, J = 13.7 Hz, PhCH), 3.81 (1H, brs, 2α-CH), 3.60−3.41 (2H, m, 1,5-CH), 2.01(1H, brs, 2β-OH), 1.99−1.32 (8H, m, 3,4,6,7-CH_2).

8-Benzyl-8-azabicyclo[3.2.1]octan-2α-ol (26). In a similar way to the preparation of **25**, compound **26** was obtained from **24** as a colorless oil, yield 43%: CI-MS *m*/*z* 218 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.38−7.23 (5H, m, Ph), 3.91 (1H, brs, 2β-CH), 3.60 (2H, s, $PhCH_2$), 3.20−3.10 (2H, m, 1,5-CH), 2.12 (1H, brs, 2α-OH), 1.94−1.40 (8H, m, 3,4,6,7-CH_2).

8-Benzyl-2β-acetoxy-8-azabicyclo[3.2.1]octane (27). In a similar way to the preparation of **4**, compound **27** was obtained from **25** as a colorless oil, yield 49%: CI-MS *m*/*z* 260 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.42−7.21 (5H, m, Ph), 4.60 (1H, brs, 2α-CH), 3.59 (1H, d, J = 13.7 Hz, PhCH), 3.42 (1H, d, J = 14.0 Hz, PhCH), 3.25 (2H, brs, 1,5-CH), 2.08 (3H, s, CH_3-CO), 1.99−1.32 (8H, m, 3,4,6,7-CH_2).

8-Benzyl-2α-acetoxy-8-azabicyclo[3.2.1]octane (28). In a similar way to the preparation of **4**, compound **28** was obtained from **26** as a colorless oil, yield 51%: CI-MS *m*/*z* 260 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.38−7.23 (5H, m, Ph), 4.91 (1H, brs, 2β-CH), 3.55 (2H, s, $PhCH_2$), 3.20 (1H, brs, 1-CH), 3.13 (1H, brs, 5-CH), 2.07 (3H, s, CH_3CO), 1.94−1.44 (8H, m, 3,4,6,7-CH_2).

8-Benzyl-2β-acetoxy-6β-(phenylsulfonyl)-8-azabicyclo-[3.2.1]octane (29). In a similar way to the preparation of **4**, compound **29** was obtained from **23** as colorless crystals, yield

100%: mp 125−126 °C; CI-MS *m/z* 400 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.94−7.24 (10H, m, 2Ph), 4.57 (1H, brs, 2α-CH), 4.00 (1H, d, J = 13.4 Hz, PhCH), 3.86 (1H, d, J = 14.1 Hz, PhCH), 3.90 (1H, brs, 1-CH), 3.58 (1H, t, J = 8.7, 6.6 Hz, 6α-CH), 3.45 (1H, brs, 5-CH), 2.52 (1H, m, 7β-CH), 2.23−2.12 (1H, m, 3β-H), 2.03 (3H, s, CH_3CO), 1.83 (1H, dd, J = 14.0, 9.5 Hz, 7α-CH), 1.72−1.36 (3H, m, 3α-CH, 4-CH_2). Anal. ($C_{22}H_{25}NO_4S$) C, H, N.

8-Benzyl-2α-acetoxy-6β-(phenylsulfonyl)-8-azabicyclo-[3.2.1]octane (30). In a similar way to the preparation of **4**, compound **30** was obtained from **24** as colorless crystals, yield 100%: mp 147−148 °C; CI-MS *m/z* 400 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.95−7.24 (10H, m, 2Ph), 4.99 (1H, m, 2β-CH), 4.04 (1H, d, J = 13.9 Hz, PhCH), 3.82 (1H, d, J = 13.9 Hz, PhCH), 3.79 (1H, brs, 1-CH), 3.51 (1H, t, J = 8.4, 7.0 Hz, 6α-CH), 3.39 (1H, brs, 5-CH), 2.40 (1H, m, 7β-CH), 2.15 (1H, dd, J = 14.0, 9.4 Hz, 7α-CH), 2.10−2.04 (1H, m, 3β-H), 1.98 (3H, s, CH_3-CO), 1.95−1.25 (3H, m, 3α-CH, 4-CH_2). Anal. ($C_{22}H_{25}NO_4S$) C, H, N.

