Muscarinic Activity of the Thiolactone, Lactam, Lactol, and Thiolactol Analogues of Pilocarpine and a Hypothetical Model for the Binding of Agonists to the m1 Receptor

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Pilocarpine isosteres have been synthesized and characterized with regard to their in vitro muscarinic properties. The results indicate that the carbonyl oxygen of the lactone function of pilocarpine is of primary importance for agonist activity with the ether oxygen being of lesser or secondary importance. An X-ray structure determination for the hydrogen *O,O'*-ditoluoyltartrate salt of thiolactone pilocarpine isostere **2a** has been performed. This compound has an unusual pharmacological profile exhibiting M1-agonist selectivity as well as presynaptic antagonism. As a result this compound is also viewed as having therapeutic potential for Alzheimer's disease. A model for the binding of pilocarpine and other muscarinic agonists to the third transmembrane helix of the human m1 muscarinic receptor has been developed.

There has been considerable interest recently in centrally active muscarinic agonists due to their therapeutic potential in Alzheimer's disease.[1] An inspection of the patent literature of the last few years reflects a notable effort of the pharmaceutical industry in this field. Although the natural product muscarine defines the muscarinic subtype of acetylcholine receptors, many substances or substance classes can act as muscarinic agonists. The possibility for structural diversity can be seen in three other classical agonists: arecoline, pilocarpine (both natural products), and oxotremorine (Figure 1). All three of these compounds readily penetrate the blood–brain barrier, unlike muscarine which is a quaternary ammonium compound. Arecoline suffers from a very short duration of action due to the metabolically labile ester function. Oxotremorine exhibits potent central activity and is well-suited for derivatization but is relatively toxic. Pilocarpine has long been in clinical use for glaucoma and exhibits a pharmacological profile which is somewhat unique relative to other muscarinic agonists. In particular, pilocarpine is typically characterized as a partial agonist and had been shown to enhance acetylcholine release.[2] In addition, despite its long history, pilocarpine has not been the subject of elaborate or extensive structure–activity relationship (SAR) studies.[3] Thus, pilocarpine attracted our interest and has been the subject of an extensive SAR study in our laboratories. In this first paper we present our derivatization of the lactone moiety of pilocarpine. The in vitro muscarinic properties of a series of pilocarpine isosteres are reported, and the thiolactone pilocarpine isostere, thiopilocarpine (**2a**), is characterized as having therapeutic potential.

I. Chemistry

The existing SAR for pilocarpine indicated that an intact lactone ring system with cis stereochemistry was requisite for muscarinic activity.[4] Isosteric sulfur analogues (Figure 2) of pilocarpine (**1a**) were, however, unknown, and we decided to investigate replacing either or both oxygen atoms of pilocarpine with sulfur. Lactam **5** and *N*-methyl lactam **6**, analogues of pilocarpine, had been reported[5] as having only marginal muscarinic activity. Unfortunately, no definitive stereochemical assignment was made for these lactam analogues. We were certain that the reaction conditions under which they were synthesized must have resulted in epimerization to the trans isomers **5b** and **6b**. In light of the fact that isopilocarpine (**1b**) has been shown to be much less active than pilocarpine, we set out to synthesize and characterize the true lactam analogues of pilocarpine, **5a** and **6a**. Somewhat surprisingly, the lactol analogue **7a** of pilocarpine had never been reported. In addition to this compound thiolactols **8a** and **8b** became targets for our studies.

Synthesis. Thiolactone **2** could be prepared cleanly and in excellent yield by treatment of pilocarpine with potassium thioacetate[6] in DMF at 150 °C (Scheme I). It was clear that epimerization to the isopilocarpine series must occur under these reaction conditions. As is the case for pilocarpine (**1a**) and isopilocarpine (**1b**) the cis and trans stereoisomers of thiopilocarpine **2a** and **2b** are easily differentiated and quantitated in the 360-MHz ^{1}H NMR spectrum.[7] The ethyl side chain methyl signals are fully resolved and, due to steric compression, the signal for the cis isomer **2a** is shifted ca. 0.1 ppm downfield relative to that of the trans isomer **2b**. ^{1}H NMR analysis of the crude product **2** indicated an 80:20 mixture of **2b/2a**, which corresponds to the equilibrium ratio of isopilocarpine to pilocarpine. After chromatography and nitrate salt formation the trans isomer **2b** was readily available in pure form. Synthesis of the cis **2a** isomer which expectedly epimerizes more readily than pilocarpine proved somewhat difficult. The kinetic protonation of the lithium enolate of isopilocarpine with 2,6-di-*tert*-butyl-4-methylphenol (BHT) has been reported[8] to afford a 70:30 mixture of pilocarpine/isopilocarpine. Treatment of the 80:20 **2b/2a** mixture with 1–2 equiv of lithium diisopropylamine fol-

† Molecular Modeling and Drug Design Group.

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(3) For a review of pilocarpine SAR since 1980, see: Aboul-Enein, H. Y.; Al-Badr, A. A. Prognosis of the Pilocarpine Receptor Sites. *Methods Find. Exp. Clin. Pharmacol.* **1982**, *4*, 321–329.
(4) For at least one exception to this rule, see pilocarpodiol diacetate: Mutschler, E.; Woog, H. *Arzneim-Forsch.* **1969**, *19*, 217–221.
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(7) (a) Nunes, M. A.; Brochmann-Hanssen, E. Hydrolysis and Epimerization Kinetics of Pilocarpine in Aqueous Solution. *J. Pharm. Sci.* **1974**, *63*, 716–721. (b) Neville, G. A.; Hasan, F. B.; Smith, C. P. Stereoselective epimerization of pilocarpine in aqueous solution as determined by ^{13}C nuclear magnetic resonance spectroscopy. *Can J. Chem.* **1976**, *54*, 2094–2100.
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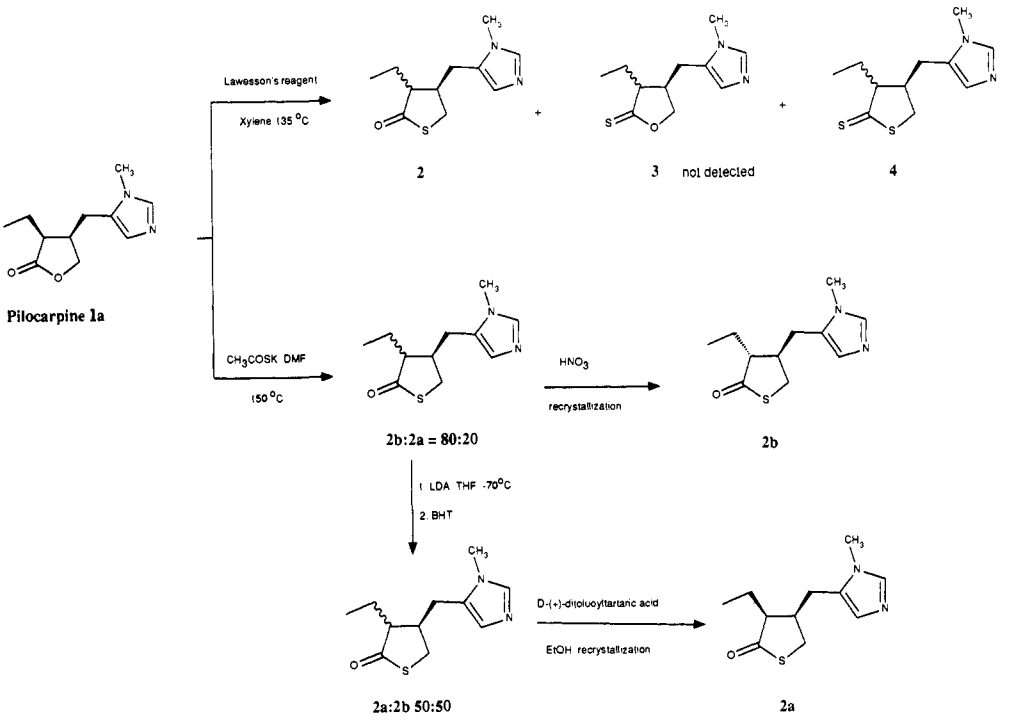
Scheme I



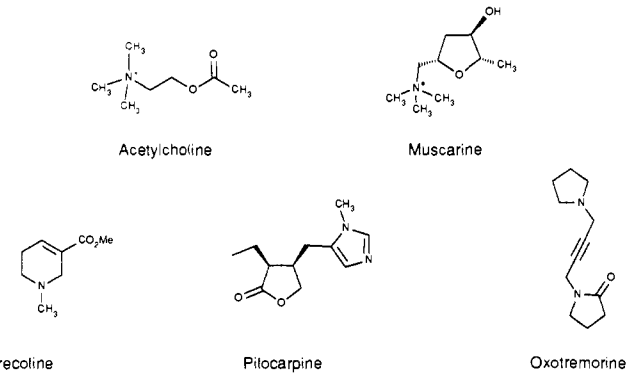
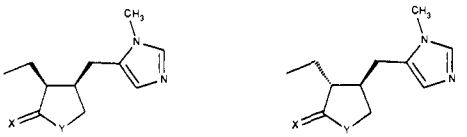


Figure 1.



X	Y		
O	O	1a	1b
O	S	2a	2b
S	O	3a	3b
S	S	4a	4b
O	NH	5a	5b
O	NMe	6a	6b
H,OH	O	7a	7b
H,OH	S	8a	8b
H,H	O	12a	12b

Figure 2.

lowed by protonation with BHT afforded at best a 50:50 mixture of cis/trans isomers **2a**/**2b**. As with pilocarpine the stereoisomers of thiopilocarpine cannot be separated[9] by simple chromatography. Purification of the cis isomer, **2a**, from a 50:50 mixture was, however, readily achieved by fractional crystallization of the hydrogen D-(+)-di-*O*,-*O'*-*p*-toluoyltartrate salt.

To prepare other sulfur analogues, pilocarpine was treated with Lawesson's reagent (Scheme I). Although it has been reported that reaction of butyrolactones with Lawesson's reagent can be used to prepare the corresponding thionolactones,[10] we were unable to isolate thionolactone **3**.[11] After chromatography of the reaction mixture, the major product isolated was dithiolactone **4** as an 80:20 trans/cis equilibrium mixture along with a small amount of thiolactone **2**. Dithiopilocarpine (**4b**) was characterized as the hydrochloride salt and could not be freed of roughly 10–15% of the corresponding cis isomer **4a** by recrystallization. We did not attempt to prepare or isolate cis isomer **4a**, which would presumably epimerize spontaneously.

