The Synthesis and Biochemical Pharmacology of Enantiomerically Pure Methylated Oxotremorine Derivatives

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Previous pharmacological studies of methylated oxotremorine derivatives bearing substituents at the 3-, 4-, and 5-positions of the pyrrolidinone ring have been conducted using racemic mixtures, and not with optically active compounds. The synthesis and radioligand binding data of optically active, methylated oxotremorine derivatives at the 3- and 4-positions are described. There are significant pharmacological differences between the 3- and 4-position derivatives. The 4-position enantiomers have weak, approximately equal affinity and antagonist-like profiles, whereas the 3-position enantiomers have significantly different affinities and partial agonist-like profiles.

Senile Dementia of the Alzheimer's Type (SDAT) is a neurodegenerative disease that results in progressive dementia and memory impairment. Autopsied brain tissue from SDAT patients has shown a consistent and significant decrease in cholinergic markers (among others), such as choline acetyltransferase and acetylcholine esterase, most notably in the nucleus basalis of Meynert, cerebral cortex, and hippocampus.[1] It has been suggested that the degeneration occurs presynaptically, while the postsynaptic muscarinic receptors remain intact.[2] These observations, in part, form the basis for the cholinergic hypothesis of age-related memory loss.[3] A series of drug discovery projects[4] have been initiated in our geriatric program to find cholinergic agents to ameliorate the cognitive symptoms and to possibly retard the behavioral sequelae[5] of this degenerative disease.

The introduction of a substitutent into a neurotransmitter mimic may decrease the affinity of the compound for its receptor by introducing steric hindrance between it and the receptor binding domain. Rather than a disadvantage, this effect may be useful as a possible method to construct molecules with the optimal degree of partial agonism and possibly with measurable pharmacological attributes such as receptor subtype or functional selectivity. The addition of a methyl group to a series of cholinergic agonists has been shown to dramatically decrease *in vivo* and *in vitro* cholinergic agonist activity. One such investigation was the synthesis and pharmacological studies of oxotremorine (Figure 1).[6] The systematic addition of a methyl group to oxotremorine produced compounds with pronounced pharmacological differences. These results provided evidence for the amount of steric tolerance that was available in oxotremorine for lipophilic substitutents within the ligand binding domain.

One drawback to the original work was that the pharmacological studies of the methylated compounds at the 3- and 4-position of the pyrrolidinone ring were conducted using racemic mixtures and not pure enantiomers. The reported data provided a detailed account of the general trend in activity for the compounds. However, the current program goal was to search for targets with selective pharmacological attributes, and the comparison of new optically active targets with racemic standards was not satisfactory. As a result, synthetic strategies to the optically active forms of the 3- and 4-methylated derivatives of oxotremorine and its congeners were sought. This report describes the syntheses of the target molecules (Figure 2) using either chiral auxiliary methodology or chiral starting materials derived from natural sources. The preliminary biochemical pharmacology of these target structures is also presented.

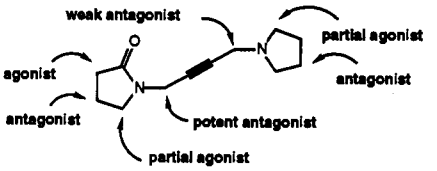


Figure 1. Structure–activity relationships for methylated oxotremorine derivatives.

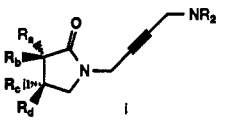
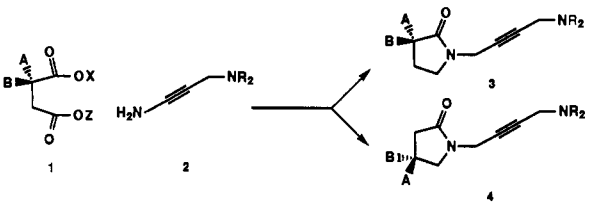


Figure 2. Targeted structures. One of the substituents R_a, R_b, R_c, R_d is CH_3, the remaining are H.

Scheme I



Chemistry

Simple optical resolution of racemic targets **I** would have been an attractive approach since equal amounts of the two enantiomers were needed for the study. However, the success of this methodology appeared doubtful since the optical center and the amine moiety were not in close proximity. Conceptually the synthesis of the four different methylated derivatives could be achieved by the differential attack of diamine **2** with an enantiomeric pair of carbonyl differentiated succinates **1** (Scheme I). This type of approach was sucessfully used in the preparation of the hydroxylated oxotremorine derivatives from D- and L-malic acids.[4b] However, in this case it was more convenient to treat the synthesis of the the 3- and 4-methylated derivatives separately.

The 3-substituted pyrrolidinone derivatives **12ab** and **13ab** were prepared from the chiral auxiliary oxazolidi-

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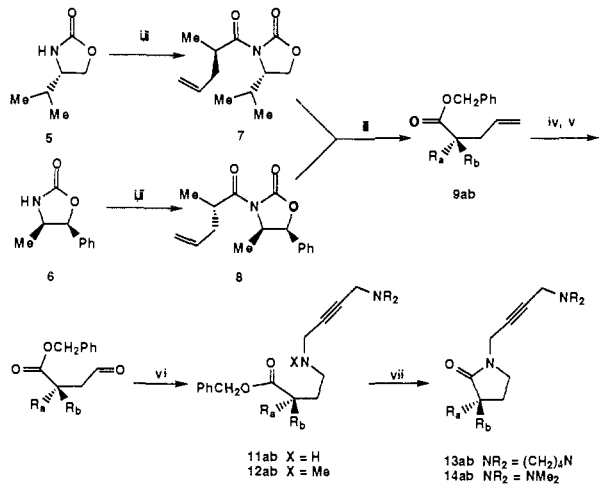
Scheme II[a]



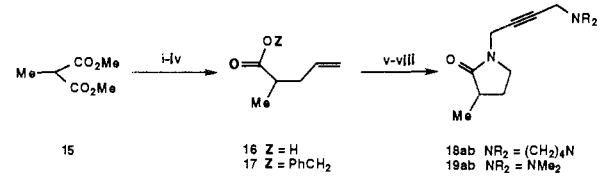
[a] (i) *n*-BuLi, CH_3CH_2COCl, THF, −78 °C; (ii) LDA, CH_2=$CHCH_2$-Br, THF, −78 °C; (iii) *n*-BuLi, $PhCH_2OH$; (iv) O_3, CH_2Cl_2, −78 °C; (v) Zn, HOAc; (vi) $NaBH_3CN$, HOAc, **24** or **25**; (vii) MeOH, HCl.

nones **5** and **6**[7] (Scheme II). Acylation of oxazolidinones **5** and **6** with propionyl chloride using *n*-butyllithium as base, followed by alkylation of the intermediates with allyl bromide using lithium diisopropylamide as base, afforded the corresponding methylated pentenoates **7** and **8**. The absolute configuration and optical rotation of the pure enantiomers **7** and **8** have been previously determined.[8] Treatment of **7** and **8** with a THF solution of benzyl alcohol and 1.5 equiv of *n*-butyllithium produced benzyl esters **9ab**. Ozonolysis of **9ab** in methylene chloride followed by a reductive workup with zinc and acetic acid gave aldehydes **10ab**, which were used directly after plug filtration through silica gel. Reductive amination of **10ab** with diamines **24** and **25** in the presence of sodium cyanoborohydride in a mixture of acetic acid and methanol gave initially the ring opened diamine **11ab** which ring closed to the target methylated oxotremorine derivative **13ab** and **14ab**, respectively.

The transformation of **5** and **6** into **13ab** and **14ab** was straightforward with overall isolated yields ranging from 30 to 40%. Some comments on the sequence of steps are required. Removal of the chiral auxiliary and formation of the benzyl ester prior to the reductive amination was necessary. Diamines **24** and **25** were found to react with the oxazolidinone moiety as well as the aldehyde functionality to produce a series of byproducts. Removal of formaldehyde, a byproduct in the ozonolysis reaction, by plug filtration through silica gel was important. If the formaldehyde was not removed, the major product in the reductive amination was the *N*-methyl derivative of the ring opened compound **11ab**. The side product **12ab** had an R_f value similar to the product which complicated the product isolation and purification.

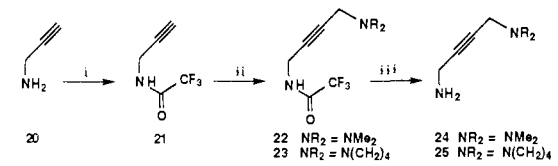
The racemic methylated compounds could be obtained by mixing an equal amount of the two enantiomers or by direct synthesis. The direct synthesis proved quite simple [8] (Scheme III). Alkylation of dimethyl methylmalonate **15** with allyl bromide using sodium methoxide as base, followed by saponification and decarboxylation, afforded 2-methyl-4-pentenoic acid **16**. Esterification with benzyl alcohol gives the racemic form of **9ab**, compound **17**. Conversion of **17** into the racemic targets **18** and **19** paralleled the chemistry used to prepare the optically active materials.

