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Muscarinic Receptor Binding Profile of Para-Substituted Caramiphen Analogues

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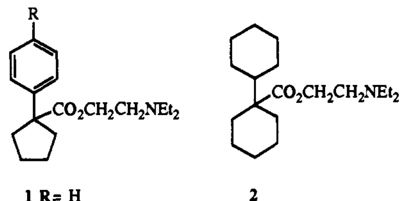
Para-substituted analogues of the antimuscarinic agent caramiphen were synthesized and evaluated for their ability to bind to the M_1 and M_2 subtypes of the muscarinic receptor. The purpose of the set was to look for a possible relationship in binding affinity or receptor subtype selectivity with aromatic substituent parameters such as Hammett's σ or Hansch's π values. It is felt this could be determined initially with only four properly chosen substituents. In this approach, substituents were chosen which have an extreme value for σ and for π, in a positive and negative direction, in all combinations. The substituents chosen for examination were amino ($-\sigma$, $-\pi$); 1-pyrrolidinyl ($-\sigma$, $+\pi$); 1-tetrazolyl ($+\sigma$, $-\pi$), and iodo ($+\sigma$, $+\pi$). It was determined in this research that caramiphen binds with high affinity (K_i = 1.2 nM) and is selective for the M_1 over M_2 muscarinic receptor subtype (26-fold). An examination of para-substitution reveals that compounds with electron-withdrawing ($+\sigma$) substituents showed M_1 selectivity, while the derivatives with electron-donating groups ($-\sigma$) were nonselective in the binding assays. On the basis of this finding, the nitro and cyano derivatives were prepared and found to be M_1 selective. The $+\sigma$ derivatives showed a decrease in M_2 affinity while the *p*-nitro and *p*-iodo derivatives retained approximately equal affinity as caramiphen for the M_1 site. The nitro- and iodocaramiphen derivatives were as potent (M_1, K_i = 5.52 and 2.11 nM, respectively) and showed a greater selectivity of M_1 over M_2 binding than the M_1 prototypical agent pirenzepine (M_1, K_i = 5.21 nM).

Introduction

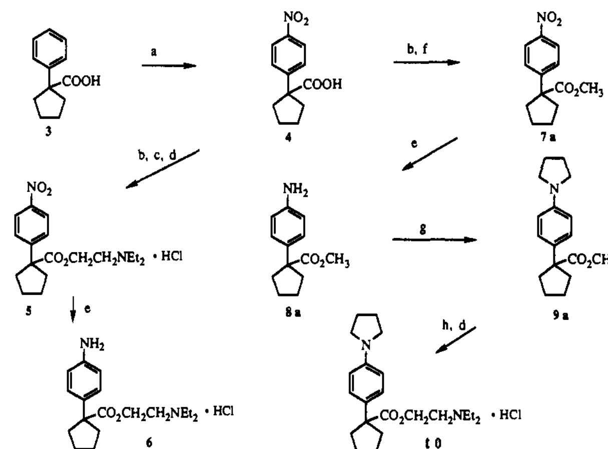
Caramiphen (1) is an antimuscarinic agent which is a more effective antidote to organophosphate acetylcholinesterase poisoning than atropine.[1-7] Our research group has previously been involved in designing new antimuscarinic agents for improved treatment of poisoning by cholinesterase inhibitors[8] and in studying structure–affinity relationships with the goal of developing high affinity, site-selective ligands for subtypes of the muscarinic receptor.



Currently three subtypes of muscarinic acetylcholine (ACh) receptors, M_1, M_2, and M_3, are defined on the basis of the action of antagonists.[9-12] The pharmacological characterization of the receptor subtypes is based on the spectrum of affinities for a number of antagonists. M_1 receptors have high affinity for pirenzepine and are mainly located in the central nervous system and peripheral ganglia. M_2 receptors are found in cardiac cells and are characterized by a high affinity for methoctramine, AF-DX-116, and gallamine. M_3 receptors are located in smooth muscle and exocrine glands and display high affinity for 4-DAMP, hexahydrosiladifenidol, and *p*-fluorohexahydrosiladifenidol. Both M_2 and M_3 receptor subtypes show low affinity for pirenzepine. Dicyclomine (2) and trihexyphenidyl bind with high affinity to M_1 receptors and with low affinity to the M_2 subtype.[13-15] Atropine fails to distinguish between these receptor subtypes in a binding assay.

Caramiphen has not been reported to be a selective muscarinic agent. Because of the structural similarity of caramiphen to dicyclomine (phenyl as opposed to cyclohexyl ring), we examined the M_1/M_2 selectivity of this compound and report that caramiphen is an M_1 selective agent. The presence of the aromatic ring affords the opportunity to examine the effect of various substituents and

Scheme I[a]



[a] (a) Fuming HNO_3, 0 °C, 4 h; (b) $SOCl_2$, C_6H_6, reflux; (c) $HOCH_2CH_2NEt_2$, C_6H_6, reflux; (d) HCl(g), ether; (e) H_2, 10% Pt/C; (f) MeOH, reflux; (g) $Br(CH_2)_4Br$, K_2CO_3, DMF, 75–80 °C, 12 h; (h) $HOCH_2CH_2NEt_2$, Na, $C_6H_5CH_3$, reflux.