The key intermediate for the synthesis (Scheme II) of the lactam pilocarpine analogue **5a** was the known ring-opened hydroxy ester **9**,[12] which belongs to a series of pilocarpine prodrugs. The use of acyclic ester intermediates is of particular advantage since they epimerize much less readily than the strained *cis*-lactone pilocarpine ring system. The hydroxyl function of **9** was smoothly converted to azide **10** by Mitsunobu reaction with hydrazoic acid. Catalytic hydrogenation of **10** then gave amine **11**, which was not isolated but cyclized directly by warming in ethanol with acetic acid to give the desired lactam **5a** in excellent yield. Comparison of **5a** with **5b**, which was prepared by the published route, showed them to be dif-

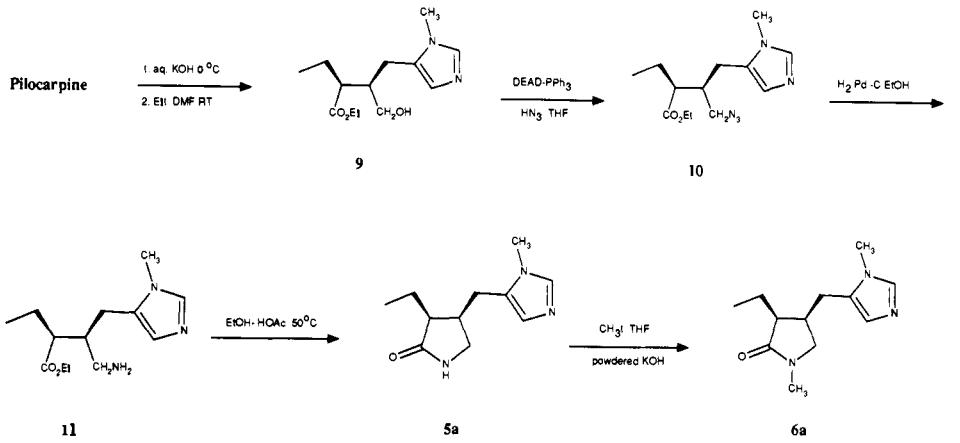
(9) Modification of the solvent system of a known[8] analytical HPLC method for resolving pilocarpine from isopilocarpine (70:30 hexane/isopropanol with 0.5% NH_4OH to 1:1:1 hexane/methyl-*tert*-butyl ether/isopropanol with 0.5% NH_4OH) allowed baseline separation of **2a** from **2b**.

(10) Scheibye, S.; Kristensen, J.; Lawesson, S. O. Studies on organphosphorus compounds-XXVII, Synthesis of thiono-, thiolo-, and dithiolactones. *Tetrahedron* **1979**, *35*, 1339–1343.

(11) The fact that dithiolactone 4 could not be prepared from the reaction of thiolactone 2b with Lawessons reagent indicates that 3 is an intermediate in the formation of 4. 2 presumably arises from the thermodynamically favored isomerization of 3.

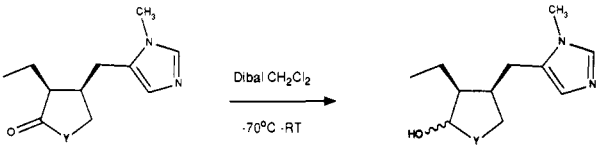
(12) Bundgaard, H.; Falch, E.; Larsen, C.; Mikkelson, T. Pilocarpine Prodrugs I. Synthesis, Physicochemical Properties and Kinetics of Lactonization of Pilocarpic Acid Esters. *J. Pharm. Sci.* **1986**, *75*, 36–43.

Scheme II



9 10 11 5a 6a

Scheme III

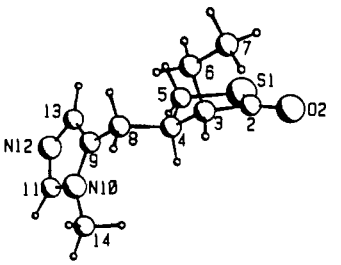


ferent compounds. It is clear from the mild conditions used to prepare **5a** that it must be indeed the cis stereoisomer and that the forcing conditions[5] described for the preparation of **5b** had caused epimerization. This was verified in the ^{1}H NMR. The methyl resonance of the ethyl group of **5a** experiences the analogous downfield shift relative to **5b** as seen for **1a** and **2a** relative to **1b** and **2b**, respectively. N-Methylation of **5a** using sodium hydride and methyl iodide in DMF at room temperature proceeded with partial isomerization to give a 75:25 **6a**/**6b** cis/trans mixture. The desired reaction without epimerization, albeit in relatively poor yield, could be achieved using a phase-transfer method[13] (THF/KOH/MeI).

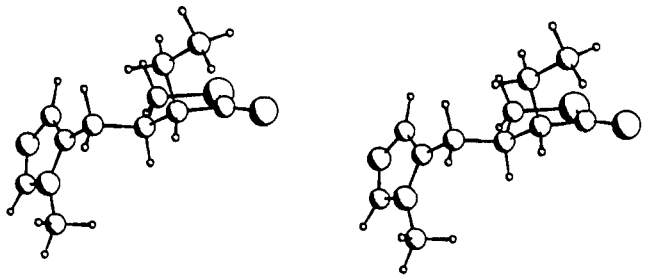
Lactol analogue **7a** was prepared (Scheme III) by Dibal reduction of pilocarpine, which proceeded unselectively to give a mixture of starting material, lactol **7a**, and overreduction product pilocarpodiol in 1:2:1 ratio. Lactol **7a** was comprised of an inseparable 2:1 epimeric mixture at the anomeric center according to ^{1}H NMR analysis in which the ring-opened hydroxyaldehyde form was not observed. Thiolactol analogues **8a** (4:1 anomeric mixture) and **8b** (1:1 anomeric mixture) were prepared in almost quantitative yield by Dibal reduction of **2a** and **2b**, respectively. As with **7a** the epimers of **8a** and **8b** were not separable by simple chromatography.

II. X-ray Crystallography

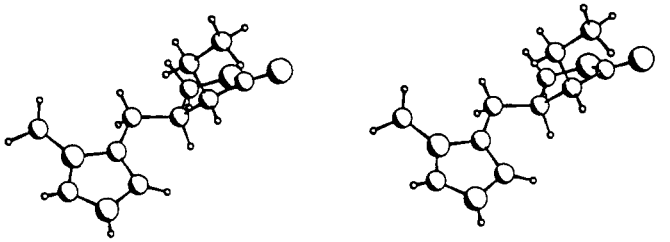
An X-ray structure determination has been performed on an orthorhombic crystal of the hydrogen D-(+)-di-*O*,*O*′-*p*-toluoyltartrate salt of thiopilocarpine (**2a**). An $I > 2.5\sigma(I)$ was found for 4221 of the 5945 measured reflections. The structure was solved by direct methods, using SHELX-86.[14] All hydrogen atoms bonded to N and O were located from difference Fourier maps. Hydrogen atoms bonded to C were included in idealized calculated positions. The final *R* factor was 0.060. A drawing of the structure is given in Figure 3. The imidazole ring nitrogen N(12) is protonated and forms a hydrogen bond to a tartrate carboxyl oxygen atom [N···O distance, 2.662 (6) Å], possibly mimicking a strong protein–drug interaction. The second carboxylic acid function is undissociated with its carbonyl oxygen, forming a so-called "very strong hydrogen bond" with the carboxylate oxygen of an adjacent tartrate molecule [O···O distance, 2.521 (5) Å]. X-ray structures for pilocarpine germanate[15] and pilocarpine hydrochloride[16] have been published. The thiolactone ring of **2a** adopts a similar envelope conformation to that found in pilocar-



2a (SDZ ENS 163)



2a (SDZ ENS 163)



Pilocarpine

Figure 3. Crossed view stereoscopic drawings (PLUTO[42]) of thiopilocarpine **2a** (the hydrogen ditoluoyltartrate moiety omitted for simplicity) and pilocarpine hydrochloride.[16]

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(14) Sheldrick, G. M. University of Göttingen, 1984.
(15) Fregerslev, S.; Rasmussen, S. E. The Crystal Structure of Pilocarpine-trichlorogermanate(II) Hemihydrate. *Acta Chem. Scand.* **1968**, *22*, 2541–2556.
(16) Codding, P. W.; James, M. N. G. The Structure of Pilocarpine Hydrochloride, $C_{11}H_{17}N_2O_2^+ \cdot Cl^-$: A Muscarinic Alkaloid. *Acta Crystallogr.* **1984**, *B40*, 429–439.

Table I. Selected Torsion Angles (deg) of X-ray Structures of 2a and Two Different Salts of Pilocarpine

	2a, X = S	pilocarpine, X = O	
		HCl	GeCl3
C(5)–X(1)–C(2)–C(3)	-4.0	1.1	-1.9
C(2)–X(1)–C(5)–C(4)	-22.0	-20.4	22.8
X(1)–C(2)–C(3)–C(4)	28.2	18.2	-20.0
X(1)–C(2)–C(3)–C(6)	-95.0	-103.4	-152.2
C(2)–C(3)–C(4)–C(5)	-44.2	-28.4	31.2
C(2)–C(3)–C(4)–C(8)	-168.3	-149.7	-87.3
C(2)–C(3)–C(6)–C(7)	-56.1	-58.0	-178.7
C(3)–C(4)–C(5)–X(1)	41.6	30.4	-32.2
C(3)–C(4)–C(8)–C(9)	-179.1	-74.5	-177.7
C(4)–C(8)–C(9)–N(10)	94.9	179.8	-165.3

pine hydrochloride (Figure 3). In both structures the ethyl group is pseudoaxially oriented with the imidazole-containing side chain adopting a pseudoequatorial orientation. The torsion angles of the bonds to the imidazole ring are however quite different for the two structures. The relevant torsion angles for **2a** and pilocarpine hydrochloride are, respectively, C(3)–C(4)–C(8)–C(9) = -179° compared to -75° and C(4)–C(8)–C(9)–N(10) = 95° compared to 180°. Selected torsion angles showing the conformation of **2a** and the two different salts of pilocarpine are given in Table I. In contrast to **2a** and pilocarpine hydrochloride, pilocarpine germanate adopts an alternate ring pucker conformation with the ethyl group pseudoequatorial and the imidazole containing side chain pseudoaxial to the five-membered ring.

III. Pharmacology

Methods. Binding. The affinity of all compounds to muscarinic receptors was determined as previously described[17] by their ability to displace [^{3}H]pirenzepine (Pir) and [^{3}H]-*cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-dioxolane (CD), in rat cortical tissue. Pirenzepine[18] is an antagonist which is selective for the M1 or neuronal subtype of muscarinic receptors and CD[19] is a nonselective, potent muscarinic agonist.

Functional Tests. Three pharmacological models were used to characterize postsynaptic functional responses. Contraction of the guinea pig ileum is a standard classical test for muscarinic activity in smooth muscle and can be considered a measure of peripheral action. The slow depolarization of the rat cervical ganglion[17] and the firing rate of the rat hippocampal CA_1 neurons[20] provide measures of neuronal or central muscarinic activity. Muscarine was always used as the internal standard, with its efficacy or maximal response being defined as 100%. Finally, the effect on electrically evoked acetylcholine (Ach) release from rat hippocampal slices[21] serves as a measure of central presynaptic activity. In this model agonists act to inhibit Ach release dose-dependently. Blockade of the effect of the standard agonist oxotremorine was used to measure antagonist activity at the presynaptic level.

Background. In the older literature muscarinic agonist activity was generally defined in terms of parasympathetic activity or effects. Examples of these are smooth muscle contraction, causing, for example, diarrhea, glandular effects such as sweating and salivation, and effects on heart rate and blood pressure. Tests for neuronal or central muscarinic activity or function have been developed only recently. Selective antagonists have demonstrated the existence of three pharmacologically[22] distinct subtypes of muscarinic receptors. These subtypes denoted M1, M2, and M3 have different cellular locations and mediate varied physiological responses. *Generally* speaking, M1 receptors are located on neurons, M2 receptors are located in cardiac tissue and have been shown to be responsible for the modulation of central Ach release,[23] and M3 receptors are located on smooth muscles and glands. Verification of the existence of muscarinic receptor subtypes has been provided by cloning experiments which have revealed the existence of at least five molecularly distinct subtypes m1–m5.[24] In our laboratories the ganglion depolarization and the hippocampal firing rate serve as M1 models, the ileum contraction as an M3[25] model, and the hippocampal Ach-release test as an M2 model. The CD binding provides a general measure of central muscarinic agonist activity and the Pir binding yields an indication of central muscarinic antagonist activity.

