Synthesis and Structure–Activity Relationships of New Muscarinic Antagonists

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Abstract □ In an attempt to develop more selective muscarinic acetylcholine receptor (m-AcChR) antagonists, (*R*)-1-azabicyclo[2.2.2]oct-3-yl thioxanthene-9-carboxylate, (*R,S*)-thiochromane-4-carboxylate, and (*R,S*)-chromane-4-carboxylate were synthesized. Evaluation of the binding affinities of these compounds to muscarinic receptors indicates that replacing the oxygen by sulfur in the xanthenyl and chromanyl moieties does not significantly change selectivity, but does reduce the affinity of **5** and enhance the affinity of **9a**.

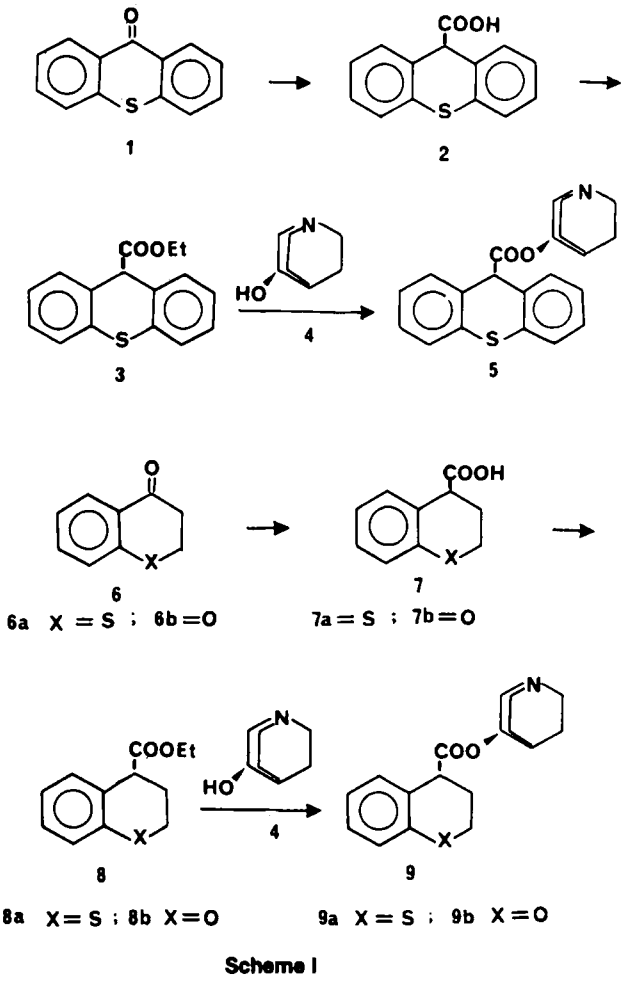
In the course of investigations on structure–activity relationships in the field of muscarinic receptor antagonists, we have synthesized a number of analogues of 3-quinuclidinyl benzilate (QNB) and reported their pharmacologic properties.[1-4] The affinities of atropine, scopolamine, 3-quinuclidinyl benzilate (QNB), and analogues of QNB were determined for the muscarinic acetylcholine receptors (M_1- and M_2-receptors). One of these antagonists, 3-quinuclidinyl xanthene-9-carboxylate exhibited greater affinity for the M_1-receptor than for the M_2-receptor. It has the same affinity for the M_1-receptor as QNB and M_1-selectivity comparable to that of pirenzepine.[5]

In an effort to improve the selectivity for M_1-receptors, we synthesized a series of analogues of 3-quinuclidinyl benzilate, namely 3-quinuclidinyl thioxanthene-9-carboxylate (**5**), thiochromane-4-carboxylate (**9a**), and chromane-4-carboxylate (**9b**). The equilibrium association constants, K_A, for muscarinic receptors were determined.