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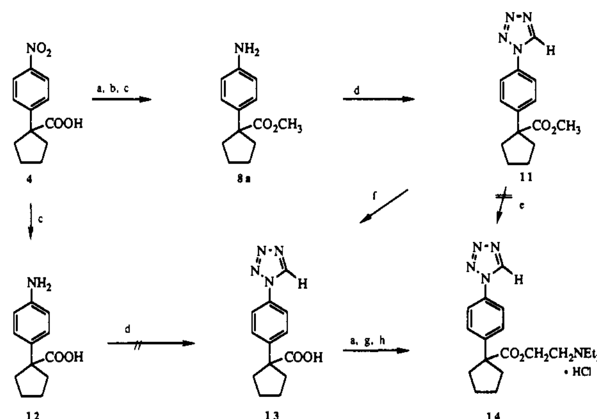
different aromatic substituent parameters such as Hammett's σ and Hansch's π values on binding affinity and receptor subtype selectivity. In the numerous cases of both the electronic (σ) and lipophilic (π) characters of aromatic ring substituents playing roles in the equation that relates structural properties to activity, both the sign and magnitude of the coefficients must be considered. The use of two parameters in a multiple-regression analysis (Hansch analysis) by the usual methods for determining quantitative structure–activity relationships (QSAR) would require 12 or more compounds in order to obtain a valid equation. In the current research, the important factor was to determine only if the parameters σ and/or π had a significant influence, if any, on the binding affinity or receptor subtype selectivity rather than to establish the quantitative relationship. It seemed this could be determined with only four properly chosen substituents. In this approach, a substituent is selected which has an extreme value for σ and for π in a positive or negative direction, in all combinations. Comparison of the activity of the four substituted derivatives with the parent compound should reveal the importance, if any, of one or both of these parameters. The compounds chosen for use in this approach were the amino ($-\sigma$, $-\pi$), 1-tetrazolyl ($+\sigma$, $-\pi$), 1-pyrrolidinyl ($-\sigma$, $+\pi$), and iodo ($+\sigma$, $+\pi$) derivatives.

Chemistry

The synthesis of the amino (6) and 1-pyrrolidinyl (10) compounds is shown in Scheme I. The nitration of 1-phenylcyclopentanecarboxylic acid (3) with fuming nitric acid gave 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (4) as the main product.[16] In method A the acid chloride was formed by using thionyl chloride and then allowed to react with 2-(diethylamino)ethanol to give amino ester 5 in high yield.[16] An alternative method (method B) to synthesize 5 was to react potassium 1-(*p*-nitrophenyl)cyclopentanecarboxylate[16] with freshly prepared 2-(diethylamino)ethyl chloride[17] in 2-propanol. Amino compound 6 was prepared by catalytic reduction of the corresponding nitro derivative 5. Pyrrolidinyl derivative 10 was prepared by the transesterification method. The intermediate nitro carboxylic acid 4 was initially protected as methyl ester 7a. The nitro methyl ester was then catalytically reduced to amino methyl ester 8a in high yield. The *p*-pyrrolidinyl ring was formed by using a modified method of Kalir et al.[18] The reaction of 8a with 1,4-dibromobutane in DMF gave the pyrrolidinyl methyl ester 9a, in 63% yield. The transesterification of pyrrolidinyl methyl ester 9a with 2-(diethylamino)ethanol and a catalytic amount of sodium, in toluene, gave the target pyrrolidinyl compound 10 in ca. 50% yield. This compound could be readily isolated as the mono hydrochloride salt and purified by recrystallization. Initially in this project the ethyl esters of these intermediates were synthesized. The transesterification step using ethyl 1-[*p*-1-pyrrolidinylphenyl)cyclopentanecarboxylate (9b) was unsuccessful. Apparently the carbonyl is sufficiently hindered such that the addition of one extra methylene in the alcohol portion of the ester adds enough steric hindrance to prevent the transesterification.

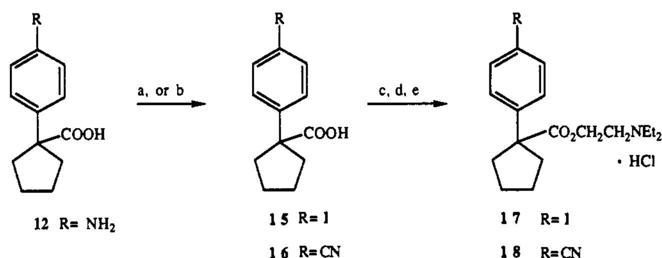
The approach to the tetrazole analogue 14 is outlined in Scheme II. The synthesis of the 1-aryltetrazoles was

Scheme II[a]



[a] (a) $SOCl_2$, C_6H_6, reflux; (b) MeOH, reflux; (c) H_2, 10% Pt/C; (d) (i) $HC(OEt)_3$, HOAc, 70–75 °C; (ii) NaN_3; (e) Na, $HOCH_2CH_2NEt_2$, C_6H_6, reflux; (f) 6 N HCl; (g) $HOCH_2CH_2NEt_2$, C_6H_6, reflux; (h) HCl(g), ether.

Scheme III[a]



[a] (a) (i) HCl, $NaNO_2$, (ii) KI, I_2; (b) (i) HCl, $NaNO_2$, (ii) KCN, CuCN; (c) $SOCl_2$, C_6H_6, reflux; (d) $HOCH_2CH_2NEt_2$, C_6H_6, reflux; (e) HCl(g), ether.