IV. Results

Postsynaptic Activity. Pilocarpine is typically characterized as a partial agonist due to a diminished maximum response or efficacy relative to classical full agonists such as muscarine and carbachol. The agonist potency (pD_2) of pilocarpine is in general between 1 and 2 orders of magnitude less than that of these standard potent ligands (Table II). In the ileum model pilocarpine displays

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Table II. Data from Functional Tests and Binding for Compounds 1–8 and Other Known Muscarinic Agents[a]

compd	X	Y	pD_2 ileum	% eff	pD_2 ganglion	% eff	pD_2 hippo	% eff	pA_2 Ach rel	IC_{50} CD	IC_{50} Pir	IC_{50} Pir: IC_{50} CD
1a	O	O	5.9	84	6.5	130	5.6	100	5.0	80.0	960	12.0
1b	O	O	4.4	77	5.0	125	5.1	100		430	1800	4.2
2a	O	S	5.3	70	6.5	130	5.3	50	5.5	54.0	435	8.1
2b	O	S	4.8	35	4.7	110	5.4	30	5.3	320	775	2.4
4b	S	S	5.6	23	5.2	38			6.2	45.0	51.0	1.1
5a	O	NH	4.2	66	5.2	110	4.5	95	<4	845	5150	6.1
5b	O	NH	4.6	90	5.6	120	4.7	105	<4	800	9950	12.4
6a	O	NCH_3	4.2	30	5.0	100				4600	10200	2.2
6b	O	NCH_3	<4		4.4	85	4.1	30		10000	10000	1.0
7a	H, OH	O	5.2	22	5.9	65	<4		6.4	45.0	110	2.4
8a	H, OH	S	<4		4.4	70	<4		5.4	705	695	1.0
8b	H, OH	S	<4		4.7	37	<4		5.6	330	285	0.9
12	H, H	O	4.5	34	5.7	60	<4			445	1800	4.0
13			5.4	93	6.1	132	5.6	100		100	288	2.9
pilosinine			<4		4.2	82				2890	10000	3.5
MFT			6.8	115	7.1	63	5.8	129		5.6	1410	252
oxotremorine			7.1	100	8.0	55	7.1	100		2.8	113	40.4
muscarine			6.8	100	7.4	100	5.9	100		12.0	5680	473
CD			7.5	100	7.8	100	6.3	100		6.4	2100	328
carbachol			6.7	100	7.2	100	6.0	100		16.5	11850	718
arecoline			6.5	100	6.6	80	6.6	100		20.0	1740	87.0

[a] Efficacy values for the functional tests are relative to muscarine at 100%. The SEM for all pD_2 and pA_2 determinations is less than or equal to 0.2. The maximal SEM for the binding pIC_{50} values is 5%. Hill coefficients for the binding are included in the supplementary material.

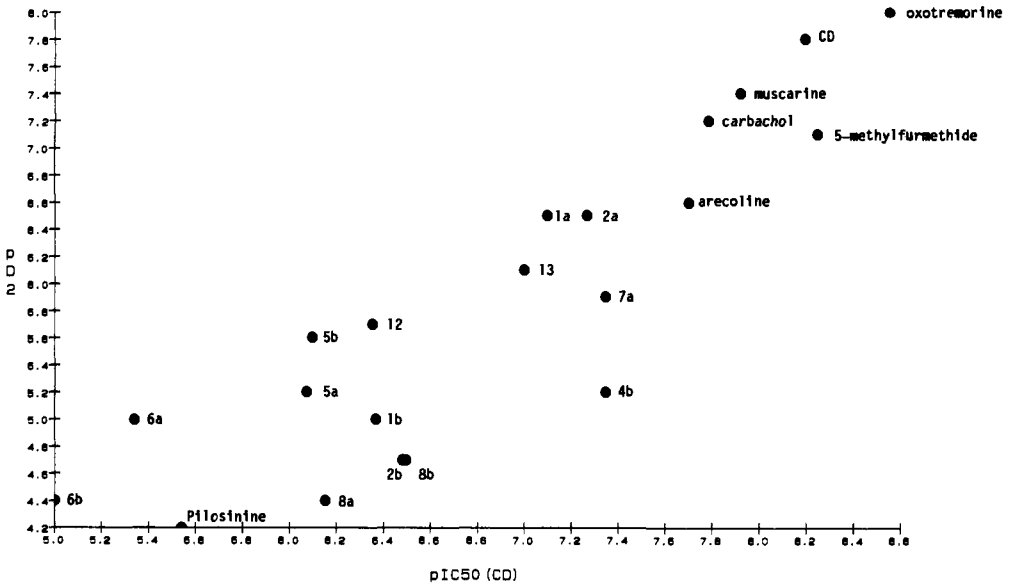

Figure 4. Correlation of CD binding (pIC_{50}) with agonist potency (pD_2) in the ganglion model (R = 0.89).

partial agonism in accord with the characterization above. In the ganglion depolarization and hippocampal firing rate models, however, pilocarpine acts as a full agonist. In the ganglion model the maximal response is, in fact, greater than that of muscarine. Thus, with respect to postsynaptic neuronal activity considered to be an M1-mediated response, pilocarpine may be characterized as a full agonist.

For the series of analogues 1–8 (Table II), pilocarpine, as parent compound, is the most potent with respect to postsynaptic agonism. Isopilocarpine is much less active than pilocarpine in binding and in all functional models being particularly weak in the ileum model. Indeed isopilocarpine has been noted for its lack of parasymptomimetic activity.[3] Thiopilocarpine (**2a**) is equipotent (pD_2) and of equal efficacy to pilocarpine in the ganglion model but considerably weaker in the ileum and hippocampal models, where reduction both in potency and efficacy is observed. Isothiopilocarpine (**2b**) is slightly less active than isopilocarpine (**1b**) in all models and is much less active than **2a** in the ileum and ganglion models. Interestingly, the pairwise differences for **1a**:**1b** and **2a**:**2b** in hippocampal activity are not so large. Although dithiolactone **4b** has pD_2 values similar to those for isopilocarpine, it displays very low efficacies in all models and so is a very weak partial agonist. Astonishingly, the cis and trans lactam isomers **5a** and **5b** are roughly equipotent both in functional tests and binding, contrasting strongly with the results obtained for **1a** vs **1b** and **2a** vs **2b**. The agonist potencies and binding affinities of **5a** and **5b** are, however, quite modest, roughly equaling those of isopilocarpine. The *cis*-*N*-methyl lactam **6a** is more potent than trans isomer **6b**, but is only marginally active, albeit selective, as a full agonist in the ganglion model. The only significant postsynaptic activity observed for the lactol analogues **7a** and **8a**,**b** was a moderate partial agonist activity for pilocarpine lactol **7a** in the ganglion model.

The correlation of the CD binding with the agonist potency for analogues 1–8 along with several other known muscarinic agonists (see Table II) is good in all three models. A graph of CD binding vs ganglion potency is

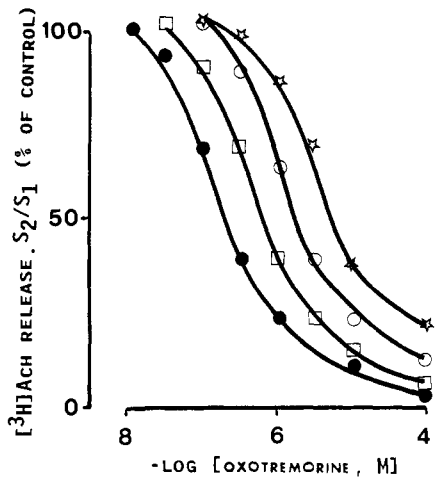

Figure 5. Concentration effect curves of oxotremorine in the absence of **2a** (black circles) in the presence of 10 μM **2a** (open boxes), 30 μM **2a** (open circles), and 100 μM **2a** (stars). Results are mean values of four experiments with SEM < 5%.

shown in Figure 4. R values of 0.94 (n = 17), 0.89 (n = 21), and 0.92 (n = 14) were found for the correlations of CD binding with functional potency in the ileum, ganglion, and hippocampal models, respectively. No correlation was found between pirenzepine binding and functional potency in these three models. In the final column of Table II the binding ratio IC_{50} of pirenzepine:IC_{50} of *cis*-dioxolane is tabulated. The binding ratio of the nonselective antagonist *N*-methylscopolamine to the full agonist oxotremorine-M, log (IC_{50}NMS/IC_{50}OXO-M), has been shown[26] to correlate with the efficacy of cortical PI turnover. We have attempted to correlate the Pir:CD ratio with the efficacies measured in our functional tests. Interestingly, a modest correlation was obtained for the ileum efficacy (R = 0.80, n = 17) with lower correlations for both ganglion (R = 0.30, n = 19) and hippocampal efficacies (R = 0.61, n = 14).

Presynaptic Activity. The muscarinic agonist oxotremorine acts to decrease the electrically evoked Ach release in hippocampal slices by 80%. Pilocarpine acts as a partial agonist with a maximal effect of 30% decrease in basal acetylcholine release. Thiopilocarpine (**2a**) acts as an antagonist in the release model with a pA_2 of 5.5 (Figure 5). Interestingly the trans isomer, isothiopilocarpine (**2b**), is only slightly less active than **2a** as an antagonist in this test. A correlation of the pA_2 (Table II) values with the binding activity (as pIC_{50}) revealed a good correlation (R = 0.94, n = 7) with antagonist binding (Pir) (Figure 6) and a poor correlation with agonist (CD) binding as expected. Isodithiopilocarpine (**4b**), pA_2 = 6.2, is a relatively potent antagonist in this release model. The *trans*-lactams **5b** and **6b** were inactive in this test. The *cis*-lactams **5a** and **6a** were not tested since the binding data provided no indication of activity. Lactol **7a**, a weakly active partial agonist in our functional tests for postsynaptic activity, proved to be the most potent antagonist of the series with a pA_2 of 6.4. Thiolactols **8a** and **8b**, which were virtually inactive in postsynaptic functional tests, also acted as presynaptic antagonists, being roughly equipotent to **2a**.

V. Discussion

Pilocarpine is an interesting muscarinic ligand due to its varied action as either a full or partial agonist depending on the tissue studied. An increase in agonist potency could not be realized in the series of analogues **1–8**. In general a large decrease in agonist potency and or efficacy was observed. However, selective attenuation of agonist potency was possible in the case of thiopilocarpine (**2a**), which is equipotent and of equal efficacy to pilocarpine in the ganglion model but considerably less potent in the ileum model and of lesser efficacy in the hippocampus model. Using the ganglion as a criterion for central or M1 activity and the ileum as a model of M3-mediated peripheral activity, thiopilocarpine can be viewed as an agonist which enjoys an enhanced central (M1) selectivity relative to pilocarpine. A shift of the presynaptic release (M2) effect for pilocarpine from partial agonism to antagonism was achievable by different structural modifications (e.g. **2a** vs **7a**). However, only in the case of **2a** was it possible to retain significant postsynaptic activity in the process. Thus, **2a** is a rather unique muscarinic ligand which can act as a full agonist, a partial agonist, or an antagonist depending on the tissue studied.

