79-19-6; 1-methyl-2-(4'-cyanophenyl)imidazo[1,2-*a*]pyridinium tosylate, 123510-87-2; 2-phenylimidazo[1,2-*a*]pyridine, 4105-21-9; hydrazine, 302-01-2; hydrazide, 25415-88-7; *S*-methyl thiosemicarbazide hydriodide, 35600-34-1; *N*-aminoguanidinium bicarbonate, 2582-30-1; *p*-toluenesulfonic acid, 104-15-4; 1*H*-imidazoline-2(3*H*)-thione, 872-35-5; 3,4,5,6-tetrahydro-2(1*H*)-pyrimidinethione, 2055-46-1; *N*-amino-*N'*-hydroxyguanidium tosylate, 36826-58-1; *N*-amino-*N'*-nitroguanidine, 18264-75-0; *N*-aminomorpholine, 4319-49-7; 1,2,4-triazol-3-ylhydrazine, 38767-33-8; 3,4-diamino-1,2,4-triazole, 38104-45-9; 3,4,5-triamino-(4*H*)-1,2,4-triazole, 473-96-1; (4-amino-4*H*-1,2,4-triazol-3-yl)hydrazine, 6421-06-3; 4-aminopyrimid-2-ylhydrazine, 584-13-4; 2-pyridylhydrazine, 4930-98-7; pyrimid-2-ylhydrazine, 7504-94-1; 4-aminopyrimid-2-ylhydrazine, 123510-88-3; (4,6-diaminopyrimid-2-yl)hydrazine, 123510-89-4; diaminopyridinium iodide, 4931-36-6; thiazol-2-ylhydrazine, 30216-51-4; 3,4-diamino-1,2,4-triazole-5-thione, 3529-50-8; 5-aminopyrazol-2-ylhydrazine, 123510-91-8.

Supplementary Material Available: Tables showing melting point and elemental composition and a summary of biological data for all compounds tested (27 pages). Ordering information is given on any current masthead page.

Synthesis of Some 3-(1-Azabicyclo[2.2.2]octyl) 3-Amino-2-hydroxy-2-phenylpropionates: Profile of Antimuscarinic Efficacy and Selectivity

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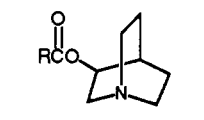
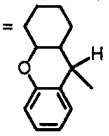
A series of 3-quinuclidinyl atrolactate [3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-2-phenylpropionate, QNA] derivatives in which the methyl group of the parent is substituted with a tertiary amino substituent was prepared and tested for antimuscarinic activity. In general, potency was markedly decreased, although the morpholinyl and thiomorpholinyl derivatives retained significant activity. These compounds were also examined for muscarinic receptor subtype selectivity. Their subtype selectivities were comparable to that of (*R,R*)-QNA. The results of this investigation suggest possible differences in the accessory binding sites of the proteinaceous receptor subtypes.

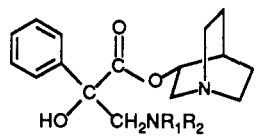
A principle utilized for derivation of structurally novel anticholinergics[1] entails appropriate substitution[2,3] of the acetyl group of an agonist and incorporation of the ethanolamine chain into a rigid cyclic system. Exemplary of such compounds are ones in which the acetyl group of 3-(1-azabicyclo[2.2.2]octyl) acetate (3-quinuclidinyl acetate, aceclidine, **1a**), a potent and selective muscarinic receptor agonist,[4-6] is properly substituted. This has resulted in the potent antimuscarinic agents 3-quinuclidinyl benzilate (QNB, **1b**),[7,8] 3-quinuclidinyl xanthene-9-carboxylate (QNX, **1c**),[9] and 3-quinuclidinyl atrolactate (QNA, **1d**).[10,11]



