Synthesis and Antimuscarinic Activity of Some 1-Cycloalkyl-1-hydroxy-1-phenyl-3-(4-substituted piperazinyl)-2-propanones and Related Compounds

Carl Kaiser,*,† Vicki H. Audia,† J. Paul Carter,† Daniel W. McPherson,† Philip P. Waid,† Valerie C. Lowe,‡ and Lalita Noronha-Blob‡

Scios Nova Inc., 6200 Freeport Centre, Baltimore, Maryland 21224-6522

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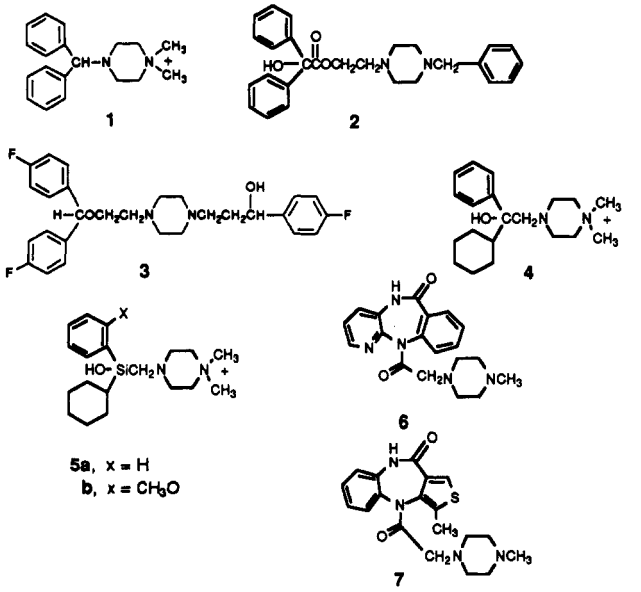
A new class of substituted 1-phenyl-3-piperazinyl-2-propanones with antimuscarinic activity is reported. As part of a structure–activity relationship study of this class, various structural modifications, particularly ones involving substitution of position 1 and the terminal piperazine nitrogen, were investigated. The objective of this study was to derive new antimuscarinic agents with potential utility in treating urinary incontinence associated with bladder muscle instability. These compounds were examined for M_1, M_2, and M_3 muscarinic receptor selectivity in isolated tissue assays and for in vivo effects on urinary bladder contraction, mydriasis, and salivation in guinea pigs. Potency and selectivity in these assays were influenced most notably by the nature of the substituent group on the terminal nitrogen of the piperazine moiety. Benzyl substitution was particularly advantageous in producing compounds with functional M_3 receptor (smooth muscle) and bladder selectivity; it provided several candidates for clinical study. In vivo, 3-(4-benzylpiperazinyl)-1-cyclobutyl-1-hydroxy-1-phenyl-2-propanone (**24**) demonstrated 11- and 37-fold separations in its effect on bladder function versus mydriatic and salivation responses, respectively. The corresponding 2-chlorobenzyl derivative **25** was more than 178-fold selective for M_3 versus M_1 and M_2 muscarinic receptors. 3-(4-Benzylpiperazinyl)-1,1-diphenyl-1-hydroxy-2-propanone (**51**) was 18-fold selective for M_3 versus M_1 and 242-fold selective for M_3 versus M_2 receptors. It was also selective in guinea pigs, where it displayed 20- and 41-fold separations between bladder function and effect on mydriasis and salivation, respectively. In general, the results of this study are consistent with the proposition that the described piperazinylpropanones interact with muscarcinic receptors in a hydrogen-bonded form that presents a conformation similar to that apparently adopted by classical antimuscarinic agents.

Molecular cloning studies have identified five unique gene sequences, m_1–m_5, coding for muscarinic receptors;[1] however, on the basis of their response to selective antagonists, to date only three major subtypes of these receptors, i.e., M_1, M_2, and M_3, have been pharmacologically classified.[2] Receptors having high affinity for pirenzepine and (+)-telenzepine are designated M_1; they are present in sympathetic ganglia and in parts of the central nervous system such as the cerebral cortex and hippocampus. Those in cardiac cells that have strong affinity for AF-DX 116, methoctramine, and himbacine are termed M_2. M_3 receptors, located particularly in glandular and smooth muscle tissue, have high affinity for DAMP, hexahydrosiladifenidol, and its *p*-fluoro derivative.[3-8]

Antimuscarinic activity has been noted for a variety of compounds containing a piperazine ring. Benzhydrylpiperazines (**1**) are nearly equipotent with atropine in an isolated guinea pig ileum preparation.[9] The piperazinylalkyl glycolate **2** is claimed to have about one-half the antispasmodic potency of atropine.[10] Anticholinergic activity has also been claimed for some benzhydryloxypiperazines, e.g., **3**.[11] Hexocyclium (**4**)[12] has potent antimuscarinic properties; it is 63-fold selective for rat ganglionic versus hippocampal M_1 muscarinic receptors.[13] A silicon analog of **4**, i.e., silahexocyclium (**5a**), was the most potent anticholinergic tested in an isolated rat sympathetic ganglion preparation,[13] and it showed a 16-fold selectivity for M_3 ileal muscarinic receptors over those (M_2) in the atria.[14] In contrast, the related *o*-methoxy derivative **5b** is a potent M_1 selective muscarinic receptor antagonist.[15] The tricyclic piperazines pirenzepine (**6**)[16,17] and telenzepine (**7**)[18] are prototypical potent and selective M_1 receptor antagonists.

Chart I



In the course of continuing research[19,20] directed toward the development of M_3 selective agents with potential

† Division of Medicinal Chemistry.
‡ Division of Pharmacology.

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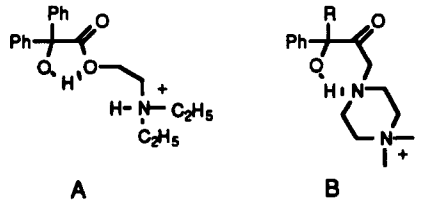
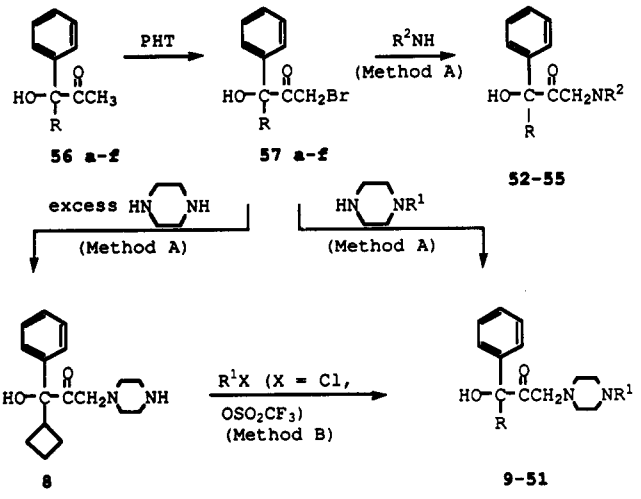


A B

Figure 1. Comparison of preferred receptor-bound conformation of benactyzine (A)[22] with a possible conformation of substituted 1-hydroxy-1-phenyl-3-(1-piperazinyl)-2-propanones (B).