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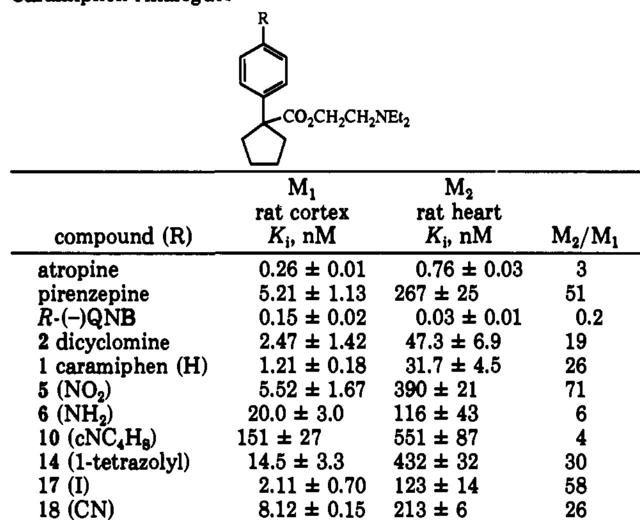
Table I. Muscarinic Binding Affinity of Para-Substituted Caramiphen Analogues[a]



compound (R)	M_1 rat cortex K_i, nM	M_2 rat heart K_i, nM	M_2/M_1
atropine	0.26 ± 0.01	0.76 ± 0.03	3
pirenzepine	5.21 ± 1.13	267 ± 25	51
R-(−)QNB	0.15 ± 0.02	0.03 ± 0.01	0.2
2 dicyclomine	2.47 ± 1.42	47.3 ± 6.9	19
1 caramiphen (H)	1.21 ± 0.18	31.7 ± 4.5	26
5 (NO_2)	5.52 ± 1.67	390 ± 21	71
6 (NH_2)	20.0 ± 3.0	116 ± 43	6
10 (cNC_4H_8)	151 ± 27	551 ± 87	4
14 (1-tetrazolyl)	14.5 ± 3.3	432 ± 32	30
17 (I)	2.11 ± 0.70	123 ± 14	58
18 (CN)	8.12 ± 0.15	213 ± 6	26

[a] Data are the mean ± SEM.

accomplished by using the method of Kamitani and Saito.[19] In this approach the tetrazole is prepared by reacting an aniline or its salt with ethyl orthoformate and sodium azide in acetic acid. Methyl 1-(*p*-1-tetrazolylphenyl)-cyclopentanecarboxylate (**11**) was formed in 65% yield by reacting aniline **8a** with ethyl orthoformate and sodium azide in acetic acid at 70–75 °C. All attempts to form target compound **14** by transesterification of **11** with 2-(diethylamino)ethanol failed. The addition of a catalytic amount of sodium metal resulted in massive decomposition. It was felt a more favorable route to form **14** would be from tetrazolyl acid **13**. Amino acid **12** was formed by catalytic reduction of nitro acid **4** in high yield.[20] All attempts to form tetrazole **13** from amino acid **12** by the methods previously described were unsuccessful. Hydrolysis of **11** in 6 N HCl readily gave tetrazolyl acid **13** in 82% yield. The acid chloride of **13** was formed with thionyl chloride and then allowed to react with 2-(diethylamino)ethanol to readily give **14**. Formation of tetrazole **14** directly from aniline **6** was attempted by using the reaction conditions previously described. This reaction gave low yields of a crude product which could not be purified by crystallization. Column chromatography (silica gel, $CHCl_3$–MeOH 9:1) of this crude product yielded only a small quantity of pure **14**. Intermediates 1-(*p*-iodophenyl)cyclopentanecarboxylic acid (**15**) and 1-(*p*-cyanophenyl)cyclopentanecarboxylic acid (**16**) were synthesized via the diazonium salt (diazotization of amino acid **12**) (Scheme III). Final basic esters **17** and **18** were prepared by allowing 2-(diethylamino)ethanol to react with the appropriate acid chloride.

Results and Discussion

Radioligand binding data for the reference muscarinic agents and the para-substituted caramiphen derivatives are shown in Table I. Compounds were evaluated for binding by displacing [^{3}H]pirenzepine from M_1 sites in rat cortex homogenates and [^{3}H]-(−)-quinuclidinyl benzilate (QNB) from rat heart homogenates as a measure of M_2 receptor affinity. The results show that caramiphen (**1**) binds with high affinity (K_i = 1.21 nM) and with selectivity (26-fold) for the M_1 subtype of the muscarinic receptor. Caramiphen demonstrates a slightly higher affinity for M_1 sites than pirenzepine (K_i = 5.21 nM) but does not distinguish between the M_1 and M_2 subtypes to the degree of pirenzepine (51-fold greater selectivity for M_1 receptors). This is due to caramiphen's moderate affinity for the M_2 site (31.7 nM), while pirenzepine displays low affinity (K_i = 267 nM) for the M_2 site. Comparing caramiphen (**1**) to dicyclomine (**2**) reveals that the two compounds display similar binding affinities and selectivity ratios at M_1 and M_2 receptor subtypes, suggesting common modes of binding of the two compounds.

An examination of the para-substituted compounds indicates affinity varies dramatically with the substituent. Substituent constants for a number of parameters are shown in Table II. Electron-donating substituents (**6** and **10**) abolish the subtype selectivity. The amino-substituted compound (**6**) retains moderate affinity for the M_1 site while the pyrrolidinyl analogue is weak at both M_1 and M_2 receptor subtypes.