Scheme III[a]



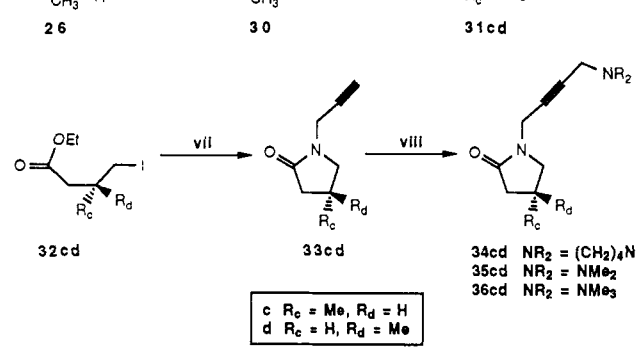
[a] (i) NaOMe, CH_2=$CHCH_2Br$; (ii) NaOH, EtOH, H_2O; (iii) 180 °C; (iv) $PhCH_2OH$, TsOH; (v) O_3, CH_2Cl_2, 78 °C; (vi) Zn, HOAc; (vii) $NaBH_3CN$, HOAc, **25** or **26**; (viii) MeOH, HCl.

Scheme IV[a]



[a] (i) $(CF_3CO)_2O$, CH_2Cl_2; (ii) HNR_2, $(CH_2O)_n$, CuCl, HOAc, dioxane; (iii) HCl, EtOH, heat or $NaBH_4$, EtOH.

Scheme V[a]



[a] (i) Dihydropyran, HCl; (ii) LAH, THF; (iii) TsCl, Et_3N; (iv) NaCN, EtOH; (v) MeOH, HCl; (vi) TMSI, EtOH; (vii) propargylamine, Na_2CO_3, EtOH; (viii) HNR_2, CH_2O, CuCl, HOAc, dioxane, heat.

The diamine intermediates **24** and **25** were readily prepared in multigram scale[9] (Scheme IV). Treatment of propargylamine **20** with trifluoroacetic anhydride afforded trifluoroacetamide **21** which was reacted with either dimethylamine or pyrrolidine and paraformaldehyde in a mixture of acetic acid and dioxane to give the aminoacetamide **22** and **23**. Removal of the trifluoroacetamide group from **22** and **23** was achieved using either sodium borohydride in methanol, a nonacidic method, or ethanolic hydrogen chloride to afford the diamines **24** and **25**.

The synthesis of 4-methylated oxotremorine derivatives **34cd** and **35cd** used (*R*)- and (*S*)-4-methylbutyrolactones **31cd** as the key intermediates where the optical center was introduced from natural sources (Scheme V). The (*S*)-enantiomer **31d** was obtained commercially, and the (*R*)-enantiomer was readily prepared from (*S*)-3-hydroxy-2-methylpropionate **26**.[10] The four-step sequence for the conversion of **26** into **31d** was somewhat lengthy but proceeded in high yield. Protection of the hydroxyl group in **26** as the tetrahydropyranyl ether **27** followed by reduction of the ester group with lithium aluminum hydride afforded the monoprotected diol **28**. Conversion of the alcohol in **28** to the methanesulfonate **29** with methanesulfonyl chloride and triethylamine and displacenment of the methanesulfonate with cyanide yielded nitrile **30**. Treatment of **30** with methanolic hydrogen

Table I. Physical Properties of Methylated Oxotremorine Derivatives

compd	amine	config	$[\alpha]^{26}_D$,[a] deg	form[c]	method	yield, %	mp, °C
13a	Pyr	(*S*)-3	−19 (*c* = 1.33)	FB	A	49	oil
	Pyr	(*S*)-3	−7 (*c* = 1.025)	S			90–91
18	Pyr	(±)-3		FB			oil
	Pyr	(±)-3		S			oil
13b	Pyr	(*R*)-3	+20 (*c* = 1.560)	FB	A	54	oil
	Pyr	(*R*)-3	+7 (*c* = 1.36)	S			88–89
34c	Pyr	(*S*)-4	+9 (*c* = 1.46)	FB	B	75	oil
	Pyr	(*S*)-4	+3 (*c* = 1.0)	S			116–118
34d	Pyr	(*R*)-4	−9 (*c* = 1.03)	FB	B	85	oil
	Pyr	(*R*)-4	−3 (*c* = 1.0)	S			111–112
14a	NMe_2	(*S*)-3	−21 (c = 0.989)	FB	A		oil
19	NMe_2	(±)-3		FB	C		oil
14b	NMe_2	(*R*)-3	+22 (*c* = 0.980)	FB	A		oil
35c	NMe_2	(*S*)-4	+9 (*c* = 1.62)	FB	B	67	oil
	NMe_2	(*S*)-4	+4 (c = 0.97)	S			151–152
35d	NMe_2	(*R*)-4	−8 (c = 1.1)	FB	B	62	oil
	NMe_2	(*R*)-4	−3 (*c* = 1.0)	S			153–154
36c	NMe_3	(*S*)-4	+3 (c = 1.0)	S			148–149
36d	NMe_3	(*R*)-4	−2 (c = 1.0)	S			146–147

[a] Optical rotations are carried out in methylene chloride for the free bases and in methanol for the amine salts. [b] Pyr = pyrrolidinyl. [c] S = salt, FB = free base.

chloride afforded **31d** in 54% isolated yield which was significantly higher than the reported procedure where potassium hydroxide followed by acidification[11] was used.

Treatment of butyrolactones **31cd** with trimethylsilyl iodide in ethanol[11] followed by the addition of triethyl orthoformate afforded the iodo esters **32cd.** Reaction of iodo esters **32cd** with propargylamine in ethanol containing sodium carbonate as base gave, upon cyclization, the propargylpyrrolidinones **33cd**. The 4-methylated oxotremorine derivatives **34cd** and **35cd** were then prepared by reaction of **33cd** with paraformaldehyde and either pyrrolidine or dimethylamine in a mixture of acetic acid and dioxane containing a catalytic amount (5%) of cuprous chloride. Treatment of **35cd** with methyl iodide produced the corresponding methiodides **36cd.**

Pharmacology

Displacement of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) in muscarinic receptor binding assays has been used extensively as a rapid and efficient screen for test compounds that interact at cholinergic receptors.[12] Little information regarding the potential intrinsic activity or pharmacological selectivity of the compound for muscarinic receptor subtypes is obtained in this assay. Meaningful estimates of intrinsic activity and pharmacological selectivity can nevertheless be determined from the apparent equilibrium dissociation constants (K_i) for test compounds.[13,19] This information can be obtained using a series of assays that employ tritiated agonists as well as antagonists to label receptor populations in several different tissues.

A method for using the relative affinities (K_i values) of test compounds to displace [^{3}H]-*N*-methylscopolamine ([^{3}H]NMS, antagonist) and [^{3}H]oxotremorine-M ([^{3}H]-Oxo-M, agonist) as an index of their efficacy at cortical muscarinic receptors (NMS/OXO-M ratio) has been described.[13] Alternatively, [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB, antagonist) can be used to label both the high- and low-affinity states[14] and [^{3}H]-*cis*-[(methyltrimethylammonio)methyl]-1,3-dioxolane ([^{3}H]CD, agonist) to label the high affinity agonist state of the muscarinic receptor in competition assays.[15] The experimental conditions were chosen to maximize the difference between agonists and antagonists, and the ratio of K_i values for the two assays provides a useful index of intrinsic activity. Muscarinic antagonists display similar affinity in both binding assays (K_i ratio is ~ 1.0). Full muscarinic agonists displace [^{3}H]CD from the muscarinic receptor with high affinity relative to their displacement of [^{3}H]QNB. Thus, full agonists such as carbachol exhibit large ratios (K_i ratios ~ 500–2000). Compounds that display partial muscarinic agonist activity, such as oxotremorine, in functional assays have intermediate ratios (K_i ratio ~ 50–500). The ratio of K_I values determined in the two assays correlates well with the ability of agonists to stimulate cortical phosphatidylinositol turnover.[14]

To evaluate pharmacological selectivity of these analogs, displacement of [^{3}H]pirenzepine ([^{3}H]PZ) from rat cortical receptors was used in conjunction with displacement of [^{3}H]QNB from rat cardiac muscarinic receptors. Pirenzepine (PZ) is an antagonist that selectively labels a subpopulation of muscarinic receptors (M_1) in central and peripheral tissues relative to the classical antagonist QNB. Approximately 80% of rat cortical receptors labeled by [^{3}H]QNB bind PZ with high affinity (M_1) while the remaining receptors bind PZ with low affinity (M_2).[16] Using a low concentration of [^{3}H]PZ, the M_1 subpopulation of muscarinic receptors can be preferentially labeled. Rat cardiac receptors are essentially of the M_2 subtype with low affinity for PZ.[17] With this nearly homogeneous population, the M_2 subtype of receptors can be separately labeled using rat cardiac tissue and [^{3}H]QNB.