1a: R = CH_3
b: R = $Ph_2C(OH)$
c: R =

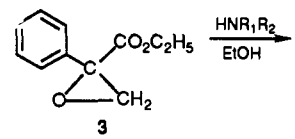


d: R = $PhC(CH_3)(OH)$



2

Scheme I



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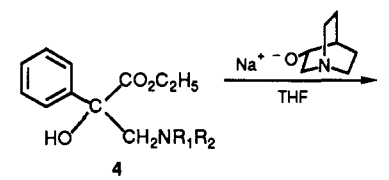

4

Examination of a large number of analogues and derivatives of QNB (**1b**), QNX (**1c**), and QNA (**1d**)[9-13] has resulted in the observation of remarkable muscarinic M_1 receptor subtype selectivity for some of the optical isomers of QNA in particular.[14] To further study this subtype selectivity, a series of QNA derivatives **2** bearing a tertiary amine functionality on the atrolactic acid methyl group, i.e., 3-(1-azabicyclo[2.2.2]octyl) 3-amino-2-hydroxy-2-

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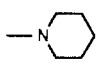
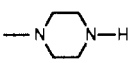
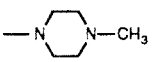
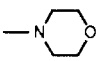
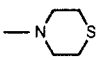
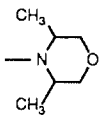
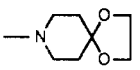
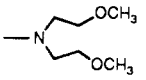
Table I. 3-(1-Azabicyclo[2.2.2]octyl) 3-Amino-2-hydroxy-2-phenylpropionates (2)

compd	NR_1R_2	formula	anal.[a]	mp, °C
2a		$C_{20}H_{28}N_2O_3 \cdot HCl$	C, H, N, Cl	248–251 dec
2b		$C_{21}H_{30}N_2O_3 \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl[b]	142–147
2c		$C_{20}H_{29}N_3O_3 \cdot 3HCl \cdot H_2O$	C, H, N, Cl	200–210 dec
2d		$C_{21}H_{31}N_3O_3 \cdot 3HCl$	C, H, N, Cl	198–205
2e		$C_{20}H_{28}N_2O_4 \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	215–220 dec
2f		$C_{20}H_{28}N_2O_3S \cdot 2HCl \cdot 0.25H_2O$	C, H, N, Cl[c]	182–185 dec
2g		$C_{22}H_{32}N_2O_4 \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	205–210 dec
2h		$C_{23}H_{32}N_2O_5$	C, H, N	43–47
2i		$C_{22}H_{34}N_2O_5 \cdot 2HCl$	C, H, N, Cl	171–174
2j	**2e**·CH_3I	$C_{20}H_{28}N_2O_4 \cdot CH_3I$	C, H, N, I	130 dec

[a] All compounds gave elemental analyses within 0.4% of calculated values unless indicated otherwise. [b] Cl: calcd, 15.47; found, 14.70. [c] H: calcd, 6.77; found, 7.26. Cl: calcd, 15.62; found, 15.11.

phenylpropionates, was prepared and examined for antimuscarinic activity. Affinity and selectivity for muscarinic receptors were determined in a radioligand binding assay (inhibition of [^{3}H]QNB) and in several functional in vitro assays. The results of this study are described in this article.

Chemistry. Substituted 3-(1-azabicyclo[2.2.2]octyl) 3-amino-2-hydroxy-2-phenylpropionates (**2a–j**, Table I) were prepared as outlined in Scheme I. Accordingly, ethyl 2-phenyl-2,3-epoxypropionate (ethyl α-phenylglycidate, **3**)[15,16] was treated with the appropriate secondary amine to afford the corresponding ethyl α-(aminomethyl)-mandelate **4**, which was transesterified with potassium 3-quinuclidinolate to give the amino-substituted derivatives (**2a–j**) of QNA.