The data from the para-electron-withdrawing functions reveals all the compounds tested in this study demonstrate at least a 26-fold greater selectivity for the M_1 receptor subtype as opposed to the M_2 subtype. Receptor selectivity results from the loss of the moderate M_2 receptor binding affinity as was observed with caramiphen (M_2, K_i = 31.7 nM). Substitution in the para position with $+\sigma$ groups (i.e. **5** and **14**) resulted in compounds which bind with weak affinity at the M_2 site (M_2, K_i = 390 and 432 nM, respectively). Compounds **17** (K_i = 123 nM) and **18** (K_i = 213 nM) were also much weaker than caramiphen at the M_2 site. However, these derivatives retain high affinity at the M_1 site. Iodocaramiphen (**17**) ($+\sigma$, $+\pi$) binds with high affinity at the M_1 site (K_i = 2.1 nM) and displays a 58-fold greater selectivity for the M_1 than M_2 site. Tetrazolyl derivative ($+\sigma$, $-\pi$) **14**, (M_1, K_i = 14.5 nM), although weaker than **17**, displays a significant preference for M_1 sites (30-fold). Because of the significance of these findings, intermediate nitrocaramiphen (**5**) (+0.78 σ; −0.28 π) was evaluated in the binding assays. Compound **5** is in the same σ/π quadrant as *p*-1-tetrazolylcaramiphen (**14**). The nitro derivative binds with equally high affinity at M_1 sites (K_i = 5.52 nM) but with a greater receptor selectivity than **17**. Both nitrocaramiphen (**5**, 71-fold) and iodocaramiphen (**17**, 58-fold) bind with equal affinity, but with a greater selectivity than pirenzepine for M_1 muscarinic receptors. Influenced by the promising results of nitro derivative **5**, cyanocaramiphen (**18**) was synthesized and evaluated in the binding assays. The cyano (+0.66 σ; −0.57 π) was chosen because the aromatic substituent parameters of cyano are similar to those of the nitro function. Additionally, cyano and nitro are similar in "steric bulk" and would have comparable spatial requirements to probe this region of the receptor (molar refraction (MR) values for nitro = 7.36, for cyano = 6.33).[21] Compound **18** was sim-

Table II. Aromatic Substituent Constants[a]

substituent	σ_p	π	MR	V_w
amino	−0.66	−1.23	5.42	10.54
1-pyrrolidinyl	−0.90	+1.18	24.85	43.16
1-tetrazolyl	+0.50	−1.04	18.33	
iodo	+0.18	+1.12	13.94	19.64
nitro	+0.78	−0.28	7.36	16.80
cyano	+0.66	−0.57	6.33	14.70

[a] σ_p, π, and MR values taken from ref 21. Constants for diethylamine are used as an approximation for 1-pyrrolidinyl. V_w values taken from ref 22. The value for 1-pyrrolidinyl was calculated, by using individual group increments and corrected for intramolecular crowding in the ring structure.

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ilar to 5 in binding though somewhat less selective (26-fold vs 71-fold). The difference in subtype selectivity of these two compounds is due to a modest increase in binding affinity for the M_2 site by cyano derivative 18.

When this project was begun, it was not known if the muscarinic receptor subtypes could accomodate additional "steric bulk" in the para position. The lipophilic region where the phenyl ring binds may be a pocket only large enough for the phenyl ring itself. This had never been determined with structural analogues. From the results of the binding data it is apparent that this binding region on the receptor can accomodate additional volume from the drug molecule. It is evident the contribution which the substituent imparts to binding can drastically affect affinity and selectivity. Although para substitution results in loss of binding affinity at both M_1 and M_2 receptor subtypes, the loss of affinity is more pronounced at the M_2 site with $+\sigma$ substituents with proper steric bulk. Shown in Table II are two parameters used as a measure for steric substituent effects, molar refraction (MR) and van der Waals volume (V_w). Comparison of the results of the 1-pyrrolidinyl (10) and 1-tetrazolyl (14) derivatives serves as an example of the importance of σ and steric bulk. The 1-tetrazolyl ($+\sigma$) moiety is a large group and derivative 14 retains affinity for the M_1 site (M_1, K_i = 14.5 nM) compared to the bulky 1-pyrrolidinyl ($-\sigma$) derivative 10 (M_1, K_i = 151 nM). Using MR as an approximation of molar volume (steric bulk) the pyrrolidinyl (MR = 24.85)[23] substituent occupies a space about the same as a phenyl ring (MR = 25.36),[21] while the tetrazole moiety is slightly smaller (MR = 18.33).[21] MR values must be used cautiously in structure–affinity correlations or multiple-regression analysis. For example, iodo derivative 17 is one of the more potent and selective compounds in this study. The iodo moiety is actually smaller than its MR value indicates because an MR value contains an electronic contribution. It is directly proportional to the polarizability. Often van der Waals volume gives a better correlation. With V_w the iodo group (V_w = 19.64) occupies a space slightly larger than a nitro group (V_w = 16.80). In order to establish the degree of steric tolerance of substituents at this position more derivatives would be necessary.

In conclusion, the important finding in this research is that caramiphen is an M_1-selective agent in receptor binding assays. Examination of para substitution with four properly chosen substituents reveals that derivatives with electron-withdrawing substituents ($+\sigma$) showed M_1 subtype selectivity equal to or greater than that of caramiphen. Nitro- and iodocaramiphen bind with high affinity for M_1 sites and show a greater separation in binding selectivity of M_1 vs M_2 receptors than the M_1 selective agent pirenzepine. The discovery of the influence of aromatic electron-withdrawing substituents and of proper steric bulk on the binding affinity is important information which could be utilized for the design of new, more selective, high-affinity muscarinic ligands.