Scheme I



where: **a**, R = *c*-C_3H_5; **b**, R = *c*-C_4H_7; **c**, R = *c*-C_5H_9; **d**, R = *c*-C_6H_{11}; **e**, R = *i*-C_4H_9; **f**, R = C_6H_5

therapeutic utility, for example in treating urinary incontinence associated with bladder muscle instability, several piperazinylpropanone derivatives having potent antimuscarinic properties were identified.[21] On the basis of the subtype, albeit not M_3, selectivity demonstrated by some of the described antimuscarinic piperazine derivatives and considering that the piperazinylpropanones, as indicated in Figure 1, might present a constrained conformation resembling the one preferred by the protypical antimuscarinic drug benactyzine,[22] a series of similar piperazines (**8–51**, Table I) and related compounds (**52–55**, Table I) was prepared and studied pharmacologically. The new compounds were tested for functional muscarinic receptor subtype selectivity in rabbit vas deferens (nerve, M_1),[23] guinea pig atrial (cardiac, M_2),[2,14,24] and guinea pig ileal (smooth muscle, M_3)[3,4,25] preparations. To evaluate the potential of these compounds to cause side effects, such as dry mouth (xerostomia) and blurred vision (resulting from mydriasis), which are commonly associated with nonselective antimuscarinics,[26] the new series was also examined for in vivo effects on urinary bladder contraction, mydriasis, and salivary secretion in guinea pigs. The results of these studies that led to the identification of several bladder selective M_3 muscarinic receptor antagonists are reported in this article.

Chemistry. Substituted piperazinylpropanone derivatives **8–51** and related compounds **52–55** were prepared by two general methods. As illustrated in Scheme I (method A), the appropriate 1-substituted 1-hydroxy-1-phenyl-2-propanone (**56**)[19] was brominated with pyrrolidone hydrotribomide (PHT) to provide the corresponding 3-bromopropanone **57**. Amination of **57** with piperazine, a substituted piperazine, or other amine afforded **8**, **9**, **11–15**, **18**, **20–24**, **33**, **37–39**, and **41–55**. In an alternative method, as indicated in Scheme I (method B), **10**, **16**, **17**, **19**, **25–32**, **34–36**, and **40** were derived by alkylation of 1-cyclobutyl-1-hydroxy-1-phenyl-3-(1-piperazinyl)-2-propanone (**8**) with a benzyl chloride, 2-thienylmethyl chloride, furfuryl chloride, chloroacetonitrile, or phenethyl triflate.

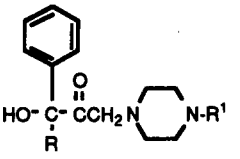
Results and Discussion

The piperazinylpropanone derivatives and related compounds in this series (**8–55**) were examined for antagonist activity in tests selective for the pharmacologically defined M_1, M_2, and M_3 muscarinic receptors. M_1 receptor antagonist potency was measured as the test compound's ability to reverse the inhibitory effect of the selective M_1 agonist McN-A-343[27,28] on electrically stimulated contractions of isolated rabbit vas deferens. Efficacy in this paradigm is expressed as an affinity constant, K_b,[29] the calculated molar concentration of the test compound needed to cause a 2-fold increase in the ED_{50} of the agonist, i.e., the concentration that inhibits the contractions by 50%. M_2 antagonist activity is also expressed as an affinity constant; it represents the ability of the test compound to double the predetermined ED_{50} of carbachol for attenuating the rate of contraction of isolated guinea pig right atria.[26,30] M_3 receptor antagonist activity is described as the ability of the test compound to decrease the response of guinea pig ileum muscle strips to carbachol.[20,26]

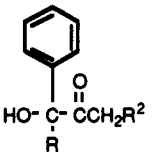
As the major objective of the present study was to discover new bladder-relaxing agents free of typical antimuscarinic side effects, compounds were also examined in a guinea pig cystometrogram (CMG) model and for their liability to cause mydriasis[26,31] and xerostomia.[26,32] In the CMG assay the strength of functional detrusor muscle contraction was measured as peak intravesical bladder pressure ($P_{ves}P$). ID_{50} values are the calculated dose of antagonist that inhibits $P_{ves}P$ by 50%.[19] The liability of antagonists to produce mydriasis[26,31] or to decrease carbachol-stimulated salivation[26,32] was evaluated in guinea pigs. ED_{50} values for mydriasis production represent the calculated subcutaneously administered dose of test compound that increases the pupil diameter to 50% of the maximum increase produced by atropine. Salivation ID_{50}s are the subcutaneously administered dose of antagonist that inhibits carbachol-induced salivation in 50% of the test animals.[26] The results of pharmacological testing of **8–55** for M_1, M_2, M_3, CMG, mydriatic, and salivation effects are tabulated in Table II.

Binding to muscarinic receptors is apparently initiated by an interaction between the cationic head of the antagonist molecule and an anionic site at the receptor surface. Effects of terminal nitrogen substitution on muscarinic receptor affinity indicate that the size and shape of the cationic group play critical roles in drug–receptor interaction;[33] however, apparently this has not been investigated in detail in tests selective for the muscarinic receptor subtypes. Consistent with the suggestion that piperazinylpropanones of the present series may adopt a conformation such that the terminal piperazine nitrogen, as depicted in Figure 1, assumes the role of the antimuscarinic cationic head,[33–36] the tertiary amine **9** was a more potent antimuscarinic at M_1, M_2, and M_3 sites than was the secondary amine **8**.[36] The carbamate **47**, in which this nitrogen is nonbasic, and the weakly basic anilines **20–23** are devoid of significant antimuscarinic activity. Also, consistent with earlier data derived in non-

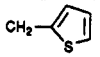
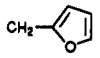
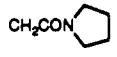
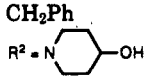
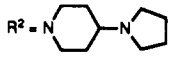
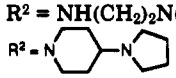
Table I. Physical Data for 1-Cycloalkyl-1-hydroxy-1-phenyl-3-(4-substituted piperazinyl)-2-propanones and Related Compounds (8–55)[a]