2β-Acetoxy-8-azabicyclo[3.2.1]octane (6). Compound **27** (82 mg, 0.32 mmol) was dissolved in THF (5 mL), and $Pd(OH)_2$−C (20 mg) was added. The mixture was stirred under H_2 and monitored by TLC until all the starting material had disappeared. The catalyst was removed by filtration, and the solvent was evaporated in a vacuum. The residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give **6** (54 mg, 100%) as a colorless oil. It was converted to a fumarate salt as white crystals. **6**·fumarate: mp 122−132 °C. Free base: CI-MS *m/z* 170 (MH^+); ^{1}H NMR ($CDCl_3$) δ 4.72 (1H, brs, 2α-CH), 4.12 (1H, brs, 1-CH), 3.76 (1H, brs, 5-CH), 2.13 (3H, s, CH_3CO), 2.17−1.26 (9H, m, NH, 3,4,6,7-CH_2). **6**·fumarate: Anal. ($C_9H_{15}NO_2 \cdot C_4H_4O_4$) C, H, N.

2α-Acetoxy-8-azabicyclo[3.2.1]octane (7). In a similar way to the preparation of **6**, compound **7** was obtained from **28** as a colorless oil, yield 73%. It was converted to a fumarate salt as white crystals. **7**·fumarate: mp 190−192 °C. Free base: CI-MS *m/z* 170 (MH^+); ^{1}H NMR ($CDCl_3$) δ 5.02−4.97 (1H, m, 2β-CH), 4.10 (1H, brs, 1-CH), 3.79 (1H, brs, 5-CH), 1.98 (3H, s, CH_3CO), 2.17−1.26 (9H, m, NH, 3,4,6,7-CH_2). **7**·fumarate: Anal. ($C_9H_{15}NO_2 \cdot C_4H_4O_4$) C, H, N.

2β-Acetoxy-6β-(phenylsulfonyl)-8-azabicyclo[3.2.1]-octane (8). In a similar way to the preparation of **6**, compound **8** was obtained from **29** as colorless crystals, yield 64%. It was also converted to an oxalate salt as white crystals. **8**: mp 110−112 °C; CI-MS *m/z* 310 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.94−7.59 (5H, m, Ph), 4.67 (1H, brs, 2α-CH), 3.99 (1H, brs, 1-CH), 3.75 (1H, d, J = 5.21 Hz, 5-CH), 3.53 (1H, dd, J = 8.8, 5.1 Hz, 6α-CH), 2.32 (1H, m, 7β-CH), 2.12 (3H, s, CH_3CO), 2.10 (1H, s, NH), 2.00−1.92 (1H, m, 3β-H), 1.96 (1H, dd, J = 14.3, 9.0 Hz, 7α-CH), 1.90−1.58 (2H, m, 3α,4β-CH), 1.42−1.35 (1H, m, 4α-H). Anal. ($C_{15}H_{19}NO_4S$) C, H, N. **8**·oxalate: mp 138−140 °C. Anal. ($C_{15}H_{19}NO_4S \cdot C_2H_2O_4 \cdot 0.2H_2O$) C, H, N.

2β-Acetoxy-6α-(phenylsulfonyl)-8-azabicyclo[3.2.1]-octane (9). In a similar way to the preparation of **6**, compound **9** was obtained from **30** as a colorless oil, yield 86%. It was converted to an oxalate salt as a white powder. **9**·oxalate: mp 156−159 °C. Free base: CI-MS *m/z* 310 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.94−7.59 (5H, m, Ph), 4.85 (1H, m, 2β-CH), 3.92 (1H, brs, 1-CH), 3.70 (1H, brs, 5-CH), 3.45 (1H, dd, J = 8.3, 5.5 Hz, 6α-CH), 2.33−2.10 (3H, m, NH, 3β,7β-CH), 2.01 (3H, s, CH_3CO), 2.01−1.27 (4H, m, 3α,7α-CH, 4-CH_2). **9**·oxalate: Anal. ($C_{15}H_{19}NO_4S \cdot C_2H_2O_4 \cdot 0.3H_2O$) C, H, N.

