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6β-Acyloxy(nor)tropanes: Affinities for Antagonist/Agonist Binding Sites on Transfected and Native Muscarinic Receptors

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A series of esters of 6β-hydroxynortropane and the *N*-methyl analogue 6β-tropanol were synthesized and screened versus binding of an antagonist (quinuclidinyl benzilate) and an agonist (oxotremorine-M) at sites on human m_1-, m_2-, m_3-, and m_4-muscarinic receptors in transfected cell membranes and on native M_1-muscarinic receptors in rat brain membranes and native M_2-muscarinic receptors in rat heart membranes. Most 6β-acyloxy(nor)tropanes had higher affinity versus oxotremorine-M binding compared to quinuclidinyl benzilate binding at transfected m_1- and native M_1-receptors, indicative of agonist activity. 6β-Acetoxynortropane had K_i values versus oxotremorine-M binding at m_1-, m_2-, and m_4-receptors ranging from 4 to 7 nM. *N*-Methylation reduced affinity greatly as did increasing the size of the acyl moiety. The affinity of 6β-benzoyloxynortropane and other analogues with larger acyl moieties was little affected by *N*-methylation or in some cases was increased. 6β-Acyloxy(nor)tropanes and classical muscarinic agonists, such as muscarine and oxotremorine, had higher affinity versus oxotremorine-M binding compared to quinuclidinyl benzilate binding at native M_2-muscarinic receptors of heart, but not at transfected m_2-muscarinic receptors. Antagonist/agonist binding ratios were not obtained for transfected m_3-receptors, since significant oxotremorine-M binding could not be detected. 6β-Acyloxy(nor)tropane, two other (nor)tropanes, and the classical muscarinic agonists had higher affinity versus agonist binding compared to antagonist binding for transfected m_4-receptors. The antagonist/agonist binding ratio method is clearly not always reliable for predicting agonist activity at muscarinic receptors.

Introduction

Muscarinic acetylcholine receptors play key roles in the central and peripheral nervous system, and there have been intensive efforts to develop selective/specific agonists or antagonists for each of the major subtypes.[1,2] Functionally, all subtypes are G-protein-coupled receptors with the M_1-, M_3-, and M_5-muscarinic receptors being stimulatory to phospholipase C, while the M_2- and M_4-receptors are inhibitory to adenylyl cyclase. All five muscarinic subtypes are expressed in brain, thus complicating efforts to develop selective agents based on assays with brain preparations.[3] Cerebral cortical preparations are rich in M_1-receptors, heart preparations in M_2-receptors, pancreatic preparations in M_3-receptors, and striatal preparations in M_4-receptors. The affinities of agents versus binding of an antagonist, such as [^{3}H]-quinuclidinyl benzilate (QNB), and versus binding of an agonist, such as [^{3}H]oxotremorine-M (Oxo-M), differ greatly at least in cerebral cortical preparations.[4−9] Agonists show very low affinities versus [^{3}H]QNB and much higher affinities versus [^{3}H]Oxo-M, the latter binding presumed to be at the same M_1-receptors of rat cerebral cortex that [^{3}H]QNB binds. Antagonist/agonist ratios of greater than 100 have been taken to indicate a full agonist, while ratios of 10 or less indicate an antagonist and ratios of 10−100 suggest partial agonism. Such a ratio approach has focused on rat brain membranes rich in M_1-receptors. There has been one report[10] on the ratio approach using a mixture of M_1-, M_2-, M_3-, and M_4-receptors and a recent report on the ratio approach for M_2-receptors of rat heart membranes.[11] Apparently, the ratio approach has not been investigated for M_3- or M_4-receptors.

Cells transfected with specific muscarinic receptors provide preparations for binding studies and functional assays uncomplicated by the presence of multiple subtypes of muscarinic receptors.[12] For initial screening and identification development of lead compounds as agonists, partial agonists, or antagonists, such binding assays with membranes from transfected cells should be rapid and reproducible. Thus, a series of analogues of the very potent muscarinic agonist 6β-acetoxynortropane[13] in which the acyl moiety has been varied from acetyl to proprionyl, butyryl, cyclohexanecarbonyloxyl, benzoyl, etc., has been prepared and screened using binding assays with the antagonist ligand [^{3}H]QNB and the agonist ligand [^{3}H]Oxo-M to human m_1-, m_2-, m_3-, and m_4-muscarinic receptors in membranes of transfected cells. Arecoline, muscarine, oxotremorine, and carbamylcholine were also assayed as standard muscarinic agonists. The present results with native M_1-muscarinic receptors of rat cerebral cortex and the transfected m_1-muscarinic receptors indicate that all such acyl analogues are much less active than 6β-acetoxynortropane (**1A**) and that, on the basis of antagonist/agonist binding ratios, most are expected to be agonists at M_1-muscarinic receptors. The antagonist/agonist binding ratios for the transfected m_2-muscarinic

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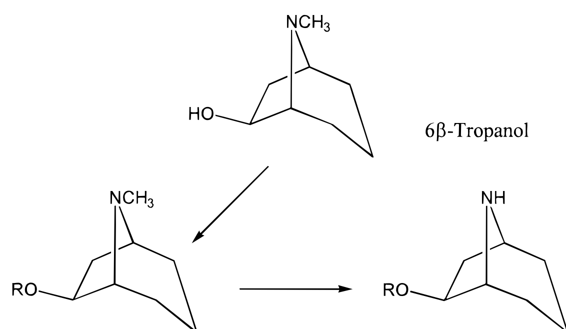
Chart 1



Tropanes	Nortropanes
1B: R = CH_3CO	1A: R = CH_3CO
2B: R = CH_3CH_2CO	2A: R = CH_3CH_2CO
3B: R = $CH_3(CH_2)_2CO$	3A: R = $CH_3(CH_2)_2CO$
4B: R = $(CH_3)_2NCO$	4A: R = $(CH_3)_2NCO$
5B: R = CH_3SO_2	5A: R = CH_3SO_2
6B: R = Cl_3CCH_2OCO	
7B: R = c-$C_6H_{11}CO$	7A: R = c-$C_6H_{11}CO$
8B: R = C_6H_5CO	8A: R = C_6H_5CO
9B: R = p-$NO_2C_6H_4CO$	9A: R = p-$NO_2C_6H_4CO$
10B: R = o-$CH_3OC_6H_4CO$	10A: R = o-$CH_3OC_6H_4CO$
11B: R = p-$CH_3OC_6H_4CO$	11A: R = p-$CH_3OC_6H_4CO$
12B: R = C_6H_5NHCO	12A: R = C_6H_5NHCO

receptor varied greatly within this series of potential agonists and for the standard muscarinic agonists. However, a subset of 6*β*-acyl(nor)tropanes and the standard muscarinic agonists did afford the antagonist/agonist binding ratios expected of agonists with native M_2-muscarinic receptors of rat heart. Thus, it appears that the method of antagonist/agonist binding ratios for muscarinic receptors is not reliable for transfected human m_2-receptors but may prove useful with native M_2-receptors. Antagonist/agonist binding ratios could not be obtained for transfected m_3-receptors but did appear likely to be predictive of agonist and low efficacy partial agonist activity for transfected m_4-receptors.