VI. SAR Discussion

Background. Given the proven existence of receptor subtypes, the value of interpreting the activity of a muscarinic agonist using a generalized pharmacophore such as the one proposed by Schulman[27] for muscarinic agonists possessing an NCCOCC chain may be questioned. Nevertheless, the homology of the seven putative transmembrane helical regions[24] for the cloned muscarinic receptor subtypes where agonist binding presumably occurs is very high. It appears that three basic elements are common to any muscarinic agonist binding site: an anionic site which binds the trimethylammonium function of acetylcholine, an ester binding site where the acetylcholine ester function binds, and a hydrophobic site where the terminal acetyl methyl group binds. In the Schulman model the anionic site is represented by a point P located 3.0 Å from the quaternary nitrogen atom and colinear with the C–N main-chain bond. The ester binding site is defined by a point Q located on the COC bisector in the direction of the ether oxygen lone pairs at a distance of 1.2 Å from oxygen. Their respective ligand attachments are represented by points N and O which correspond to the nitrogen and ether oxygen atoms of acetylcholine. The distance PQ = 6.6–6.8 Å and the dihedral angle PNOQ = 100–117° are the essential elements of the pharmacophore. Finally, the optimal distance for P to a terminal atom (usually a methyl group), PC = 8.5 Å, defines a hydrophobic binding site. There are four aspartic acid residues which are conserved over all known muscarinic receptor sequences, and it is clear that one of these, which remains to be assigned unequivocally,[28] represents the anionic site. At the ester site

(26) Freedman, S. B.; Harley, E. A.; Iversen, L. L. Relative affinities of drugs acting at cholinoceptors in displacing agonist and antagonist radioligands: the NMS/Oxo-M ratio as an index of efficacy at cortical muscarinic receptors. *Br. J. Pharmacol.* **1988**, *93*, 437–445.

(27) Schulman, J. M.; Sabio, M. L.; Disch, R. Recognition of Cholinergic Agonists by the Muscarinic Receptor. 1. *Acetylcholine* and Other Agonists with the NCCOCC Backbone. *J. Med. Chem.* **1983**, *26*, 817–823.

(28) (a) Curtis, C. A. M.; Wheatley, M.; Bansal, S.; Birdsall, N. J. M.; Eveleigh, P.; Pedder, E. K.; Poyner, D.; Hulme, E. C. Propylbenzilylcholine Mustard Labels an Acidic Residue in Transmembrane Helix 3 of the Muscarinic Receptor. *J. Biol. Chem.* **1989**, *264*, 489–495. (b) Sokolovsky, M.; Galron, R. Carboxyl Residue(s) at the Ligand-Binding Site of Rat Muscarinic Receptors. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1203–1208. (c) Fraser, C. M.; Wang, C. D.; Robinson, D. A.; Gocayne, J. D.; Venter, J. C. Site-Directed Mutagenesis of m_1 Muscarinic Acetylcholine Receptors: Conserved Aspartic Acids Play Important Roles in Receptor Function. *Mol. Pharmacol.* **1990**, *36*, 840–847.

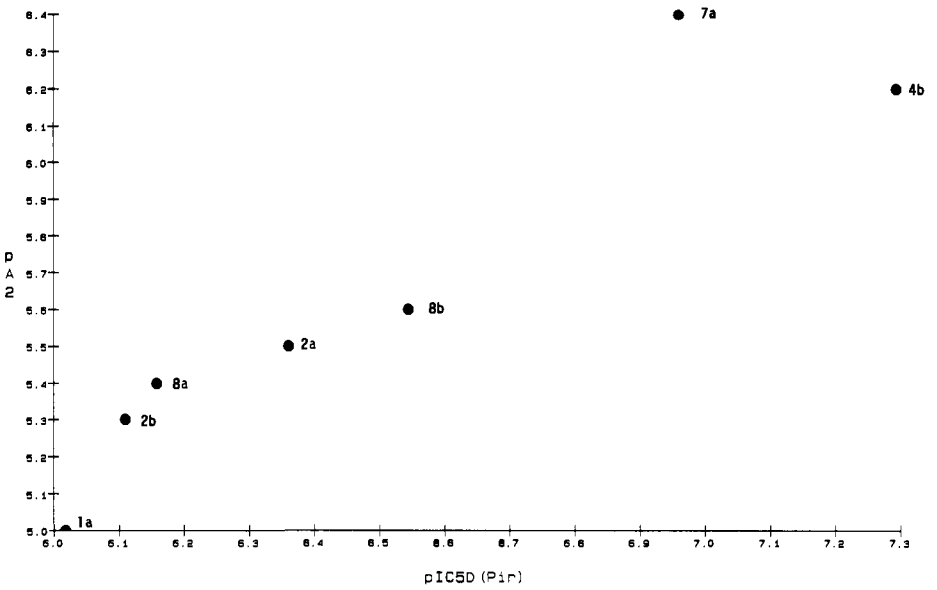

Figure 6. Correlation of antagonist activity (pA_2, $R = 0.94$) in hippocampal acetylcholine release with pirenzepine binding (pIC_{50}).

a hydrogen bond donating residue is postulated to interact with the hydrogen bond accepting ester function. There has been, to our knowledge, no speculation regarding the molecular nature of the putative hydrophobic binding interaction. The assumption made by Schulman that a relatively fixed geometry can be assigned to the conformationally mobile binding functionalities of the receptor is fundamental to the generation of a precise pharmacophore. Although all potent muscarinic agonists have functionalities capable of interacting at the fundamental binding sites, the considerable range in their potencies, efficacies, and molecular structures is indicative of dynamic interaction between receptor and ligand. It is our opinion that a pharmacophore of defined geometry may at best be used to interpret SAR data within a particular substance class and then a common binding motif to the defined pharmacophore may not be assumed in all cases. We hope to illustrate this in the case of pilocarpine analogues.

Agonist SAR. In a review[3] of pilocarpine SAR until 1982 four sites of attachment were proposed for binding to a receptor site. These were sites for the protonated imidazole function, the ether oxygen, the carbonyl oxygen, and the ethyl side chain. The relative importance of the carbonyl and ether oxygens was not addressed. The biological data obtained for the series of pilocarpine analogues **1–8** now establish this relative importance.

The first evidence that the carbonyl oxygen is essential for agonist binding and function is provided by the tetrahydrofuran pilocarpine analogue **12**[5] (Figure 7). This compound exhibits only marginal binding, is virtually inactive in ileum and hippocampus models (see Table II), and is a partial agonist in the ganglion model. It would appear from this result that *the primary ester binding interaction is with the carbonyl oxygen* and that the ether oxygen's contribution to binding and agonist action (efficacy) is of a weak or secondary nature. This is borne out by our results with **2a**. A moderate overall decrease in agonist activity is observed for **2a** relative to pilocarpine compared to the dramatic decrease in all postsynaptic functional tests observed for **12**. Since the sulfur atom of **2a** is a very poor hydrogen-bond acceptor,[29] it follows that

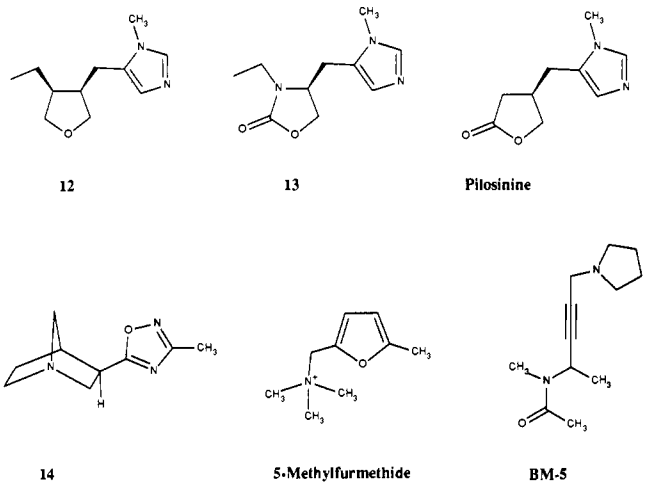

Figure 7.

the carbonyl oxygen of pilocarpine is primarily responsible for binding at the ester site. The decrease in agonist activity observed for **2a** relative to pilocarpine may be explained, however, by the existence of a weak hydrogen bond between the pilocarpine ether oxygen and the receptor.

The existence of a weak hydrogen bond to the pilocarpine ether oxygen is supported by the results obtained with the lactam analogue **5a**. If the carbonyl function of pilocarpine were solely responsible for binding at the ester binding site with no interaction taking place near the ether oxygen, then one would not expect such a dramatic drop in activity as is observed for the lactam analogue **5a**. The oxazolidinone pilocarpine isostere **13**[30] (Figure 7) has been reported to be equipotent to pilocarpine. Thus, two distinct ring systems, thiolactone and oxazolidinone, which have geometries slightly different from pilocarpine, are effective analogues. Therefore, an explanation of the drop

(29) Abraham, M. H.; Duce, P. P.; Prior, D. V.; Barrat, D. G.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1355.

(30) (a) Sauerberg, P.; Chen, J.; Woldemussie, E.; Rapoport, H. Cyclic Carbamate Analogues of Pilocarpine. *J. Med. Chem.* **1989**, *32*, 1322–1326. (b) Our material was prepared according to the following: Gonzalez, F. B.; Baz, J. P.; Espina, M. R. I. Synthesis of (+)-Pilocarpine Analogs with a 2-oxozolidinone structure. *Tetrahedron Lett.* **1989**, *30*, 2145–2148.

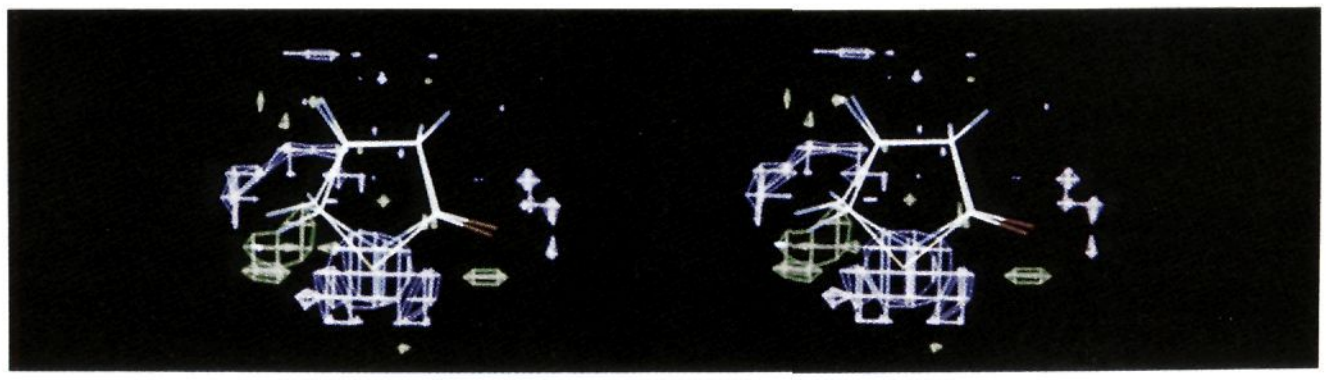

Figure 8. Difference volumes of γ-thiolactone and γ-lactam ring systems (crossed stereoscopic picture). The region occupied only by the thiolactone is depicted in violet and that only by the lactam in green. Volumes were obtained with SYBYL by the MVOLUME function applied to the superimposed models.

in activity for **5a** as being due to geometrical and or conformational differences between lactone and lactam rings would not seem tenable. An explanation purely based on steric interference of the lactam hydrogen of **5a** at the binding site is refuted by the results with **2a**. Although the volume (van der Waals) occupied by a sulfur atom is less than that filled by the combined nitrogen and hydrogen volumes, the geometries for the thiolactone and lactam ring systems are different. As a result, when a common volume comparison (Figure 8) is made between substructures of **2a** and **5a** in which the carbonyl group and ring carbons are constrained to overlap with a root mean square of 0.22 Å, it can be seen that the steric requirement at the 1-position is in fact slightly greater for **2a** than for **5a**. If a steric repulsion were present, then **2a** should be less active then is in fact the case. If, however, a receptor hydrogen-bond donor is geometrically situated at a point enabling a weak hydogen bond to the ether oxygen of pilocarpine, then an electrostatic repulsion may result with the lactam hydrogen of **5a**. To avoid this repulsion the receptor may change its conformation to relieve this effect upon binding **5a**. Alternatively, **5a** may bind to the receptor in an orientation as well as in a conformation which is different from that of pilocarpine. Finally, distortion of both ligand and receptor orientation may occur. This distortion then leads to suboptimal receptor–ligand interaction as observed for **5a**.