Results

Chemistry—The required thioxanthene-9-carboxylic acid (**2**), (*R,S*)-thiochromane-4-carboxylic acid (**7a**), and (*R,S*)-chromane-4-carboxylic acid (**7b**) were synthesized from thioxanthene-9-one (**1**), thiochromane-4-one (**6a**), and 4-chromanone (**6b**) by modified procedures (Scheme I).[6,7] Thus, **1**, **6a**, and **6b** were reacted with trimethylsilyl cyanide, and reductive hydrolysis of the resulting trimethylsilyl cyanohydrins afforded **2**, **7a**, and **7b**, respectively. Then ethyl esters **3**, **8a**, and **8b** were prepared by heating under reflux in ethanol, **2**, **7a**, or **7b**, respectively, with a catalytic amount of trifluoromethanesulfonic anhydride. The (*R*)-enantiomer of 3-quinuclidinol (**4**) was prepared by the method described by Grob et al.[8] and Ringdahl et al.[9] The different (*R*)-3-quinuclidinyl thioxanthene-9-carboxylate (**5**), (*R,S*)-thiochromane-4-carboxylate (**9a**), and (*R,S*)chromane-4-carboxylate (**9b**) were prepared by transesterification of (*R*)-3-quinuclidinol (**4**) and the ethyl ester of the desired thioxanthene-9-carboxylic acid (**3**), (*R,S*)-thiochromane-4-carboxylic acid (**8a**), or (*R,S*)-chromane-4-carboxylic acid (**8b**; Scheme I).

Pharmacology—The equilibrium association constant (K_A) for (*R*)-3-quinuclidinyl thioxanthene-9-carboxylate (**5**), (*R,S*)-thiochromane-4-carboxylate (**9a**), and (*R,S*)-chromane-4-carboxylate (**9b**) were determined by competitive ligand binding assay of *R*-(−)-[^{3}H]QNB using dog ventricular muscle (VM; predominantly M_2) and rat corpus striatum (CS; predominately M_1) as the sources of the muscarinic acetylcholine receptor (m-AcChR). Each K_A (Table I) is the result of at least five determinations and the apparent association constants were determined using the LIGAND programs of Munson and Rodbard.[10]



Scheme I

Discussion

In Table I, data are presented which show the relative affinities of 3-quinuclidinyl benzilate (QNB), 3-quinuclidinyl xanthene-9-carboxylate (QNX), (*R*)-3-quinuclidinyl thioxanthene-9-carboxylate (**5**), (*R,S*)-thiochromane-4-carboxylate (**9a**), and chromane-4-carboxylate (**9b**) for the m-AcChR from dog ventricular muscle (VM) and rat corpus striatum (CS).

Replacing the oxygen in the xanthene ring of 3-quinucli-

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dinyl xanthene-9-carboxylate (QNX, the planar analogue of QNB) by sulfur (5) resulted in a large loss of affinity for the m-AcChR obtained from VM and CS. However, 5 exhibited an 8.5-fold higher affinity for the receptor from CS than that obtained from VM. Elimination of one benzene ring in the xanthene moiety [namely, (*R*)-3-quinuclidinyl (*R,S*)-chromane-4-carboxylate (**9b**)] reduces the affinity for the m-AcChR from both VM and CS, while retaining CS selectivity (fivefold). Compound **9a** is an analogue of **9b** in which the chromanyl oxygen is replaced by sulfur. Compound **9a** has the same selectivity as **9b**, but exhibits twofold higher affinity.

In summary, these data show that replacing oxygen by sulfur in xanthenyl and chromanyl moieties does not significantly change the M_2:M_1 selectivity of the compounds. The results also indicate that replacement of oxygen by sulfur in the xanthenyl moiety reduces the affinity of the antagonist, while the same change in the chromanyl moiety enhances the affinity twofold. Compounds of this type may be useful as the basis for further structure–activity relationship studies to define the binding sites of the m-AcChR and to develop more selective m-AcChR antagonists.

Experimental Section

The melting points were obtained on a Fisher-Johns apparatus. The IR spectra of the compounds, neat or in KBr pellet, were obtained on a Beckman model IR 20A spectrophotometer. The analyses were performed by Galbraith Laboratories, Inc. (P.O. Box 4187, Knoxville, TN). The results obtained are within ± 0.4% of the theoretical values. 4-Chromanone, thiochroman-4-one, thioxanthen-9-one, and 3-quinuclidinol were obtained from Aldrich.

Thioxanthene-9-carboxylic acid (2)—Thioxanthen-9-one **1** (42.45 g, 0.2 mol) in methylene chloride (2 L) was allowed to react with trimethylsilyl cyanide (50 g, 0.50 mol) in the presence of zinc iodide (1 g) at room temperature with stirring for 4 d. The mixture was washed with saturated sodium bicarbonate and water. The solvent was removed under reduced pressure. The crude trimethylsilyl cyanohydrin was added to a mixture of tin (II) chloride dihydrate (200 g), glacial acetic acid (250 mL), and hydrochloric acid (250 mL), and was heated under reflux with stirring for 120 h. The mixture was evaporated under reduced pressure and extracted with methylene chloride, and the solvent was removed. The residue was suspended in 4M potassium hydroxide and extracted with methylene chloride. The aqueous layer was acidified with 6M hydrochloric acid and extracted with ethyl acetate. The extract was dried with magnesium sulfate and the solvent was removed under reduced pressure. The residue recrystallized from ethyl acetate:hexane to yield 10 g (20.6%); mp 198 °C dec.; TLC [silica gel, toluene:HOAc (9:1)] R_f 0.71; IR (KBr): 1690 (C=O) cm^{-1}.