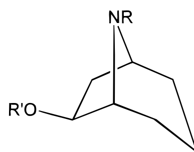
Chemistry

The compounds have been numbered in an **A**-series for the nortropanes and a corresponding **B**-series for the tropanes (see Chart 1). Compounds **1A** and **1B** has been previously described.[13] Compounds **1B**, **2B**, **3B**, **7B**, **8B**, **9B**, **10B**, and **11B** were prepared from 6*β*-tropanol[13,14] by reaction with corresponding alkylcarbonyl chloride or arylcarbonyl chloride in CH_2Cl_2 or ether, catalyzed by Et_3N. The dimethylcarbamate **4B** was prepared by reacting 6*β*-tropanol with dimethylcarbamyl chloride and NaH in THF. The phenylcarbamate **12B** was prepared from 6*β*-tropanol with phenyl isocyanate in ether, catalyzed by a trace amount of Na. Compound **5B** was prepared from 6*β*-tropanol with reaction of methanesulfonyl chloride in CH_2Cl_2, catalyzed by Et_3N. Compound **6B** was prepared from 6*β*-tropanol by reaction with 2,2,2-trichloroethyl chloroformate and K_2CO_3 in refluxing toluene. All the above compounds with the exception of **6B** were converted to nortropanes (**2A**, **3A**, **4A**, **5A**, **7A**, **8A**, **9A**, **10A**, **11A**, and **12A**) with 2,2,2-trichloroethyl chloroformate in refluxing toluene, followed by treating the carbamate (not isolated) with zinc in acetic acid.

Results and Discussion

The binding data for the presumed major M_1-receptor of rat cerebral cortical membranes are presented in Table 1. 6*β*-Acetoxynortropane (**1A**), as previously reported,[13] had a very high affinity versus agonist ([^{3}H]-Oxo-M) binding to presumed M_1-muscarinic receptors in rat cerebral cortical membranes, while having a 11000-fold lower affinity versus antagonist ([^{3}H]QNB) binding, indicative of full agonist activity, as previously confirmed with human m_1-receptors coupled to phosphoinositide breakdown in transfected cells (EC_{50} 23 nM).[13] The presence of an *N*-methyl group (**1B**) reduced affinity by about 400-fold versus agonist ([^{3}H]Oxo-M) binding, but the antagonist/agonist binding ratio was still high at about 300. Increasing the size of the acyl moiety by one (**2A**) or two (**3A**) methylene groups reduced affinity versus agonist binding compared to **1A**, by 15- and 80-fold, respectively. The presence of an *N*-methyl group (**2B**, **3B**) caused a further marked decrease in affinity versus agonist binding. The antagonist/agonist binding ratio for **3B** was only 28, suggestive of partial agonist activity. The carbamates (**4A**, **4B**, **12A**, **12B**) and the sulfonyl analogues (**4A**, **4B**) showed very low affinity versus agonist binding with K_i values in the micromolar range for such analogues. The presence of an *N*-methyl group had little or no effect on affinity. Analogues with acyl moieties larger than butyryloxy, namely cyclohexanecarbonyloxy (**7A**, **7B**), benzoyloxy (**8A**, **8B**), and substituted benzoyloxy (**9A**, **9B**, **10A**, **10B**, **11A**, **11B**) moieties, also showed low affinity, ranging from 0.4 μM (**9A**) to 14 μM (**9B**). The presence of an *N*-methyl group either had no effect or increased or decreased affinity versus agonist binding. The antagonist/agonist binding ratios for most of these low-affinity analogues would be estimated to be less than 50, suggestive of partial agonist activity, while it was less than 10 for **8A**, **8B**, and **9A**, suggestive of antagonist activity. It would appear that an acetoxy group (**1A**) confers by far the greatest agonist affinity for the nortropanes, while for the tropane series with an *N*-methyl group the size of the acyl moiety is much less important with analogues containing the acetoxy (**1B**), butyryloxy (**3B**), cyclohexanecarbonyloxy (**7B**), and benzoyloxy (**8A**) moieties having equivalent affinity. It would also appear that larger acyl moieties and the *N*-methyl group reduce the antagonist/agonist binding ratio. The four muscarinic agonists tested, namely arecoline, muscarine, oxotremorine, and carbamylcholine, all show nanomolar (12−30 nM) affinity versus agonist binding and all showed very high (>1000) antagonist/agonist binding ratios with M_1-muscarinic receptors of rat brain membranes (Table 1). However, it should be noted that the generally accepted antagonist/agonist binding ratio for M_1-receptors in brain membranes[4−9] may be compromised by significant binding of [^{3}H]agonists to more than one muscarinic receptor subclass.[15,16]

Table 1. Affinities of 6β-Acyloxy(nor)tropanes for Muscarinic Receptors in Rat Cerebral Cortical Membranes



compd	R	R′	K_i (μM) or % inhibition binding[a] [^{3}H]QNB	[^{3}H]Oxo-M	ratio[b]
1A	H	CH_3CO	22 ± 3[c]	0.0020 ± 0.0002	11000
1B	CH_3	CH_3CO	280 ± 33[c]	0.86 ± 0.06	330
2A	H	CH_3CH_2CO	32 ± 4	0.030 ± 0.002	1100
2B	CH_3	CH_3CH_2CO	23%	1.5 ± 0.1	>70
3A	H	$CH_3(CH_2)_2CO$	30 ± 3	0.16 ± 0.01	190
3B	CH_3	$CH_3(CH_2)_2CO$	34 ± 7	1.2 ± 0.2	28
4A	H	$(CH_3)_2NCO$	21%	1.7 ± 0.1	>60
4B	CH_3	$(CH_3)_2NCO$	16%	5.9 ± 0.5	>60
5A	H	CH_3SO_2	4%	2.7 ± 0.1	>100
5B	CH_3	CH_3SO_2	0%	2.2 ± 0.1	>100
6B	CH_3	Cl_3CCH_2OCO	23 ± 2	0.72 ± 0.04	31
7A	H	*c*-$C_6H_{11}CO$	25 ± 3	1.9 ± 0.2	13
7B	CH_3	*c*-$C_6H_{11}CO$	8.5 ± 2.1	0.68 ± 0.05	13
8A	H	C_6H_5CO	0.80 ± 0.20	0.65 ± 0.08	1.2
8B	CH_3	C_6H_5CO	1.8 ± 0.5	0.86 ± 0.04	2.1
9A	H	*p*-$NO_2C_6H_4CO$	2.2 ± 0.4	0.43 ± 0.04	5.1
9B	CH_3	*p*-$NO_2C_6H_4CO$	12%	14 ± 1	>10
10A	H	*o*-$CH_3OC_6H_4CO$	53 ± 6	2.8 ± 0.1	19
10B	CH_3	*o*-$CH_3OC_6H_4CO$	31 ± 1	1.4 ± 0.1	22
11A	H	*p*-$CH_3OC_6H_4CO$	21%	1.7 ± 0.1	>60
11B	CH_3	*p*-$CH_3OC_6H_4CO$	25%	7.3 ± 0.4	>15
12A	H	C_6H_5NHCO	17%	4.3 ± 0.4	>25
12B	CH_3	C_6H_5NHCO	34%	5.1 ± 0.3	>20
arecoline			43%	0.031 ± 0.002	>3000
muscarine			3%	0.014 ± 0.001	>10000
oxotremorine			58 ± 1	0.013 ± 0.001	4500
carbachol[d]			5%	0.012 ± 0.001	>10000

[a] K_i values are means ± SEM ($n = 3$) for inhibition of binding of [^{3}H]QNB or [^{3}H]Oxo-M to muscarinic receptors in rat cerebral cortical membranes. Either the K_i value or the percent inhibition at 100 μM is reported. The K_d for [^{3}H]QNB was 0.26 nM and the B_{max} 2500 fmol/mg of protein. The K_d for [^{3}H]Oxo-M was 0.75 nM and the B_{max} 320 fmol/mg of protein. [b] Ratios are calculated or estimated from affinity versus antagonist [^{3}H]QNB divided by affinity versus agonist [^{3}H]Oxo-M. [c] Values from ref 13. [d] Carbamylcholine.