Results and Discussion

Substitution of the methyl carbon of QNA (**1a**) with a tertiary nitrogen bearing overall bulk approximately equivalent to that of the phenyl substituent in QNB has a negative influence on its interaction with muscarinic receptors. This is evidenced by the displacement of [^{3}H]QNB binding, as well as by inhibition of carbachol-induced contraction of guinea pig ileum longitudinal muscle (Table II). One of a number of possible explanations is that the bulk volume resulting from substitution of one methylene removed from the benzylic carbon of the substituted mandelic acid esters of 3-quinuclidinol may extend into a region of bulk intolerance in the proteinaceous muscarinic receptors. This view may be supported by the report of only antispasmodic activity for a series of tropic acid esters of ethanolamines.[17] Similarly, in a somewhat related series, Wagner-Jauregg and his associates[18] studied some 2-(dimethylamino)ethyl esters of phenylacetic acid in which the benzylic carbon was substituted with a phenylalkyl group. As the length of the alkyl group increased, spasmolytic activity decreased.

Table II. Biological Data for 3-(1-Azabicyclo[2.2.2]octyl) 3-Amino-2-hydroxy-2-phenylpropionates (**2**)

compd	[^{3}H]QNB binding rat ileum, K_i, nM[a–c]	inhibition of carbachol-induced contraction of guinea pig ileum, K_b, nM[a–c]
2a	12230 ± 6690 (3)	167 ± 80 (5)
2b	8280 ± 2360 (3)	430 ± 175 (3)
2c	2380 ± 395 (3)	>1000 (3)
2d	9190 ± 3720 (3)	>1000 (3)
2e	195 ± 116 (3)	23 ± 5.4 (4)
2f	158 ± 84 (3)	7.5 ± 1.0 (4)
2g	1620 ± 637 (4)	664 ± 48 (4)
2h	5240 ± 1060 (3)	641 ± 237 (5)
2i	12600 ± 3610 (3)	>1000 (3)
2j	3980 ± 1390 (4)	458 ± 119 (3)
QNA[d]	6.0 ± 1.0 (4)	1.0 ± 0.1 (3)
atropine	0.45 ± 0.05 (3)	0.5 ± 0.1 (6)

[a] See Experimental Section for description of procedure and definition of K_i and K_b. [b] Number in parentheses is number of experiments/animals. [c] Values represent the means ± standard error of measurement. [d] (*R,R*)-QNA.[14]

The strikingly greater affinity of the morpholinyl (**2e**) and the thiomorpholinyl (**2f**) derivatives of QNA may be significant. Perhaps their enhanced binding relative to that of the isomeric piperidinyl derivative **2b** may reflect interaction with an accessory binding site on the receptor, possibly as a consequence of a complementarily located hydrogen bond donor on the receptor or a receptor subtype that can interact with the free pair of electrons on the morpholinyl oxygen or the thiomorpholinyl sulfur. It may be significant that the thiochromane-4-carboxylic acid ester of 3-quinuclidinol has a greater affinity for muscarinic receptors than does the related chromane.[13] Perhaps the

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Table III. Affinity Profiles in M_1, $M_{2\alpha}$, and $M_{2\beta}$ Muscarinic Receptor Selective Tissues[a]

	functional in vitro studies, K_b, nM					
	guinea pig ileal muscle ($M_{2\beta}$)	guinea pig atrial muscle ($M_{2\alpha}$)	rabbit vas deferens (M_1)	selectivity ratios		
compd				$M_1/M_{2\alpha}$	$M_1/M_{2\beta}$	$M_{2\beta}/M_{2\alpha}$
(*R*,*R*)-QNA	1.0 ± 0.1	4.0 ± 0.9	0.95 ± 0.03	0.23	0.95	0.25
2e	23 ± 5.4	169 ± 69	18 ± 4.3	0.11	0.78	0.13
2f	7.5 ± 1.0	493 ± 128	10 ± 2	0.02	1.0	0.02

[a] See Experimental Section for description of test procedures. K_b values are the means ± SEM of 4–10 experiments.

larger sulfur atom locates a free pair of electrons in a vicinity closer to that of these electron pairs in **2e** and **2f** than does the smaller oxygen of the chromane derivative.