Anal.—($C_{14}H_{10}O_2S$) C,H,S.

(*R,S*)-Thiochroman-4-carboxylic acid (7a)—This compound was prepared from thiochroman-4-one **6a** (32.84 g, 0.2 mol) in a similar fashion to 2. It was recrystallized from hexane to give 29 g (74.75%) of 7a; mp 76 °C; IR (KBr): 1680 (C=O) cm^{-1}.

Anal.—($C_{10}H_{10}O_2S$) C,H,S.

(*R,S*)-Chromane-4-carboxylic acid (7b)—This product was obtained from 4-chromanone **6b** (44.45 g, 0.3 mol) and trimethylsilyl cyanide (35.7 g, 0.36 mol) in the same manner as 2. The resulting acid was recrystallized from water to give 48 g (89%) of 7b; mp 90–92 °C; IR (KBr): 1680 (C=O) cm^{-1}.

Anal.—($C_{10}H_{10}O_3$) C,H.

Ethyl thioxanthene-9-carboxylate (3)—To a solution of 2 (9.68 g, 0.04 mol) in ethanol (250 mL) was added 5 mL of trifluoromethanesulfonic anhydride. The mixture was heated at reflux for 6 h. The solvent was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic layer was separated, washed with water, and dried over anhydrous $MgSO_4$. Removal of the solvent afforded 9.2 g (85.2%) of 3, mp 98 °C (lit.[11] 100 °C); TLC [silica gel, toluene:HOAC (9:1)] R_f 0.64; IR (KBr): 2975, 1720, and 740 cm^{-1}.

(*R,S*)-Ethyl thiochromane-4-carboxylate (8a)—This compound was prepared from 7a (19.4 g, 0.1 mol) and ethanol (250 mL) by use of the procedure described above for the preparation of 3: yield 20.4 g (92%); TLC [silica gel, toluene:HOAC (9:1)] R_f 0.71; IR (neat): 2960, 1710, and 725 cm^{-1}.

Anal.—($C_{12}H_{14}O_2S$) C,H,S.

(*R,S*)-Ethyl chromane-4-carboxylate (8b)—This compound was obtained from 7b (17.8 g, 0.1 mol) and ethanol (250 mL) according to the procedure for the synthesis of compound 3: yield 20 g (97%); TLC [silica gel, toluene:HOAc (9:1)] R_f 0.78; IR (neat): 2960, 1730, and 740 cm^{-1}.

Anal.—($C_{12}H_{14}O_3$) C,H.

(*R*)-1-Azabicyclo[2.2.2]oct-3-yl thioxanthene-9-carboxylate (5)—A solution of 5.12 g (0.04 mol) of (*R*)-3-quinuclidinol 4 in 200 mL of anhydrous benzene was heated at reflux for 1 h (Dean-Stark trap used to remove traces of water) then 0.4 g of sodium was added and the mixture was refluxed with stirring for 1 h. After removal of the remaining sodium, 10.8 g (0.04 mol) of 3 was added and the reaction mixture was heated again at reflux for 24 h. After the solvent was removed, the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water, and dried over $MgSO_4$. After removal of the solvent, an oil remained. The oxalate salt was recrystallized from ethanol:petroleum ether to yield 7.49 g (40.3%), mp 105–111 °C; TLC [silica gel, MeOH:NH_4OH (98:2)] R_f 0.44; IR (oxalate, KBr): 2950, 1720, and 1615 cm^{-1}.

Anal.—[$C_{21}H_{21}NO_2S \cdot (CO_2H)_2 \cdot H_2O$] C,H,N,S.

(*R*)-1-Azabicyclo[2.2.2]oct-3-yl(*R,S*)-thiochromane-4-carboxylate (9a)—This compound was obtained in a manner similar to that of 5 from 4 (2.55 g, 0.02 mol) and 8a (5.12 g, 0.023 mol), and an oil was obtained. The oxalate salt was recrystallized from the ethanol to yield 4.9 g (54.2%), mp 142–148 °C; TLC [oxalate; silica gel, MeOH:NH_4OH (98:2)] R_f 0.43; IR(oxalate, KBr): 2980, 1720, and 1640 cm^{-1}.