Experimental Section

Synthesis. Proton magnetic resonance spectra (^{1}H NMR) were obtained at 60 mHz with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard; infrared spectra (IR) were recorded with either a Nicolet 5ZDX FT-IR spectrophotometer or a Beckman Acculab 8 grating spectrophotometer. Spectral data were consistent with the assigned structures. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab and values are within 0.4% of the theoretical value.

2-(Diethylamino)ethyl 1-(*p*-Nitrophenyl)cyclopentanecarboxylate Hydrochloride (5). Method A. To a solution of 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (4) (5.0 g, 22.6 mmol) in 25 mL of sodium-dried benzene was added 5 mL of thionyl chloride. The solution was stirred at reflux for 4 h. The solution was concentrated under reduced pressure and then reconcentrated with three 25-mL portions of benzene to remove the remaining thionyl chloride. The residue was dissolved in 40 mL of dry benzene and decolorized with charcoal. Then, 5.38 g (45.3 mmol) of freshly distilled 2-(diethylamino)ethanol was added in 75 mL of dry benzene, and the mixture was heated under reflux for 4 h. The solution was cooled to room temperature, poured into 250 mL of water and made basic with a 10% Na_2CO_3 solution. The layers were separated, and the aqueous phase was washed twice (50-mL portions) with diethyl ether. The combined organic layers were concentrated under reduced pressure, dissolved in about 100 mL of diethyl ether, and extracted four times with 100-mL portions of water. The ether layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from methanol–diethyl ether yielded 6.53 g (78%) of 5 as a white solid, mp 180–182 °C (lit.[17] mp 182–182.5 °C). IR (Nujol): 1720 cm^{-1} (C=O). ^{1}H NMR (D_2O): δ 1.35 (t, 6, CH_2CH_3), 1.8 (br s, 6, cyclopentyl), 2.4–2.8 (complex m, 2, cyclopentyl), 3.2 (q, 4, CH_2CH_3), 3.5 (m, 2 CH_2NEt_2), 4.5 (m, 2, $-OCH_2-$), 7.55 (d, 2, ArH), 8.05 (d, 2, ArH). Anal. ($C_{18}H_{26}N_2O_2{\cdot}HCl$): C, H, N.

Method B. Potassium 1-(*p*-nitrophenyl)cyclopentanecarboxylate[17] (2.3 g, 8.4 mmol) dissolved in 25 mL of absolute ethanol was added in one portion to freshly prepared 2-(diethylamino)ethyl chloride[18] (1.68 g, 12.4 mmol) dissolved in 10 mL of absolute ethanol. The solution immediately became turbid, and a precipitate began to separate. The mixture was then stirred at reflux for 12 h. After the solution was cooled, the precipitated inorganic salt was removed by filtration, washed with ethanol, and discarded. The filtrate was evaporated to dryness at reduced pressure. The resulting oil was dissolved in diethyl ether (100 mL), washed with three 50-mL portions of water, and dried over anhydrous magnesium sulfate. The hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from methanol–diethyl ether yielded 1.8 g (58%) of 5 as a white solid, mp 182–184 °C (lit.[17] mp 182–182.5 °C). This compound was identical in its physical and spectral properties with 5 prepared from 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (method A).

2-(Diethylamino)ethyl 1-(*p*-Aminophenyl)cyclopentanecarboxylate Hydrochloride (6). A dry methanol solution (60 mL) of 5 (5.95 g, 16.1 mmol) was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration through a bed of Celite. The solvent was evaporated under reduced pressure, yielding a crude yellow solid. Recrystallization from cold dry methanol–ethyl acetate yielded 4.35 g (80%) of 6 as a light tan solid, mp 135–137 °C. IR (Nujol): 3460, 3360, 3200 cm^{-1} (NH_2), 1720 cm^{-1} (C=O). ^{1}H NMR (D_2O): δ 1.2 (t, 6, CH_2CH_3), 1.8 (br s, 6, cyclopentyl), 2.2–2.7 (complex m, 2, cyclopentyl), 3.05 (q, 4, CH_2CH_3), 3.4 (br, 2, CH_2NEt_2), 4.4 (br, 2, $-OCH_2-$), 6.85 (d, 2, ArH), 7.25 (d, 2, ArH). Anal. ($C_{18}H_{28}N_2O_2{\cdot}HCl{\cdot}0.5H_2O$): C, H, N.

Methyl 1-(*p*-Nitrophenyl)cyclopentanecarboxylate (7a). To a solution of 6.25 g (26.6 mmol) of 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (4) in 25 mL of sodium-dried benzene was added 5 mL of thionyl chloride, and the solution was heated at reflux for 4 h. The solution was concentrated under reduced pressure and reconcentrated with three 25-mL portions of benzene. The solution was then heated at reflux with 75 mL of dry methanol for 4 h. After the solution was cooled, the solvent was removed under reduced pressure. The resulting crude tan solid was recrystallized from methanol, yielding 5.14 g (78%) of 7 as light

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(23) MR value for diethylamine was taken from ref 21 as an approximation for the size of 1-pyrrolidinyl. The actual value would decrease slightly from this value due to the intramolecular crowding effect in the ring structure.

tan needles, mp 72–73 °C. Anal. ($C_{13}H_{15}NO_4$): C, H, N.

Ethyl 1-(p-Nitrophenyl)cyclopentanecarboxylate (7b). Compound **7b** was prepared from 4 (3.5 g, 14.2 mmol) and dry ethanol in a manner similar to that used for **7a**. Evaporation of the ethanol under reduced pressure yielded an amber oil. The oil was distilled under vacuum, yielding 2.8 g (75%) of an amber oil, bp 110–115 °C (0.25 mm). Anal. ($C_{14}H_{17}NO_4$): C, H, N.