Table 2. Affinities of 6β-Acyloxy(nor)tropanes for Human Muscarinic Receptors in Membranes from Transfected CHO cells

	K_i (μM) or % inhibition binding[a]							
	m_1-muscarinic		m_2-muscarinic		m_3-muscarinic		m_4-muscarinic	
compd	[^{3}H]QNB	[^{3}H]Oxo-M	[^{3}H]QNB	[^{3}H]Oxo-M	[^{3}H]QNB	[^{3}H]Oxo-M	[^{3}H]QNB	[^{3}H]Oxo-M
1A	21 ± 1	0.0046 ± 0.0005	0.0026 ± 0.0005	0.0070 ± 0.0006	0.68 ± 0.03		0.033 ± 0.010	0.0040 ± 0.0003
1B	11%	2.3 ± 0.2	2.7 ± 0.2	4.4 ± 1.5	15 ± 1		8.3 ± 1.0	0.90 ± 010
2A	36%	0.19 ± 0.05	3.1 ± 0.4	0.19 ± 0.03	11.4 ± 0.4		0.66 ± 0.07	0.085 ± 0.012
2B	46%	0.23 ± 0.05	2.4 ± 0.5	1.2 ± 0.1	7.4 ± 0.9		0.96 ± 0.12	1.4 ± 0.1
6B	27 ± 1	1.0 ± 0.2	0.82 ± 0.19	0.92 ± 0.07	6.7 ± 0.6		0.042 ± 0.022	2.3 ± 0.5
7A	20 ± 1	0.97 ± 0.13	3.6 ± 0.8	9.0 ± 0.3	7.5 ± 0.8		0.84 ± 0.02	2.2 ± 0.7
7B	7.0 ± 0.1	0.45 ± 0.23	0.73 ± 0.11	1.6 ± 0.5	3.0 ± 0.3		0.30 ± 0.02	0.70 ± 0.07
8A	2.8 ± 0.2	0.12 ± 0.04	0.30 ± 0.03	1.7 ± 0.6	1.3 ± 0.2		0.19 ± 0.01	0.50 ± 0.07
8B	7.7 ± 0.5	0.27 ± 0.06	0.26 ± 0.02	1.3 ± 0.2	3.2 ± 0.2		0.41 ± 0.05	0.91 ± 0.26
9A	0.78 ± 0.01	0.029 ± 0.01	0.36 ± 0.07	0.31 ± 0.08	0.45 ± 0.03		0.048 ± 0.002	0.45 ± 0.03
arecoline	13%	0.014 ± 0.001	0.040 ± 0.011	0.040 ± 0.007	42%		2.4 ± 0.5	0.026 ± 0.004
muscarine	2%	0.056 ± 0.012	0.025 ± 0.020	0.072 ± 0.017	28%		1.4 ± 0.4	0.023 ± 0.001
oxotremorine	9%	0.017 ± 0.003	0.66 ± 0.07	0.038 ± 0.02	28%		0.16 ± 0.02	0.0015 ± 0.0001
carbamylcholine	2%	0.17 ± 0.03	0.020 ± 0.001	0.038 ± 0.004	4%		2.6 ± 0.7	0.024 ± 0.005

[a] K_i values are means ± SEM ($n = 3$) for inhibition of binding of [^{3}H]QNB or [^{3}H]Oxo-M to muscarinic receptors in membranes of CHO cells transfected with m_1-, m_2-, m_3-, or m_4-muscarinic receptors. Either the K_i value or the percent inhibition at 100 μM is reported. The antagonist/agonist binding ratios are reported in Table 4. For K_d and B_{max} values for [^{3}H]QNB, see ref 13. The K_d values for [^{3}H]Oxo-M were as follows: m_1 = 1.9 nM, 1800 fmol/mg of protein; m_2 = 2.2 nM, 230 fmol/mg of protein; m_4 = 1.4 nM, 2800 fmol/mg of protein.

A representative 10-member series of acyl derivatives of 6β-tropanol were now screened in a similar manner versus antagonist ([^{3}H]QNB) and agonist ([^{3}H]Oxo-M) binding in membranes of Chinese hamster ovary (CHO) cells transfected with human m_1-, m_2-, m_3-, or m_4-muscarinic receptors (Table 2). The results with the transfected m_1-receptor (Table 2) were similar to those obtained for the M_1-receptor of rat cerebral cortical membranes (Table 1). The 6β-acetoxynortropane (**1A**) was by far the most active with low namomolar affinity in both the brain M_1-receptor and transfected m_1-receptor binding assays. There were some minor differences between the two systems with **2A** and **2B** showing comparable affinities for the m_1-transfected receptors, while a marked decrease in affinity because of the presence of the *N*-methyl in **2B** was seen in the brain

Table 3. Affinities of 6β-Acyloxy(nor)tropanes versus Antagonist and Agonist Binding to M_2-Muscarinic Receptors in Rat Heart Membranes[a]

compd	K_i (μM) or % inhibition of binding		antagonist/ agonist ratio
	[3H]QNB	[3H]Oxo-M	
1A	0.40 ± 0.08	0.00095 ± 0.0001	420
1B	17 ± 1	0.092 ± 0.021	180
8A	1.4 ± 0.03	0.44 ± 0.06	3.2
8B	1.4 ± 0.2	0.79 ± 0.12	1.8
arecoline	1.9 ± 0.2	0.0053 ± 0.0004	360
muscarine	4.4 ± 0.2	0.0083 ± 0.0002	530
oxotremorine	0.33 ± 0.07	0.0060 ± 0.0002	55
carbamylcholine	2.9 ± 0.7	0.053 ± 0.011	55

[a] Values are means ± SEM ($n = 3$) for inhibition of binding of the antagonist [3H]QNB and of the agonist [3H]Oxo-M to muscarinic receptors of rat heart membranes. The K_d for [3H]QNB was 0.050 nM and the B_{max} 3600 fmol/mg of protein. The K_d for [3H]Oxo-M was 0.55 nM and the B_{max} 4300 fmol/mg of protein.

membrane system. The other difference was that the benzoyloxy analogues (**8A**, **8B, 9A**) had significantly greater affinity versus [3H]Oxo-M binding to the transfected m_1-receptors than for the native M_1-receptors in the brain membranes. Indeed, the *p*-nitrobenzoyloxy analogue **9A** was only 6-fold less potent than the acetoxy analogue **1A** at the transfected m_1-receptors. Species differences between rat (M_1) and human (m_1) muscarinic receptors[12] might account for such disparities. Another possible explanation is that [3H]Oxo-M binds significantly to muscarinic subtypes in rat brain membranes[16] other than the predominant M_1-receptor labeled by antagonists.[15] It should be noted that other laboratories have not been able to detect [3H]Oxo-M binding to membranes from cells transfected with m_1-receptors as pointed out to us by one of the referees. Apparently, in such cases all the m_1-receptors have been in the low-affinity agonist binding state. Why in most cases transfected m_1-receptors and also m_3-receptors (see below) coupled through G-binding proteins to phospholipase C should exist virtually in the low-affinity state uncoupled to the G-protein is unknown, unless levels of transfected receptors greatly exceed levels of G-proteins.

When assayed with transfected m_2-muscarinic receptors, the results with the 10 members of the 6β-acyl series of (nor)tropanes and with the 4 standard muscarinic agonists were unexpected. The antagonist/agonist binding ratio was 1 or less than 1 for arecoline, muscarine, and carbamylcholine and was only 17 for oxotremorine. This lack of a diagnostic high ratio was also evident for 6β-acetoxynortropane (**1A**), which has been shown in functional assays to be a potent full agonist for M_2-receptors (see ref 13). The antagonist/agonist ratio was actually reversed for this full agonist, which was nearly 3-fold more potent versus antagonist binding compared to agonist binding at the transfected m_2-receptors. Similarly, seven of the (nor)tropanes had antagonist/agonist binding ratios of 1 or less. Nortropane **2A** had a 16-fold higher affinity and tropane **2B** a 2-fold higher affinity versus agonist binding compared to antagonist binding. Clearly, the antagonist/agonist binding ratios were unsatisfactory for transfected m_2-muscarinic receptors. In the present series, on the basis of only agonist binding, the benzoyloxy analogues (**8A**, **9A**) showed about 10-fold selectivity for m_1-receptors, compared to m_2-receptors. In contrast, the acetoxy analogues (**1A**, **1B**) were about equipotent at the two receptors.