8-Benzyl-6β-carbomethoxy-8-azabicyclo[3.2.1]oct-3-en-2-one (31). A mixture of 1-benzyl-3-oxidopyridinium chloride (11.1 g, 50 mmol), methyl acrylate (25 mL), Et_3N (10 mL), and hydroquinone (100 mg) in THF (65 mL) was refluxed with stirring overnight, cooled to room temperature, and filtered. The solvent was evaporated in a vacuum, and the residue was chromatographed (CH_2Cl_2/MeOH = 40/1). Crude **31** was obtained and recrystallized twice from EtOH to give **31** (6.31 g, 47%) as yellow crystals: mp 90−91 °C; CI-MS *m/z* 272 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.33−7.23 (5H, m, Ph), 6.96 (1H, dd, J = 9.8, 5.0 Hz, 4-CH), 6.10 (H, d, J = 9.8 Hz, 3-CH), 4.06 (1H, d, J = 5.0 Hz, 5-CH), 3.83 (1H, d, J = 13.5 Hz, PhCH), 3.72 (1H, d, J = 13.5 Hz, PhCH), 3.74 (3H, s, OCH_3), 3.66 (1H, d, J = 7.6 Hz, 1-CH), 2.96−2.87 (2H, m, 6α,7β-CH), 1.93 (1H, dd, J = 13.7, 9.4 Hz, 7α-H). Anal. ($C_{16}H_{17}NO_3$) C, H, N.

6β-Carbomethoxy-8-azabicyclo[3.2.1]octan-2-one (10) and 8-Benzyl-6β-carbomethoxy-8-azabicyclo[3.2.1]octan-2-one (32). Compound **31** (5.54 g, 20.4 mmol) was dissolved in EtOH (15 mL), and 10% Pd−C (500 mg) was added. The mixture was stirred at room temperature under H_2 and monitored by TLC until all the starting material disappeared. The catalyst was removed by filtration, and the solvent was evaporated in a vacuum. The residue was chromatographed (CH_2Cl_2/MeOH = 40/1) to give **32** (2.53 g, 46.3%) as colorless crystals and **10** (1.82 g, 48.8%) as a colorless oil. Compound **10** was converted to an oxalate salt as white crystals. **10**·oxalate: mp 170−175 °C. Free base: CI-MS *m/z* 184 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.85−3.75 (2H, m, 1,5-CH), 3.75 (3H, s, OCH_3), 3.00 (1H, dd, J = 9.2, 5.1 Hz, 6α-CH), 2.39−1.89 (7H, m, NH, 3,4,7-CH_2). **10**·oxalate: Anal. ($C_9H_{13}NO_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N. **32**: mp 70−72 °C; CI-MS *m/z* 274 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.30−7.23 (5H, m, Ph), 3.79−3.66 (4H, m, 1,5-CH, $PhCH_2$), 3.73 (3H, s, OCH_3), 3.48 (1H, d, J = 7.4 Hz, 1-CH), 2.98 (1H, dd, J = 9.5, 5.7 Hz, 6α-CH), 2.71 (1H, m, 7β-CH), 2.06 (1H, dd, J = 14.0, 9.5 Hz, 7α-CH), 2.39−1.55 (4H, m, 3,4-CH_2). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

6β-Carbomethoxy-2,2-(1′,2′-ethylenedithiano)-8-azabicyclo[3.2.1]octan-2-one (11). Compound **10** (960 mg, 5.25 mmol) was dissolved in 1,2-ethanedithiol (5 mL), and $BF_3 \cdot Et_2O$ (2.5 mL) was slowly added dropwise with stirring. The mixture was stirred at room temperature under N_2 overnight, then poured into H_2O (20 mL), and extracted with Et_2O (2 × 20 mL). The aqueous layer was neutralized with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 20 mL). After the removal of CH_2Cl_2 in a vacuum, the residue was chromatographed (CH_2Cl_2/MeOH = 40/1) to give **11** (743 mg, 54.5%) as a colorless oil. It was converted to a fumarate salt. **11**·fumarate: mp °C. Free base: CI-MS *m/z* 260 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.69 (3H, s, OCH_3), 3.62 (1H, brs, 1-CH), 3.30−3.25 (3H, m, 5-CH, S-CH_2), 2.90−2.71 (4H, m, NH, 6α-CH, S-CH_2), 2.22−1.67 (6H, m, 3,4,7-CH_2). **11**·fumarate: Anal. ($C_{11}H_{17}NO_2S_2 \cdot C_4H_4O_4$) C, H, N.