At least three subtypes of muscarinic acetylcholine receptors (M_1, $M_{2\alpha}$, $M_{2\beta}$) have been identified according to the classification of Mutschler and Lambrecht.[19] Selectivity of compounds **2e** and **2f** was evaluated at M_1 receptors in the rabbit vas deferens,[20] smooth muscle $M_{2\beta}$ receptors in the guinea pig ileum,[21,22] and cardiac $M_{2\alpha}$ receptors in the guinea pig atria (Table III). Pretreatment with compounds **2e** and **2f** caused parallel rightward shifts of the concentration–response curves for cholinergic agonists in all three tissues. Quinuclidinyl esters, **2e** and **2f**, like the parent compound (*R*,*R*)-QNA, possessed higher affinities for M_1 receptors on the vas deferens and $M_{2\beta}$ receptors on the ileum than at cardiac $M_{2\alpha}$ receptors (Table III). The selectivity of compounds **2e** and **2f** is comparable to that of (*R*,*R*)-QNA.[14]

In summary, although the compounds described in this study are markedly less potent antimuscarinics than QNA, they do suggest possible differences in the binding sites of muscarinic receptor subtypes.

Experimental Section

Melting points were determined on microscope slides with a Bristoline apparatus; they are uncorrected. IR spectra were recorded with a Beckman FT 1300 spectrophotometer. ^{1}H NMR spectra were recorded with either a Varian EM-360A 60-MHz or a General Electric QE 300-MHz spectrometer; Me_4Si was used as the internal standard. (*R*,*R*)-QNA was prepared at the Nova Pharmaceutical Corp.[14]

Chemistry. General Methods. Ethyl 2-Hydroxy-3-(4-morpholinyl)-2-phenylpropionate (4e). This procedure illustrates the general method utilized for preparation of the ethyl 3-amino-2-hydroxy-2-phenylpropionate precursors **4** to the 3-(1-azabicyclo[2.2.2]octyl) 3-amino-2-hydroxy-2-phenylpropionates (**2**). To a solution of ethyl α-phenylglycidate[15,16] (4.96 g, 25.8 mmol) in 50 mL of ethanol was added 6.79 g (78.0 mmol) of morpholine, and the solution was heated at reflux for 18 h and then cooled to 25 °C and diluted with ether. The organic mixture was washed twice with a saturated aqueous sodium chloride solution, dried over sodium sulfate, and concentrated to afford a yellow liquid. Flash column chromatography (silica, chloroform/triethylamine, 100:2) gave 4.56 g (63.2%) of a pale yellow liquid: IR (neat) 3495, 1730, 1450, 1232 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.2 (t, 3 H), 2.5–2.70 (m, 4 H), 2.75 (d, 1 H, *J* = 13.4 Hz), 3.35 (d, 1 H, *J* = 13.4 Hz), 3.6–3.7 (m, 4 H), 4.2–4.3 (m, 2 H), 4.6 (bs, 1 H), 7.3–7.4 (m, 5 H) ppm. It was used for the next step without further purification.