Methyl 1-(p-Aminophenyl)cyclopentanecarboxylate (8a). A methanol solution (60 mL) of **7a** (2.25 g, 9.0 mmol) was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration through a bed of Celite. The solvent was evaporated under reduced pressure. Recrystallization of the residue from methanol yielded 1.45 g (77%) of **8a** as white needles, mp 129.5–131 °C. Anal. ($C_{13}H_{17}NO_2$): C, H, N.

Ethyl 1-(p-Aminophenyl)cyclopentanecarboxylate (8b). An ethanol solution (60 mL) of **7b** (2.2 g, 8.4 mmol) was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration through a bed of Celite. The solvent was evaporated under reduced pressure, yielding a yellow oil. The oil was distilled under vacuum, yielding 1.46 g (75%) of a yellow oil, bp 128–132 °C (0.2 mm), which solidified on standing to yellow prisms, mp 62–63 °C. Anal. ($C_{14}H_{19}NO_2$): C, H, N.

Methyl 1-(p-1-Pyrrolidinylphenyl)cyclopentanecarboxylate (9a). To a stirred solution of **8a** (2.7 g, 12.3 mmol) in 40 mL of dry DMF containing 3.0 g of anhydrous potassium carbonate (at 75–80 °C under a nitrogen atmosphere) was added 1,4-dibromobutane (2.66 g, 12.3 mmol) in 5 mL of DMF, dropwise over a 15-min period. The reaction mixture was then stirred at 75–80 °C for 12 h. The solution was poured over crushed ice water, and the precipitate was collected by filtration. Recrystallization from methanol yielded 2.1 g (63%) of **9a** as light tan needles, mp 115–117 °C. Anal. ($C_{17}H_{23}NO_2$): C, H, N.

Ethyl 1-(p-1-Pyrrolidinylphenyl)cyclopentanecarboxylate (9b). Compound **9b** was prepared in a similar manner to that used for **9a** with **8b** (1.6 g, 6.9 mmol) and 1,4-dibromobutane (1.5 g, 6.9 mmol). Recrystallization from methanol yielded 1.3 g (66%) of **9b** as a white solid, mp 78–79 °C. Anal. ($C_{18}H_{25}NO_2$): C, H, N.

2-(Diethylamino)ethyl 1-(p-1-Pyrrolidinylphenyl)cyclopentanecarboxylate Hydrochloride (10). A mixture of methyl 1-(p-1-pyrrolidinylphenyl)cyclopentanecarboxylate (**9a**) (4.0 g, 14.7 mmol), freshly distilled 2-(diethylamino)ethanol (3.43 g, 29.3 mmol), and 0.1 g of sodium in 125 mL of dry toluene was heated under reflux with stirring for 20 h, during which massive precipitation occurred. After the reaction was cooled to room temperature, the solid was collected by filtration, washed with benzene and dissolved in water. The toluene solution was extracted three times with 100-mL portions of a 10% HCl solution. The aqueous layer was made basic with the addition of solid sodium carbonate in portions, combined with the aqueous solution of the precipitate, and extracted four times with diethyl ether (50-mL portions). The combined ether extracts were dried over anhydrous magnesium sulfate. The hydrochloride salt was prepared by the slow addition of an ethereal solution of gaseous HCl to the base in ether. Recrystallization from chloroform–ether yielded 2.75 g (49%) of **10** as a tan solid, mp 208–211 °C. ^1H NMR ($D_2O \cdot CF_3COOD$): δ 1.2 (t, 6, CH_2CH_3), 1.8 (br, 6, cyclopentyl), 2.0–2.6 (m, 6, $-CH_2-$), 3.1 (q, 4, CH_2CH_3), 3.2–3.8 (m, 6, NCH_2), 4.2–4.5 (m, 2, OCH_2), 7.4 (s, 4, ArH). Anal. ($C_{22}H_{34}N_2O_2 \cdot HCl$): C, H, N.

Methyl 1-(p-1-Tetrazolylphenyl)cyclopentanecarboxylate (11). To a stirred solution of methyl 1-(p-aminophenyl)cyclopentanecarboxylate (**8a**) (2.1 g, 9.6 mmol) in 20 mL of glacial acetic acid, at 70–75 °C under nitrogen, was added 1.42 g (9.6 mmol) of freshly distilled ethyl orthoformate in 5 mL of glacial acetic acid. The mixture was stirred for 4 h at 70–75 °C. Then, solid sodium azide (1.87 g, 28.8 mmol) was added portionwise, and the reaction was continued for an additional 24 h. After cooling to room temperature, the solution was poured into 250 mL of ice water. The precipitate was collected by filtration and recrystallized from methanol, yielding 1.9 g (74%) of **11** as light yellow needles, mp 118–119 °C. ^1H NMR ($CDCl_3$): δ 1.7 (br s, 6, cyclopentyl), 2.3–2.6 (complex m, 2, cyclopentyl), 3.5 (s, 3, OCH_3), 7.4–7.8 (complex m, 4, ArH), 9.7 (s, 1, TetH). Anal. ($C_{14}H_{16}N_4O_2$): C, H, N.