6β-Carbomethoxy-2α-(ethylthiano)-8-azabicyclo[3.2.1]-octane (12) and 6β-Carbomethoxy-8-azabicyclo[3.2.1]-octane (13). Compound **11** (752 mg, 2.90 mmol) was dissolved in THF (30 mL), and Raney nickel (5 g) was added. The mixture was refluxed with stirring under H_2 for 1 h. After cooling to room temperature, the catalyst was removed by filtration, and the solvent was evaporated in a vacuum. The residue was chromatographed (CH_2Cl_2/MeOH = 40/1) to give first **12** (80 mg, 12%) as a colorless oil and then **13** (71 mg, 14%) as a colorless oil. Both were converted to fumarate salts. **12**·fumarate: mp 150−151 °C. Free base: CI-MS *m/z* 230 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.70 (3H, s, OCH_3), 3.59 (2H, brs, 1,5-CH), 2.77 (1H, dd, J = 8.8, 4.7 Hz, 6α-CH), 2.56 (2H, dd, J = 14.2, 6.2 Hz, S−CH_2), 2.35−2.27 (2H, m, NH, 7β-CH), 1.98−1.55 (5H, m, 7β-CH, 3,4-CH_2), 1.26 (3H, t, J = 7.2 Hz, CH_3). **12**·fumarate: Anal. ($C_{11}H_{19}NO_2S \cdot C_4H_4O_4$) C, H, N. **13**·fumarate: mp 158−160 °C. Free base: CI-MS *m/z* 170 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.71 (4H, s, NH, OCH_3), 3.42 (2H, brs, 1,5-CH), 2.91 (1H, dd, J = 8.8, 5.1 Hz, 6α-CH), 2.56 (2H, dd, J = 14.2, 6.2 Hz, S-CH_2), 2.14 (1H, m, 7β-CH), 2.02 (1H, dd, J = 13.1, 9.2 Hz, 7α-CH), 1.83−1.46 (6H, m, 3,4,5-CH_2). **13**·fumarate: Anal. ($C_9H_{15}NO_2S \cdot C_4H_4O_4$) C, H, N.

8-Benzyl-6β-carbomethoxy-8-azabicyclo[3.2.1]octan-2β- and -2α-ols (33 and 34). In a similar way to the preparation of **22** and **23**, compounds **33** (36%) and **34** (44%) were obtained from **32** as a colorless oil and colorless crystals, respectively. **33**: CI-MS *m/z* 276 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.30−7.26 (5H, m, Ph), 3.74 (3H, s, OCH_3), 3.58−3.44 (4H, m, 1,2α-CH, $PhCH_2$), 3.33 (1H, brs, 5-CH), 2.86 (1H, dd, J = 9.5, 5.6 Hz, 6α-CH), 2.65 (1H, m, 7β-CH), 1.90−1.80 (2H, m, OH, 7α-CH), 1.59−1.28 (4H, m, 3,4-CH_2). **34**: mp 110−112 °C; CI-MS *m/z* 276 (MH^+); 7.33−7.21 (5H, m, Ph), 3.90−3.84 (1H, m, 2β-CH), 3.73 (3H, s, OCH_3), 3.66 (1H, d, J = 13.6 Hz, PhCH), 3.58 (1H, d, J = 13.7 Hz, PhCH), 3.53 (1H, brs, 1-CH), 3.23

(1H, brs, 5-CH), 2.86 (1H, dd, J = 9.7, 5.9 Hz, 6α-CH), 2.46 (1H, m, 7β-CH), 2.12 (1H, dd, J = 13.9, 9.9 Hz, 7α-CH), 1.94−1.20 (5H, m, OH, 3,4-CH_2). Anal. ($C_{16}H_{21}NO_3$) C, H, N.

8-Benzyl-2β-acetoxy-6β-carbomethoxy-8-azabicyclo-[3.2.1]octane (35). In a similar way to the preparation of **4**, compound **35** was obtained from **33** as a colorless oil, yield 82%: CI-MS m/z 318 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.42−7.21 (5H, m, Ph), 4.65 (1H, brs, 2α-CH), 3.76 (3H, s, OCH_3), 3.68 (1H, J = 13.9 Hz, PhCH), 3.58 (1H, J = 14.0 Hz, PhCH), 3.66 (1H, brs, 1-CH), 3.43 (1H, d, J = 4.9 Hz, 5-CH), 2.90 (1H, dd, J = 9.3, 5.9 Hz, 6α-CH), 2.61 (1H, m, 7β-CH), 2.08 (3H, s, CH_3), 1.83 (1H, dd, J = 14.0, 9.6 Hz, 7α-CH), 1.76−1.27 (4H, m, 3,4-CH_2).