Guanine nucleotides shift M_2 muscarinic receptor affinity for agonists to a single low affinity state.[18] Displacement of [^{3}H]QNB from rat cardiac M_2 was performed in the absence and presence of 10 μM guanosine 5′-(β,γ-imidotriphosphate) (Gpp(NH)p) to assess binding of compounds to all affinity states and to the low-affinity state, respectively, of the muscarinic receptors.[19]

Muscarinic receptor (M_1) selectivity was estimated from the apparent K_i ratio of K_i QNB (heart) and K_i PZ (cortex), and potential M_2 agonist activity was determined in heart tissue from the apparent K_i ratio of K_i QNB + Gpp(NH)p and K_i QNB − without Gpp(NH)p. The results for the title compounds and a series of six standard ligands for the muscarinic receptor ranging from full agonist-like

Table II. Nonspecific Agonist Binding Properties of the Methylated Oxotremorine Derivatives and Standards

compd	R_n	NR_2	K_i, μM cortex rat[a] [^{3}H]QNB	K_i, μM cortex rat[a] [^{3}H]CD	ratio [^{3}H]QNB/[^{3}H]CD[b]
13[e]	R_a	Pyr	2.9 ± 0.67	0.055 ± 0.010	51
18[e]	$R_{a/b}$	Pyr	0.71 ± 0.03	0.015 ± 0.001	48
13b[e]	R_b	Pyr	0.43 ± 0.13	0.023 ± 8.9	19
34c[e]	R_c	Pyr	4.7 ± 0.66	2.5 ± 0.29	1.9
34d[e]	R_d	Pyr	2.3 ± 2.2	1.5 ± 0.11	1.6
14a	R_a	NMe_2	>100	0.63 ± 0.15	
19	$R_{a/b}$	NMe_2	22 ± 3.6	0.26 ± 0.010	85
14b	R_b	NMe_2	8.9 ± 1.1	0.071 ± 0.005	125
35c[e]	R_c	NMe_2	>1000	>10	
35d[e]	R_d	NMe_2	150	>10	
36c	R_c	NMe_2	>100	0.98 ± 0.21	
36d	R_d	NMe_2	>100	0.45 ± 0.14	
37, carbachol			5.0 ± 0.32	0.004 ± 0.001	1220
38, Oxo-M			0.91 ± 0.27	0.00078 ± 0.0001	1200
39, Oxo-2			2.9 ± 0.04	0.0063 ± 0.0004	460
40, oxotremorine			0.06 ± 0.01	0.0005 ± 0.0001	120
41, Oxo-Pip			0.12 ± 0.05	0.056 ± 0.011	2.2
42, BM-5			0.08 ± 0.02	0.004 ± 0.001	19.5

[a] Mean of 2–7 determinations. [b] The [^{3}H]QNB data is from rat cortex. [c] From one determination or a mean of two determinations. [d] The [^{3}H]QNB data is from rat heart. [e] Identical results (2–4 determinations) were obtained in parallel assays with the free base of the compound.

(carbachol, Oxo-M) to muscarinic antagonist-like (Oxo-Pip) from the five *in vitro* assays are presented in Tables II and III.

In general, the data for both the 3-methyl- and the 4-methyl-substituted pyrrolidinone derivatives parallel the previously described observations for the racemic compounds. The 3-methyl derivatives retain agonist-like activity, albeit weaker in both affinity and efficacy, relative to oxotremorine, and the 4-methyl derivatives possess antagonist-like effects similar to Oxo-Pip[20] but with significantly weaker affinity. In addition, the dimethylamine derivatives, when compared to the corresponding pyrrolidino derivatives, have weaker affinities (potency) for the receptors but have greater agonist-like effects (efficacy). The major advantage to these data are the subtle differences that are observed between the optical isomers.

Discussion

Our drug discovery program over the past several years has searched for substituted oxotremorine derivatives as therapeutic drug leads for the treatment of SDAT. The cholinergic hypothesis of memory deficits implies that the desired drug candidate should be an agonist-like compound which is selective for the disease-affected receptors, presumably postsynaptic and M1, while other muscarnic receptors in the periphery and brain, presumably M2, would be unaffected by the agonist-like compound with little or no side effects. The systematic efforts of Ringdahl[6] to pharmacologically characterize the effect of methyl substitution about the muscarinic agonist oxotremorine provided a starting point for this current work. Substitution at the 3- and 4-position on the pyrrolidinone ring of oxotremorine became the major focus and unfortunately, in the origional studies only racemic material substituted at these positions was characterized. To provide a reference point for future work the synthesis and pharmacological characterization of optically pure 3- and 4-methylpyrrolidinone ring substituted oxotremorine derivatives was undertaken. A ligand-based biochemical profile of the optically active forms of the methylated derivatives, when compared to carbachol and the unsubstituted oxotremorine derivatives, provides some insight into the type of target compound that should be selected as a potential therapeutic agent.

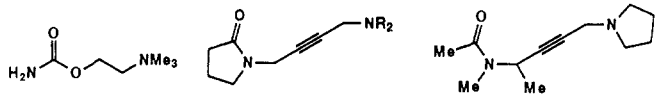


Figure 3. Muscarinic standards.

The series of six muscarinic agents that were selected as standards ranged from full agoinist-like compounds (carbachol, Oxo-M) to the reported antagonist-like compound, Oxo-Pip[20] (Figure 3, Tables II and III). In addition, three of the standards (oxotremorine, Oxo-2 and Oxo-M) are the nonmethylated references of the title compounds. The full muscarinic agonists (carbachol, Oxo-M), having [^{3}H]QNB/[^{3}H]CD ratios of ca. 1200, elicited strong guanine nucleotide shifts (G-shifts of 63 and 23, respectively), suggesting strong agonist-like effects in the heart, which are presumably M_2 mediated. The partial agonists oxotremorine and Oxo-2, having [^{3}H]QNB/[^{3}H]CD ratios of 120 and 420, produced equally large G-shifts (32 and 23) in the heart, suggesting strong M_2 agonist-like effects. The muscarinic antagonist Oxo-Pip, having a predicted antagonist [^{3}H]QNB/[^{3}H]CD ratio of 2.2, produced only a weak shift (5.0) in the concentration–response curve with the addition of the stable guanine nucleotide analog.

The oxotremorine related standard BM-5[21] has been characterized as both a weak partial M_1 agonist and as a M_2 antagonist. Hypothetically, this profile defines a putative prototype SDAT therapeutic agent. In the current series of biochemical assays, BM-5 produced a weak partial agonist profile, ([^{3}H]QNB/[^{3}H]CD ratio of 20) and it produced a weaker G-shift (2.5) than the antagonist Oxo-Pip (5.0). The current data provide evidence complementary to the data reported for BM-5 as a mixed M_1 partial agonist and M_2 antagonist.

The apparent K_i ratio of K_i [^{3}H]QNB (heart) to K_i [^{3}H]-PZ (cortex) does not provide a direct measure of a compound's M_1 selectivity or agonist-like effects. This ratio, when used in comparison to the ratios of other test compounds, provides the relative ranking for interaction at M_1 and M_2 receptors. The smaller the ratio the greater the M_2 interaction and the larger the ratio the greater the M_1 effects. Oxotremorine and the oxotremorine-like

Table III. Biochemical Data for M1 and M2 Selectivity of Methylated Oxotremorine Derivatives and Standards

compd	R_n	NR_2	[^{3}H]PZ K_i, μM rat[c]	[^{3}H]QNB K_i, μM heart rat[c]	[^{3}H]QNB + GppNHp K_i, μM heart	ratio [^{3}H]QNB + GppNHp/ [^{3}H]QNB	ratio [^{3}H]QNB + GppNHp/[^{3}H]PZ	ratio [^{3}H]QNB/ [^{3}H]PZ[d]
13a[e]	R_a	Pyr	2.2 ± 0.17	0.11 ± 0.1	3.3 ± 1.7	32.	1.5	0.05
18[e]	$R_{a/b}$	Pyr	0.29 ± 0.14	0.08 ± 0.003	1.1 ± 0.07	15.	3.9	0.26
13b[e]	R_b	Pyr	0.25 ± 0.06	0.09 ± 0.05	0.43 ± 0.17	4.9	1.8	0.36
34c[e]	R_c	Pyr	1.3 ± 0.33	13. ± 3.7	27. ± 2.3	2.0	21.	11.
34d[e]	R_d	Pyr	0.51 ± 0.1	3.8 ± 1.0	6.0 ± 1.3	1.6	12	12.
14a	R_a	NMe_2	>1078 ± 15	21 ± 6.1	30.	1.4.	0.4	0.27
19	$R_{a/b}$	NMe_2	212 ± 1.0	1.3 ± 0.53	13 ± 2.5	10.	1.1	0.11
14b	R_b	NMe_2	6.8 ± 0.49	0.24 ± 0.04	9.3 ± 3.4	38	1.4	0.04
35c[e]	R_c	NMe_2	>100	>100	>100	>100		
35d[e]	R_d	NMe_2	15	>100	>100	>100		
36c	R_c	NMe_2	>109.1 ± 0.75	21 ± 8.5	52 ± 9.0	2.4	5.7	2.3
36d	R_d	NMe_2	>108.3 ± 0.41	1.9 ± 0.62	>100	0.23		
37, carbachol			5.8.	2.3	160	69	28	0.39
38, Oxo-M			1.2 ± 0.09	0.06 ± 0.02	1.5 ± 0.02	25	1.3	0.05
39, Oxo-2			6.8 ± 0.69	0.07 ± 0.02	1.6 ± 0.01	23.	0.2	0.01
40, oxotremorine			0.12 ± 0.02	0.005 ± 0.002	0.16 ± 0.02	32	1.3	0.04
41, Oxo-Pip			0.13 ± 0.03	0.07 ± 0.03	0.35 ± 0.11	5.0	2.7	0.54
42, BM-5			0.04 ± 0.01	0.07	0.18	2.5	5.1	1.8