1-(p-1-Tetrazolylphenyl)cyclopentanecarboxylic Acid (13). A suspension of methyl 1-(p-1-tetrazolylphenyl)cyclopentanecarboxylate (**11**) (1.4 g, 5.2 mmol) in 10 mL of 6 N HCl was stirred at vigorous reflux for 4 h. After cooling on an ice bath, the white solid was collected by filtration and recrystallized from MeOH–H_2O, yielding 1.1 g (82%) of **13** as a white solid, mp 175–177 °C. Anal. ($C_{13}H_{14}N_4O_2$): C, H, N.

2-(Diethylamino)ethyl 1-(p-1-Tetrazolylphenyl)cyclopentanecarboxylate Hydrochloride (14). Compound **14** was prepared from 1-(p-1-tetrazolylphenyl)cyclopentanecarboxylic acid (**13**) (800 mg, 3.1 mmol) and 2-(diethylamino)ethanol (730 mg, 6.2 mmol) by the same general procedure (method A) used for **5**. The hydrochloride salt was prepared by slowly adding a cold ether–HCl(g) solution to a cold ethereal solution of the base. Recrystallization from MeOH–ethyl acetate yielded 375 mg (31%) as an off-white solid, mp >106 °C dec. ^1H NMR (D_2O): δ 1.35 (t, 6, CH_2CH_3), 1.85 (br s, 6, cyclopentyl), 2.3–2.8 (complex m, 2, cyclopentyl), 3.2 (q, 4, CH_2CH_3), 3.55 (m, 2, CH_2NEt_2), 4.55 (t, 2, $-OCH_2-$), 7.7–8.1 (m, 4, ArH), 9.9 (s, 1, TetH). Anal. ($C_{19}H_{27}N_5O_2 \cdot HCl \cdot 0.4H_2O$): C, H, N.

1-(p-Iodophenyl)cyclopentanecarboxylic Acid (15). To a rapidly stirred suspension of 1-(p-aminophenyl)cyclopentanecarboxylic acid (**12**) (3.0 g, 14.6 mmol) in 10 mL of 6 N HCl at −5 °C was added $NaNO_2$ (1.1 g, 16.1 mmol) in 5 mL of H_2O dropwise over a 5-min period. Stirring was continued for 30 min at −5 °C. Potassium iodide (2.43 g, 14.6 mmol) and a small crystal of I_2 in 5 mL of H_2O was slowly added dropwise to the solution of the diazonium chloride. This dark red solution was constantly stirred and shaken while being allowed to warm to room temperature. The solution was then stirred for 1 h at room temperature and 1 h at 90 °C. After cooling of the reaction mixture on an ice bath, the solid mass was collected by filtration and washed with H_2O. The dark semisolid was dissolved in a 10% NaOH solution and filtered to remove any insoluble material. The crude acid was precipitated by slow addition of 10% HCl solution. Recrystallization from acetone–H_2O yielded 3.1 g (67%) as a fine tan solid, mp 177–179 °C. Anal. ($C_{12}H_{13}O_2I$): C, H.

1-(p-Cyanophenyl)cyclopentanecarboxylic Acid (16). The diazonium chloride was formed from 1-(p-aminophenyl)cyclopentanecarboxylic acid (**12**) (1.6 g, 7.8 mmol) and sodium nitrite (600 mg, 8.5 mmol) in about 8 mL of 1 N HCl in a similar manner as described for the preparation of **15**. While cold, and with stirring, the solution of diazonium chloride was carefully neutralized with solid anhydrous Na_2CO_3. A stirred solution of CuCN (840 mg, 9.4 mmol) and KCN (610 mg, 9.4 mmol) in ca. 20 mL of H_2O was warmed on a hot plate to 60–65 °C. The cold neutralized diazonium solution was then added in small quantities at a time, with vigorous shaking of the stirred flask after each addition and maintanence of the temperature of the mixture at 60–70 °C. The reaction was continued for 1 h and then the product isolated in a similar manner as described for **15**. Recrystallization from MeOH–H_2O yielded 700 mg (42%) of a fine brown solid, mp 78–81 °C.

2-(Diethylamino)ethyl 1-(p-Iodophenyl)cyclopentanecarboxylate Hydrochloride (17). Compound **17** was prepared from 1-(p-iodophenyl)cyclopentanecarboxylic acid (**15**) (1.25 g, 4.0 mmol) and 2-(diethylamino)ethanol (0.94 g, 8.0 mmol) by the general procedure (method A) used for **5**. The hydrochloride salt was prepared by adding an ether–HCl(g) solution slowly to an ethereal solution of the base. Recrystallization from MeOH–ether yielded 650 mg (36%) of an off-white solid, mp 134–137 °C. IR (Nujol): 1720 cm^{-1} (C=O). ^1H NMR (free base, $CDCl_3$): δ 0.8–1.25 (m, 6, CH_2CH_3), 1.8 (br s, 6, cyclopentyl), 2.35–2.9 (complex m, 8, $-CH_2-$), 4.15 (t, 3, $-OCH_2-$), 7.4 (d, 2, ArH), 7.8 (d, 2, ArH). Anal. ($C_{18}H_{26}INO_2 \cdot HCl$): C, H, N.