3-(1-Azabicyclo[2.2.2]octyl) 2-Hydroxy-3-(4-morpholinyl)-2-phenylpropionate Dihydrochloride Hemihydrate (2e). This procedure illustrates the general method for preparation of the 3-(1-azabicyclo[2.2.2]octyl) 3-amino-2-hydroxy-2-phenylpropionates **2**. A solution of 3-quinuclidinol (5.78 g, 45.4 mmol) in 150 mL of benzene was refluxed azeotropically for 1 h and then cooled to 25 °C, and 1 g (0.045 mol) of sodium was added in small pieces. The mixture was refluxed for 1 h and then allowed to cool slightly and transferred to a solution of 4.24 g (15.2 mmol) of **4e** in 150 mL of benzene. The resulting mixture was refluxed azeotropically for 18 h and then cooled and concentrated. The residue was washed with ethyl acetate and filtered. The ethyl acetate solution was washed with water followed by a saturated sodium chloride solution, dried over sodium sulfate, and evaporated to dryness to afford an orange oil. The product was purified by flash column chromatography (silica, chloroform/triethylamine, 100:2, followed by chloroform/methanol/triethylamine, 97:1:2) and passing the product obtained through a plug of Florisil. This afforded the product as a pale yellow solid (3.76 g, 68.4%): mp 92–93 °C; IR (KBr) 3379, 1720, 1450 cm^{-1}; TLC (RP-C18, methanol/0.5 M ammonium acetate, 8:2) R_f = 0.63; NMR ($CDCl_3$) δ 1.3–2.0 (m, 5 H), 2.5–2.9 (m, 10 H), 2.7 (d, 1 H, *J* = 13.4 Hz), 3.2 (m, 1 H, *J* = 13.4 Hz), 3.3 (d, 1 H), 3.7 (m, 4 H), 4.8 (bs, 1 H), 4.9 (m, 1 H), 7.2–7.6 (m, 5 H) ppm. Anal. Calcd for $C_{20}H_{28}N_2O_4$: C, 65.98; H, 7.86; N, 7.69. Found: C, 66.01, 65.93; H, 7.73, 7.78; N, 7.61.

A solution of the product in 10 mL of methanol was brought to pH 2 with ethereal hydrogen chloride. Ether was added to afford a cloudy solution which upon cooling deposited crystals of **2e**.

This general procedure was employed to prepare other 3-(1-azabicyclo[2.2.2]octyl) 3-amino-2-hydroxy-2-phenylpropionates (Table I): 3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-2-phenyl-3-pyrrolidinylpropionate dihydrochloride (**2a**), 3-(1-azabicyclo-[2.2.2]octyl) 2-hydroxy-2-phenyl-3-piperidinylpropionate dihydrochloride sesquihydrate (**2b**), 3-azabicyclo[2.2.2]octyl) 2-phenyl-3-piperazinylpropionate trihydrochloride hydrate (**2c**), 3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-3-(4-methylpiperazinyl)-2-phenylpropionate hydrochloride (**2d**), 3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-2-phenyl-3-(4-thiomorpholinyl)propionate dihydrochloride hydrate (**2f**), 3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-3-[4-(2,6-dimethylmorpholinyl)]-2-phenylpropionate dihydrochloride hemihydrate (**2g**), 3-(1-azabicyclo[2.2.2]octyl) 3-(1,4-dioxa-8-azaspiro[4.5]decanyl)-2-hydroxy-2-phenylpropionate (**2h**), and 3-(1-azabicyclo[2.2.2]octyl) 2-hydroxy-3-[*N*,*N*-bis(2-methoxyethyl)amino]-2-phenylpropionate dihydrochloride (**2i**).

3-(1-Methyl-1-azabicyclo[2.2.2]octyl) 2-Hydroxy-3-(4-morpholinyl)-2-phenylpropionate Iodide (2j). Methyl iodide (0.32 g, 2.2 mmol) was added to a solution of 7.9 g (2.2 mmol) of **2e** base in 55 mL of ether. The solution was stirred at room temperature for 24 h, and then the solvent was removed under vacuum to afford a yellow solid. The solid was washed with ether and the product recrystallized from ether/ethanol to afford yellow crystals (0.95 g, 86%) of **2j**: mp 130 °C dec; NMR ($CDCl_3$) δ 1.9 (m, 2 H), 2.1 (m, 2 H), 2.5 (d, 1 H), 2.6 (m, 4 H), 2.9 (t, 1 H), 3.2–4.0 (m, 14 H), 4.3 (m, 1 H), 5.2 (bs, 1 H), 7.3–7.5 (m, 3 H), 7.6 (m, 1 H), 7.7 (m, 1 H); IR (KBr) 3394, 1733, 1116 cm^{-1}; TLC (RP-C18, methanol/0.5 M ammonium acetate, 8:2) R_f = 0.72.