[a] Mean of 2–7 determinations. [b] The [^{3}H]QNB data is from rat cortex. [c] From one determination or a mean of two determinations. [d] The [^{3}H]QNB data is from rat heart. [e] Identical results (2–4 determinations) were obtained in parallel assays with the free base of the compound.

agonists (Oxo-M and Oxo-2) have relatively low [^{3}H]QNB/[^{3}H]PZ ratios which suggest a preference for heart muscarinic receptors and therefore M_2 selectivity. These data are supported by the large *G*-shift values for these compounds. The antagonist Oxo-Pip has a 10–50-fold weaker preference for the heart or M_2 receptors and, therefore, should be more M_1 selective. The increased preference for M_1 receptors is further supported by the weak *G*-shift ratio (5.0) or M_2 agonist-like effects. Oxo-Pip, however, is a functional antagonist (too little agonist character) and lies outside the scope of our interest. Taken together, compounds with full agonist-like effects have stronger preference for M_2 receptors. In a series of previously reported compounds, as the agonist-like effects decrease (partial agonism) the preference for M_2 receptors decreases faster than the preference for M_1 receptors.[22]

The weak partial agonist/antagonist BM-5, which was characterized pharmacologically using functional assays, can serve as a prototype for the SDAT therapeutic agent. The compound is a weak partial agonist ([^{3}H]QNB/[^{3}H]-CD ratio = 20), it produced little if any G-shift, suggesting M_2 antagonist-like profile, and relative to other oxotremorine-like compounds has a preference (selectivity) for M_1-like receptors.

The methylated derivatives, with no exception, produced weaker effects (higher K_i values) in all five receptor binding assays (Tables II and III) relative to their unsubstituted congeners. Steric size has a greater effect on the high affinity agonist binding site labeled by [^{3}H]CD than the binding sites labeled by [^{3}H]QNB. This trend is expressed in the lower ratios ([^{3}H]QNB/[^{3}H]CD) for the 3-methyl derivatives (**13a,13b, 18, 14a, 14b, 19**) and more significantly for the 4-methyl derivatives (**34c, 34d, 35c, 35d, 36c, 36d**). These agonist/antagonist profiles were predicted in the original racemic study; the 3-methylated derivatives were muscarinic agonists, albeit weaker than oxotremorine, and the 4-methylated derivatives were functional antagonists.[6]

The effect of the amine moiety is also parallel to that observed in the literature[23] for oxotremorine, Oxo-2, and Oxo-M in guinea pig ileum studies. The dimethylamino analog Oxo-2 had about one-fifth the potency of oxotremorine, both as a stimulant of the ileum and as a tremorogenic agent. This reduction in potency was entirely the result of the loss of affinity (consistent throughout), at least in the ileum, because Oxo-2 had 6-fold greater intrinsic efficacy than oxotremorine (increased [^{3}H]QNB/[^{3}H]CD ratio).

A comparison of oxotremorine and enantiomers **13a** and **13b** may provide some insight into to obtaining muscarinic agonists which are less M_2 selective (or more M_1 selective). The addition of an (*R*)-methyl group to oxotremorine (**13b**) produced a 2-fold decrease in affinity for the receptors labeled by [^{3}H]PZ in the cortex (M_1), whereas the addition of an (*S*)-methyl group (**13a**) produced a significantly larger 20-fold decrease in affinity. With cardiac muscarinic receptors ([^{3}H]QNB heart), the addition of either an (*R*)- or an (*S*)-methyl group (**13a** or **13b**) gave an equal 20-fold reduction in affinity. The preference of **13b** for pirenzepine labeled receptors (M_1) in the cortex is also expressed as the decrease in agonist-like effects in the heart (M_2). The effect of the stable guanine nucleotide, Gpp(NH)p, on **13a** (*G*-shift of 32) is equal and parallel to that for oxotremorine (*G*-shift of 32). The *G*-shift for **13b** (4.9) is significantly weaker than for oxotremorine (32) and is similar to that observed for BM-5 (2.5). These data suggest that **13b**, like BM-5, has significantly weaker agonist-like effects than **13a** and oxotremorine in the heart (M_2) while maintaining a similar affinity for pirenzipine-labeled receptors (M_1) in the brain.

In summary, **13b** has a biochemical profile similar to BM-5 and is significantly different from its enantiomer **13a** and oxotremorine. Only after the optically pure (and not racemic) compounds were prepared and tested biochemically were the subtle differences in subtype selectivity and reduced affinity discovered for the enantiomeric compounds.

Experimental Section

All melting points were determined using a Mel-Temp apparatus and are uncorrected. All NMR spectra were measured in $CDCl_3$ or CD_3OD using a General Electric QE 300 spectrometer. The coupling constants are recorded in hertz (Hz), and the chemical shifts are reported in parts per million δ (ppm) downfield from tetramethylsilane (TMS) which is used as an internal standard. IR spectra were recorded on a Nicolet 20 SXB FT-IR spectrophotometer. Low-resolution mass spectra (MS-LR) were recorded on a Finnigan MAT-90 mass spectrometer and high-resolution mass spectra (MS-HR) were recorded on either a

Finnigan MAT-90 for electron impact (EI) or a VG ZAB-SE mass spectrometer for fast atom bombardment (FAB). Specific rotations ($[\alpha]^{26}_{D}$, deg) were recorded on a Perkin-Elmer 241 polarimeter with the sample concentration in grams per milliliter of solution. Thin-layer chromatography was conducted using silica gel (Analtech) or aluminum oxide (Analtech) plates. For flash column chromatography, Kieselgel 60 (230–400 mesh ASTM, E. M. Science), for preparative HPLC Waters Prep-500 HPLC system with normal-phase silica gel cartridge and for gravity column chromatography alumina deactivated with 4.5% H_2O was used. Celite (Johns Mansville) was used as a filter aid and Magnesol (FMC Corp), hydrous magnesium silicate, was used for plug filtration. (*S*)-Methyl L-β-hydroxyisobutyrate and (*S*)-β-Methyl-γ-butyrolactone were purchased from Fluka Chem. Co.

Benzyl (*R*)-2-Methyl-4-oxabutanoate (10b). A stream of O_3 was bubbled through a solution of benzyl (*R*)-2-methyl-4-pentenoate **9b**[7] (3.32 g,16.3 mmol) in MeOH (150 mL) which was cooled to −78 °C until a blue color persisted. The excess ozone was removed by first purging with oxygen and then with argon. Zinc dust (2.47 g) and acetic acid (3 ml) were added, and the mixture was allowed to warm to room temperature with stirring for 1 h. The resulting precipitate was removed by filtration, and the solvent was evaporated *in vacuo*. The residue was triturated with ether, and the filtrate was evaporated to dryness. The residue was purified by plug filtration (SiO_2; eluent, ether) to give 3.10 g (92%) of 10b as a colorless oil which was used in the next step directly without further purification.

Benzyl (*S*)-2-Methyl-4-oxabutanoate (10a). The analogous reaction of **9a** (4.22 g, 20.7 mmol) gave a 97% yield of 10a as a colorless oil.

(*R*)-3-Methyl-1-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidinone (13b). Benzyl (*R*)-2-methyl-4-oxobutanoate 10b (3.10 g, 15.0 mmol) was addedto a mixture of of 4-(1-pyrrolidinyl)-2-butynyl-1-amine dihydrochloride **24** (5.02 g, 23.8 mmol), sodium acetate (4.17 g, 50.9 mmol), and acetic acid 4 (mL) in MeOH (50 mL) at room temperature. After stirring for 15 min, sodium cyanoborohydride (395 mg, 6.29 mmol) was added in several portions and then stirred for 3 h. The reaction was quenched by adding sodium carbonate, and the mixture was concentrated in vacuo. The residue was partitioned between water and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2. The combined organic phase was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography (Al_2O_3, 300 g; eluent, 33–50% v/v EtOAc/hexanes) gave 1.78 g (54%) of **13b** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.12 (m, 2 H), 3.39 (m, 4 H), 2.59 (m, 4 H), 2.47 (m, 1 H), 2.26 (m, 1 H), 1.81 (m, 4 H), 1.60 (m, 1 H), 1.20 (d, *J* = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 176.7, 79.8, 77.9; 52.7, 44.4, 43.3, 36.6, 32.4, 26.9, 23.7, 16.2; IR (neat) 1693 cm^{-1}. Anal. ($C_{13}H_{20}N_2O$) C, H, N.