2-(Diethylamino)ethyl 1-(p-Cyanophenyl)cyclopentanecarboxylate Hydrochloride (18). Compound **18** was prepared from 1-(p-cyanophenyl)cyclopentanecarboxylic acid (**16**) (150 mg, 0.6 mmol) and 2-(diethylamino)ethanol (200 mg, 1.7 mmol) by the same general procedure (method A) used for **5**. The hydrochloride salt was prepared by adding an ether–HCl(g) solution to a cold ethereal solution of the base. Recrystallization from MeOH–ether yielded 125 mg (60%) as a white solid, mp 154–158 °C. IR (Nujol): 1720 cm^{-1} (C=O), 2225 cm^{-1} (CN). ^1H NMR

(free base, $CDCl_3$): δ 1.1 (t, 6, CH_2CH_3), 1.9 (br s, 6, cyclopentyl), 2.4–2.9 (complex m, 8, $-CH_2-$), 4.2 (t, 3, $-OCH_2-$), 7.4–7.8 (m, 4, ArH). Anal. ($C_{19}H_{28}N_2O_2\cdot HCl\cdot 0.5H_2O$): C, H, N.

Radioligand Binding Assays. Measurement of binding to muscarinic receptor subtypes was performed with minor modifications of the methods of Watson et al.[24,25] Male Sprague–Dawley rats were anesthetized with CO_2 and sacrificed by decapitation. Tissues were dissected and placed in the appropriate assay buffers. The M_1 binding assay used [^{3}H]pirenzepine (PZ, 87 Ci/mmol, New England Nuclear) as the ligand, rat cortex as the source of tissue, and 50 mM HEPES–KOH as the assay buffer, while the M_2 binding assay used [^{3}H]-(–)-quinuclidinyl benzilate (QNB, 32 Ci/mmol, New England Nuclear) as the ligand, rat heart as the source of tissue, and 50 mM Tris-HCl as the assay buffer. Tissues were homogenized with a Polytron at setting 6 for 15 s at a concentration of 10 mg/mL ([^{3}H]PZ) or minced and then homogenized at setting 8 for 15 s at a concentration of 8 mg/mL ([^{3}H]QNB). The homogenates were centrifuged at 48000*g* for 10 min at 4 °C, resuspended, and washed twice. Assay tubes contained 500 μL of tissue, 100 μL of ligand, and sufficient buffer for a final volume of 1 mL for [^{3}H]PZ binding and 2 mL for [^{3}H]QNB binding. Final ligand concentrations were 0.5 nM for [^{3}H]PZ binding and 0.1 nM for [^{3}H]QNB binding. Nonspecific binding for both assays was defined by 1 μM atropine. Displacement experiments at 13 concentrations of nonlabeled drug were performed in triplicate. Incubations were conducted for 1 h at room temperature for [^{3}H]PZ binding, and at 37 °C for [^{3}H]QNB binding. Incubations were terminated by filtration through Whatman GF/B filters that were presoaked with 0.1% polyethylenimine for at least 1 h, followed by three 4-mL washes with ice-cold assay buffer. Following addition of scintillation cocktail, samples were allowed to equilibrate for at least 8 h. The amount of bound radioactivity was determined by liquid scintillation spectrometry using a Beckman LS 5000TA liquid scintillation counter with an efficiency for tritium of ca. 60%. Inhibition constants (K_i values) for the binding of test compounds to recognition sites were calculated with the EBDA/LIGAND program.[26] Data are the mean of at least three separate experiments performed in triplicate.

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Registry No. 1, 77-22-5; 4, 52648-77-8; 5, 135569-16-3; 5·HCl, 98636-73-8; 6, 135569-18-5; 6·HCl, 135569-17-4; 7a, 135569-19-6; 7b, 135569-34-5; 8a, 135569-20-9; 8b, 135569-35-6; 9a, 135569-21-0; 9b, 135569-36-7; 10, 135569-23-2; 10·HCl, 135569-22-1; 11, 135569-24-3; 12, 91640-63-0; 13, 135569-25-4; 14, 135569-27-6; 14·HCl, 135569-26-5; 15, 135569-28-7; 16, 135569-29-8; 17, 135569-31-2; 17·HCl, 135569-30-1; 18, 135569-33-4; 18·HCl, 135569-32-3; $HOCH_2CH_2NEt_2$, 100-37-8; $ClCH_2CH_2NEt_2$, 100-35-6; $Br(CH_2)_4Br$, 110-52-1; potassium 1-(4-nitrophenyl)cyclopentanecarboxylate, 89490-69-7.

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Synthesis and Muscarinic Cholinergic Receptor Affinities of 3-Quinuclidinyl α-(Alkoxyalkyl)-α-aryl-α-hydroxyacetates

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Seven analogues of 3-quinuclidinyl benzilate (QNB) in which one phenyl ring was replaced by an alkoxyalkyl moiety were synthesized and their affinities for the muscarinic cholinergic receptor determined. An oxygen in the β-position of the moiety was not well-tolerated. By contrast, an oxygen in the γ-position did not change the affinity for the muscarinic receptor. However, when a bromine was placed on the remaining phenyl ring, the affinity was significantly reduced in striking contrast to results obtained on halogenation of QNB.

We have developed radiohalogenated (*R,R*)- and (*R*,-*S*)-1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (4IQNB) as agents for imaging muscarinic acetylcholine receptors (m-AChR) in the central nervous system.[1,2] When labeled with ^{125}I, these radiotracers provide highly selective receptor localization.[3,4] The (*R,R*)-[^{123}I]-4IQNB has been used to obtain images of the distribution of the m-AChR in healthy individuals[5,6] and patients with dementias.[7,8] Pharmacokinetic studies[9] in rat indicate that the concentration of radiohalogenated 4IQNB which localizes in the brain is, in part, a function of the concentration of m-AChR. A positive correlation has also been shown between the concentration of m-AChR in various structures in the brain and the concentration of radioiodinated 4IQNB in these structures at single times

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