8-Benzyl-2α-acetoxy-6β-carbomethoxy-8-azabicyclo-[3.2.1]octane (36). In a similar way to the preparation of **4**, compound **36** was obtained from **34** as a colorless oil, yield 93%: CI-MS m/z 318 (MH^+); ^{1}H NMR ($CDCl_3$) δ 7.35−7.21 (5H, m, Ph), 4.92 (1H, p, 2β-CH), 3.73 (3H, s, OCH_3), 3.65 (2H, d, J = 3.7 Hz, $PhCH_2$), 3.52 (1H, brs, 1-CH), 3.36 (1H, brs, 5-CH), 2.78 (1H, dd, J = 9.5, 5.7 Hz, 6α-CH), 2.50 (1H, m, 7β-CH), 2.11 (1H, dd, J = 13.9, 9.7 Hz, 7α-CH), 2.02 (3H, s, CH_3), 1.98−1.27 (4H, m, 3,4-CH_2).

6β-Carbomethoxy-8-azabicyclo[3.2.1]octan-2β-ol (14). In a similar way to the preparation of **6**, compound **14** was obtained from **33** as a colorless oil, yield 87%. It was converted to a fumarate salt. **14**·fumarate: mp 170−173 °C. Free base: CI-MS m/z 186 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.74 (3H, s, OCH_3), 3.70−3.33 (3H, m, 1, 2α,5-CH), 2.86 (1H, dd, J = 9.5, 5.6 Hz, 6α-CH), 2.65 (1H, m, 7β-CH), 1.90−1.28 (7H, m, NH, OH, 7α-CH, 3,4-CH_2). **14**·fumarate: Anal. ($C_9H_{15}NO_3 \cdot C_4H_4O_4$) C, H, N.

6β-Carbomethoxy-8-azabicyclo[3.2.1]octan-2α-ol (15). In a similar way to the preparation of **6**, compound **15** was obtained from **34** as a colorless oil, yield 100%. It was converted to a fumarate salt. **15**·fumarate: mp 159−161 °C. Free base: CI-MS m/z 186 (MH^+); ^{1}H NMR ($CDCl_3$) δ 3.90−3.84 (1H, m, 2β-CH), 3.73 (3H, s, OCH_3), 3.53−3.23 (2H, m, 1,5-CH), 2.75 (1H, dd, J = 9.7, 5.9 Hz, 6α-CH), 2.45 (1H, p, 7β-CH), 2.12 (1H, dd, J = 13.9, 9.9 Hz, 7α-CH), 1.94−1.20 (6H, m, NH, OH, 3,4-CH_2). **15**·fumarate: Anal. ($C_9H_{15}NO_3 \cdot C_4H_4O_4$) C, H, N.

2β-Acetoxy-6β-carbomethoxy-8-azabicyclo[3.2.1]-octane (16). In a similar way to the preparation of **6**, compound **16** was obtained from **35** as a colorless oil, yield 98%. It was converted to a fumarate salt. **16**·fumarate: mp 130−135 °C. Free base: CI-MS m/z 228 (MH^+); ^{1}H NMR ($CDCl_3$) δ 4.68 (1H, brs, 2α-CH), 3.72 (3H, s, OCH_3), 3.70−3.63 (2H, m, 1,5-CH), 2.93 (1H, dd, J = 9.5, 4.8 Hz, 6α-CH), 2.86 (1H, p, 7β-CH), 2.24 (2H, m, NH, 3β-CH), 2.13 (3H, s, CH_3CO), 1.93 (1H, dd, J = 13.7, 9.2 Hz, 7α-CH), 1.87−1.26 (3H, m, NH, OH, 3α-CH, 4-CH_2). **16**·fumarate: Anal. ($C_{11}H_{17}NO_3 \cdot C_4H_4O_4$) C, H, N.