The sesquioxalate salt of **13b** was prepared by the addition of oxalic acid (1.5 equiv) to a solution of **13b** (0.653 g, 2.97 mmol) in MeOH (5 mL) at room temperature. After stirring for 15 min, the solvent was evaporated *in vacuo* to dryness. The white solid was recrystallized from MeOH / EtOAc to give the sesquioxalate salt of **13b** as white crystals: mp 88–89 °C; ^{1}H NMR (CD_3OD) δ 4.18 (m, 2 H), 4.13 (m, 2 H), 3.45 (m, 4 H), 3.31 (hept, *J* = 1.7 Hz, 2 H), 2.48 (m, 1 H), 2.32 (m, 1 H), 2.10 (m, 4 H), 1.67 (m, 1 H), 1.16 (d, *J* = 7.1 Hz, 3 H); IR (KBr) 1724, 1683, 1646 cm^{-1}. Anal. ($C_{16}H_{23}N_2O_7$) C, H, N.

(*S*)-3-Methyl-1-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidinone (13a). The analogous reaction of **10a** (4.02 g, 19.5 mmol) gave a 49% yield of **13a** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.12 (m, 2 H), 3.39 (m, 4 H), 2.59 (m, 4 H), 2.46 (m, 1 H), 2.26 (m, 1 H), 1.81 (m, 4 H), 1.64 (m, 1 H), 1.20 (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 177, 79.9, 78.0, 52.7, 44.5, 43.3, 36.6, 32.4, 27.0, 23.8, 16.2; IR (neat) 1694 cm^{-1}. Anal. ($C_{13}H_{20}N_2O$) C, H, N.

The sesquioxalate salt of **13a** was prepared by the addition of oxalic acid (1.5 equiv) to a solution of **13a** (1.42 g, 6.45 mmol) in MeOH (5 mL) at room temperature. After the mixture was stirred for 15 min, the solvent was evaporated *in vacuo* to dryness. The white solid was recrystallized from MeOH/EtOAc to give the sesquioxalate salt of **13a** as white crystals: mp 90–91 °C; ^{1}H NMR (CD_3OD) δ 4.18 (m, 2 H), 4.13 (m, 2 H), 3.44 (m, 4 H), 3.31 (hept, *J* = 1.6 Hz, 2 H), 2.49 (m, 1 H), 2.32 (m, 1 H), 2.10 (m, 4 H), 1.68 (m, 1 H), 1.16 (d, *J* = 7.1 Hz, 3 H); IR (KBr) 1724, 1688, 1638 cm^{-1}. Anal. ($C_{13}H_{20}N_2O$) C, **H**, N.

(*S*)-1-[(1-(Dimethylamino)-2-butyn-4-yl]-3-methyl-2-pyrrolidinone (14a). The analogous reaction of **10a** gave **14a** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.21 (d, *J* = 7.1 Hz, 3 H), 1.64 (m, 1 H), 2.23–2.32 (m, 1 H), 2.28 (s, 6 H), 2.47 (m, 1 H), 3.24 (m, 2 H), 3.37–3.44 (m, 2 H), 4.14 (d, *J* = 1.3 Hz, 2 H);IR (neat) 1694 cm^{-1}. Anal. ($C_{11}H_{18}N_2O$) C, H, N.

(*R*)-1-[(1-(Dimethylamino)-2-butyn-4-yl]-3-methyl-2-pyrrolidinone (14b). The analogous reaction of **10a** gave **14b** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.21 (d, *J* = 7.1 Hz, 3 H), 1.64 (m, 1 H), 2.23–2.32 (m, 1 H), 2.28 (s, 6 H), 2.47 (m, 1 H), 3.24 (m, 2 H), 3.37–3.44 (m, 2 H), 4.14 (d, *J* = 1.3 Hz, 2 H); IR (neat) 1694 cm^{-1}. Anal. ($C_{11}H_{18}N_2O$) C, H, N.

Benzyl 2-Methyl-4-pentenoate [(±)-16]. Oxalyl chloride (48.2 mL, 55 mmol) was added dropwise to a solution of 2-methyl-4-pentenoic acid[8] and 0.3 mL of DMF in CH_2Cl_2 (150 mL) which was stirred at 0 °C. The mixture was stirred at room temperature for 3 h and then concentrated *in vacuo* to dryness to give 2-methyl-4-pentenoyl chloride (28.5 g, 97%) as a yellow oil.

A solution of 2-methyl-4-pentenoyl chloride 28.5 g in CH2Cl2 (50 mL) was added dropwise to a solution of benzyl alcohol (23.8g, 0.22 mol) and pyridine (18.7 mL, 0.23 mol) in CH_2Cl_2 (250 mL) which was stirred at 0 °C. The reaction was stirred at room temperature for 4 h and washed with cold hydrochloric acid (150 mL, 2 N) and saturated aqueous $NaHCO_3$ (100 mL). The CH_2Cl_2 solution was dried ($MgSO_4$) and concentrated *in vacuo* to dryness to give the crude **16** (42.1 g) as a yellow oil. Purification of the crude oil by Kugulrohr distillation (70–75 °C, 0.1 mmHg) afforded **16** (35.2g, 80%) as a colorless oil: IR (thin film) 1735 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.18 (d, *J* = 7 Hz, 3 H), 2.20 (m, 1 H), 2.44 (m, 1 H), 2.58 (m, 1 H), 5.01 (m, 2 H), 5.11 (s, 2 H), 5.73 (m, 1 H), 7.25–7.37 (m, 5 H); MS (CI-LR) MH$^+$ at *m/z* 205. Anal. ($C_{13}H_{20}O_2 \cdot 0.1H_2O$) C, H, N.

Benzyl 2-Methyl-4-oxabutanoate [(±)-17]. The analogous reaction of **9a** (4.22 g, 20.7 mmol) gave a 97% yield of **17** as a colorless oil.

3-Methyl-1-(1-pyrrolidinyl-2-butyn-4-yl)-2-pyrrolidinone [(±)-18]. The analogus reaction of **10a** gave **18** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (d, *J* = 7.2 Hz, 3H), 1.65 (m, 1H), 1.18 (m, 1 H), 2.26 (m, 1 H), 2.47 (m, 1 H), 2.59 (m, 4 H), 3.39 (m, 4 H), 4.12 (m, 2 H).

The sesquioxalate salt of **18** as colorless crystals: ^{1}H NMR (CD_3OD) δ 1.16 (d, *J* = 6.9 Hz, 3 H), 1.68 (m, 1 H), 2.10 (m, 4 H), 2.32 (m, 1 H), 2.50 (m, 1 H), 3.31 (hept, *J* = 1.7 Hz, 2 H), 3.42 (m, 4 H), 4.13 (t, *J* = 2.0 Hz, 2 H), 4.18 (t, *J* = 1.8 Hz, 2 H).

1-[1-(Dimethylamino)-2-butyn-4-yl]-3-methyl-2-pyrrolidinone [(±)-19]. The analogous reaction of **10b** (2.4 g, 14.7 mmol) gave a 60% yield of **19** as a colorless oil: IR (thin film) 1692 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.21 (d, *J* = 7 Hz, 3 H), 1.65 (m, 1 H), 2.28 (s,6H), 2.47 (m, 1 H), 3.24 (t, *J* = 1 Hz, 2 H), 3.41 (m, 3 H), 4.14 (br s, 2 H); MS (LR-CI) MH *m/z* 195. Anal. ($C_{11}H_{18}N_2O$) C, H, N.

***N*-(2-Propynyl)-2,2,2-trifluoroacetamide (21).** A solution of trifluoroacetic anhydride (63 g, 0.3 mol) in CH_2Cl_2 (50 mL) was added dropwise to a solution of propargylamine **20** (15.0 g, 0.27 mol) in CH_2Cl_2 (200 mL) which was stirred at 0 °C for 1 h followed by the addition of water (100 mL). The mixture was washed with 1 N HCl (150 mL) and saturated aqueous $NaHCO_3$ (150 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to dryness. The residue was distilled under vacuum to give 32.1 g of **21** as a colorless oil: bp 40–45 °C (0.2 mmHg); IR (KBr) 1713 cm^{-1}; NMR ($CDCl_3$) δ 2.35 (t, *J* = 7 Hz, 1 H), 4.15–4.18 (m, 2 H), 6.6 (br s, 1 H); MS (EI-LR) MH$^+$ *m/z* 151. Anal. ($C_5H_4F_3NO$) C, H, N.

***N*-[1-(Dimethylamino)-2-butyn-4-yl]-2,2,2-trifluoroacetamide Hydrochloride (22).** The analogous reaction of **23** gave **22** as colorless crystals: mp 112–114 °C; IR (KBr) 1715, 1731 cm^{-1}; NMR (DMSO-d_6) δ 2.75 (s, 6 H), 4.07 (d, *J* = 1.8 Hz, 2 H), 4.14 (d, *J* = 5.2Hz, 2 H), and 10.16 (br s , 1 H); MS (EI-LR) MH$^+$ at *m/z* 208. Anal. ($C_8H_{10}F_3N_2O \cdot HCl \cdot 0.25H_2O$) C, H, N.