The slightly greater activity of the trans lactam isomer **5b** over the cis isomer **5a** is surprising. **5b** and isopilocarpine are roughly equipotent. It may be that isopilocarpine binds to the receptor in a motif different from that of pilocarpine. In this altered binding mode one may easily envisage the loss of any hydrogen-bonding interaction between the lactone ether oxygen and the receptor. As a result, unlike the case for **5a** and pilocarpine, **5b** may be able to bind in a fashion analogous to that of isopilocarpine. Thus, **5b** may be viewed as being a good analogue of isopilocarpine with similar muscarinic activity. Support for an altered binding motif for isopilocarpine is provided by the example of pilosinine. Pilosinine (Figure 7, Table II), which lacks the ethyl side chain of pilocarpine, displays only marginal muscarinic activity. This indicates the existence of an important binding interaction for the ethyl group of pilocarpine at the putative hydrophobic binding site of the receptor. If isopilocarpine were to bind to the receptor in a motif analogous to that of pilocarpine, it is difficult to imagine its ethyl group being able to interact at the same hydrophobic site. Since a tight fit is evidenced by muscarinic agonists, with little toleration for extra atoms, a different binding mode for isopilocarpine in which some hydrophobic binding is maintained while the other binding interactions are attenuated seems likely. The only marginal activity seen for **6a** and the inactivity of **6b** indicates that a tight fit exists in agonist–receptor binding which is very sensitive to steric effects. Although receptor and or ligand distortion can accomodate the electronic and modest steric demand of **5ab**, the steric demand of the methyl group of **6ab** is too great and cannot be accomodated.

The postsynaptic functional activity for **7a** must be interpreted carefully with regard to SAR. In ileum and ganglion functional models **7a** acts as a weak partial agonist with moderate potency. In the release model **7a** displays antagonist activity. The antagonist binding (Pir) of **7a** is significantly greater than that of pilocarpine and the agonist (CD) binding is slightly greater. Although a correlation between agonist potency and CD binding has been demonstrated (Figure 4), partial agonists are known to exhibit anomalous behavior. A partial agonist may effectively displace an agonist ligand, thus demonstrating high affinity, but nevertheless elicit only weak functional responses.[31] As a result, it is somewhat difficult to evaluate the effect of exchanging the carbonyl group of pilocarpine for the hydroxyl function of **7a** on the interaction at the agonist binding site. An adjustment of both oxygen functionalities of the lactone moiety in **8a,b**, where the ether oxygen is replaced with sulfur and the carbonyl oxygen by hydroxyl, leads to compounds virtually devoid of any functional agonistic activity. In addition, for **8a** both agonist and antagonist binding are relatively weak. In this case the drop in activity of **8a** relative to **2a** clearly demonstrates that the hydroxyl group of **8a** cannot bind effectively at the ester binding site of the muscarinic receptor.

In summary, the carbonyl oxygen of the lactone function is essential to the agonist function of pilocarpine while the ether oxygen is of secondary importance. The results indicate a tight fit for pilocarpine at an agonist binding site with a stringent geometric requirement for agonist activity.

Muscarinic Receptor Model. The question of which ester oxygen atom of acetylcholine itself is responsible for binding to the muscarinic receptor has already been a topic of dispute. In the Schulman model the ether oxygen atom binds to a putative receptor hydrogen-bond donor. This was criticized by Snyder who pointed out that the carbonyl oxygen is a better hydrogen-bond acceptor.[32] In the case of pilocarpine, we have clearly shown that it is the carbonyl oxygen which is primarily responsible for binding and agonist function. Nevertheless, our results indicate that the lactone ether oxygen is involved in a secondary binding interaction. More recently, the Merck group has reported

(31) For examples with serotonergic agonists, see: Hoyer, D.; Boddeke, E.; Schoeffter, P. Second Messengers in the definition of 5-HT receptors. In *Serotonin: molecular biology, receptors and functional effects*; Fozard, J. R., Saxena, P. R.; Eds.; Birkhäuser Verlag: Berlin, in press.

(32) Snyder, J. P. Molecular Models for Muscarinic Receptors. *Trends Pharmacol. Sci.* **1985**, *6*, 464–466.

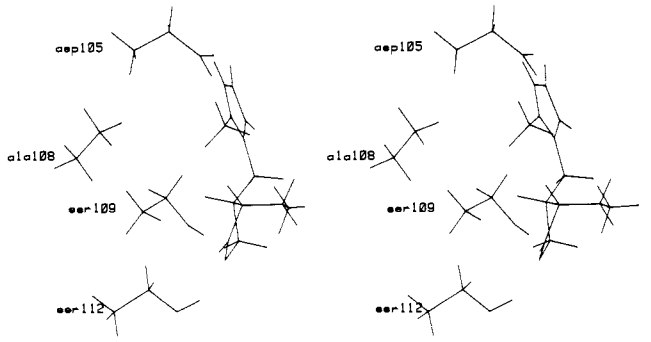

Figure 9. Crossed stereoscopic view of the binding model of protonated pilocarpine and the simplified human m1 muscarinic receptor. The model of the receptor consists of amino acid side chains Asp 105, Ala 108, Ser 109, and Ser 112 with their C_α-atoms placed on a standard α-helix. Pilocarpine is docked manually to Asp 105, Ser 109, and Ser 112. Subsequent geometry optimization was carried out with MAXIMIN2 (SYBYL) using Gasteiger–Hueckel charges. The final geometry shows close contacts of (i) one of the two oxygens of Asp 105 with the imidazolium nitrogen, (ii) the oxygen of Ser 109 with the carbonyl oxygen, and (iii) the oxygen of Ser 112 with the ether oxygen.

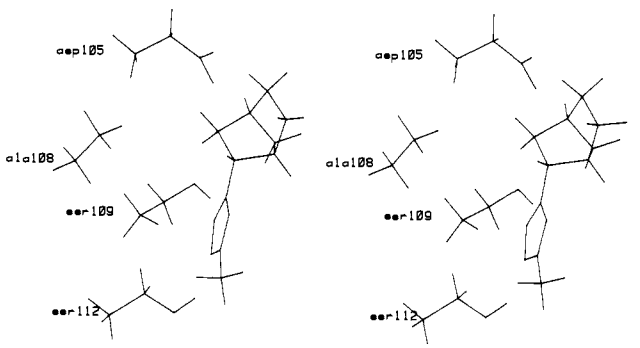

Figure 10. Crossed stereoscopic view of the binding model of protonated oxadiazole 14 and the simplified human m1 muscarinic receptor (derived as in Figure 9). The final geometry shows close contacts of (i) one of the two oxygens of Asp 105 with the ammonium nitrogen of 14, (ii) the oxygen of Ser 109 with the 4′ oxadiazole ring nitrogen, and (iii) the oxygen of Ser 112 with the 2′ oxadiazole ring nitrogen. The methyl group of 14 fits into the region between the side chains of Ser 109 and Ser 112.

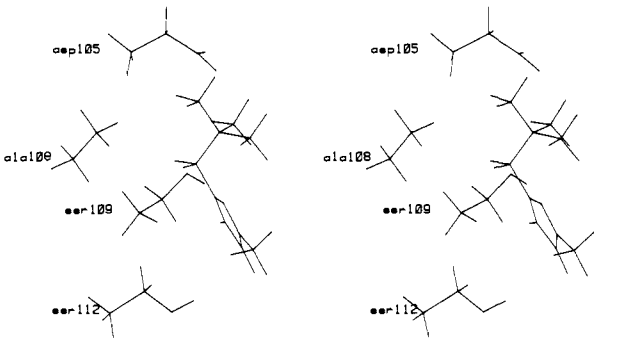

Figure 11. Crossed stereoscopic view of the binding model of methylfurmethide (MFT) and the simplified human m1 muscarinic receptor (derived as in Figure 9). The final geometry shows close contacts of (i) both Asp 105 oxygens with the quaternary ammonium nitrogen and (ii) the oxygen of Ser 109 to the furan oxygen.

a series of extremely potent oxadiazole[33] muscarinic agonists, which provide evidence for the existence of two hydrogen-bond donors at the ester binding site. These receptor hydrogen-bond donors have been represented by two unspecified receptor serine residues. We agree with this two hydrogen bond donor hypothesis and have developed a dynamic model for the binding of several muscarinic agonists of diverse structural classes (pilocarpine, muscarine, 5-methylfurmethide, oxotremorine, and arecoline) to the third putative membrane spanning helix of the human m1 receptor.[24] The essential elements of the model are an anionic binding site localized at aspartate 105 and an ester binding site with Ser 109 and Ser 112 acting as hydrogen-bond donors. Consideration of the conformational mobility of the receptor is fundamental to the model. In our modeling of the binding of muscarinic agonists to the third membrane-spanning helix of the m1 receptor, an idealized α-helix (ϕ = −58°, ψ = −47°, ω = 180°) is assumed on which the C_α and C_β atoms of residues Asp105, Ala108, Ser109, and Ser112 are fixed. In a further simplification the backbone bonds C_α–N and C_α–C are then replaced by C_α–H bonds. The side chains are given torsional freedom. This allows the modeling of favorable binding geometries of this side chain quadrad for various agonists in one or another active conformation and relative orientation with respect to the helix. Binding motifs with plausible inter- and intramolecular geometries were routinely derived by graphical manipulation followed by energy minimization with the SYBYL/PS390[34] molecular modeling system of the reduced helix model interacting with a particular ligand conformer. A binding motif for pilocarpine is depicted in Figure 9. Pilocarpine is modeled in a conformation very similar to that observed in the hydrochloride X-ray structure, with the sole difference being a torsion angle C(4)–C(8)–C(9)–N(10) of −120.4° instead of 179.8° for the imidazole side chain. The protonated imidazole N(12) nitrogen forms a salt bridge with the carboxylate function of Asp105 with an NH···O distance of 1.8 Å. Ser109 forms a hydrogen bond to the carbonyl oxygen (distance OH···C=C, 1.8 Å) and Ser112 hydrogen bonds to the ether oxygen (distance OH···O, 1.9 Å). In the SAR discussion the ether oxygen has been shown to be of secondary importance for the binding of pilocarpine and the carbonyl oxygen to be of primary consequence. Consequently, our model implies that the intrinsic hydrogen-bonding strength for the carbonyl oxygen is considerably greater than that for the ether oxygen. We do not, however, rule out the possible existence of a binding mode for pilocarpine in which the carbonyl oxygen accepts two hydrogen bonds[35] and the ether oxygen does not participate in hydrogen bonding. Such a binding mode could theoretically become favorable in the case of thiolactone analogue **2a**, which lacks the ether oxygen. When

(33) (a) Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, L. L.; Kneen, C.; Macleod, A. M.; Merchant, K. J.; Snow, R. J.; Baker, R. Novel Quinuclidine-Based Ligands for the Muscarinic Cholinergic Receptor. *J. Med. Chem.* **1990**, *33*, 1128–1138. (b) Saunders, J.; Freedman, S. B. The design of full agonists for the cortical muscarinic receptor. *Trends Pharmacol. Sci. Suppl.* **1989**, 70–75 (Subtypes of Muscarinic Receptors IV).