There was no marked selectivity of carbamylcholine, pilocarpine, or oxotremorine as inhibitors versus agonist binding compared to antagonist binding for M_2-receptors in rat heart membranes.[18] However, a high antagonist/agonist binding ratio was reported recently for muscarinic agonists, including oxotremorine-M, and a low ratio for antagonists, such as atropine, with M_2-muscarinic receptors of rat heart.[11] When assayed with native M_2-muscarinic receptors of rat heart membranes, 6β-acetoxynortropane (**1A**) and its *N*-methyl derivative (**1B**) showed very high antagonist/agonist ratios, as did arecoline and muscarine, indicative of full agonist activity (Table 3). 6β-Acetoxynortropane (**1A**) is a full agonist at native M_2-receptors (see ref 13). The classical agonists oxotremorine and carbamylcholine had antagonist/agonist ratios of about 55 at the rat heart M_2-receptors. The 6β-benzoyloxynortropane (**8A**) and its *N*-methyl derivative (**8B**) had low ratios of 3.2 and 1.8, respectively, suggestive of antagonist activity.

For the transfected m_3-muscarinic receptors only antagonist ([3H])QNB) binding could be detected. The reason for lack of significant levels of high-affinity binding of agonist ([3H]Oxo-M) in these membranes is unknown. Both GDPβS and a peptide, adenoregulin, the latter known to enhance high-affinity binding at G-protein-coupled receptors,[19] did not result in detectable [3H]Oxo-M binding. In a recent study, it was concluded, based on affinity of agonists versus binding of an antagonist radioligand to transfected human muscarinic receptors and the lack of effect of the guanine nucleotide GppNHp,[20] that virtually all of the m_1- and m_3-receptors were in the low-affinity agonist binding state. This apparently is also the case for the transfected m_3-receptors in the present study. Apparently only [3H]-antagonist binding has been reported for pancreas and submandibular gland, which contain mainly M_3-muscarinic receptors.[21,22] There is one report of [3H]Oxo-M binding to what were probably M_3-muscarinic receptors in cultured bovine pulmonary arterial endothelial cell membranes.[23] The [3H]Oxo-M binding sites were present at a 4-fold lower level than [3H]QNB binding sites in such membranes. In the present report on inhibition of [3H]QNB binding at the transfected m_3-receptors, the standard cholinergic agonists showed very low affinity, while the series of (nor)tropanes inhibited binding with K_i values of 3−15 μM with the exception of **1A**, **8A**, and **9A** with K_i values of about 0.5−1.3 μM. 6β-Acetoxynortropane (**1A**) has been reported to be a potent partial

Table 4. Antagonist/Agonist Binding Ratios for 6β-Acyloxy(nor)tropanes at Human m_1-, m_2-, and m_4-Receptors in Transfected CHO Cells[a]

compd	antagonist/agonist ratio		
	m_1	m_2	m_4
1A	4600	0.37	8.3
1B	>50	0.61	9.2
2A	>600	16	7.8
2B	450	2.0	0.69
6B	27	0.89	0.019
7A	21	0.40	0.38
7B	16	0.46	0.43
8A	23	0.18	0.38
8B	29	0.20	0.45
9A	27	1.2	0.11
arecoline	>7000	1.0	92
muscarine	≫2000	0.35	61
oxotremorine	>6000	17	110
carbamylcholine	≫600	0.52	110

[a] Ratios calculated or estimated from data of Table 2.

agonist in stimulation of inositol monophosphate accumulation in m_3-receptor-transfected cells.[13]

When assayed with transfected m_4-muscarinic receptors, the four standard muscarinic agonists all showed markedly higher affinities versus agonist binding compared to antagonist binding (Table 2). The antagonist/agonist binding ratios ranged from 60 to 100 (Table 4). 6β-Acetoxynortropane **(1A)** at transfected m_4-receptors had the highest affinity of the (nor)tropanes with a K_i value versus [^{3}H]Oxo-M binding of 4 nM, comparable to its nanomolar affinity at m_1- and m_2-receptors. The antagonist/agonist binding ratio, however, was only 8. The *N*-methyl derivative **1B** had a 250-fold lower affinity than **1A** in the agonist binding assay but also showed an antagonist/agonist binding ratio of about 9. The propionyloxy analogue **2A** was the only other (nor)-tropane with high affinity (K_i 85 nM) against [^{3}H]Oxo-M binding and also showed an antagonist/agonist binding ratio of about 8. The other (nor)tropanes had K_i values ranging from 0.45 to 2.3 μM against agonist binding at the m_4-receptors, and the antagonist/agonist binding ratios were either near unity or in some cases much less than unity. In the case of the *p*-nitrobenzoyloxy analogue **9A** it was 10-fold more potent versus antagonist binding compared to agonist binding. The analogue **6B** was truly remarkable in being 54-fold selective for antagonist binding. The results would suggest that many of the (nor)tropanes will prove to be antagonists or low-efficacy partial agonists at m_4-muscarinic receptors.

A limited group of (nor)tropanes was assessed for inhibition of adenylate cyclase in the cells transfected with m_4-receptors. 6β-Acetoxynortropane (**1A**), as previously reported,[13] was a full agonist with an apparent IC_{50} of 34 ± 5 nM. The *N*-methyl derivative **1B** had very low functional activity causing only a 38% inhibition at 30 μM. On the basis of relative affinities of **1A** and **1B** versus agonist binding at the transfected m_4-receptors, **1B** would have been expected to have an IC_{50} of about 9 μM. The two benzoyloxy analogues **8A** and **8B** caused only about a 10% inhibition at 30 μM and may represent very low-efficacy partial agonists. Both had antagonist/agonist ratios at transfected m_4-receptors less than unity.

The present exploratory study indicates that the very high muscarinic potency of 6β-acetoxynortropane (**1A**) is greatly reduced by N-methylation or by replacement of the acetoxy by larger acyl groups for all four muscarinic receptors. Activity has been assessed by measuring affinity of the (nor)tropanes versus antagonist ([^{3}H]-QNB) and agonist ([^{3}H]Oxo-M) binding to M_1-muscarinic receptors in rat brain membrane and for a subset of (nor)tropanes to transfected human m_1-, m_2-, m_3-, and m_4-receptors and to native M_2-muscarinic receptors in rat heart membranes.

The antagonist/agonist binding ratio method has become well-accepted to be predictive of agonist, partial agonist, and antagonist activity for native M_1-muscarinic receptors of brain membranes.[4−9] The present results indicate that transfected human m_1-muscarinic receptors, at least in the present transfected CHO cell line, can also provide ratios predictive of activity, based on comparison of data from brain M_1-receptors (Table 1) and transfected m_1-receptors of the present CHO cell line (Table 2). The antagonist/agonist binding ratios for native M_2-muscarinic receptors of heart membranes also appear to be predictive of activity as agonist/antagonist activity based on a recent report[11] and present data (Table 3). However, ratios for transfected human m_2-receptors had no apparent predictive value.

Significant oxotremorine-M binding to transfected m_3-receptors could not be detected. To our knowledge [^{3}H]-oxotremorine-M binding has not been reported for native M_3-muscarinic receptors except perhaps for membranes of endothelial cells.[23]

At transfected m_4-receptors, the five standard muscarinic agonists and three of the (nor)tropanes exhibited higher affinities against [^{3}H]Oxo-M binding compared to [^{3}H]QNB binding, suggesting that antagonist/agonist binding ratios of about 10 or greater at transfected m_4-receptors may prove to be predictive of agonist activity. Ratios of 1 or less may prove to be predictive of low efficacy partial agonist activity or antagonist activity. Thus, muscarine (ratio 61) and nortropane **1A** (ratio 9) were full agonists, tropane **1B** a partial agonist (ratio 8), and nortropane **8A** and tropane **8B** very low-efficacy partial agonists (ratios 0.38 and 0.45) in functional assays with cells transfected with m_4-receptors (see above and ref 13).