2,2,2-Trifluoro-*N*-(1-pyrrolidin-2-butyn-4-yl)acetamide Hydrochloride (23). A mixture of **21** (50.0 g, 0.33 mol), pyrrolidine (47.1 g, 0.66 mol), paraformaldehyde (25g, 0.83mol), acetic acid (50 mL), cuprous chloride (0.5 g, 6 mmol), and dioxane (500 mL) was refluxed for 20 min. The reaction mixture was concentrated

in vacuo and made basic to pH 10–11 with concentrated ammonium hydroxide. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered through a pad of Celite, and concentrated *in vacuo* to dryness. The residue was purified by vacuum distillation (110–120 °C, 0.3 mmHg) to give 58.1 g (75%) of **23** as a yellow oil which was used directly in the next step. A sample of the oil was dissolved in 1 N methanolic hydrogen chloride. The resulting solid was collected and recrystallized from MeOH/ether to give the hydrochloride salt of **23** as colorless crystals: mp 125–127°C; IR (KBr) 1731 cm^{-1}; NMR (DMSO-d_6) δ 1.94 (br s, 4 H), 3.3 (br s, 2 H), 4.11 (s, 2 H), and 4.13 (s, 2 H); MS (EI-LR) MH^+ at *m/z* 233. Anal. ($C_{10}H_{13}F_3N_2O$·HCl) C, H, N.

***N,N*-Dimethyl-2-butyn-1,4-diyldiamine Dihydrochloride (24).** The analogous reaction of **25** gave **24** as colorless crystals: mp 181–183 °C; Anal. ($C_8H_{12}N_2$·2HCl) C, H, N.

4-(1-Pyrrolidinyl)-2-butyn-1-ylamine Dihydrochloride (25). A solution of **23** (50.0 g, 214 mmol) and 4 N hydrochloric acid (270 mL) was refluxed for 2 h. Concentrated hydrochloric acid (70 mL) was added, and the mixture was refluxed overnight. The reaction was concentrated *in vacuo*, and the residue was washed with CH_2Cl_2. The aqueous layer was basified with 10 N NaOH and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give 28 g of a dark oil. The oil was treated with 450 mL of 1 N methanolic hydrogen chloride and concentrated *in vacuo*. The resulting solid was recrystallized from MeOH/ether to give 31.2 g (52%) of **25** as hygoscopic off-white crystals: mp 192–193 °C; NMR (DMSO-d_6) δ 1.97 (br s, 4 H), 3.29–3.39 (m, 4 H) 3.43 (m, 1 H), 3.8 (s, 2 H) 4.2 (s, 2 H). Anal. ($C_8H_{14}N_2$·2HCl) C, H, N.

(*S*)-Methyl 3-(Tetrahydro-2-pyranyloxy)-2-methylpropionate (27). A solution of (*S*)-(+)-methyl L-β-hydroxyisobutyrate **26** (10.93 g, 92.5 mmol), 3,4-dihydro-2*H*-pyran (9.34 g, 111 mmol), and *p*-toluenesulfonic acid monohydrate (0.22 g, 1.16 mmol) in ether (150 mL) was stirred at room temperature overnight. The reaction mixture was washed with aqueous $NaHCO_3$ and dried over $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified by distillation to give 17.73 g (95%) of **27** as a colorless oil: bp 63–66 °C (0.35–0.4 mmHg); $[\alpha]^{26}_D$ = +16° (*c* = 0.852, ether) [lit.[10] $[\alpha]^{21}_D$ = –16.3° (*c* = 1.39, ether)]; ^{1}H NMR ($CDCl_3$) δ 4.61(m, 1 H), 3.94–3.41 (m, 4 H), 3.70 (s, 3 H), 2.77 (m, 1 H), 1.81–1.49 (m, 7 H), 1.20 (d, *J* = 7.1 Hz, 1.5 H), 1.19 (d, *J* = 7.1 Hz, 1.5 H); IR (neat) 1741 cm^{-1}.

(*R*)-3-(Tetrahydro-2-pyranyloxy)-2-methyl-1-propanol (28). A solution of lithium aluminum hydride (70 mL, 1 N, 70 mmol) in ether was slowly added via syringe pump to a solution of **27** (17.7 g, 87.6 mmol) in dry ether (50 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The excess lithium aluminum hydride was decomposed by the successive addition of water (1.5 mL), 15% NaOH (4 mL), and water (9 mL) to the stirred ice-cooled mixture. After stirring for 1.5 h, the mixture was filtered and washed with ether. The combined filtrate and washings were dried over K_2CO_3. After concentration the residue was distilled to give 13.6 g (89%) of **28** as acolorless oil: bp 87–89 °C (0.9 mmHg); $[\alpha]^{26}_D$ = +1° (*c* = 1.885, ether) [lit.[10] $[\alpha]^{21}_D$ = –1.2° (*c* = 1.471, ether)]; ^{1}H NMR ($CDCl_3$) δ 4.59 (m, 1 H), 3.86 (m, 2 H), 3.70–3.50 (m, 4.5 H), 3.35 (dd, *J* = 7.4 , 9.5 Hz, 0.5 H), 2.34 (br s, 1 H), 2.05 (m, 1 H), 1.76 (m, 2 H), 1.55 (m, 4 H), 0.91 (d, *J* = 7.0 Hz, 1.5 H) and 0.90 (d, *J* = 7.0 Hz, 1.5 H); IR (neat) 3430, 2942, 1032 cm^{-1}.

(*S*)-3-(Tetrahydro-2-pyranyloxy)-2-methylpropyl *p*-Toluenesulfonate (29). In one portion *p*-TsCl (19.2 g, 101 mmol) was added to a solution of **28** (13.5 g, 77.6 mmol) in pyridine (120 mL) at 0 °C. The reaction mixture was kept in a refrigerator overnight and then poured into ice–water. The aqueous solution was extracted with ether, and the combined extract was washed with aqueous $CuSO_4$, $NaHCO_3$, and brine. The ether solution was dried over $MgSO_4$ and concentrated *in vacuo* to give 24.1 g (95%) of **29** as a pale-yellow oil which was employed in the next step without further purification.

(*R*)-4-(Tetrahydro-2-pyranyloxy)-3-methylbutanenitrile (30). A solution of **29** (39.9 g, 0.121 mol) and NaCN (10 g, 0.204 mol) in DMSO (120 mL) was stirred for 2 days at room temperature. It was then diluted with water and extracted three times with 200 mL of petroleum ether (30–70 °C). The combined petroleum ether extract was dried over $MgSO_4$ and concentrated *in vacuo* to dryness. The residue was purified by HPLC (eluant, 1:4 v/v EtOAc/hexanes) to give 15.4 g (70%) of **30** as a colorless oil: $[\alpha]^{26}_D$ = +24° (*c* = 1.875, ether) [lit.[10] $[\alpha]^{21.5}_D$ = –27.8° (*c* = 1.41, ether)]; ^{1}H NMR ($CDCl_3$) δ 4.53 (m, 1 H), 3.83 (m, 1 H), 3.76 (dd, *J* = 5.0, 9.8 Hz, 0.5 H), 3.55 (m, 1.5 H), 3.38 (dd, *J* = 4.8, 9.9 Hz, 0.5 H), 3.21 (dd, *J* = 8.3, 9.7 Hz, 0.5 H), 2.49 (m, 2 H), 2.17 (m, 1 H), 1.71 (m, 2 H), 1.55 (m, 4 H), 1.11 (d, *J* = 6.8 Hz, 1.5 H), and 1.10 (d, *J* = 6.8 Hz, 1.5 H); IR (neat) 2942, 2246, 1455, 1035 cm^{-1}.

(*R*)-(+)-β-Methyl-γ-butyrolactone (31c). A solution of **30** (6.80 g, 37.1 mmol) in concentrated HCl (5 mL) and MeOH (50 mL) was heated to gentle reflux for 7 h. The mixture was concentrated *in vacuo* and then diluted with water (50 mL). The aqueous solution was neutralized with $NaHCO_3$, stirred at room temperature for 1 h, and extracted with CH_2Cl_2 (50 mL). The CH_2Cl_2 solution was dried over Na_2SO_4 and concentrated *in vacuo* to dryness. The residue was purified by HPLC (eluant, 1:4 EtOAc/hexanes) to give 1.69 g (46%) of **31c** as a colorless oil: $[\alpha]^{26}_D$ = +24° (*c* = 1.235, MeOH) [lit.[10] $[\alpha]^{21}_D$ = –24.96° (*c* = 1.771, MeOH)]; ^{1}H NMR ($CDCl_3$) δ 4.42 (dd, *J* = 7.3, 8.8 Hz, 1 H), 3.88 (dd, *J* = 6.4, 8.8 Hz, 1 H), 2.65 (m, 2 H), 2.14 (m, 1 H), 1.17 (d, *J* = 6.6 Hz, 3 H); IR (neat) 2378, 2243, 1774 cm^{-1}. Anal. ($C_5H_8O_2$) C, H, N.