2α-Acetoxy-6β-carbomethoxy-8-azabicyclo[3.2.1]-octane (17). In a similar way to the preparation of **6**, compound **17** was obtained from **36** as a colorless oil, yield 100%. It was converted to a fumarate salt. **17**·fumarate: mp 138−139 °C. Free base: CI-MS m/z 228 (MH^+); ^{1}H NMR ($CDCl_3$) δ 4.84 (1H, m, 2β-CH), 3.71 (3H, s, OCH_3), 3.61−3.58 (2H, m, 1,5-CH), 2.79 (1H, dd, J = 8.9, 4.8 Hz, 6α-CH), 2.21 (1H, dd, J = 13.5, 9.1 Hz, 7α-CH), 2.05 (3H, s, CH_3CO), 2.00−1.92 (3H, m, NH, 3β,7β-CH), 1.78−1.26 (3H, m, 3α-CH, 4-CH_2). **17**·fumarate: Anal. ($C_{11}H_{17}NO_3 \cdot C_4H_4O_4$) C, H, N.

Other Agents. Muscarine, methacholine, arecoline, ecgonidine methyl ester, and ecgonine methyl ester were from Research Biochemicals International (Natick, MA). Carbamylcholine, atropine, and (−)-nicotine were from Sigma Chemical Co. (St. Louis, MO). The [^{3}H]quinuclidinyl benzilate (sp. act. 46 Ci/mmol), [^{3}H]nicotine (sp. act. 75 Ci/mmol), [^{3}H]-adenine (sp. act. 29 Ci/mmol), and [^{3}H]inositol (sp. act. 21 Ci/mmol) were from New England Nuclear (Boston, MA). Other compounds were from standard commercial sources.

Cultured Cells. Four lines of CHO cells, each expressing a different homogeneous human muscarinic receptor population, were provided by Dr. Jurgen Wess (National Institutes of Health, Bethesda, MD) and grown in Dulbecco's modified Eagle medium with 10% fetal bovine serum, 100 units/mL penicillin, and 100 g/mL streptomycin. Cells were grown at 37 °C in an atmosphere enriched in CO_2. For description of transfection protocols and characterization of cells, see ref 6.

Membranes. The cerebral cortex of rat brains (Pel Freez Biologicals, Rogers, AR) were homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.4) using a Brinkman polytron (setting 6, 10 s). The homogenate was centrifuged for 15 min at 35000g at 4 °C, and the pellet was washed once by recentrifugation in Tris buffer. The final pellet was resuspended in Tris buffer and stored at −70 °C. Prior to assay, membranes were diluted to a concentration of 1−3 mg/mL in a binding buffer consisting of 20 mM HEPES buffer (pH 7.4) containing 1 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, and 2 mM $CaCl_2$.

The CHO cells were harvested at 80−100% confluence. Cells were washed with phosphate-buffered (pH 7.4) saline solution and then scraped into 10 mL of ice-cold binding buffer (see above). The tissue was homogenized using a Brinkman polytron (setting 6, 10 s) and centrifuged for 15 min at 16000g at 4 °C. The pellet was resuspended in the binding buffer and homogenized again at the same setting. Aliquots (2 mL) were stored at −70 °C. Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL), using bovine albumin as a standard.

Binding Assays. Inhibition of [^{3}H]nicotine binding was assayed essentially as described.[15] Briefly, assays contained 0.2 nM [^{3}H]nicotine, 300 μL of HEPES binding buffer (see above), test agents, 100 μL of membrane suspension, and 200 μM diisopropyl fluorophosphate in a final volume of 0.5 mL. Nonspecific binding was determined with 1 μM nicotine. Assays were initiated by addition of membrane and were for 120 min at 0−4 °C in triplicate. Assays were terminated by filtration through Whatman GF/B filters presoaked in 0.3% poly(ethylenimine) for 30 min using a Brandel M24R cell harvester (Brandel, Gaithersburg, MD). Filters were washed twice with ice-cold 50 mM Tris-HCl buffer (pH 7.4), then placed in vials with 4 mL of Hydrofluor scintillation fluid, and counted for tritium.

Inhibition of binding of [^{3}H]quinuclidinyl benzilate was assayed with rat cerebral cortical membranes essentially as described.[15] Briefly, assays contained 0.2 nM [^{3}H]quinuclidinyl benzilate, 100 μL of membrane suspension, and test agents in 20 mM HEPES buffer (pH 7.4) containing 10 mM $MgCl_2$ and 100 mM NaCl in a final volume of 0.5 mL. Nonspecific binding was determined with 1 μM atropine. Assays were initiated by addition of membranes and were for 30 min at 37 °C in triplicate. Filtration, washing, and scintillation counting were as described for [^{3}H]nicotine binding.