The antagonist/agonist binding ratio method is based on the assumption that the state of the muscarinic receptor is static during binding assays; i.e., that there is virtually no interconversion between receptors in states with either a high or low affinity for agonists. Thus, the K_i value for an agonist versus [^{3}H]Oxo-M would reflect only affinity for the high-affinity agonist state, while the K_i value for an agonist versus [^{3}H]QNB would reflect a composite affinity versus both low- and high-affinity agonist states, both of which would have high affinity for QNB. When the low-affinity state is predominate as it is in brain membranes,[24,25] the K_i of an agonist versus [^{3}H]QNB would represent almost exclusively the affinity of the agonist toward the predominate low-affinity agonist binding state. However, in heart membranes the apparent levels of high-affinity agonist binding state can represent about one-half of the total sites labeled by an antagonist[18] or, as in the present study and one prior study,[11] would appear to be equivalent to the total sites labeled by an antagonist. Similarly, the levels of the high-affinity state have been

reported to be about one-half that of total sites for transfected m_4-receptors.[17] In the present study, the B_{max} of [^{3}H]QNB binding to membranes with transfected m_1-, m_2-, and m_4-receptors appeared in each case similar to the B_{max} for [^{3}H]Oxo-M binding (see footnote to Table 2). However, B_{max} values for the two ligands were measured in different batches of cells. The question arises as to how the agonist/antagonist binding ratio method could be predictive of agonist activity for heart M_2-receptors and the present transfected m_1- and m_4-receptors where all or a large proportion of the receptors labeled by antagonist appear to be in the high-affinity agonist state. Perhaps there can be interconversion of low- and high-affinity agonist states of muscarinic receptors during binding assays, or perhaps there are both low- and high-affinity antagonist binding states.[26] Regardless, the present data provide a cautionary note relevant to any use of the agonist/antagonist binding ratio method with transfected human muscarinic receptors.

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 were reported as δ values (ppm) relative to Me_4Si as an internal standard. MS (*m*/*z*) for chemical ionization (CI) were recorded on a Finnigan-1015D mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc. Unless otherwise indicated, all flash chromatographic separations were with Merck silica gel 60, 230−400 mesh columns using the described solvents. All reactions involving nonaqueous solutions were performed under an inert atmosphere and with anhydrous solvents unless otherwise noted. All compounds prepared in this paper are racemates. Free bases were converted to HCl salts using methanolic HCl and ether.

8-Methyl-6β-propionyloxy-8-azabicyclo[3.2.1]octane (2B) (6β-Propionyloxytropane). 6β-Tropanol (141 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (2 mL), followed by addition of Et_3N (170 μL) and propionyl chloride (111 mg, 1.2 mmol, 105 μL). The mixture was stirred at room temperature overnight and poured into saturated $NaHCO_3$ solution (5 mL). The CH_2Cl_2 layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). After the removal of solvent under reduced pressure, the residue was chromatographed (CH_2Cl_2/MeOH = 9/1) to give **2B** (195 mg, 99.5%) as a colorless oil. It was converted to hydrochloride salt as white crystals. **2B**·HCl: mp 61−62 °C. Free base: CI-MS *m*/*z* 198 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 5.14 (t, 1H, *J* = 5.9 Hz, 6α-CH), 3.33 (brs, 1H, 1-CH), 3.10 (brs, 1H, 5-CH), 2.48 (s, 3H, NCH_3), 2.32 (dd, 2H, *J* = 15.7, 7.9 Hz, CH_2CO), 2.12 (dd, 2H, *J* = 6.3, 3.4 Hz, 7-CH_2), 1.81−1.18 (m, 6H, 2,3,4-CH_2), 1.13 (t, 3H, *J* = 7.8, *CH_3*CH_2O). **2B**·HCl: Anal. ($C_{11}H_{19}NO_2$·HCl·1.1H_2O) C, H, N.

8-Methyl-6β-butyryloxy-8-azabicyclo[3.2.1]octane (3B) (6β-Butyryloxytropane). In a similar way to the preparation of **2B**, compound **3B** was obtained from 6β-tropanol as a colorless oil, yield 80%. **3B**: CI-MS *m*/*z* 212 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 5.14 (t, 1H, *J* = 5.9 Hz, 6α-CH), 3.32 (brs, 1H, 1-CH), 3.09 (brs, 1H, 5-CH), 2.48 (s, 3H, NCH_3), 2.28 (t, 2H, *J* = 7.9 Hz, CH_2CO), 2.12 (dd, 2H, *J* = 6.3, 3.4 Hz), 1.80−1.20 (m, 8H, CH_3*CH_2*, 2,3,4-CH_2), 0.94 (t, 3H, *J* = 7.8 Hz, *CH_3*CH_2CH_2CO).

8-Methyl-6β-(*N,N*-dimethylcarbamyloxy)-8-azabicyclo[3.2.1]octane (4B) (6β-(*N,N*-Dimethylcarbamyloxy)tropane). 6β-Tropanol (141 mg, 1.0 mmol) was dissolved in THF (2 mL) and cooled to 0 °C, followed by addition of NaH (400 mg, 10 mmol, 60% dispersion in mineral oil). The mixture was stirred at room temperature for 10 min, followed by addition of dimethylcarbamyl chloride (538 mg, 5.0 mmol, 460 μL). The mixture was stirred at room temperature overnight, then 5% HCl solution (10 mL) was added very slowly. The mixture was washed with diethyl ether (2 × 10 mL). The aqueous layer was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 10 mL). After the removal of solvent under reduced pressure, the residue was chromatographed (CH_2Cl_2/MeOH = 9/1) to give **4A** (127 mg, 60%) as a colorless oil. It was converted to hydrochloride salt as white crystals. **4B**·HCl: mp 170−171 °C. Free base: CI-MS *m*/*z* 213 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 5.08 (dd, 1H, *J* = 6.8, 3.9 Hz, 6α-CH), 3.32 (brd, 1H, *J* = 3.9 Hz, 1-CH), 3.14 (brs, 1H, 5-CH), 2.90 (brs, 6H, $(CH_3)_2N$), 2.49 (s, 3H, NCH_3), 2.17−2.07 (m, 2H, 7-CH_2), 1.79−1.26 (m, 6H, 2,3,4-CH_2). **4B**·HCl: Anal. ($C_{11}H_{20}N_2O_2$·HCl·0.8H_2O) C, H, N.

8-Methyl-8-azabicyclo[3.2.1]octyl 6β-Methansulfonate (5B). 6β-Tropanol (141 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (2 mL), followed by addition of Et_3N (170 μL) and methanesulfonyl chloride (139 mg, 1.23 mmol, 94 μL). The mixture was stirred at room temperature overnight and poured into saturated $NaHCO_3$ solution (5 mL). The CH_2Cl_2 layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). After the removal of solvent under reduced pressure, the residue was chromatographed (CH_2Cl_2/MeOH = 9/1) to give **5B** (219 mg, 100%) as a colorless oil. It was converted to hydrochloride salt as white crystals. **5B**·HCl: mp 190−191 °C. Free base: CI-MS *m*/*z* 220 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 5.16 (dd, 1H, *J* = 6.8, 2.9 Hz, 6α-CH), 3.39−3.35 (m, 2H, 1,5-CH), 3.03 (s, 3H, CH_3O_2SO), 2.50 (s, 3H, NCH_3), 2.37 (ddd, 1H, *J* = 14.6, 6.8, 2.9 Hz, 7α-CH), 2.19 (dd, 1H, *J* = 14.6, 7.8 Hz, 7α-CH), 1.86−1.23 (m, 6H, 2,3,4-CH_2). **5B**·HCl: Anal. ($C_9H_{17}NO_3S$·HCl) C, H, N.