Ethyl (*R*)-4-Iodo-3-methylbutyrate (32c). Iodotrimethylsilane (6.04 g, 30.2 mmol) was slowly added to a solution of **31c** (1.5 g, 15. mmol) in EtOH (30 mL) at –20 °C. After stirring at –20 °C for 30 min, triethyl orthoformate (2.5 g, 15. mmol) was added, and the resulting solution was brought to gentle reflux overnight. The solution was concentrated *in vacuo*, and the residue was purified by column chromatography (SiO_2, eluant, 1:20 v/v EtOAc/hexanes) to give 3.09 g (79%) of **32c** as a pale yellow oil: $[\alpha]^{26}_{365}$ = +6° (*c* = 1.98, MeOH); ^{1}H NMR ($CDCl_3$) δ 4.15 (q, *J* = 7.2 Hz, 2 H), 3.27 (m, 2 H), 2.46 (dd, *J* = 6.5, 15.7 Hz, 1 H), 2.23 (dd, *J* = 7.2, 15.7 Hz, 1 H), 2.03 (m, 1 H), 1.27 (t, *J* = 7.1 Hz, 3 H), 1.06 (d, *J* = 6.6 Hz, 3 H); IR (neat) 1733 cm^{-1}. Anal. ($C_7H_{13}IO_2$) C, H, I.

Ethyl (*S*)-4-Iodo-3-methylbutyrate (32d)[3]. Analogous reaction of iodotrimethylsilane (44. g, 0.22 mol) with (*S*)-β-methyl-γ-butyrolactone(11.2 g, 0.110 mol) gave an 88% yield of product as a pale yellow oil: $[\alpha]^{26}_{365}$ = –6° (*c* = 1.44, MeOH); ^{1}H NMR ($CDCl_3$) δ 4.15 (q, *J* = 7.1 Hz, 2 H), 3.27 (m, 2 H), 2.46 (dd, *J* = 6.5, 15.7 Hz, 1 H), 2.23 (dd, *J* = 7.2 Hz, 15.7 Hz, 1 H), 2.03 (m, 1 H), 1.27 (t, *J* = 7.2 Hz, 3 H), 1.06 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 172.0, 60.5, 41.1, 31.8, 20.5, 15.9, 14.2; IR (neat) 1733 cm^{-1}. Anal. ($C_7H_{13}IO_2$) C, H, N.

(*R*)-4-Methyl-1-(2-propynyl)-2-pyrrolidinone (33c). A solution of **32c** (4.81 g, 18.6 mmol) in EtOH (20 mL) was slowly added over 6.5 h to a solution of propargylamine (6.14 g, 112 mmol) and Na_2CO_3 (1.98 g, 18.6 mmol) in EtOH (10 mL) at 90 °C. The reaction mixture was cooled and concentrated *in vacuo* to dryness. Water (70 mL) was added to the residue and extracted three times with CH_2Cl_2 (80 mL). The extract was dried over Na_2SO_4 and concentrated *in vacuo* to dryness. The residue was purified by column chromatography (SiO_2, eluent, 1:2 to 1:1 v/v EtOAc/hexanes) to give 2.40 g (94%) of **33c** as a pale yellow oil: $[\alpha]^{26}_D$ = –5°(*c* = 0.7, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 4.10 (apparent t, *J* = 2.2 Hz, 2 H), 3.60 (dd, *J* = 7.7, 9.5 Hz, 1 H), 3.06 (dd, *J* = 6.0, 9.5 Hz, 1 H), 2.55 (m, 2 H), 2.22 (t, *J* = 2.5 Hz, 1 H), 2.03 (dd, *J* = 6.3, 16.0 Hz, 1 H), 1.15 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.1, 77.7, 72.1, 53.6, 39.1, 31.8, 26.2, 19.8; IR (neat) 2120, 1688cm^{-1}; MS (HREI) calcd for $C_8H_{11}NO$ 137.0841, found 137.0846. Anal. ($C_8H_{11}NO$) H, N; C: calcd, 70.04; found, 67.21.

(*S*)-4-Methyl-1-(2-propynyl)-2-pyrrolidinone (33d). The analogous reaction of **32d** (1.89 g, 7.33 mmol) with propargylamine (1.21 g, 22 mmol) and Na_2CO_3 (0.389 g, 3.67 mmol) gave a 71% yield of product **33d** as a pale yellow oil: $[\alpha]^{26}_D$ = +6° (*c* = 2.44, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 4.10 (apparent t, *J* = 2.2 Hz, 2 H), 3.60 (dd, *J* = 7.7, 9,5 Hz, 1 H), 3.06 (dd, *J* = 6.0, 9.5 Hz, 1 H), 2.59 (m, 2 H), 2.22 (t, *J* = 2.6 Hz, 1 H), 2.03 (dd, *J* = 6.3, 16.0 Hz, 1 H), 1.14 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.0, 77.6, 72.0, 53.5, 39.0, 31.7, 26.1, 19.7; IR (neat) 2119, 1689, 1626 cm^{-1}; MS (HR-EI) calcd for $C_8H_{11}NO$ 137.0841, found 137.0481. Anal. ($C_8H_{11}NO$) H, N; C: calcd, 70.04; found, 69.44.

(*S*)-4-Methyl-1-(1-pyrrolidinyl-2-butyn-4-yl)-2-pyrrolidinone (34d) (General Procedure). A mixture of **33d** (0.584 g,

4.26 mmol), paraformaldehyde (0.34 g, 11.3 mmol), pyrrolidine (0.61 g, 8.5 mmol), CuCl (50 mg), and acetic acid (2 mL) in 30 mL of *p*-dioxane was stirred at room temperature for 15 min and then refluxed for 1.5 h. The reaction mixture was concentrated *in vacuo* to dryness. The residue was purified by column chromatography (Al_2O_3 eluant, 1:2 to 1:1 v/v EtOAc/hexanes) to give 0.80 g (85%) of **34d** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.11 (t, J = 2.0 Hz, 2 H), 3.59 (dd, J = 7.8, 9.5 Hz, 1 H), 3.40 (t, J = 2 Hz, 2 H), 3.04 (dd, J = 5.9, 9.5 Hz, 1 H), 2.59 (m, 4 H), 2.50 (m, 2 H), 2.02 (dd, J = 6.3, 16. Hz, 1 H), 1.81 (m, 4 H), 1.14 (d, J = 6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.9, 79.8, 77.9, 53.7, 52.7, 43.3, 39.2, 32.1, 26.2, 23.7, 19.8; IR (neat) 1694 (C=O) cm^{-1}. Anal. ($C_{13}H_{20}N_2O$) C, H, N.

The sesquioxalate salt of **34d** was prepared by the addition of oxalic acid (1.5 equiv) to a solution of **34d** (0.80 g, 3.63 mmol) in MeOH (10 mL) at room temperature. After stirring for 15 min, the solution was concentrated *in vacuo* to dryness. The resulting white solid was recrystallized from MeOH/EtOAc to give the sesquioxalate salt of **34d** as white crystals: ^{1}H NMR (CD_3OD) δ 4.18 (s, 2 H), 4.13 (t, J = 1.7 Hz, 2 H), 3.65 (dd, J = 7.8, 9.5 Hz, 1 H), 3.42 (br s, 4 H), 3.31 (hept, J = 1.6 Hz, 2 H), 3.10 (dd, J = 5.8, 9.5 Hz, 1 H), 2.53 (m, 2 H), 2.11 (m, 4 H), 2.02 (dd, J = 5.5, 15.6 Hz, 1 H), 1.13 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (CD_3OD) δ 177.0, 165.3, 84.6, 74.2, 55.3, 54.5, 44.4, 39.9, 32.8, 27.6, 24.4, 19.8; IR (KBr) 1721 (C=O) cm^{-1}. Anal. ($C_{16}H_{23}N_2O_7$) C, H, N.

(*R*)-4-Methyl-1-(1-pyrrolidinyl-2-butyn-4-yl)-2-pyrrolidinone (34c). The analogous reaction of **34d** gave the product **34c** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.11 (t, J = 2.0 Hz, 2 H), 3.59 (dd, J = 7.8, 9.5 Hz, 1 H), 3.40 (t, J = 2.1 Hz, 2 H), 3.04 (dd, J = 6.0, 9.5 Hz, 1 H), 2.60 (m, 4 H), 2.50 (m, 2 H), 2.03 (dd, J = 6.3, 16.1 Hz, 1 H), 1.81 (m, 4 H), 1.14 (d, J = 6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.0, 79.7, 78.0, 53.7, 52.7, 43.2, 39.2, 32.1, 26.2, 23.7, 19.8; IR (neat) 1692 (C=O) cm^{-1}. Anal. ($C_{13}H_{20}N_2O$) C, H, N.

The sesquioxalate salt of **34c** as white crystals: mp 116–118 °C; ^{1}H NMR (CD_3OD) δ 4.14 (s, 2 H), 4.01 (t, J = 1.8 Hz, 2 H), 3.58 (dd, J = 7.7, 9.4 Hz, 1 H), 3.36 (br s, 4 H), 3.03 (dd, J = 5.9, 9.4 Hz, 1 H), 2.53 (m, 2 H), 2.10 (m, 4 H), 2.02 (dd, J = 5.5, 15.6 Hz, 1 H), 1.15 (d, J = 6.5 Hz, 3 H); ^{13}C NMR (CD_3OD) δ 177.0, 165.3, 84.6, 74.2, 55.3, 54.5, 44.4, 39.9, 32.8, 27.6, 24.4, 19.8; IR (KBr) 1721, 1681 cm^{-1}. Anal. ($C_{16}H_{23}N_2O_7$) C, H, N.