(34) *SYBYL Molecular Modeling System*; Tripos Associates: St. Louis, MO.

(35) For an example of an X-ray structure (Cambrige Database) exhibiting two hydrogen bonds to the carbonyl function of a butyrolactone, see: Cooley, C.; Duncan, R. F.; Kirk, D. N.; Patel, S.; Wynn, S.; Buckingham, M. J.; Hawkes, G. E.; Hursthouse, M. B.; Galas, A. M. R.; Lawson, A. M.; Setchell, D. R. Structural Analysis of the Urinary Lignan 2,3-Bis-(3-hydroxybenzyl)butanolide ("Enterolactone"). A 400MHz Nuclear Magnetic Resonance Study for the Solution State and X-Ray Study for the Crystal State. *J. Chem. Soc., Perkin Trans. 2* **1984**, 489.

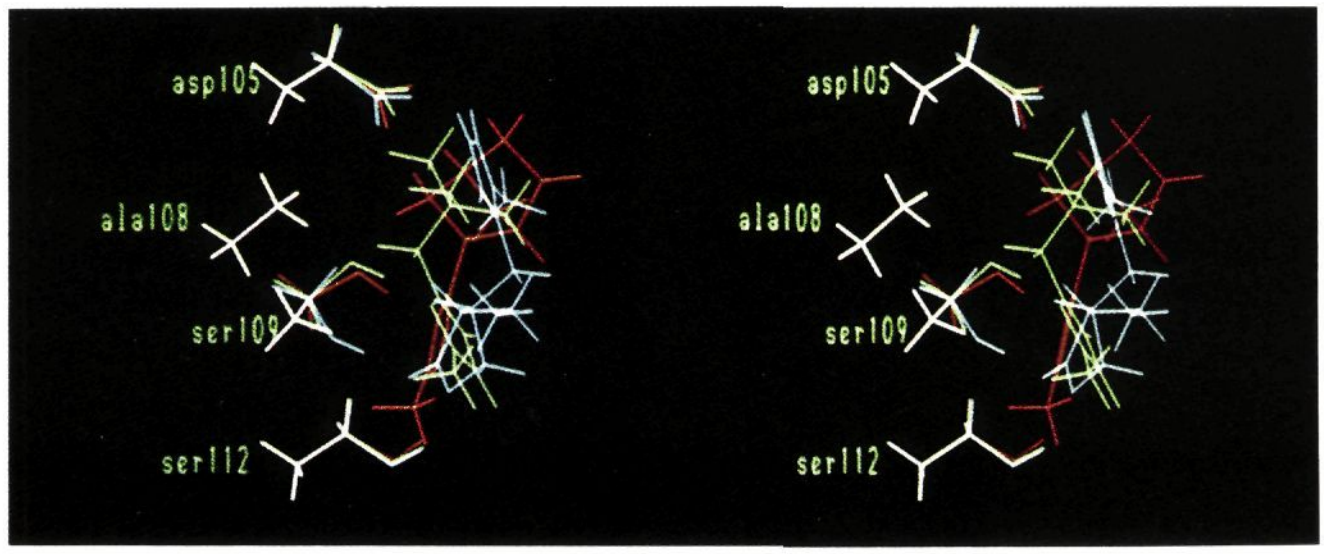

Figure 12. Superposition of the binding models depicted in Figure 9 (cyan), Figure 10 (red), and Figure 11 (green) with respect to the C_α-atoms of Asp 105, Ala 108, Ser 109, and Ser 112. The conformational variability of the receptor is particularly exemplified by Ser 109, which adapts very different geometries depending on the particular agonist ligand. Note that the pharmacophoric groups of the ligands do not superimpose.

Table III. Schulman Parameters PNOQ, PQ, and PC_t for Pilocarpine, **14** and **15**

	N	O	C_t	PQ, Å	PNOQ, deg	PC_t, Å
pilocarpine	NH	C—O—C	CH_3	10.0	96.0	7.5
oxadiazole	NH	C=N—C	CH_3	5.2	−30.8	8.0
	NH	O—N=C	CH_3	9.2	134.8	8.0
methyl-furmethide	$N(CH_3)_3$	C—O—C	CH_3	5.5	67.0	7.5

an energetically favorable conformation of the *1R,3R,4R* isomer[36] of oxadiazole **14** (Figure 7) is fitted to the model (Figure 10), a salt bridge is found with an NH···O distance of 1.6 Å. Both Ser109 and Ser112 form hydrogen bonds of optimal geometry (each distance 1.9 Å) to the nitrogens of the heterocycle. Since this compound displays extremely potent binding, both hydrogen bonds are postulated to be intrinsically of high strength. In the case of 5-methylfurmethide **15** (Figure 7) there is only a single oxygen present for hydrogen bonding. In our receptor model **15** can interact (Figure 11) in a conformation very similar to its X-ray structure[37] by forming a salt bridge between the ammonium head and Asp105, where a carboxylate oxygen is situated above one of the three CH_3–CH_3 edges at a distance of 3.5 Å from nitrogen. Ser109 donates a hydrogen bond to the furan oxygen, the distance being 1.8 Å.

The parameters PNOQ, PQ, and PCt of the Schulman muscarinic pharmacophore for pilocarpine, **14**, and **15** as modeled above are listed in Table III. As such, a marked variance is seen for these parameters. In addition, no one set of parameters matches the proposed pharmacophore geometry which was developed for agonists with the NCCOCC backbone. The superposition of the three receptor:ligand binding motifs (Figure 12) with respect to the three C_α atoms of the reduced helix exemplifies the case which cannot be taken into account by a pharmacophore superposition or a parameterization. In constructing a general pharmacophore it is assumed that the binding moieties of the receptor may be assigned fixed locations. The pharmacophoric groups of the conformationally mobile ligand are then constrained to overlap (a situation which may be defined by internal geometrical parameters, i.e. a parameterization). Neglecting the conformational mobility of the receptor is a vast oversimplification, the pitfalls of which are evident from the superposition in Figure 12. In our model the dynamic nature of the receptor is particularly exemplified by the side chain of Ser 109, which adopts very different conformations depending on the agonist ligand bound. Furthermore, there is *no overlap of the pharmacophoric groups* for these three ligands in this dynamic model of receptor–ligand interaction. Due to its conformational flexibility the receptor attains varying geometries depending on the arrangement of pharmacophoric groups present in active analogues and allows for different orientations of the agonists. Given this situation, a structure–activity relationship needs to be extrapolated from models of receptor–ligand interactions agonist by agonist. The increasing amount of information on receptor sequence and structure is making this a feasible undertaking.

Finally, it must be stressed that the muscarinic receptor–agonist binding model presented here is hypothetical and has its limitations. In a cavity enclosed by seven transmembrane helices where a ligand binds, it can be assumed that there are van der Waals contacts to more than just one helix. It is to be noted that a hydrophobic binding site, the significance of which for agonists has been disputed,[33a] has not as yet been unequivocally identified.

Antagonist SAR. An interpretation of the SAR data with respect to antagonist activity is difficult. To our knowledge no general pharmacophore of well-defined[38] geometry has been proposed for muscarinic antagonists, and it is unclear whether the antagonist binding site is spatially distinct from the agonist binding site for a given receptor subtype. Evidence both for distinct and common binding sites[39] has been presented and so it would seem that antagonists can but most not bind at the agonist binding site. Furthermore, the differentiation of muscarinic subtypes M1, M2, and M3 by antagonists clearly does not allow precise definition of a general antagonist phar-

(36) The *1S,3S,4S* enantiomer of **14** fits well with the same binding interactions as the *1R,3R,4R* enantiomer but somewhat less favorably.

(37) Chothia, C.; Baker, R. W.; Pauling, P. Conformation of Acetylcholine at Muscarinic Nerve Receptors: Crystal and Molecular Structure of 2-Trimethylammoniummethyl-5-Methylfuran Iodide (5-Methylfurmethide Iodide). *J. Mol. Biol.* **1976**, *105*, 517–526.

(38) For a crude model and references therein, see: Gordon, R. K.; Breuer, E.; Padilla, F. N.; Smejkal, R. M.; Chiang, P. K. Distance Geometry of α-Substituted 2,2-Diphenylpropionate Antimuscarinics. *Mol. Pharmacol.* **1990**, *36*, 766–772.

(39) Gualtieri, F.; Romanelli, M. N.; Teodori, E. On the Site of Interaction of Some Muscarinic Agonists and Competitive Antagonists. *Il Farmaco* **1989**, *44*, 897–909.

macophore. Given this complexity, antagonist SAR must also be performed carefully at best within a particular substance class and with consideration of receptor subtypes.

Some of the pilocarpine analogues presented here are exceptional in that, in functional tests of M1 and M3 activity, full or partial agonist activity is observed, while in the release model of M2 activity antagonism is observed. Thus, the structural requirements for antagonist activity for pilocarpine analogues relate to the M2 subtype. The general requirement for a cationic moiety which binds to an anionic receptor site is common to antagonists and agonists. Aside from this there appears to be no specific requirement for antagonist activity. As a general rule, however, antagonists have nonpolar moieties such as phenyl which are able to participate in hydrophobic binding. Pilocarpine functions as a weak partial agonist in the release model and so lies on the agonist/antagonist borderline for the M2 receptor. Exchange of the ether lactone oxygen for sulfur may be seen to increase the hydrophobicity to a point where complete antagonism results, as seen for **2a**. It would seem that hydrogen bonding is not necessary for moderate antagonist binding since dithiopilocarpine (**4b**) is even more potent than thiopilocarpine as an antagonist. This and the fact that the cis stereochemistry does not significantly increase antagonist activity for **2a** relative to **2b** can be explained either by a distinct antagonist binding site or by a different binding motif for antagonists at the agonist binding site. An intriguing result comes from lactol **7a**, which is less hydrophobic than pilocarpine yet is in fact the most potent M2 antagonist of the series. The dramatic shift of **7a** to antagonism can only be explained by invoking a strong specific hydrogen-bonding interaction at an antagonist binding site. In conclusion our results bear out the large structural variation allowed for antagonist activity.

VII. Conclusion

There is currently intense interest in muscarinic agonists which are selective for receptors located on neurons in the CNS as potential therapeutic drugs for Alzheimer's disease. This stems from the cholinergic hypothesis of Alzheimer's disease which maintains that the cholinergic deficit characteristic of the disease is related to the disease symptoms. The corollary to this is that an enhancement of cholinergic transmission should be therapeutic. One way to achieve this is by direct stimulation with an agonist. The clinical[40] use of muscarinic agonists has up to date been hindered by a lack of selectivity associated with limiting peripheral side effects. Nevertheless, at least theoretically, it may be possible to identify centrally active muscarinic agonists which are M1 or neuron selective and as a result have less limiting side effects. Increased cholinergic transmission can also be achieved through enhancement of acetylcholine release. It has been established that the autoreceptor controlling acetylcholine release is of the M2 subtype.[23] Selective central presynaptic M2 receptor blockade should therefore result in an overall increase in cholinergic transmission. The desirability of a drug having the dual synergistic actions of postsynaptic stimulation and presynaptic antagonism clearly follows from the above discussion. This concept has already been forwarded and oxotremorine analogue BM-5[41] (Figure 7) has been reported to be just such a compound which has these dual actions. In this work we have presented a novel compound, thiopilocarpine (**2a**), which is both a presynaptic antagonist and M1-*selective* postsynaptic agonist, making it an ideal drug for augmenting central cholinergic transmission. Thiopilocarpine, which has the internal classification SDZ ENS 163, is currently in clinical trials for senile dementia of the Alzheimer type.