8-Methyl-8-azabicyclo[3.2.1]octyl 6β-(2′,2′,2′-Trichloroethylcarbonate) (6B). 6β-Tropanol (141 mg, 1.0 mmol) was dissolved in toluene (7 mL), followed by addition of K_2CO_3 (138 mg, 1 mmol) and 2,2-trichloroethyl chloroformate (265 mg, 1.25 mmol, 172 μL). The mixture was refluxed with stirring for 2 days. After cooling to room temperature, the mixture was poured into H_2O (10 mL) and extracted with diethyl ether (3 × 10 mL). After the removal of solvent under reduced pressure, the residue was chromatographed (CH_2Cl_2/MeOH = 40/1) to give **6B** (195 mg, 62%) as a colorless oil. It was converted to hydrochloride salt as white crystals. **6B**·HCl: mp 201−203 °C. Free base: CI-MS *m*/*z* 318 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 5.12 (dd, 1H, *J* = 7.9, 4.0 Hz, 6α-CH), 4.77 (s, 2H, Cl_3CH_2OCO), 3.37 (brs, 1H, 1-CH), 3.25 (brs, 1H, 5-CH), 2.49 (s, 3H, NCH_3), 2.27 (ddd, 1H, *J* = 14.7, 7.8, 4.0 Hz, 7α-CH), 2.18 (dd, 1H, *J* = 14.8, 7.8 Hz, 7α-CH), 1.81−1.25 (m, 6H, 2,3,4-CH_2). **6B**·HCl: Anal. ($C_{11}H_{16}Cl_3NO_3$·HCl) C, H, N.

8-Methyl-6β-cyclohexanecarbonyloxy-8-azabicyclo[3.2.1]octane (7B) (6β-Cyclohexanecarbonyloxytropane). In a similar way to the preparation of **2B**, compound **7B** was obtained from 6β-tropanol as a colorless oil, yield 99.5%. It was converted to hydrochloride salt as white crystals. **7B**·HCl: mp 216−217 °C. Free base: CI-MS *m*/*z* 212 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 5.13 (t, 1H, *J* = 5.9, 4.8 Hz, 6α-CH), 3.32 (brs, 1H, 1-CH), 3.07 (brs, 1H, 5-CH), 2.47 (s, 3H, NCH_3), 2.32−2.23 (m, 1H, CHCO), 2.10 (t, 2H, *J* = 4.9 Hz, 7-CH_2), 2.01−1.19 (m, 16H, 8xCH_2). **7B**·HCl: Anal. ($C_{15}H_{25}NO_2$·HCl) C, H, N.

8-Methyl-6β-benzoyloxy-8-azabicyclo[3.2.1]octane (8B) (6β-Benzoyloxytropane). 6β-Tropanol (141 mg, 1.0 mmol) was dissolved in diethyl ether (10 mL), followed by addition of Et_3N (2.2 mmol, 306 μL) and benzoyl chloride (310 mg, 2.2 mmol, 256 μL). The mixture was stirred at room temperature overnight and poured into saturated $NaHCO_3$ solution (10 mL). The ether layer was separated and the aqueous layer was extracted with diethyl ether (2 × 10 mL). After the removal of solvent under reduced pressure, the residue was chromatographed (CH_2Cl_2/MeOH = 9/1) to give **8B** (245 mg, 100%) as a colorless oil. It was converted to hydrochloride salt as white crystals. **8B**·HCl: mp 212−214 °C. Free base: CI-MS *m*/*z* 246 (MH$^+$); ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 2H, *J* = 7.8 Hz, 2′,6′-CH), 7.65 (t, 1H, *J* = 7.8, 6.8 Hz, 4′-CH), 7.50 (t, 2H, *J* = 7.8, 6.8 Hz), 5.51 (dd, 1H, *J* = 7.8, 3.9 Hz, 6α-CH), 4.03 (brd, 1H, *J* = 6.8 Hz, 1-CH), 3.87 (brs, 1H, 5-CH), 3.00 (s, 3H, NCH_3), 2.76−2.55 (m, 2H, 7-CH_2), 1.98−1.55 (m, 6H, 2,3,4-CH_2). **8B**·HCl: Anal. ($C_{15}H_{19}NO_2$·HCl) C, H, N.

8-Methyl-6β-(*p*-nitrobenzoyloxy)-8-azabicyclo[3.2.1]-octane (9B) (6β-(*p*-Nitrobenzoyloxy)tropane). In a similar way to the preparation of **8B**, compound **9B** was obtained from 6β-tropanol as yellow crystal, yield 78%. **9B**: mp 92−93 °C; CI-MS *m/z* 291 (MH^+); ^1H NMR ($CDCl_3$) δ 8.29 (d, 2H, J = 9.8 Hz, 2′,6′-CH), 8.20 (d, 2H, J = 8.7 Hz, 3′,5′-CH), 5.42 (dd, 1H, J = 6.9, 3.9 Hz, 6β-CH), 3.42 (brs, 1H, 1-CH), 3.28 (brs, 1H, 5-CH), 2.55 (s, 3H, NCH_3), 2.35−2.22 (m, 2H, 7-CH_2), 1.87−1.30 (m, 6H, 2,3,4-CH_2). Anal. ($C_{15}H_{18}N_2O_4$) C, H, N.

8-Methyl-6β-(*o*-methoxybenzoyloxy)-8-azabicyclo[3.2.1]-octane (10B) (6β-(*o*-Methoxybenzoyloxy)tropane). In a similar way to the preparation of **8B**, compound **10B** was obtained from 6β-tropanol as a colorless oil, yield 95%. It was converted to hydrochloride salt as white crystals. **10B**·HCl: mp 172−173 °C. Free base: CI-MS *m/z* 276 (MH^+); ^1H NMR ($CDCl_3$) δ 7.78 (d, 1H, J = 9.8 Hz, 6′-CH), 7.55 (t, 1H, J = 8.8, 7.8 Hz, 5′-CH), 7.03 (t, 1H, J = 7.8 Hz, 4′-CH), 7.01 (d, 1H, J = 8.8 Hz, 3′-CH), 5.49 (dd, 1H, J = 7.8, 3.9 Hz, 6β-CH), 3.98 (brs, 1H, 1-CH), 3.88 (s, 3H, OCH_3), 3.85 (brs, 1H, 5-CH), 3.01 (s, 3H, NCH_3), 2.77−2.14 (m, 2H, 7-CH_2), 1.96−1.46 (m, 6H, 2,3,4-CH_2). **10B**·HCl: Anal. ($C_{16}H_{21}NO_3\cdot HCl\cdot H_2O$) C, H, N.

8-Methyl-6β-(*p*-methoxybenzoyloxy)-8-azabicyclo[3.2.1]-octane (11B) (6β-(*p*-Methoxybenzoyloxy)tropane). In a similar way to the preparation of **8B**, compound **11B** was obtained from 6β-tropanol as a colorless oil, yield 79%. It was converted to hydrochloride salt as white crystals. **11B**·HCl: mp 164−65 °C. Free base: CI-MS *m/z* 276 (MH^+); ^1H NMR ($CDCl_3$) δ 7.98 (d, 2H, J = 8.8 Hz, 2′,6′-CH), 6.92 (d, 2H, J = 8.8 Hz, 3′,5′′-CH), 5.37 (dd, 1H, J = 7.8, 3.9 Hz, 6β-CH), 3.86 (s, 3H, OCH_3), 3.41 (brd, 1H, J = 5.8 Hz, 1-CH), 3.28 (brs, 1H, 5-CH), 2.56 (s, 3H, NCH_3), 2.34−2.19 (m, 2H, 7-CH_2), 1.83−1.32 (m, 6H, 2,3,4-CH_2). **11B**·HCl: Anal. ($C_{16}H_{21}NO_3\cdot HCl\cdot 2.5H_2O$) C, H, N.