8-51



52-55

compd[b]	R	R^1	method[c]	yield, %	mp, °C	formula[d]	recrystn solvent
8	c-C_4H_7	H	A	83	167–169	$C_{17}H_{24}N_2O_2$·2HCl[e]	EtOH
9	c-C_4H_7	CH_3	A	41	191–193	$C_{18}H_{26}N_2O_2$·2HCl	MeOH/Et_2O
10	c-C_4H_7	C_2H_5	B	90	182–184	$C_{19}H_{28}N_2O_2$·2HCl	EtOH
11	c-C_4H_7	*n*-Pr	A	67	187–189	$C_{20}H_{30}N_2O_2$·2HCl	MeOH
12	c-C_4H_7	$(CH_2)_2OH$	A	68	197–199	$C_{19}H_{28}N_2O_3$·2HCl	MeOH/Et_2O
13	c-C_4H_7	c-C_4H_7	A	63	189–191	$C_{21}H_{30}N_2O_2$·2HCl	EtOH
14	c-C_4H_7	$CH_2CH{=}CH_2$	A	54	180–182	$C_{20}H_{28}N_2O_2$·2HCl	MeOH/Et_2O
15	c-C_4H_7	$CH_2C{\equiv}CH$	A	93	197–199	$C_{20}H_{26}N_2O_2$·2HCl	EtOH
16	c-C_4H_7	$CH_2C(CH_3){=}CH_2$	B	48	193–195	$C_{21}H_{30}N_2O_2$·2HCl	MeOH/Et_2O
17	c-C_4H_7	$CH_2CH{=}C(CH_3)_2$	B	61	180–182	$C_{22}H_{32}N_2O_2$·2HCl	MeOH/Et_2O
18	c-C_4H_7	$CH_2CH{=}CHPh$	A	80	210–212	$C_{26}H_{32}N_2O_2$·2HCl	MeOH
19	c-C_4H_7	$(CH_2)_2CH{=}CH_2$	B	68	184–186	$C_{21}H_{30}N_2O_2$·2HCl	MeOH/Et_2O
20	c-C_4H_7	Ph	A	71	180–182	$C_{23}H_{28}N_2O_2$·2HCl	EtOH/Et_2O
21	c-C_4H_7	Ph-4-NO_2	A	72	134–136	$C_{23}H_{27}N_3O_4$[e]	EtOH
22	c-C_4H_7	Ph-2-OC_2H_5	A	35	198–200	$C_{25}H_{32}N_2O_3$·2HCl	EtOH/Et_2O
23	c-C_4H_7	Ph-2-OCH_3	A	37	198–200	$C_{24}H_{30}N_2O_3$·2HCl	EtOH/Et_2O
24	c-C_4H_7	CH_2Ph	A	73	214–216	$C_{24}H_{30}N_2O_2$·2HCl	EtOH
25	c-C_4H_7	CH_2Ph-2-Cl	B	55	207–209	$C_{24}H_{29}ClN_2O_2$·2HCl	MeOH/Et_2O
26	c-C_4H_7	CH_2Ph-3-Cl	B	75	202–204	$C_{24}H_{29}ClN_2O_2$·2HCl	MeOH/Et_2O
27	c-C_4H_7	CH_2Ph-4-Cl	B	67	220–222	$C_{24}H_{29}ClN_2O_2$·2HCl	MeOH/Et_2O
28	c-C_4H_7	CH_2Ph-3-OCH_3	B	38	196–198	$C_{25}H_{32}N_2O_3$·2HCl	MeOH/Et_2O
29	c-C_4H_7	CH_2Ph-4-OCH_3	B	37	209–211	$C_{25}H_{32}N_2O_3$·2HCl	MeOH
30	c-C_4H_7	CH_2Ph-2-CH_3	B	38	201–203	$C_{25}H_{32}N_2O_2$·2HCl[f]	MeOH/Et_2O
31	c-C_4H_7	CH_2Ph-4-CH_3	B	65	208–210	$C_{25}H_{32}N_2O_2$·2HCl[f]	MeOH/Et_2O
32	c-C_4H_7	CH_2Ph-4-NO_2	B	54	211–213	$C_{24}H_{29}N_3O_4$·2HCl[f]	MeOH/Et_2O
33	c-C_4H_7	CH_2Ph-3,4-OCH_2O	A	55	208–209	$C_{25}H_{30}N_2O_4$·2HCl	EtOH/Et_2O
34	c-C_4H_7		B	36	196–198	$C_{22}H_{28}N_2O_2S$·2HCl[e]	EtOH/Et_2O
35	c-C_4H_7		B	40	181–183	$C_{22}H_{28}N_2O_3$·2HCl	EtOH/Et_2O
36	c-C_4H_7	CH_2CN	B	57	177–179	$C_{19}H_{25}N_3O_2$·2HCl	MeOH/Et_2O
37	c-C_4H_7		A	32	222–224	$C_{23}H_{33}N_3O_3$·2HCl[f]	EtOH
38	c-C_4H_7	$CH_2COCPh(OH)$-c-C_4H_7	A	33	231–233	$C_{30}H_{38}N_2O_2$·2HCl	MeOH/Et_2O
39	c-C_4H_7	$CHPh_2$	A	73	187–189	$C_{30}H_{34}N_2O_2$·2HCl[e]	EtOH
40	c-C_4H_7	$(CH_2)_2Ph$	B	75	200–202	$C_{25}H_{32}N_2O_2$·2HCl[f]	MeOH/Et_2O
41	c-C_6H_{11}	CH_3	A	43	190–195	$C_{20}H_{30}N_2O_2$·2HCl	MeOH/Et_2O
42	c-C_6H_{11}	$(CH_2)_2OH$	A	59	164–166	$C_{21}H_{32}N_2O_3$·2HCl[f]	MeOH/Et_2O
43	c-C_6H_{11}	$CH_2CH{=}CH_2$	A	74	191–192	$C_{22}H_{32}N_2O_2$·2HCl[f]	EtOH/Et_2O
44	c-C_6H_{11}	$CH_2C{\equiv}CH$	A	61	198–200	$C_{22}H_{30}N_2O_2$·2HCl[f]	EtOH/Et_2O
45	c-C_6H_{11}	CH_2Ph	A	95	201–203	$C_{26}H_{34}N_2O_2$·2HCl[e]	EtOH/Et_2O
46[g]	c-C_6H_{11}	CH_2Ph	A	68	202–203	$C_{26}H_{33}FN_2O_2$·2HCl[h]	MeOH
47	c-C_6H_{11}	$CO_2C_2H_5$	A	57	143–145	$C_{22}H_{32}N_2O_4$·2HCl	EtOH/Et_2O
48	*i*-C_4H_9	CH_2Ph	A	85	205–206	$C_{24}H_{32}N_2O_2$·2HCl	MeOH/Et_2O
49	c-C_3H_5	CH_2Ph	A	37	204–206	$C_{23}H_{28}N_2O_2$·2HCl	MeOH
50	c-C_5H_9	CH_2Ph	A	74	209–210	$C_{25}H_{32}N_2O_2$·2HCl	MeOH/Et_2O
51	Ph	CH_2Ph	A	50	208–210	$C_{28}H_{28}N_2O_2$·2HCl	MeOH/Et_2O
52	c-C_4H_7		A	68	186–187	$C_{18}H_{25}NO_3$·HCl	EtOH/Et_2O
53	c-C_4H_7		A	32	240–242	$C_{22}H_{32}N_2O_2$·2HCl	MeOH/Et_2O
54	c-C_6H_{11}	$R^2 = NH(CH_2)_2N(CH_3)_2$	A	59	175–177	$C_{19}H_{30}N_2O_2$·2HCl	MeOH/Et_2O
55	c-C_6H_{11}		A	76	245–247	$C_{24}H_{36}N_2O_2$·2HCl	MeOH/Et_2O