(*S*)-1-[1-(Dimethylamino)-2-butyn-4-yl]-4-methyl-2-pyrrolidinone (35d). The analogous reaction of **34d** gave the product **35d** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.13 (t, J = 1.8 Hz, 2 H), 3.60 (dd, J = 7.7, 9.5 Hz, 1 H), 3.24 (t, J = 2.0 Hz, 2 H), 3.05 (dd, J = 5.9, 9.5 Hz, 1 H), 2.52 (m, 2 H), 2.28 (s, 6 H), 2.03 (dd, J = 6.3, 16.0 Hz, 1 H), 1.14 (d, J = 6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.9, 79.0, 78.8, 53.7, 48.0, 44.2, 39.2, 32.1, 26.2, 19.8; IR (neat) 1692 cm^{-1}. Anal. ($C_{11}H_{18}N_2O$) C, H, N.

The ethanedioate salt of **35d** was prepared by the treatment of **35d** (1.36 g, 7.0 mmol) with oxalic acid (1 equiv) in MeOH (15 mL). Recrystallization of the resulting solid from MeOH/EtOAc gave the ethanedioate salt of **35d** as white crystals: mp 153–154°C; ^{1}H NMR (CD_3OD) δ 4.10 (t, J = 1.9 Hz, 2 H), 3.97 (t, J = 2.2 Hz, 2 H), 3.56 (dd, J = 7.7, 9.5 Hz, 1 H), 3.21 (hept, J = 1.6 Hz, 2 H), 3.00 (dd, J = 2.9, 6.6 Hz, 1 H), 2.82 (s, 6 H), 2.43 (m, 2 H), 1.92 (dd, J = 5.5, 15.5 Hz, 1 H), 1.04 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (CD_3OD) δ 176.9, 166.5, 85.8, 73.5, 55.3, 47.8, 42.8, 39.9, 32.8, 27.6, 19.8; IR (KBr) 1702 cm^{-1}. Anal. ($C_{13}H_{20}N_2O_5$) C, H, N.

***N*-[(*S*)-4-(4-Methyl-2-oxo-1-pyrrolidinyl)-2-butynyl]-*N*,*N*,*N*-trimethylaminium Iodide (36d).** MeI (0.1 mL, 1.61 mmol) was added to a solution of **35d** (126 mg, 0.65 mmol) in ether (0.5 mL) at room temperature. The mixture was allowed to stand overnight. A yellow solid formed, and the solvent was evaporated *in vacuo* to leave a solid residue. The residue was recrystallized from CH_3CN to give **36d** as a pale-yellow crystal: mp 146–147 °C; ^{1}H NMR (CD_3OD) δ 1.09 (d, J = 6.3 Hz, 3 H), 1.93 (m, 1 H), 2.45 (m, 2 H), 3.03 (dd, J = 5.8, 9.2 Hz, 1 H), 3.18 (s, 9 H), 3.57 (dd, J = 7.8, 9.3 Hz, 1 H), 4.15 (apparent d, J = 1.8 Hz, 2 H), 4.31 (t, J = 2.1 Hz, 2 H); IR (KBr) 2234, 1704, 1677 cm^{-1}. Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

(*R*)-4-Methyl-1-[1-(dimethylamino)-2-butyn-4-yl]-2-pyrrolidinone (35c). The analogous reaction of **34d** gave the product **35c** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.13 (s, 2 H), 3.60 (dd, J = 7.6, 9.5 Hz, 1 H), 3.24 (t, J = 2.0 Hz, 2 H), 3.06 (dd, J = 5.9, 9.5 Hz, 1 H), 2.52 (m, 2 H), 2.28 (s, 6 H), 2.04 (m, 1 H), 1.14 (d, J = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.9, 79.0, 78.7, 53.7, 47.9, 44.2, 39.2, 32.0, 26.2, 19.8; IR (neat) 1694 cm^{-1}. Anal. ($C_{11}H_{18}N_2O$) C, H, N.

The ethanedioate salt of **35c** as white crystals: mp 151–152 °C; ^{1}H NMR (CD_3OD) δ 4.19 (s, 2 H), 4.07 (t, J = 2.2 Hz, 2 H), 3.66 (dd, J = 7.7, 9.5 Hz, 1 H), 3.31 (hept, J = 1.6 Hz, 2 H), 3.10 (dd, J = 5.9, 9.5 Hz, 1 H), 2.92 (s, 6 H), 2.52 (m, 2 H), 2.01 (m, 1 H), 1.13 (d, J = 6.6 Hz, 3 H); IR (KBr) 1700 cm^{-1}. Anal. ($C_{13}H_{20}N_2O_5$) C, H, N.

***N*-[(*R*)-4-(4-Methyl-2-oxo-1-pyrrolidinyl)-2-butynyl]-*N*,*N*,*N*-trimethyl-1-ammonium Iodide (36c).** The analogous reaction of **35c** (127 mg, 0.65 mmol) with MeI (0.1 ml, 1.61 mmol) gave **36c** (recrystallized from CH_3CN) as yellow crystals: mp 148–149 °C; ^{1}H NMR (CD_3CN) δ1.10 (d, J = 6.5 Hz, 3 H), 2.45 (m, 2 H), 3.01 (dd, J = 5.9, 9.2 Hz, 1 H), 3.15 (s, 9 H), 3.57 (dd, J = 7.8, 9.3 Hz, 1 H), 4.15 (s, 2 H), 4.26 (apparent d, J = 1.5 Hz, 2 H); IR (KBr) 2234, 1704, 1677 cm^{-1}. Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

Muscarinic Receptor Binding Assays. The receptor binding assays were adapted from published procedures.[14-17] The cerebral cortex from male Wistar rats (200–250-g body weight) was homogenized in 50 volumes (wet wt/vol) of ice-cold 10 mM (8.1 mM Na_2HPO_4, 1.9 mM KH_2PO_4) sodium–potassium phosphate buffer (NaKPB), pH 7.4. The resulting homogenate was then diluted 1:3000, 1:300, or 1:400 (original wet wt/vol) with ice-cold NaKPB for use in the [^{3}H]QNB, [^{3}H]CD, and [^{3}H]PZ assays, respectively. The final protein content per 2.0 mL of incubation mixture was ~ 0.1 mg, 0.75 and 0.55 mg, respectively.

The heart from male Wistar rats (200–250-g body weight) was placed in ice-cold 10 mM (8.1 mM Na_2HPO_4, 1.9 mM KH_2PO_4) sodium–potassium phosphate buffer (NaKPB), pH 7.4, and gently squeezed to flush blood from the ventricles. Excess buffer was blotted from the heart which was weighed and placed into 50 volumes (wet wt/vol) of ice-cold buffer where it was minced with a scissor. The mince was then homogenized using a Polytron, setting 8, with PT-10 saw-tooth generator for 60 s. The homogenate was then centrifuged at 48000*g* for 15 min at 4 °C. The supernatant was discarded and the pellet resuspended in 50 volumes (wet wt/vol) of fresh buffer using the Polytron as above. The homogenate was then filtered through two layers of gauze and diluted to a final tissue concentration of 1:750 (wet wt/vol), giving a final protein concentration of 0.15–0.2 mg/2.0 mL in the assay tubes.

Binding assays were initiated by adding 1.80 mL of the appropriate tissue homogenate to tubes containing 100 μL of tritiated ligand, 50 μL of unlabeled compound (final concentration 1×10^{-3} to 1×10^{-12} M) and 50 μL of buffer or atropine (final concentration = 10 μM) to define nonspecific binding. For [^{3}H]-QNB binding to cardiac membranes, Gpp(NH)p was added to the diluted homogenate to give a final concentration of 10 μM. The concentrations of radioligand were [^{3}H]QNB (30.0 Ci/mmol) 250 pM, [^{3}H]CD (55.5 Ci/mmol) 1 nM, and [^{3}H]PZ (84.1 Ci/mmol) 0.1 nM. Samples were incubated at 25 °C for 120 min ([^{3}H]QNB and [^{3}H]CD) or 60 min ([^{3}H]PZ). [^{3}H]CD and [^{3}H]-PZ have been shown to adhere readily to both glass and plastic surfaces. To eliminate this problem (and the chance for introducing artifacts into the results), stock vials, pipett tips, and all glass tubes were routinely treated with Prosil-28, a siliconizing agent, and oven-dried prior to use in an assay. Additionally, the GF/B glass fiber filters were presoaked in an aqueous polyethylenimine (PEI) solution (0.1%, pH 7.0) prior to use. All points of the inhibition curve (including total and nonspecific binding) were always measured on single PEI treated filter strips to minimize filter-to-filter variability. The [^{3}H]CD and [^{3}H]PZ solutions were prepared fresh in buffer just prior to use in the assay to avoid decomposition. The incubations were terminated by rapid filtration (Brandel 24 sample cell harvester), and tubes were rinsed twice with 5 mL of ice-cold NaKPB buffer. Receptor-bound radioligand was determined by liquid scintillation spectrometry. The data were analyzed by nonlinear regression (LIGAND) and apparent K_i values determined by fitting a one-site competitive model to the competition data.[24]

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