Pharmacology. [^{3}H]QNB Binding to Particulate Fraction of Rat Ileum. Fresh rat ileum was cleaned of connective tissue and luminal contents, homogenized (Polytron setting no. 5.5 for 15 s) in a 10× volume (w/v) of 50 mM Tris-HCl (pH 7.7 at room temperature) and centrifuged at 20 000 rpm for 10 min at 4 °C. The tissue pellet was washed twice by resuspension and centrifugation as above and suspended in 50 mM Tris-HCl to a concentration of 6 mg/mL.

Inhibition of the specific binding of [^{3}H]QNB to a particulate preparation of rat ileum was performed by using triplicate incubations in a total assay volume of 2 mL containing 0.2 nM [^{3}H]QNB (New England Nuclear, Boston, MA) (sp act. = 35.2 Ci/mmol), 6 mg of tissue, 50 mM Tris-HCl (pH 7.7 at room temperature), and 10 concentrations of the test compounds. Nonspecific binding was defined by 10^{-6} M atropine. Following

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a 2-h incubation at room temperature, the binding reaction was stopped by vacuum filtration (Whatman GF/B filters). Samples were counted (Beckman Ready Safe scintillation fluid) in a Beckmann LSM-11 counter. The IC_{50} values were calculated by using computer-assisted log-logit analyses. The apparent affinity constants (K_i) were calculated according to the method of Cheng and Prusoff.[23] The apparent K_d for [^{3}H]QNB in this tissue preparation was calculated from two separate saturation experiments, and an average value of 0.2 nM was obtained.

Inhibition of Carbachol-Induced Contraction of Guinea Pig Ileum Longitudinal Muscle. Distal ileum (5 cm above the ileocecal junction) was excised from male Hartley guinea pigs (300–500 g) and placed immediately in oxygenated Tyrodes solution of the following composition (mM): NaCl, 137; KCl, 2.7; $NaHCO_3$, 11.9; NaH_2PO_4, 0.36; $MgCl_2 \cdot 6H_2O$, 1.0; $CaCl_2 \cdot 2H_2O$, 1.8; dextrose, 5.6. Longitudinal muscle strips (3–4 cm) prepared as described[22] were suspended in a 10 mL of organ bath that was gassed continuously (5% CO_2 in O_2) and maintained at 37 °C. Eight tissue segments were used in parallel from each animal and were allowed to equilibrate for 60 min prior to testing. Initial tension was set at 0.5 g. Isometric contractions were recorded by means of a electromechanical transducer on a potentiometric recorder.

Antimuscarinic studies were conducted by constructing concentration response curves to carbachol in the absence and presence of several concentrations of antagonist. Carbachol concentration curves (0.01–10 μM) were constructed in a cumulative fashion. A higher concentration of carbachol was added to the tissue bath after the contraction elicited by the previous carbachol addition had reached a steady value. A control concentration curve to carbachol was first constructed, the tissue was washed several times, and then additional curves were constructed in the presence of increasing concentrations of test antagonist (added 5 min prior to carbachol). Responses were expressed as a percentage relative to the maximum contraction elicited by carbachol in the absence of antagonist. K_b values are calculated from the equation:

$$K_b = [\text{antagonist}]/(DR - 1)$$

where DR is the dose ratio calculated as the ED_{50} for the agonist in the presence of the antagonist divided by the EC_{50} for the control.