[a] See text, Scheme I, for description of general structure. [b] All compounds are isomeric mixtures. [c] See the Experimental Section, general methods. [d] All compounds were analyzed for C, H, and N; values were within 0.4% of those calculated. [e] Hydrate. [f] Hemihydrate. [g] 1-(4-Fluorophenyl) substituent. [h] Sesquihydrate.

subtype-selective antimuscarinic tests, the *N*-methyl derivative **9** is more potent than its ethyl (**10**), *n*-propyl (**11**), and allyl (**14**) relatives, as is the case for N-substituted tropine esters.[36,37]

Other studies indicate that relatively large substituents may be introduced on the cationic head of prototypical antimuscarinics with little loss of activity.[38] These results indicate that considerable bulk is tolerated by at least some subpopulations of muscarinic receptors in a vicinity complementary to that at which the cationic head binds.

Table II. Pharmacological Data for 1-Cycloalkyl-1-hydroxy-1-phenyl-3-(4-substituted piperazinyl)-2-propanones and Related Compounds (8–55)[a]

	muscarinic receptor tests, isolated tissue			antimuscarinic activity, in vivo		
compd[b]	vas deferens rabbit, K_b, nM ± SEM (M_1)[b,c]	atria, guinea pig, K_b, nM ± SEM (M_2)[b,c]	ileum, guinea pig, K_b, nM ± SEM (M_3)[b,c]	cystometrogram ED_{50}, mg/kg, sc ± SEM[b,c]	mydriasis ED_{50}, mg/kg, sc[b]	salivation inh, ID_{50}, mg/kg, sc[b,d]
8	36 ± 5	940 ± 192	87 ± 7	0.49 ± 0.18 (4)	9.36 (5–12)	1.65 (1.3–2.3)
9	19 ± 4	79 ± 7	13 ± 2	0.126 ± 0.023 (4)	0.75 (0.5–1.4)	0.72 (0.52–1)
10	42 ± 6	223 ± 39	124 ± 21	0.87 ± 0.19 (7)	0.91 (0.5–1.5)	2.06 (1.7–3.2)
11	236 ± 41	1189 ± 269	673 ± 113	3.02 ± 0.34 (4)	2.71 (1–5)	31.1 (28–34)
12	186 ± 35	2354 ± 450	185 ± 43	1.38 ± 0.08 (4)	4.65 (3–9)	4.93 (2.6–9.5)
13	302 ± 17	1802 ± 234	230 ± 27	1.64 ± 0.25 (3)	4.01 (2.5–5.8)	18.3 (12–24)
14	98 ± 4	299 ± 43	60 ± 17	2.68 ± 0.94 (4)	41.3 (36–58)	20.4 (15–32)
15	462 ± 51	>30000	536 ± 103	3.96 ± 1.5 (4)	54.4 (42–65)	46.9 (34–57)
16	54 ± 6	551 ± 40	100 ± 21	2.10 ± 0.41 (3)	31 (25–40)	17 (13–20)
17	96 ± 18	305 ± 23	22 ± 4	0.49 ± 0.1 (3)	5.4 (4.1–7.3)	4.9 (2.6–9.5)
18	11 ± 2	2.3 ± 0.7	9.92 ± 0.3	0.17 ± 0.02 (3)	3.38 (1.8–6)	1.2 (0.9–2.6)
19	219 ± 20	*e*	35.6 ± 3.5	3.00 ± 0.6 (4)	21.2 (14–36)	27.2 (14–37)
20		>30000 (2)	624 ± 141	>30 (3)		
21		>10000 (2)	>10000 (2)			
22		>10000 (2)	>10000 (2)			
23		>10000 (2)	>10000 (2)			
24	25 ± 2	60 ± 4	15 ± 0.5	0.15 ± 0.05 (3)	4.5 (2.5–5.4)	14.7 (10–17)
25	>10000 (2)	>10000	56 ± 2			
26	20 ± 2.5	380 ± 78	27 ± 1	6.89 ± 0.4 (3)	7.3 (5.8–9.2)	8.3 (6.4–10)
27	48 ± 5	515 ± 49	16 ± 2.5	2.08 ± 0.19 (3)	5.3 (4–7)	9.8 (6–11)
28	35 ± 1	136 ± 24	17 ± 1.6			
29	110 ± 2.6	263 ± 28	27.9 ± 4	0.47 ± 0.1 (4)	3.5 (2–6.5)	10.8 (8–13)
30	3347 ± 150	>1000 (2)	199 ± 22	10.4 ± 2.3 (4)		
31	11 ± 2	82 ± 5	11 ± 0.8	0.38 ± 0.09 (3)	21.4 (17–28)	8.4 (6–10)
32	495 ± 18	2695 ± 444	105 ± 5	4.63 ± 0.8 (4)	63 (48–91)	21 (9–50)
33	30 ± 1	45 ± 1.5	8 ± 0.4	0.29 ± 0.04 (3)	4.3 (3–7)	4.0 (3–7)
34	77 ± 4	491 ± 79	43.8 ± 9	1.14 ± 0.23 (6)	1.2 (0.9–3)	25 (21–39)
35		>30000 (2)	750 ± 79			
36	1997 ± 530	>30000 (2)	2710 ± 534	5.94 ± 1.2 (5)	17.3 (13–22)	35.7 (30–41)
37	1764 ± 35	>3000 (2)	210 ± 25	>10 (4)		
38		>1000 (2)	207 ± 20			
39		>100000 (2)	>10000 (2)			
40	1435 ± 148	>1000 (2)	302 ± 47	16.1 ± 3.7 (4)		
41	18 ± 2	210 ± 26	15 ± 2	0.25 ± 0.04 (10)	1.04 (0.8–1.3)	1.84 (1.0–2.1)
42	117 ± 10	530 ± 89	53 ± 4	0.84 ± 0.18 (4)	2.45 (1.6–4)	>10
43	23 ± 0.5	425 ± 75	25.6 ± 2	2.88 ± 0.82 (3)	14.6 (11–17)	21 (14–32)
44	566 ± 34	5671 ± 922	260 ± 90	10.4 ± 1.5 (4)		
45	16 ± 2	282 ± 66	12 ± 3	0.44 ± 0.02 (3)	9.7 (1.5–15)	4.38 (2.2–8.8)
46	9 ± 1	441 ± 63	20 ± 2	1.47 ± 0.17 (3)	28.1 (21–32)	13.4 (10–16)
47		>30000 (2)	>10000 (2)			
48	257 ± 13	>3000 (2)	171 ± 12	2.03 ± 0.5 (3)	65.9 (52–77)	43.4 (31–55)
49	9 ± 2	225 ± 5	24 ± 2.6	0.61 ± 0.05 (3)	1.4 (1–1.7)	9.9 (6–11)
50	11 ± 1.5	78 ± 7	2.6 ± 0.3	0.19 ± 0.05 (4)	9.7 (5–14)	0.24 (0.18–0.30)
51	111 ± 10	1455 ± 147	6 ± 1	2.4 ± 0.4 (4)	47.7 (40–54)	>100
52	1084 ± 76	2338 ± 370	472 ± 101	>30 (3)		
53		2437 ± 169	>10000 (2)			
54	18 ± 3	216 ± 48	5.5 ± 2	0.10 ± 0.03 (4)	0.35 (0.16–0.6)	0.13 (0.12–0.16)
55	1041 ± 210	4507 ± 83	349 ± 85	4.18 ± 1.16 (4)		
atropine	0.4 ± 0.1	1.5 ± 0.2	1.7 ± 0.26	0.15 ± 0.01 (5)	0.05 (0.03–0.07)	0.14 (0.13–0.16)