Experimental Section

General. Tetrahydrofuran was distilled from sodium before use. Medium-pressure liquid chromatography (MPLC) was performed using 0.040–0.063 mm silica gel (Merck silica gel 60). Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. NMR spectra were determined on a Bruker WH360 spectrometer using tetramethylsilane as an internal standard. Chemical shifts are recorded in parts per million δ. Mass spectra were recorded on AEI MS30 and Varian MAT 212 (70 eV) machines. FAB MS spectra were recorded using thioglycerine as a matrix.

(+)-(3*R*-*trans*)-3-Ethyl-4,5-dihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-2(3*H*)-thiophenethione Hydrochloride (4b). Pilocarpine hydrochloride (6.70 g, 48 mmol) and Lawesson's reagent (24.8 g, 61 mmol) in 600 mL of xylene were stirred for 7 h at 135 °C under argon. The mixture was cooled to room temperature, the xylene decanted off, and the resinous brownish residue dissolved in 400 mL of dichloromethane/methanol (95:5). This solution was washed with 2 N sodium carbonate solution (2 × 150 mL), dried over sodium sulfate, and concentrated to viscuous oil. MPLC of the oil over silica gel (CH_2Cl_2/MeOH/NH_4OH, 93:7:0.7) gave 4.63 g of 4 as an 80:20 trans/cis epimeric mixture. The corresponding hydrochloride was prepared in ethanol and recrystallized from ethanol/ether to give **4b** hydrochloride contaminated with ca. 10% of cis isomer **4a**. Mp: 158–161 °C. $[\alpha]^{20}_D = -23.6°$ (c = 0.50, EtOH). ^{1}H NMR (DMSO-d_6, 360 MHz): δ 9.08 (s, 1), 7.63 (s, 1), 3.81 (s, 3), 3.68 (dd, 1, J = 5.5, 12.0 Hz), 3.45 (dd, 1, J = 7.0, 12.0 Hz), 2.85–3.03 (m, 3), 2.72 (q, 1, J = 7.0 Hz), 1.96 (m, 1), 1.81 (m, 1), 0.88 (t, 3, J = 7.0 Hz). MS: m/e = 240 (M^+). Anal. ($C_{11}H_{17}N_2S_2Cl$): C, H, N, Cl.

(+)-(3*R*-*trans*)-3-Ethyl-4,5-dihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-2(3*H*)-thiophenone Dihydrogen Phosphate (2b). Pilocarpine (42.5 g, 0.20 mol) and potassium thioacetate (39.7 g, 0.35 mol) were stirred together for 6 h in 500 mL of dimethylformamide at 150 °C under argon. The mixture was concentrated on a rotary evaporator under high vacuum and the residue was dissolved in 1.5 L of dichloromethane/methanol (95:5). This solution was washed with saturated sodium bicarbonate (2 × 200 mL), dried over sodium sulfate, and concentrated to an oil. MPLC of the oil over silica gel (CH_2Cl_2/MeOH/NH_4OH, 93:7:0.7) gave 38.2 g (85%) of the colorless oil **2** as an 80:20 trans/cis diastereomeric mixture. Treatment of the mixture with nitric acid in ethanol gave a salt which after two recrystallizations from ethanol was >99% pure trans isomer **2b** which was converted to the free base and then to the dihydrogen phosphate salt in ethanol. Mp: 189–192 °C. $[\alpha]^{20}_D = +20.2°$ (c = 1.0, H_2O). ^{1}H NMR (DMSO-d_6, 360 MHz): δ 7.81 (s, 1), 6.92 (s, 1), 3.61 (s, 3), 3.37 (dd, 1, J = 7.0, 12.0 Hz), 3.06 (dd, 1, J = 10.0, 12.0 Hz), 2.92 (dd, 1, J = 4.5, 14.5 Hz), 2.71 (dd, 1, J = −10.0, 14.5 Hz), 2.43–2.55 (m, 1), 2.34–2.42 (m, 1), 1.65–1.78 (m, 1), 1.51–1.65 (m, 1), 0.85 (t, 3, J = 7.5 Hz). Anal. ($C_{11}H_{19}N_2O_5PS$): C, H, N, O, P.

(+)-(3*R*-*cis*)-3-Ethyl-4,5-dihydro-4-[(1-methyl-1*H*-

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imidazol-5-yl)methyl]-2(3*H*)-thiophenone Dihydrogen Phosphate (2a). Butyllithium (26.3 mL, 1.6 M in hexane, 42 mmol) was added to a stirred solution of diisopropylamine (6.6 mL, 46 mmol) in 250 mL of absolute THF at 0 °C under argon. The solution was cooled to −70 °C, and an 80:20 **2b**/**2a** mixture (7.0 g, 31 mmol) in 60 mL of THF was added dropwise. After stirring of the mixture for 30 min at −70 °C, 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) (15.6 g, 71 mmol) was added in 70 mL of THF. The cold solution was poured into 300 mL of 2 N HCl and the aqueous phase extracted twice with dichloromethane (500 mL). The aqueous phase was adjusted to pH 8 with saturated sodium bicarbonate solution and extracted with dichloromethane (3 × 250 mL). The combined organic phases were dried over sodium sulfate and concentrated to 6.5 g of an oil which was shown by NMR analysis to consist of a 50:50 mixture of **2a**/**2b**. The oil was dissolved in ethanol and 1 equiv of D-(+)-di-*O,O'*-*p*-toluoyltartaric acid was added, after which **2** crystallized out as a 70:30 cis/trans mixture. A second recrystallization from ethanol gave **2a** of 95% purity, and a third recrystallization afforded crystals of **2a** which were satisfactory for X-ray analysis. Pure **2a** could be converted to the free base and other standard salt forms, in this case the dihydrogen phosphate, with no isomerization. Mp: 193–196 °C. $[\alpha]^{20}_D$ = +37.4° (*c* = 0.65, H_2O). ^1H NMR (DMSO-d_6, 360 MHz): δ 7.79 (s, 1), 6.89 (s, 1), 3.60 (s, 3), 3.38 (dd, 1, *J* = 5.5, 12.0 Hz), 3.12 (dd, 1, *J* = 6.5, 12.0 Hz), 2.88 (seven-line multiplet, 1, *J* = 5.5 Hz), 2.63–2.74 (m, 2), 2.54 (dd, 1, *J* = 11.0, 15.0 Hz), 1.59–1.71 (seven-line multiplet, 1, *J* = 7.5 Hz), 1.44–1.58 (seven-line multiplet, 1, *J* = 7.5 Hz), 0.96 (t, 3, *J* = 7.5 Hz). Anal. ($C_{11}H_{19}N_2O_5PS$): C, H, N, O, P, S.

Ethyl 2(*S*)-Ethyl-3(*R*)-[(1-methyl-1*H*-imidazol-5-yl)-methyl]-4-azidobutyrate (10). To a stirred solution of hydroxy ester 4[12] (4.00 g, 15.7 mmol), triphenylphosphine (4.20 g, 16.0 mmol), and hydrazoic acid (16.0 mL, 1.0 M solution in toluene, 16.0 mmol) in 60 mL of dichloromethane was added diethyl diazodicarboxylate (DEAD) until the orange color persisted. After stirring for 30 min at room temperature, sodium bicarbonate solution was added and the resulting solution was extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried over sodium sulfate and concentrated to 2.95 g of an orange oil. Purification by medium-pressure chromatography (MPLC) over silica gel (dichloromethane/methanol/NH_4OH, 95:4:1) afforded 2.51 g of **10** as a colorless oil. ^1H NMR ($CDCl_3$, 360 MHz): δ 7.39 (s, 1), 6.82 (s, 1), 4.16 (q, 2, *J* = 7.0 Hz), 3.55 (s, 3), 3.39 (d, 2, *J* = 4.2 Hz), 2.62 (dd, 1, *J* = 15.6, 9.5 Hz), 2.45–2.59 (m, 2), 2.02–2.09 (m, 1), 1.61–1.69 (m, 2), 1.28 (t, 3, *J* = 7.0 Hz), 0.94 (t, 3, *J* = 7.0 Hz). MS (FAB): *m/e* = 280 (MH^+).

(+)-(3*R*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)-methyl]-2-pyrrolidone Nitrate (5a). Azide **10** (2.00 g, 7.2 mmol) was dissolved in 60 mL of absolute ethanol with 300 mg of 10% palladium on carbon and hydrogenated overnight on a Parr shaker apparatus. The mixture was filtered, 0.5 mL of acetic acid was added, and the resulting mixture was stirred overnight at 50 °C. Evaporation of the solvent gave 1.55 g of pure lactam base **5a** as a white crystalline material which was converted to 1.22 g of its subsequently characterized nitrate salt. Mp: 176–177 °C. $[\alpha]^{20}_D$ = +31.8° (*c* = 1.07, H_2O). ^1H NMR (DMSO-d_6, 360 MHz): δ 14.15 (br s, 1, protonated imidazole), 9.05 (s, 1), 7.59 (s, NH lactam, 1), 7.56 (s, 1), 3.76 (s, 3), 3.25 (dd, 1, *J* = 6.0, 10.2 Hz), 2.87 (dd, 1, *J* = 4.2, 10.2 Hz), 2.69–2.82 (m, 2), 2.52–2.59 (m, 2), 2.28 (q, 1, *J* = 7.0 Hz), 1.58 (seven-line multiplet, 1, *J* = 7.0 Hz), 1.43 (seven-line multiplet, 1, *J* = 7.0 Hz), 0.98 (t, 3, *J* = 7.0 Hz). Anal. ($C_{11}H_{18}N_4O_4$): C, H, N, O.

(+)-(3*S*,4*R*)-1-Methyl-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-2-pyrrolidone Nitrate (6a). A solution of lactam **5a** (2.45 g, 11.8 mmol), tetrabutylammonium bromide, powdered potassium hydroxide (0.87 g, 1.3 mmol), and methyl iodide (1.80 g, 20.0 mmol) in 50 mL of THF was stirred overnight under argon. The solution was concentrated and chromatographed directly to give 480 mg of product which was converted to 450 mg of its nitrate salt. Mp: 142–143 °C. $[\alpha]^{20}_D$ = +35.5° (*c* = 0.27, H_2O). ^1H NMR (DMSO-d_6, 360 MHz): 14.07 (br s, imidazolium NH), 9.07 (s, 1), 7.56 (s, 1), 3.77 (s, 3), 3.44 (dd, 1, *J* = 8.0, 10.5 Hz), 2.97 (dd, 1, *J* = 4.0, 10.5 Hz), 2.72 (s, 3), 2.61–2.78 (m, 2), 2.47–2.56 (m, 2), 2.39 (q, 1, *J* = 7.0 Hz), 1.59 (seven-line multiplet, 1, *J* = 7.0 Hz), 1.45 (seven-line multiplet, 1, *J* = 7.0 Hz), 0.97 (t, 3, *J* = 7.0 Hz). Anal. ($C_{12}H_{20}N_4O_4$): C, H, N, O.