8-Methyl-6β-(*N*-phenylcarbamyloxy)-8-azabicyclo[3.2.1]-octane (12B) (6β-(*N*-Phenylcarbamyloxy)tropane). 6β-Tropanol (71 mg, 0.5 mmol) was dissolved in diethyl ether (2 mL) and Na (∼1 mg) was added. The mixture was stirred at room temperature for 10 min, followed by addition of phenyl isocyanate (72 mg, 0.6 mmol, 65 μL). The mixture was stirred at room temperature for 10 min, then evaporated in vacuo to remove the solvent. To the residue was added H_2O (2 mL) slowly. The mixture was extracted with CH_2Cl_2 (2 × 5 mL). After the removal of solvent under reduced pressure, the residue was chromatographed (CH_2Cl_2/MeOH = 9/1) to give **12B** (130 mg, 100%) as a colorless oil. It was converted to hydrochloride salt as white crystals. **12B**·HCl: mp 220−222 °C. Free base: CI-MS *m/z* 261 (MH^+); ^1H NMR ($CDCl_3$) δ 7.39 (m, 4H, 2′,3′,5′,6′-CH), 7.06 (t, 1H, J = 6.9 Hz, 4′-CH), 6.68 (brs, 1H, PhNHCO), 5.08 (t, 1H, J = 5.8, 4.9 Hz, 6α-CH), 3.36 (brs, 1H, 1-CH), 3.19 (brs, 1H, 5-CH), 2.53 (s, 3H, NCH_3), 2.18 (t, 2H, J = 4.7, 3.9 Hz, 7-CH_2), 1.90−1.17 (m, 6H, 2,3,4-CH_2). **12B**·HCl: Anal. ($C_{15}H_{20}N_2O_2\cdot HCl\cdot 0.5H_2O$) C, H, N.

6β-Propionyloxy-8-azabicyclo[3.2.1]octane (2A) (6β-Propionyloxynortropane). Compound **2B** (165 mg, 0.84 mmol) was dissolved in toluene (3 mL), and 2,2,2-trichloroethyl chloroformate (0.6 mL, 4.4 mmol) was added. The reaction mixture was refluxed overnight, cooled and poured into 5% HCl solution (20 mL). The toluene layer was separated and the aqueous layer was extracted with toluene (2 × 10 mL). The combined toluene layers were washed with brine and dried (Na_2SO_4). After the removal of solvent in vacuum, the residue was chromatographed, first eluting with CH_2Cl_2 to remove unreacted chloroformate, then with eluent (CH_2Cl_2/MeOH = 15/1). After removal of solvent in vacuo, the residue was dissolved in AcOH (2 mL), and then zinc powder (0.5 g) was added. The mixture was stirred vigorously at room temperature for 2 days and filtered on Celite 535. The filtrate was evaporated in vacuum, and the residue was dissolved in H_2O (10 mL) and washed with diethyl ether (10 mL). The aqueous layer was neutralized with concentrated ammonium hydroxide and extracted with CH_2Cl_2 (3 × 15 mL). The combined CH_2Cl_2 layers were washed with brine, dried (Na_2SO_4) and evaporated in vacuum to give **2A** as a colorless oil (104 mg, 68%) which was treated with oxalic acid (43 mg, 0.47 mmol, in ether) to give **2A**·oxalate as a white powder. **2A**·oxalate: mp 140−141 °C. Free base: CI-MS *m/z* 184 (MH^+); ^1H NMR ($CDCl_3$) δ 5.14 (dd, 1H, J = 6.9, 3.0 Hz, 6α-CH), 3.62 (brd, 1H, J = 6.8 Hz, 1-CH), 3.30 (brs, 1H, 5-CH), 2.30 (dd, 2H, J = 15.6, 7.8 Hz, CH_2CO), 2.21 (1H, dd, J = 13.7, 6.8 Hz, 7α-CH), 1.87 (b.s, 1H, NH), 1.77 (ddd, 1H, J = 13.7, 6.9, 3.0 Hz, 7α-CH), 1.68−1.37 (m, 6H, 2,3,4-CH_2). **2A**·oxalate: Anal. ($C_{10}H_{17}NO_2C_2H_2O_4\cdot 0.2H_2O$) C, H, N.

6β-Butyryloxy-8-azabicyclo[3.2.1]octane (3A) (6β-Butyryloxynortropane). In a similar way to the preparation of **2A**, compound **3A** was obtained from **3B** as a colorless oil, yield 29%. It was converted to oxalate salt as white crystals. **3A**·oxalate: mp 129−130 °C. Free base: CI-MS *m/z* 198 (MH^+); ^1H NMR ($CDCl_3$) δ 5.14 (dd, 1H, J = 6.9, 3.0 Hz, 6α-CH), 3.63 (brd, 1H, J = 6.8 Hz, 1-CH), 3.30 (brs, 1H, 5-CH), 2.26 (t, 2H, J = 7.9 Hz, CH_2CO), 2.21 (dd, 1H, J = 14.2, 7.3 Hz, 7α-CH), 1.87−1.26 (m, 10H, NH, 7α-CH, CH_3*CH_2*, 2,3,4-CH_2), 0.94 (t, 3H, J = 7.8 Hz, *CH_3*CH_2CH_2CO). **3A**·oxalate: Anal. ($C_{11}H_{19}NO_2C_2H_2O_4$) C, H, N.

6β-(*N,N*-Dimethylcarbamyloxy)-8-azabicyclo[3.2.1]-octane (4A) (6β-(*N,N*-Dimethylcarbamyloxy)nortropane). In a similar way to the preparation of **2A**, compound **4A** was obtained from **4B** as a colorless oil, yield 91%. It was converted to oxalate salt as white crystals. **4A**·oxalate: mp 215−216 °C. Free base: CI-MS *m/z* 199 (MH^+); ^1H NMR ($CDCl_3$) δ 5.06 (dd, 1H, J = 7.6, 2.5 Hz, 6α-CH), 3.62 (brd, 1H, J = 6.8 Hz, 1-CH), 3.34 (brs, 1H, 5-CH), 2.90 (b.s, 6H, $(CH_3)_2N$), 2.20 (dd, 1H, J = 14.0, 7.3 Hz, 7α-CH), 1.85−1.38 (m, 8H, NH, 7α-CH, 2,3,4-CH_2). **4A**·oxalate: Anal. ($C_{10}H_{18}N_2O_3\cdot C_2H_2O_4\cdot H_2O$) C, H, N.

8-Azabicyclo[3.2.1]octyl 6β-Methansulfonate (5A). In a similar way to the preparation of **2A**, compound **5A** was obtained from **5B** as a colorless oil, yield 63%. It was converted to oxalate salt as white crystals. **5B**·oxalate: mp 203−205 °C. Free base: CI-MS *m/z* 296 (MH^+); ^1H NMR ($CDCl_3$) δ 5.17 (dd, 1H, J = 7.3, 2.4 Hz, 6α-CH), 3.71−3.69 (m, 1H, 1-CH), 2.57 (brs, 1H, 5-CH), 3.03 (s, 3H, CH_3O_2SO), 2.29 (dd, 1H, J = 14.6, 6.9 Hz, 7α-CH), 2.18 (brs, 1H, NH), 2.08−2.02 (m, 1H, 7α-CH), 1.73−1.59 (m, 6H, 2,3,4-CH_2). **5B**·oxalate: Anal. ($C_8H_{15}NO_3S\cdot C_2H_2O_4$) C, H, N.