Guinea Pig Atrial Preparation. The heart from male albino guinea pigs was removed and immediately placed into an oxygenated Krebs–Henseleit buffer of the following composition (mM): NaCl, 116.2; $NaHCO_3$, 22.4; KCl, 6.0; NaH_2PO_4, 0.98; $MgSO_4$ 1.0; $CaCl_2$, 2.46; dextrose, 11.1. The ventricles were dissected from the atria, which were then bisected to isolate the right atrium. Tissues were suspended with silk suture in 10-mL tissue baths containing Krebs–Henseleit buffer bubbled with 95% O_2/5% CO_2 and maintained at a temperature of 33 °C. Isometric contractions were measured with an electrochemical force displacement transducer coupled to a physiograph. Initial tension was set at 0.5 g. Right atria beat spontaneously, and resting tension was adjusted to the peak of the length tension curve for each tissue after a 30-min equilibration period. The baths were emptied and filled after this period and allowed to equilibrate for an additional 30 min. The potency of test compounds to reverse the negative chronotropic effect of carbachol was measured. Cumulative concentration response curves to carbachol (0.01–10 μM) were obtained before and after administration of three concentrations of antagonist (administered 10 min prior to carbachol). K_b values were calculated as previously described.

Rabbit Vas Deferens Preparation. Experiments in the vas deferens were performed similarly to that described by Eltze.[20] After removal of the vas deferens from male New Zealand White rabbits (2.0 kg), the prostatic portion (2.0 cm) was fixed at one end to a glass rod, while the other end was passed through two ring-shaped platinum electrodes and attached with suture to an electromechanical displacement transducer for measuring isometric contractions. The tissue was suspended under a resting tension of 0.75 g at 30 °C in a physiological solution [composition (mM): NaCl, 118.0; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; $MgSO_4 \cdot 7H_2O$, 0.6; $KHPO_4$, 1.2; $NaHCO_3$, 25.0; glucose, 11.1; plus 1×10^{-6} M yohimbine]. Electrical field stimulation was applied with a duration of 0.5 ms and a frequency of 0.1 Hz, and the voltage was adjusted to 50% of the maximum.

Antimuscarinic studies were conducted by constructing concentration effect curves to McN-A-343 [[4-[[(*m*-chlorophenyl)carbamoyl]oxy]-2-butyl]trimethylammonium chloride] in the absence and presence of several concentrations of antagonist. When contraction heights stabilized, the concentration–response curves for McN-A-343 were obtained by quantifying its inhibitory activity after cumulative addition (0.01–10 μM) to the organ bath. The concentration of McN-A-343 that inhibited electrically induced twitching by 50% was defined as the EC_{50}. A control concentration curve was first constructed; the tissue was washed several times; and then additional curves to McN-A-343 were constructed in the presence of at least three concentrations of the test antagonist. Inhibitory responses were expressed as a percentage of the maximal relaxation elicited by McN-A-343 in the absence of an antagonist. K_b values were calculated as previously described.

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Registry No. **3**, 19780-36-0; **2a**, 122846-61-1; **2a**·HCl, 122846-71-3; **2b**, 122846-62-2; **2b**·2HCl, 122846-72-4; **2c**, 122846-63-3; **2c**·3HCl, 122846-73-5; **2d**, 122846-64-4; **2d**·3HCl, 122846-74-6; **2e**, 122846-65-5; **2e**·2HCl, 122846-75-7; **2f**, 122846-66-6; **2f**·2HCl, 122846-76-8; **2g**, 122846-67-7; **2g**·2HCl, 122846-77-9; **2h**, 122846-68-8; **2i**, 122846-69-9; **2i**·2HCl, 122846-78-0; **2j**, 122846-70-2; **4a**, 122846-52-0; **4b**, 122846-53-1; **4c**, 122846-54-2; **4d**, 122846-55-3; **4e**, 122846-56-4; **4f**, 122846-57-5; **4g**, 122846-58-6; **4h**, 122846-59-7; **4i**, 122846-60-0; $(MeOCH_2CH_2)_2NH$, 111-95-5; pyrrolidine, 123-75-1; piperidine, 110-89-4; piperazine, 110-85-0; *N*-methylpiperazine, 109-01-3; morpholine, 110-91-8; thiomorpholine, 123-90-0; 3,5-dimethylmorpholine, 123-57-9; 1,4-dioxa-8-azaspiro[4.5]decane, 177-11-7; 3-quinuclidinol, 1619-34-7.

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