(+)-(2*RS*,3*R*,4*S*)-2-Hydroxy-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]tetrahydrofuran Hydrogen Fumarate (7a). Dibal (28 mL, 1 M in hexane, 28 mmol) was added to a stirred solution of pilocarpine (3.00 g, 24 mmol) in 150 mL of dichloromethane at −70 °C under argon. The reaction mixture was allowed to warm to room temperature and was stirred overnight. TLC analysis indicated the presence of starting material, lactol, and the ring-opened overreduction product pilocarpodiol. Saturated sodium sulfate solution was added until the aluminum salts precipitated, after which the reaction mixture was filtered. The salts were extracted with CH_2Cl_2/MeOH 80:20 and the combined extracts concentrated to a solid which was chromatographed to give 900 mg of **7a** as a viscous oil which was converted to its hydrogen fumarate salt in ethanol and characterized as a 2:1 mixture of diastereomers at the anomeric center. Mp: 133–134 °C. $[\alpha]^{20}_D$ = +40.9° (*c* = 0.64, H_2O). ^1H NMR (DMSO-d_6, 360 MHz): δ 7.65 (s, 0.33), 7.63 (s, 0.66), 6.70 (s, 0.66), 6.68 (s, 0.33), 6.62 (s, 2), 5.23 (d, 0.33, *J* = 4.0 Hz), 5.05 (d, 0.66, *J* = 1.5 Hz), 3.89 (m, 0.66), 3.79 (m, 0.33), 3.62 (dd, 0.33, *J* = 2.0, 9.0 Hz), 3.57 (s, 3 × 0.66), 3.56 (s, 3 × 0.33), 3.39–3.45 (m, 0.66), 2.56–2.69 (m, 2), 2.35–2.47 (m, 1), 1.83–2.01 (m, 1), 1.21–1.59 (m, 2), 0.93–0.99 (four-line multiplet for overlapping triplets, 3 H). Anal. ($C_{15}H_{22}N_2O_6$): C, H, N, O.

(+)-(2*RS*,3*R*,4*S*)-2-Hydroxy-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]tetrahydrothiophene Hydrogen Fumarate (8a). Dibal (95 mL, 1 M in hexane, 95 mmol) was added to a stirred solution of **2a** (14.2 g, 63 mmol) in 200 mL of dichloromethane at −70 °C under argon. The reaction mixture was allowed to warm to room temperature and saturated sodium sulfate solution was added until the aluminum salts began to precipitate. The mixture was filtered, and the salts were washed with CH_2Cl_2/MeOH 80:20. The combined extracts were concentrated to an oil which was chromatographed (CH_2Cl_2/MeOH/NH_4OH, 89:9:1) to give 11.5 g of **8a** as an oil which was converted to its hydrogen fumarate salt in ethanol. NMR analysis of the hydrogen fumarate showed it to consist of an 80:20 mixture of epimers at the lactol C(2) carbon. Mp: 110–112 °C. $[\alpha]^{20}_D$ = +38.3° (*c* = 0.35, H_2O). ^1H NMR (DMSO-d_6, 360 MHz): δ 7.66 (s, 20% 1), 7.63 (s, 80% 1), 6.75 (br s, 1), 6.62 (s, 2), 5.41 (d, 20% 1, *J* = 5.0 Hz), 5.21 (d, 80% 1, *J* = 1.5 Hz), 3.59 (s, 80% 3), 3.56 (s, 20% 3), 2.42–2.98 (m, 6), 1.88–1.96 (m, 1), 1.39–1.51 (m, 1), 1.08–1.20 (m, 1); overlapping triplets, 0.98 (t, 20% 3, *J* = 7.5 Hz), 0.92 (t, 80% 3, *J* = 7.5 Hz). Anal. ($C_{15}H_{22}N_2O_5S$): C, H, N, O, S.

(+)-(2*RS*,3*S*,4*S*)-2-Hydroxy-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]tetrahydrothiophene Fumarate (8b). Dibal (17.0 mL, 1.0 M in hexane, 17.0 mmol) was added to a stirred solution of **2b** (2.70 g, 12 mmol) in 100 mL of dichloromethane at −70 °C under argon. The solution was allowed to warm to 0 °C and 4 mL of saturated sodium sulfate solution was added. After aluminum sulfate precipitation was complete the mixture was filtered and the filtrate concentrated to an oil. The oil was chromatographed (MPLC) (CH_2Cl_2/MeOH/NH_4OH, 89:9:1) to give 2.33 g of product as a clear oil which upon treatment with 1 equiv fumaric in ethanol gave 1.92 g of fumarate salt **8b**. ^1H NMR analysis of fumarate **8b** showed it to consist of a 1:1 mixture of lactol epimers at C(2). Mp: 125–127 °C. $[\alpha]^{20}_D$ = +85.2° (*c* = 0.43, H_2O). ^1H NMR (DMSO-d_6, 360 MHz): δ 7.61 (s, 1), 6.73 (br s, 1), 6.61 (fumarate s, 1), 5.34 (d, 0.5, *J* = 4.0 Hz), 5.23 (d, 0.5, *J* = 4.0 Hz), 3.53 (s, 3), 2.59–2.93 (m, 3), 2.27–2.48 (m, 2), 2.09–2.18 (m, 0.5), 1.76–1.82 (m, 2), 1.31–1.59 (m, 3); overlapping triplets, 0.95 (t, 1.5, *J* = 7.5 Hz), 0.90 (t, 1.5, *J* = 7.5 Hz). MS: *m/e* = 226 (M^+). Anal. ($C_{13}H_{20}N_2O_3S$): C, H, N, O, S.

X-ray Analysis. Single-crystal X-ray data for **2a**·hydrogen D-(+)-di-*O,O'*-*p*-toluoyltartrate ($C_{11}H_{16}N_2OS \cdot C_{20}H_{18}O_8$, fw = 224.3 + 386.3 = 610.6) were obtained from a colorless crystal (recrystallized from ethanol and water) with dimensions 0.25 × 0.25 × 0.25 mm. The crystal belongs to the orthorhombic space group $P2_12_12_1$ with *a* = 17.883 (4) Å, *b* = 7.635 (2) Å, *c* = 22.948 (7) Å, *V* = 3133.2 Å^3, d_{calc} = 1.294 g/cm^3, *Z* = 4, μ = 13.44 cm^{-1}. Intensities were measured on an Enraf Nonius CAD-4 diffractometer, using monochromated Cu Kα radiation to θ < 70°, counting time 70 s. Empirical absorption correction factors from 0.85 to 1.25 were based on a 360° φ scan. Of the measured 5945 reflections 4221 had $I > 2.5\,\sigma(I)$ and were considered observed. The structure was solved by direct methods using SHELX-86.[14] All hydrogen atoms

bonded to N and O were located from difference Fourier maps. Hydrogen atoms bonded to C were included in idealized calculated positions. The final R factor was 0.06.

Pharmacology. Binding Assays. [^{3}H]-*cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-dioxolane (CD; 55 Ci/mmol) and [^{3}H]pirenzipine (Pz; 70 Ci/mmol) were obtained from New England Nuclear (Boston MA). In studies of ^{3}H-CD binding the incubation buffer was 50 mM tricine containing 2 mM $MgCl_2$ and 2 mM $CaCl_2$, adjusted to pH 7.4 with 1 M NaOH/KOH (4:1). For ^{3}H-Pz binding, 50 mM sodium potassium/phosphate buffer, pH 7.4, was used. Membranes from cerebral cortex of male OFA rats (200 g) were homogenized in 40 volumes of ice-cold 50 mM Tris-HCl (pH 7.4). The homogenate was centrifuged at 4 °C and 48000g for 10 min, and the pellet was resuspended in fresh buffer. This step was repeated twice and the pellet was stored frozen at −20 °C. Membranes equivalent to 9 mg of wet tissue was incubated in 2 mL of buffer containing drugs to be tested and radioligand. The final concentrations of ^{3}H-CD and ^{3}H-Pz were 1 and 0.3 nM, respectively. ^{3}H-CD binding assays were terminated after 30 min at 22 °C and ^{3}H-Pz binding assays were terminated after 60 min at 22 °C. Bound and free ligands were separated by filtration through Whatman GF/B glass-fiber filters, presoaked in a solution of 0.05% polyethyleneimine, with a Brandell cell harvester. Nonspecific binding in both binding assays was defined by 1 μM (−)-atropine. Binding assays were performed in duplicate, and repeated twice with a SEM < 5%.

Guinea Pig Ileum. Male guinea pigs (350–350 g, Ivanonas) were killed by a blow to the head, and a segment of the ileum was removed. A piece of longitudinal muscle strip was suspended vertically in an organ bath containing 20 mL of oxygenated Krebs solution (36 °C) at a resting tension of 7.5 mN. The preparation was allowed to equilibrate for 30 min. Muscarinic agonist-induced contractions were measured with force displacement transducers and monitored on a computer.

Rat Superior Cervical Ganglion. Male rats (Sprague–Dawley, 200–300 g) were anesthetized with urethane (1.2 g kg^{-1} ip) and superior cervical ganglia were excised. Ganglia were desheathed, suspended vertically in a heated chamber (36 °C), and superfused (1 mL min^{-1}) with oxygenated Krebs solution of the following composition (in mM): NaCl, 124.0; KCl, 3.0; NaH_2PO_4, 5.0; $CaCl_2$, 2.0; and glucose, 10.0. The preparations were allowed to equilibrate for 30–45 min. Muscarinic agonist-induced depolarizations were recorded differentially between the ganglion and its postganglionic trunk using calomel electrodes. The DC potentials were amplified by microvoltmeters and monitored on computer. After 4–6 min perfusion with agonist a concentration-dependent maximal depolarization was obtained which was used as a measure of agonist effect.

Hippocampal Slices. Male rats (Sprague–Dawley 200–300 g) were sacrificed, and the hippocampi were rapidly dissected. The isolated hippocampi were chopped into 350 μm thick slices using a McIlwain tissue chopper. The slice preparations were transferred to a tissue chamber and superfused with oxygenated Krebs solution (36 °C). After ca. 1 h of equilibration, extracellular recording (tungsten) electrodes were positioned at the CA1 region. The extracellular signals were amplified by an A-M Systems differential AC amplifier. The firing rate of the CA1 pyramidal cells was quantified by a spike counter and monitored on a computer. The responsivity of the neuronal firing rate was tested using a high concentration of carbachol (3×10^{-6}).

Acetylcholine Release. Hippocampal slices from Wistar rat were prepared by cross chopping slices at a distance of 0.3 mm with a McIlwain tissue chopper. Slices were incubated for 30 min at 23 °C in 6 mL of Krebs medium (composition in mM: NaCl, 118; KCl, 5; $CaCl_2$, 1.2; $MgCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 2; Na_2EDTA, 0.02; glucose, 11; oxygenated with 95%O_2/5%CO_2; pH 7.4) containing 0.1 μM [^{3}H]choline. After incubation with [^{3}H]choline, slices (4–5 mg wet weight) were transferred into each superfusion chamber and superfused with Krebs medium containing 10 μM hemicholinium-3 at a rate of 1.2 mL/min at 30 °C. Collection of 5-min fractions (6 mL) of the superfusate began after 60 min of superfusion. Two periods of electrical stimulation (2-Hz rectangular pulses, 2 ms, 10 mA, 2 min) were applied after 70 min (S_1) and after 125 min (S_2) of superfusion. Test substances were added 30 min before S_2 and were present in the superfusion medium for 145 min. At the end of the experiment the slices were solubilized in concentrated formic acid and tritium content was determined in superfusates and in the solubilized slices. Electrically evoked ^{3}H outflow was calculated by subtraction of basal outflow and was expressed as a percent of the tritium content at the beginning of sample collection. Drug effects were quantified as ratio of S_2/S_1 for electrically evoked outflow. Stimulated release in S_1 was between 1.2% and 2%. All experiments were run in duplicate.

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Supplementary Material Available: Tables of structure amplitudes, fractional coordinates, anisotropic thermal parameters, and bond lengths and angles for the hydrogen D-(+)-di-*O,O'-p*-toluoyltartrate salt of **2a**, Hill coefficients for the binding experiments; MOL files for receptor modeling studies, and data points for plots (Figures 4 and 6) (13 pages). Ordering information is given on any current masthead page.