Inhibition of binding of [^{3}H]oxotremorine M was assayed with rat cerebral cortical membranes essentially as described.[16] Briefly, assays contained 2 nM [^{3}H]oxotremorine macetate, 100 μL of membrane suspension, and test agents in 25 mM sodium phosphate buffer (7.4) containing 5 mM $MgCl_2$ in a final volume of 0.5 mL. Nonspecific binding was determined with 5 μM atropine. Assays were initiated by addition of membranes and were for 2 h at 25 °C in triplicate. Filtration, washing, and scintillation counting were as described above for [^{3}H]nicotine binding. Saturation assays were conducted in the same manner with a range of concentrations of [^{3}H]-oxotremorine M.

Binding of [^{3}H]quinuclidinyl benzilate with membranes of transfected CHO cells was assayed as described for [^{3}H]-*N*-ethylscopolamine binding.[6] Briefly, assays were in 25 mM sodium phosphate buffer (pH 7.4) containing 5 mM $MgCl_2$, [^{3}H]-quinuclidinyl benzilate (0.2 nM for M_1- and M_3-receptors, 0.1 nM for M_2- and M_4-receptors), test agents, and 100 μL of membrane suspension in a final volume of 0.5 mL. Nonspecific binding was determined with atropine (1 μM for M_2-, M_3-, and M_4-receptors, 10 μM for M_1-receptors). Assays were initiated by addition of membranes and were for 30 min at 37 °C in triplicate. GTP at 10 μM was present in certain experiments. Filtration, washing, and scintillation counting were as described above for [^{3}H]nicotine binding. Saturation assays were

conducted in the same manner with a range of concentrations of [^{3}H]quinuclidinyl benzilate. The final volume was 1 mL.

Inhibition of Adenylyl Cyclase. The assay of inhibition of [^{3}H]cyclic AMP formation in [^{3}H]adenine-labeled CHO cells was essentially as described.[17] Briefly, the CHO cells were labeled in 12-well plates with 2 μCi/mL [^{3}H]adenine for 24 h. Wells contained ca. 3×10^5 cells in a volume of 1 mL. Cells were then washed once with Dulbecco's modified Eagle medium containing 20 mM HEPES buffer (pH 7.4) and were then incubated in the buffered Dulbecco's media containing 1 mM isobutylmethylxanthine at room temperature for 20 min. Forskolin (10 μM) and muscarinic agents were then added to each well. After incubation for 30 min at 37 °C, the medium was aspirated and replaced with 1 mL of cold 5% aqueous trichloroacetic acid with 1 mM cyclic AMP and 1 mM ATP. After 40 min at 4 °C, the cyclic AMP in the trichloroacetic acid solution was isolated by Dowex and alumina chromatography[18] and counted for tritium in Hydrofluor scintillation fluid.

Stimulation of Phosphoinositide Breakdown. The assay of accumulation of [^{3}H]inositol monophosphate is essentially as described.[17] The CHO cells were labeled in 12-well plates with 1 μCi/mL *myo*-[^{3}H]inositol for 24 h. Wells contained ca. 3×10^5 cells in a volume of 1 mL. Cells were then washed once with Hank's balanced salt media containing 20 mM HEPES buffer (pH 7.4) and were then incubated in the buffered Hank's media containing 10 mM LiCl at room temperature for 15 min. Muscarinic agents were then added to each well. After incubation for 1 h at 37 °C, the medium was aspirated and replaced with 750 μL of cold 20 mM aqueous formic acid. After 35 min at 4 °C, the formic acid was removed and replaced with 250 μL of 60 mM NH_4OH. The inositol monophosphate in the NH_4OH solution was isolated by anion-exchange chromatography[19] and counted for tritium in Hydrofluor scintillation fluid.

Data Analysis. K_d and B_{max} values were derived from linear regression analyses of the saturation binding data using GraphPad-InPlot (GraphPad Software Inc.). K_d values were the negative slope of the Rosenthal plot, and B_{max} values were the *x*-intercept. IC_{50} values from competitive binding data were determined by computer analysis, using GraphPad-InPlot, whereby a nonlinear curve was fitted to a graph of binding (% total binding) values plotted against the log values of the corresponding drug concentrations. K_i values were calculated from IC_{50} values using the Cheng and Prusoff equation.

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