[a] See Table I for description of general structure and substituents. [b] See the Experimental Section, pharmacology for description of method, K_b, ID_{50}, and ED_{50} definitions. K_b values were derived from tissue strips from 3–5 different animals, except for inactive compounds (K_b > 10 000) where duplicate determinations were made. [c] Values in parentheses for this test indicate the number of determinations. [d] Values in parentheses denote 95% confidence limits for the mydriasis and salivation assays as described in the Experimental Section. [e] Noncompetitive.

This observation, coupled with the cited evidence suggesting that the terminal antimuscarinic piperazinylpropanone nitrogen acts as the cationic head in the binding of these compounds to muscarinic receptors, prompted study of the various substituted piperazinyl derivatives listed in Table II as a potential source of new, subtype-selective antimuscarinics.

Among the series of 1-cyclobutyl-1-hydroxy-1-phenyl-3-piperazinyl-2-propanones **9–40**, selectivity toward M_3 and M_1 versus M_2 receptors was generally noted. Replacement of the *N*-methyl group of **9** with various other substituents generally decreased potency in both the isolated tissue and in vivo paradigms. This was most strikingly observed with the cited variations (**20–23** and **47**) which affected the basicity of the terminal piperazinyl nitrogen and derivatives bearing bulky aralkyl groups, e.g., **38–40**. In contrast, the allyl congener **14** was only somewhat less potent than **9** in the isolated tissue assays; the benzyl derivative **24** was essentially equipotent with the parent **9** in the receptor and CMG tests, whereas it was markedly less effective in assays measuring mydriatic and antisalivation potential. The activity of **14** prompted examination of several other unsaturated analogs **15–19**. A propargyl derivative **15** was markedly less potent than **14**, whereas the potency of the cinnamyl relative **18** was increased in the isolated tissue tests. The M_2 (atrial) selectivity of **18** was unique among members of this series.

The pharmacological profile, i.e., M_3 and CMG selectivity, of the N-benzylated derivative **24** met the objectives of the study; it demonstrated an 11-fold and a 37-fold

separation between bladder function (CMG) and mydriasis and salivation responses, respectively. These results led to the study of several substituted benzyl derivatives (25–33) and isosteres (34 and 35). Although meta and para substitution, with the exception of the potency-decreasing effect of *p*-nitro substitution (32), had relatively little effect on activity, ortho substitution (25, 30) markedly decreased M_1 and M_2 antagonist activity while considerable M_3 efficacy was retained. Thus, 25 was a highly selective M_3 antagonist, it was more than 178-fold selective for M_3 versus M_1 and M_2 muscarinic receptors. The benzyl isosteres 34 and 35 were markedly less effective antimuscarinics than was 24.

Several other N-substituted 1-hydroxy-1-phenyl-3-piperazinyl-2-propanones bearing a cyclohexyl (41–47), isobutyl (48), cyclopropyl (49), cyclopentyl (50), or phenyl (51) substituent on the 1-position were also examined. In general, the antimuscarinic properties of the cycloalkyl derivatives (41–47, 49, and 50) corresponded closely with those of their corresponding N-substituted 1-cyclobutyl counterparts. 3-(4-Benzylpiperazinyl)-1,1-diphenyl-1-hydroxy-2-propanone (51) was notable; it was 18-fold selective for M_3 versus M_1 and 242-fold selective for M_3 versus M_2 receptors. It was also selective in vivo where it displayed 20-fold and 41-fold separations between bladder function and mydriasis and salivation effects, respectively. As in other classes of antimuscarinics,[33,34] introduction of an aliphatic group (48) at the benzylic position significantly decreased antimuscarinic efficacy.

Several congeners of the piperazinylpropanones 8–51, namely, 4-hydroxypiperidine (52), 4-pyrrolidinylpiperidines (53 and 55), and dimethylethylenediamine (54) relatives, were also studied. Antimuscarinic properties similar to those of 9 were demonstrated by 54. This suggests that 9 and 54 may bind with muscarinic receptors in a similar fashion.

In summary, the results of this study of a new series of piperazinylpropanones are consistent with the speculation that these compounds may interact with muscarinic receptors in a hydrogen-bonded conformation similar to that suggested[22] for prototypical antimuscarinics. Appropriate terminal nitrogen substitution has provided several compounds with muscarinic receptor subtype selectivity. Secondary pharmacological testing suggests potential utility of some of these compounds for treating urinary incontinence associated with bladder muscle instability.

Experimental Section

Melting points were determined with a Bristoline hot-stage microscope or a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. IR spectra were recorded on a Beckman FT 1300 spectrophotometer. ^{1}H NMR spectra were obtained on either a Varian EM 360A or a General Electric QE300 spectrometer with Me_4Si as an internal standard. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. TLC was done on precoated plates (silica gel, 60F-254) with a fluorescent indicator. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA; they are indicated by symbols of the elements and were within 0.4% of calculated values.