Anal.—[$C_{17}H_{21}NO_2S \cdot (CO_2H)_2$] C,H,N,S.

(*R*)-1-Azabicyclo[2.2.2]oct-3-yl(*R,S*)-chromane-4-carboxylate (9b)—This compound was prepared from 4 (2.55 g, 0.02 mol) and 8b (4.53 g, 0.22 mol) in a similar fashion to 5, and a brown oil was obtained. The oxalate salt was recrystallized from ethanol:petroleum ether to yield 4.15 g (55%), mp 95–105 °C; TLC [oxalate; silica gel, MeOH:NH_4OH (98:2)] R_f 0.36; IR (oxalate, KBr): 2950, 1720, and 1620 cm−1.

Anal.—[$C_{17}H_{21}NO_3 \cdot (CO_2H)_2$] C,H,N.

Tissue Preparations—The receptor was prepared as previously described.[5] The heart was removed from a freshly killed dog (within 15 min of death), and the left ventricular muscle (LVM) was dissected free of atrial muscle, major vessels, and fat. The muscle

Table I—Results of Binding Affinities for the Muscarinic Acetylcholine Receptor Antagonists for Receptor Obtained from Left Ventricular Muscle and Corpus Striatum

	K_A (× 10^9 M^{-1})[a]				
Compound	Ventricular Muscle	95%	Corpus Striatum	95%	M_2:M_1[d]
QNB[b]	3.62	2.8–4.7	5.28	3.7–7.5	0.69
QNX[c]	5.43	3.7–8.1	0.391	0.26–0.58	14.0
5	0.193	0.13–0.29	0.0226	0.017–0.030	8.5
9a	0.0227	0.015–0.033	0.00437	0.0030–0.0063	5.2
9b	0.0124	0.0079–0.020	0.00247	0.0016–0.0039	5.0

[a] Equilibrium affinity constant from LIGAND program. [b] 3-Quinuclidinyl benzoate (ref 2). [c] 3-Quinuclidinyl xanthene-9-carboxylate, hemioxalate hydrate. [d] Muscarinic acetylcholine receptors.

was frozen in liquid nitrogen and stored at −80 °C until used. Receptor prepared from tissue stored for up to six months did not deteriorate. Four- to five-gram fragments of heart were broken from the LVM, thawed in ice-cold saline, minced with scissors, and homogenized in Tris-buffered (10 mM, pH 7.4) 0.9% saline containing 10% sucrose (buffer I) using a Polytron PC-U at medium speed (2 bursts for 45 s each). The homogenate was filtered through four layers of cheesecloth and used without further purification. Receptor concentration before dilution into the assay medium was 10^{-9} M.

Brains were removed from freshly killed female Sprague-Dawley rats and immediately placed on ice. The corpus striatum (CS) was removed, immediately frozen, and stored at −80 °C until used. Receptors prepared from tissue stored for one year exhibit the same properties as that freshly prepared. Samples of 0.1–0.15 g of CS were homogenized in 20 mL of ice-cold buffer I using the Polytron PC-U (two 15-s bursts). The receptors were used without further purification. The concentration of receptor before dilution in the assay medium is essentially the same as that obtained for the LVM preparation (1 nM).

Determination of Apparent Equilibrium Association Constants—The apparent equilibrium association constants for the muscarcinic ligands presented in Table I were determined by competition with [^{3}H]QNB.[2] The compounds were dissolved in 100% EtOH and added to 5 mL of Tris–saline buffer (10 mM, pH 7.4) containing 2 × 10^{-10} M (−)-[^{3}H]QNB at a final concentration of 0.5% EtOH. Concentrations of EtOH <2% do not affect the binding parameters of QNB to the m-AcChR. Competition curves were generated using 10 concentrations of unlabeled compound from 10^{-11} to 10^{-5} M. Aliquots of 0.1 mL of tissue preparation were added, and the mixture was vortexed and incubated at room temperature for 2 h. Neither continuous agitation nor increased incubation times altered the results. The incubation mixture was rapidly filtered on a GF/C filter, washed with 10 mL of ice-cold saline, dried, placed in ACS scintillation cocktail, and counted for 5 min each. Data were analyzed using the LIGAND program.[10] Confidence intervals (95%) were calculated by the method of Munson and Rodbard.[12] The K_A values are obtained from pooled data of at least five determinations.

References and Notes

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