6β-Cyclohexanecarbonyloxy-8-azabicyclo[3.2.1]-octane (7A) (6β-Cyclohexanecarbonyloxynortropane). In a similar way to the preparation of **2A**, compound **7** was obtained from **7B** as a colorless oil, yield 36.4%. It was converted to oxalate salt as white crystals. **7A**·oxalate: mp 163−165 °C. Free base: CI-MS *m/z* 238 (MH^+); ^1H NMR ($CDCl_3$) δ 5.12 (dd, 1H, J = 7.3, 2.4 Hz, 6α-CH), 3.62 (brs, 1H, 1-CH), 3.28 (brs, 1H, 5-CH), 2.27−2.17 (m, 2H, NH, CHCO), 1.86−1.22 (m, 18H, 9xCH_2). **7A**·oxalate: Anal. ($C_{14}H_{23}NO_2\cdot C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

6β-Benzoyloxy-8-azabicyclo[3.2.1]octane (8A) (6β-Benzoyloxynortropane). In a similar way to the preparation of **2A**, compound **8A** was obtained from **8B** as a colorless oil, yield 38.7%. It was converted to oxalate salt as white crystals. **8A**·oxalate: mp 172−174 °C. Free base: CI-MS *m/z* 232 (MH^+); ^1H NMR ($CDCl_3$) δ 8.03 (d, 2H, J = 6.9 Hz, 2′, 6′-CH), 7.56 (t, 1H, J = 7.8, 5.9 Hz, 4′-CH), 7.44 (t, 2H, J = 7.8 Hz, 3′,5′-CH), 5.38 (dd, 1H, J = 7.3, 2.4 Hz, 6α-CH), 3.70 (brd, 1H, J = 6.9 Hz, 1-CH), 3.49 (brs, 1H, 5-CH), 2.33 (dd, 1H, J = 13.6, 6.8 Hz, 7α-CH), 1.99−1.43 (m, 8H, 1-NH, 7α-CH, 2,3,4-CH_2). **8A**·oxalate: Anal. ($C_{14}H_{17}NO_2\cdot C_2H_2O_4$) C, H, N.

6β-(*p*-Nitrobenzoyloxy)-8-azabicyclo[3.2.1]octane (9A) (6β-(*p*-Nitrobenzoyloxy)nortropane). In a similar way to the preparation of **2A**, compound **9A** was obtained from **9B** as a colorless oil, yield 27.1%. It was converted to oxalate salt as white crystals. **9A**·oxalate: mp 144−146 °C. Free base: CI-MS *m/z* 277 (MH^+); ^1H NMR ($CDCl_3$) δ 7.84 (d, 2H, J = 7.8 Hz, 2′,6′-CH), 6.63 (d, 2H, J = 8.8 Hz, 3′,5′-CH), 5.33 (dd, 1H, J = 7.3, 2.4 Hz, 6α-CH), 3.68 (brd, 1H, J = 6.9 Hz, 1-CH), 3.46 (brs, 1H, 5-CH), 2.30 (dd, 1H, J = 13.6, 6.8 Hz, 7α-CH), 2,17 (s, 1H, NH), 1.92 (ddd, 1H, J = 13.6, 6.8, 2.0 Hz, 7α-CH), 1.70−1.41 (m, 6H, 2,3,4-CH_2). **9A**·oxalate: Anal. ($C_{14}H_{16}N_2O_2\cdot C_2H_2O_4$) C, H, N.

6β-(*o*-Methoxybenzoyloxy)-8-azabicyclo[3.2.1]octane (10A) (6β-(*o*-Methoxybenzoyloxy)nortropane). In a similar

way to the preparation of **2A**, compound **10A** was obtained from **10B** as a colorless oil, yield 51%. It was converted to oxalate salt as white crystals. **10A**·oxalate: mp 145−146 °C. Free base: CI-MS *m/z* 262 (MH^+); ^1H NMR ($CDCl_3$) δ 7.76 (dd, 2H, J = 7.8, 2.0 Hz, 6′-CH), 7.46 (d, 1H, J = 7.6, 2.0 Hz, 3′-CH), 6.99−6.95 (m, 2H, 4′,5′-CH), 5.37 (dd, 1H, J = 6.9, 2.9 Hz, 6α-CH), 3.90 (s, 3H, CH_3O), 3.66 (brd, 1H, J = 7.8 Hz, 1-CH), 3.46 (brs, 1H, 5-CH), 2.31 (dd, 1H, J = 14.2, 7.3 Hz, 7α-CH), 1.96−1.41 (m, 8H, NH, 7α-CH, 2,3,4-CH_2). **10A**·oxalate: Anal. ($C_{15}H_{19}NO_3$·$C_2H_2O_4$) C, H, N.

6β-(*p*-Methoxybenzoyloxy)-8-azabicyclo[3.2.1]octane (11A) (6β-(*p*-Methoxybenzoyloxy)nortropane). In a similar way to the preparation of **2A**, compound **11A** was obtained from **11B** as a colorless oil, yield 58%. It was converted to oxalate salt as white crystals. **11A**·oxalate: mp 112−113 °C. Free base: CI-MS *m/z* 262 (MH^+); ^1H NMR ($CDCl_3$) δ 7.98 (d, 2H, J = 8.8 Hz, 2′,6′-CH), 6.91 (d, 2H, J = 8.8 Hz, 3′,5′-CH), 5.35 (dd, 1H, J = 7.3, 2.5 Hz, 6α-CH), 3.86 (s, 3H, CH_3O), 3.68 (brd, 1H, J = 6.9 Hz, 1-CH), 3.47 (brs, 1H, 5-CH), 2.31 (dd, 1H, J = 13.7, 6.9 Hz, 7α-CH), 2.17−1.42 (m, 8H, NH, 7α-CH, 2,3,4-CH_2). **11A**·oxalate: Anal. ($C_{15}H_{19}NO_3$·$C_2H_2O_4$·$1.2H_2O$) C, H, N.

6β-(*N*-Phenylcarbamyloxy)-8-azabicyclo[3.2.1]octane (12A) (6β-(*N*-Phenylcarbamyloxy)nortropane). In a similar way to the preparation of **2A**, compound **12A** was obtained from **12B** as white crystals, yield 74%. **23**: mp 157−159 °C; CI-MS *m/z* 247 (MH^+); ^1H NMR ($CDCl_3$) δ 7.37 (d, 2H, J = 7.9, 2′,6′-CH), 7.31 (t, 2H, J = 7.9 Hz, 3′,5′-CH), 7.06 (t, 1H, J = 7.3 Hz, 4′-CH), 6.77 (b.s, 1H, PhNHCO), 5.19 (dd, 1H, J = 7.3, 2.4 Hz, 6α-CH), 3.36 (brd, 1H, J = 6.8 Hz, 1-CH), 3.44 (brs, 1H, 5-CH), 2.25 (dd, 1H, J = 14.7, 6.9 Hz, 7α-CH), 1.94−1.25 (m, 8H, NH, 7α-CH, 2,3,4-CH_2). Anal. ($C_{14}H_1N_2O_2$·$0.4H_2O$) C, H, N.

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 12.

Binding Assays. Preparation of membranes and binding assays with [^{3}H]quinuclidinyl benzilate and [^{3}H]oxotremorine macetate were as previously described in detail for rat brain membranes[13] and for [^{3}H]quinuclidinyl benzilate binding to membranes of transfected CHO cells.[13]

Binding assays for [^{3}H]oxotremorine macetate and membranes of transfected CHO cells were as follows: Briefly assays contained 2 nM [^{3}H]oxotremorine macetate, 2.5 mM sodium phosphate buffer (pH 7.4), 5 mM $MgCl_2$, and 100 μL of membrane suspension in a final volume of 0.5 mL. Nonspecific binding was determined with 5 μM atropine. Assays were initiated by addition of the membrane suspension and were for 2 h at 25 °C in triplicate. Assays were terminated by filtration through Whatman GF/B filters, which had been 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), placed in vials with 4 mL of Hydrofluor scintillation fluid, and counted for tritium.

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.[27] Briefly, the m_4-receptor-transfected 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[28] 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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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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