Chemistry. General Methods. Method A. 3-Bromo-1-cyclobutyl-1-hydroxy-1-phenyl-2-propanone (57b). To a stirred solution of 276.3 g (1.35 mol) of 1-cyclobutyl-1-hydroxy-1-phenyl-2-propanone (56b) in 3 L of tetrahydrofuran (THF) was added 903 g (1.82 mol) of pyrrolidone hydrotribromide (PHT). After the stirred mixture was refluxed for 24 h, it was cooled to 25 °C and partitioned between 4 L of water and 4 L of ether. The organic layer was separated, washed three times with a saturated aqueous solution of sodium bicarbonate and once with brine, dried ($MgSO_4$), and concentrated. The crystalline residue was recrystallized from hexane to give 195 g (50.9%) of colorless crystals: mp 79–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.8–2.2 (m, 6 H), 3.4–3.6 (m, 1 H), 3.9–4.1 (m, 2 H), 7.2–7.7 (m, 5 H) ppm; IR (KBr) 3471, 2946, 1725, 627 cm^{-1}. Anal. ($C_{13}H_{15}BrO_2$) C, H.

Bromo ketones **57a,c–f** were prepared from the corresponding propanone derivatives[19] in a similar manner. Products were purified by flash column chromatography (silica, hexane/ethyl acetate (99:1 followed by 95:5)).

1-Cyclobutyl-1-hydroxy-1-phenyl-3-(4-methylpiperazinyl)-2-propanone Dihydrochloride (9). To a solution of 3.82 g (12.2 mmol) of **57b** in 50 mL of ether was added 3.0 g (29.9 mmol) of 1-methylpiperazine. After the mixture was stirred at ambient temperature for 16 h, it was partitioned between 100 mL of a saturated aqueous solution of sodium bicarbonate and 100 mL of ether. The ether layer was washed with water, dried ($MgSO_4$), and concentrated (at reduced pressure and a temperature sufficient to remove excess amine reagent). The residue was dissolved in a minimum volume of methanol, and 50 mL of a 1 N solution of hydrogen chloride in ether was added. The crystalline solid was filtered and recrystallized from ethanol/ether to give 1.86 g (41%) of colorless crystals: mp 191–193 °C; ^{1}H NMR (DMSO-d_6) δ 1.9 (m, 6 H), 2.1 (s, 3 H), 2.2 (s, 8 H), 3.1 (m, 3 H), 6.8 (s, 1 H), 7.1 (m, 3 H), 7.3 (d, 2 H) ppm; IR (KBr) 3296, 2939, 2422, 1727, 1450 cm^{-1}.

A similar procedure was employed for the other compounds indicated (method A) in Table I. For the preparation of 1-cyclobutyl-1-hydroxy-1-phenyl-3-piperazinyl-2-propanone dihydrochloride (8), a 10-fold excess of piperazine was employed to minimize bisalkylation. When equivalents of **57b** (10 mmol) and piperazine (5 mmol) were used, 33% of the bisalkylated product 38 was obtained.

Method B. 3-[4-(2-Chlorobenzyl)piperazinyl]-1-cyclobutyl-1-hydroxy-1-phenyl-2-propanone Dihydrochloride (25). A stirred mixture of 4.8 g (18 mmol) of 1-cyclobutyl-1-hydroxy-1-phenyl-3-piperazinyl-2-propanone [obtained by basifying an aqueous solution of dihydrochloride 8, extracting the mixture with ether, and drying ($MgSO_4$) and concentrating the ether solution], 1.68 g (20 mmol) of sodium bicarbonate, 2.53 mL (3.22 g, 20 mmol) of 2-chlorobenzyl chloride, 20 mL of methanol, and 80 mL of ethyl acetate was heated at reflux for 20 h. After the mixture was cooled to 10 °C, 100 mL of 2 N sodium hydroxide was added. The layers were separated and the aqueous part was extracted with ether. The combined ether solutions were washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on 200 g of silica gel (Merck, 230–400 mesh), eluting with ethyl acetate/hexane (gradient 30:70 to 40:60), to give 4.45 g of a viscous liquid: TLC (silica, ethyl acetate/hexane, 50:50) R_f = 0.46; ^{1}H NMR ($CDCl_3$) δ 0.7–2.56 (m, 17 H), 3.16–3.35 (m, 3 H), 7.16–7.56 (m, 9 H) ppm. A solution of the liquid in 100 mL of methanol was acidified by adding 36 mL of 1 N hydrogen chloride in ether. After the mixture was cooled to 0 °C, the crystalline product was collected and recrystallized from methanol/ether to give 4.53 g (55%) of colorless crystals of dihydrochloride 25 (Table I).

A similar procedure utilizing 8 base and 3-chloro-2-methylpropene, 4-bromo-2-methyl-2-butene, 4-bromo-1-butene, 3-chlorobenzyl chloride, 4-chlorobenzyl chloride, 2-methoxybenzyl chloride, 3-methoxybenzyl chloride, 2-methylbenzyl chloride, 4-nitrobenzyl chloride, bromoacetonitrile, and 2-phenethyl triflate afforded 16, 17, 19, 25–31, 36, and 40, respectively. For the synthesis of 34 and 35, 8 base was alkylated with 2-thienylmethyl tosylate and furfuryl tosylate, respectively, in the presence of triethylamine.

1-Cyclobutyl-3-(4-ethylpiperazinyl)-1-hydroxy-1-phenyl-2-propanone Dihydrochloride (10). To a solution of 1.5 g (27 mmol) of potassium hydroxide in 150 mL of methanol were added 4.5 g (15.6 mmol) of 8 base (derived as described in preceding experiment) and 7.2 g (46 mmol) of iodoethane. After the mixture was stirred and refluxed for 5 h, it was cooled to 20 °C and partitioned between 200 mL of 10% aqueous potassium hydroxide solution and 200 mL of ether. The ether extracts were washed with water and brine, dried ($MgSO_4$), and concentrated. The residue was dissolved in a minimum volume of ethanol, and 50

mL of 1 N hydrogen chloride in ether was added. The precipitated solid was filtered and recrystallized from ethanol to give 4.85 g (79.8%) of **10** (Table I) as colorless crystals.

Pharmacology. Rabbit vas deferens (M_1 Receptor Antagonism). As described previously,[19-21,26] electrically stimulated isometric contractions of the prostatic portion of rabbit vas deferens were dose dependently antagonized by McN-A-343[27,28] in the absence or presence of three or more increasing concentrations (5-min preincubation)[39] of the test compounds. The EC_{50} of McN-A-343 was defined as the concentration that inhibited electrically induced twitching by 50%. Affinity constants (K_b), i.e., the molar concentration of antagonist required to produce a 2-fold increase in the McN-A-343 ED_{50} value, were calculated by Schild analysis.[26,29] Schild slopes, obtained from linear regression analysis of the data, ranged from 0.8 to 1.2 for all compounds, thus suggesting competitive inhibition.

Guinea Pig Atrial Muscle (M_2 Receptor Antagonism).[26] Isolated guinea pig right atria prepared as described[30] were placed in Krebs–Henseleit buffer, and cumulative concentration rate response curves to carbachol were obtained before and after the addition of at least three increasing concentrations of test compound (5-min preincubation).[39] Responses were expressed as a percentage of the maximum inhibition of atrial rate induced by carbachol in the absence of antagonist. The molar concentration of antagonist that produced a 2-fold increase in the EC_{50} value for carbachol alone, i.e., the affinity constant (K_b) value, was calculated by Schild analysis,[29] and Schild slopes as described in the vas deferens assay indicated competitive inhibition for all compounds except **19**.

Guinea Pig Ileal Muscle. (M_3 Receptor Antagonism). Longitudinal guinea pig ileum muscle strips were prepared and suspended in oxygenated Krebs buffer as previously described[26] for guinea pig bladder detrusor muscle strips. Antimuscarinic activity was determined from concentration response curves to carbachol in the absence or presence (5-min preincubation)[39] of at least three increasing concentrations of antagonist. Contractile responses were expressed as a percentage of the maximum contraction elicited by carbachol in the absence of antagonist. Affinity constants (K_b) were calculated by Schild analysis;[29] slopes, obtained by regression analysis as described for the vas deferens preparation, indicated competitive inhibition for all compounds.

An in vivo cystometrogram (CMG) in urethane-anesthetized guinea pig was performed as described previously.[19] ID_{50} values, calculated by probit analysis, were defined as the molar concentration of the test compound that inhibited peak intravesical bladder pressure ($P_{ves}P$) by 50%.

Guinea pig mydriasis was measured as described previously[26] in a modification of a procedure employed for rats.[31] ED_{50} values and 95% confidence limits were calculated from dose response relationships by linear regression using SAS probit analysis. ED_{50} is defined as the dose eliciting 50% of maximal dilation.

Guinea pig salivation was measured by procedures modified[26] from previously described methods.[32] Briefly, guinea pigs were given various sc doses of the antagonist, and after 30 min 0.1 mg/kg of carbachol was administered ip. After 5–10 min, the animals were evaluated for their ability to respond to the agonist. ID_{50} values, i.e., the dose of antagonist that inhibited salivation in 50% of the animals, and 95% confidence limits were calculated from dose response curves using SAS probit analysis.

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References

(1) Bonner, T. I. New subtypes of muscarinic acetylcholine receptors. *Trends Pharmacol. Sci. Suppl.* **1989**, 11–15.

(2) Levine, R. R., Birdsall, N. J. M., Eds. Nomenclature for muscarinic receptor subtypes recommended by symposium. In *Subtypes of Muscarinic Receptors. IV. Proceedings of the Fourth International Symposium on Subtypes of Muscarinic Receptors (Trends Pharmacol. Sci.* **1989**, December, Suppl. vii).

(3) Lambrecht, G.; Feifel, R.; Forth, B.; Strohmann, C.; Tacke, R.; Mutschler, E. *p*-Fluoro-hexahydro-siladifenidol: The first $M_{2\sigma}$-selective muscarinic antagonist. *Eur. J. Pharmacol.* **1988**, *152*, 193–194.

(4) Lambrecht, G.; Feifel, R.; Moser, U.; Wagner-Roder, M.; Choo, L. K.; Camus, J.; Tastenoy, M.; Waelbroeck, M.; Strohmann, C.; Tacke, R.; Rodrigues de Miranda, J. F.; Christophe, J.; Mutschler, E. Pharmacology of hexahydrosiladiphenidol and related selective muscarinic antagonists. *Trends Pharmacol. Sci. Suppl.* **1989**, 60–64.

(5) Melchiorre, C.; Angeli, P.; Lambrecht, G.; Mutschler, E.; Picchio, M. T.; Wess, J. Antimuscarinic action of methoctramine, a new cardioselective M-2 muscarinic receptor antagonist, alone and in combination with atropine and gallamine. *Eur. J. Pharmacol.* **1987**, *144*, 117–124.

(6) Micheletti, R.; Montagna, E.; Giachetti, A. AF-DX 116, A Cardioselective Muscarinic Antagonist. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 628–634.

(7) Hammer, R.; Giraldo, E.; Schiavi, G. B.; Monferini, E.; Ladinsky, H. Binding profile of novel cardioselective muscarinic receptor antagonist, AF-DX 116, to membranes of peripheral tissues and brain in the rat. *Life Sci.* **1986**, *38*, 1653–1662.

(8) Scheidt, C.; Boer, R.; Eltze, M.; Riedel, R.; Grundler, G.; Birdsall, N. J. M. The affinity, selectivity and biological activity of telenzepine enantiomers. *Eur. J. Pharmacol.* **1989**, *165*, 87–96.

(9) Light, A. E.; Fanelli, R. V. Antiacetylcholine Activity of Piperazine Derivatives. *J. Am. Pharm. Assoc.* **1957**, *46*, 279–287.

(10) Biel, J. H. N-Substituted Piperazinylalkyl Glycolates. U.S. Patent 3,125,577, March 17, 1964.

(11) Gootjes, J.; van de Kamp, H. H. 4-[2-[Bis(halophenyl)methoxy]-ethyl]-α-(substituted phenyl)-1-piperazinealkanol Derivatives, Processes for Their Preparation and Pharmaceutical Preparations Containing Them. U.S. Patent 4,476,129, October 9, 1984.

(12) Weston, A. W. N,N'-Disubstituted piperazines. U.S. Patent 2,907,765, October 6, 1959; *Chem. Abstr.* **1960**, *54*, 7746e.

(13) Mutschler, E.; Moser, U.; Wess, J.; Lambrecht, G. Muscarinic Receptor Subtypes: Agonists and Antagonists. *Prog. Pharmacol. Clin. Pharmacol.* **1989**, *7*, 13–31.

(14) Mitchelson, F. Muscarinic Receptor Differentiation. *Pharmacol. Ther.* **1988**, *37*, 357–423.

(15) Lambrecht, G.; Gmelin, G.; Rafeiner, K.; Strohmann, C.; Tacke, R.; Mutschler, E. o-Methoxy-sila-hexocyclium: a new quaternary, M_1-selective muscarinic antagonist. *Eur. J. Pharmacol.* **1988**, *151*, 155–156.

(16) Bechtel, W. D.; Mierau, J.; Pelzer, H. Biochemical Pharmacology of Pirenzepine. Similarities with tricyclic antidepressants in antimuscarinic effects only. *Arzneim.-Forsch./Drug Res.* **1986**, *36*, 793.

(17) Eberlein, W. G.; Engel, W. W.; Trummlitz, G.; Schmidt, G.; Hammer, R. Tricyclic Compounds as Selective Antimuscarinics. 2. Structure–Activity Relationships of M_1-Selective Antimuscarinics Related to Pirenzepine. *J. Med. Chem.* **1988**, *31*, 1169–1174.

(18) Londong, W.; Londong, V.; Meierl, A.; Voderholzer, U. Telenzepine is at least 25 times more potent than pirenzepine—a dose response and comparative secretory study in man. *Gut* **1987**, *28*, 888–895.

(19) Carter, J. P.; Noronha-Blob, L.; Audia, V. H.; Dupont, A. C.; McPherson, D. W.; Natalie, K. J., Jr.; Rzeszotarski, W. J.; Spagnuolo, C. J.; Waid, P. P.; Kaiser, C. Analogues of Oxybutynin. Synthesis and Antimuscarinic and Bladder Activity of Some 7-Amino-1-hydroxy-5-heptyn-2-ones and Related Compounds. *J. Med. Chem.* **1991**, *34*, 3065–3074.

(20) Kaiser, C.; Spagnuolo, C. J.; Adams, T. C., Jr.; Audia, V. H.; Dupont, A. C.; Hatoum, H.; Lowe, V. C.; Prosser, J. C.; Noronha-Blob, L. Synthesis and Antimuscarinic Properties of Some N-Substituted 5-(Aminomethyl)-3,3-diphenyl-2(3*H*)furanones. *J. Med. Chem.* **1992**, *35*, 4415–4424.

(21) McPherson, D. W.; Carter, J. P. 1-Aryl-1-hydroxy-1-substituted-3-(4-substituted-1-piperazinyl)-2-propanones and Their Use in Treatment of Neurogenic Bladder Disorders. U.S. Patent 5,001,160, March 19, 1991.

(22) Flavin, M. T.; Lu, M. C.; Thompson, E. B.; Bhargava, H. N. Molecular Modification of Anticholinergics as Probes for Muscarinic Receptors. 3. Conformationally Restricted Analogues of Benactyzine. *J. Med. Chem.* **1987**, *30*, 278–285.

(23) Eltze, M. Muscarinic M_1- and M_2-receptors mediating opposite effects on neuromuscular transmission in rabbit vas deferens. *Eur. J. Pharmacol.* **1988**, *151*, 205–221.

(24) Goyal, R. K. Muscarinic receptor subtypes. Physiology and clinical implications. *New Engl. J. Med.* **1989**, *321*, 1099.

(25) Micheletti, R.; Schiavone, A.; Cereda, E.; Donetti, A. Hexocyclium derivatives with a high selectivity for smooth muscle receptors. *Br. J. Pharmacol.* **1990**, *100*, 150.

(26) Noronha-Blob, L.; Kachur, J. F. Enantiomers of Oxybutynin: *In Vitro* Pharmacological Characterization at M_1, M_2 and M_3 Muscarinic Receptors and *In Vivo* Effects on Urinary Bladder Con-

traction, Mydriasis and Salivary Secretion in Guinea Pigs. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 562–567.
(27) Willfert, B.; Davidesko, D.; Timmermans, P. B. M. W. M.; van Zwieten, P. A. Differential Role of M-1 and M-2 Receptors in Sympathetic Ganglia of the Pithed Normotensive Rat in Alpha Adrenoceptor-Mediated Vasconstriction. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 855–860.
(28) Purchased from Research Biochemicals, Inc., Natick, MA.
(29) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **1959**, *14*, 45–58.
(30) Evans, D. B.; Schenden, J. A.; Bristol, J. A. Adenosine receptors mediating cardiac depression. *Life Sci.* **1982**, *31*, 2425–2432.
(31) Parry, M.; Heathcote, B. V. A comparison of the effects of pirenzepine and atropine on gastric acid secretion, salivary secretion and pupil diameter in rat. *Life Sci.* **1982**, *31*, 1465–1471.
(32) Zwagemakers, J. M. A.; Claassen, V. Pharmacology of Secoverine, A New Spasmolytic Agent with Specific Antimuscarinic Properties. Part 1: Antimuscarinic and Spasmolytic Effects. *Arzneim.-Forsch./Drug Res.* **1980**, *30*, 1517–1525.
(33) Wess, J.; Buhl, T.; Lambrecht, G.; Mutschler, E. Cholinergic Receptors. Structure-Activity Relationships of Cholinergic Agonists and Antagonists. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study & Therapeutic Application of Chemical Compounds. Volume 3. Membranes & Receptors*; Emmett, J. C.; Ed.; Pergamon: London, 1990; pp 423–492.
(34) Kaiser, C.; Rzeszotarski, W. J. Cholinergic Receptors. In *Receptor Pharmacology and Function*; Williams, M., Glennon, R. A., Timmermans, P. B. M. W. M., Eds.; Marcel Dekker: New York, 1989; pp 453–501.
(35) Stubbins, J. F.; Hudgins, P. M.; Murphy, D. C.; Dickerson, T. L. Anticholinergic Agents Based on Ariëns' Dual Receptor Site Theory: Nonester Antagonists. *J. Pharm. Sci.* **1972**, *61*, 470.
(36) Kuznetsov, S. G.; Golikov, S. G. Synthetic Atropine-Like Substances, Second Printing. U.S. Department of Commerce, Joint Publication Research Service JPRS 19,757, Washington, DC, 1963, pp 288–300.
(37) Rama Sastry, B. V. Anticholinergics: Antispasmodic and Antiulcer Drugs. In *Burger's Medicinal Chemistry*, 4th ed.; Wolff, M. E., Ed.; Wiley-Interscience: New York, 1981; Part III, pp 361–411.
(38) Cherkez, S.; Yellin, H.; Kashman, Y.; Yaavetz, B.; Sokolovsky, M. Structure-Activity Relationship in a New Series of Atropine Analogs. II. The Effect of Asymmetric N-Substituent on the Antimuscarinic Activity. *Mol. Pharmacol.* **1978**, *14*, 781.
(39) The K_b values for a number of nonselective and selective muscarinic receptor antagonists, e.g., atropine, terodiline, pirenzepine, and AFDX-112, using a 5-min preincubation were not changed by increasing the exposure time to 20 min [Noronha-Blob, L.; Prosser, J. C.; Sturm, B. L.; Lowe, V.; Enna, S. J. (±)-Terodiline: an M_1-selective muscarinic receptor antagonist. In vivo effects at muscarinic receptors mediating urinary bladder contraction, mydriasis and salivary excretion. *Eur. J. Pharmacol.* **1991**, *210*, 135–142]. Values obtained in this manner were also shown to be consistent with those determined using longer preincubation periods.[23]