5-Methyl-2-pyrrolidone Analogues of Oxotremorine as Selective Muscarinic Agonists

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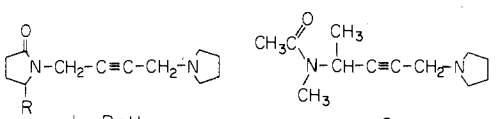
A series of *N*-(4-amino-2-butynyl)-5-methyl-2-pyrrolidones modified only in the amino group was synthesized. The compounds were agonists, partial agonists, and antagonists on the isolated guinea pig ileum. They had greater affinity and lower intrinsic efficacy at ileal muscarinic receptors than the identically modified *N*-(4-amino-2-butynyl)-2-pyrrolidones and *N*-(4-amino-2-butynyl)succinimides. Dissociation constants in the three series were correlated, suggesting that the compounds had similar mode of binding to muscarinic receptors. The 5-methyl-2-pyrrolidones were 10- to 20-fold less potent as muscarinic agonists on the guinea pig urinary bladder than on the ileum and also elicited lower relative maximal responses on the bladder. For example, the trimethylammonium (**9**) and azetidino (**10**) analogues were equipotent (EC_{50} = 0.2 μM) with the selective muscarinic stimulant *N*-(1-methyl-4-pyrrolidino-2-butynyl)-*N*-methylacetamide, BM 5 (**2**), as agonists on the ileum, but on the bladder **9** and **10** were relatively weak partial agonists, whereas **2** was an antagonist. Compound **10**, like **2** and the dimethylamino analogue **8**, also differentiated between centrally mediated muscarinic effects in vivo as it was potent in producing analgesia and hypothermia but did not elicit tremor. Instead, **10** antagonized oxotremorine-induced tremor. Thus, **10** resembled **2** in its actions except that the greater intrinsic efficacy of **10** shifted the balance between agonist and antagonist properties slightly toward agonism. Manipulation of intrinsic efficacy by minor changes in chemical structure is emphasized as a means of attaining selectivity.

Muscarinic and antimuscarinic agents capable of passing the blood–brain barrier are of potential value in treating several neurological and psychiatric conditions.[1,2] In patients suffering from Alzheimer's disease (AD), for example, there are major deficits in central cholinergic transmission. More specifically, cholinergic nerve terminals in certain areas of the brain (notably cerebral cortex and hippocampus) appear to have undergone extensive degeneration while postsynaptic muscarinic receptors are largely unaffected.[3] Consequently, muscarinic receptor agonists have been suggested as therapeutic agents in AD.[4,5] Among the muscarinic stimulants considered in a recent perspective on drug development for senile cognitive decline,[6] the oxotremorine (**1**) analogue **2** (BM 5) shows unusual selectivity in its muscarinic actions. This compound, described originally by Resul et al.,[7] was reported to act as an antagonist at certain muscarinic sites while being an agonist at most others.[7-12]

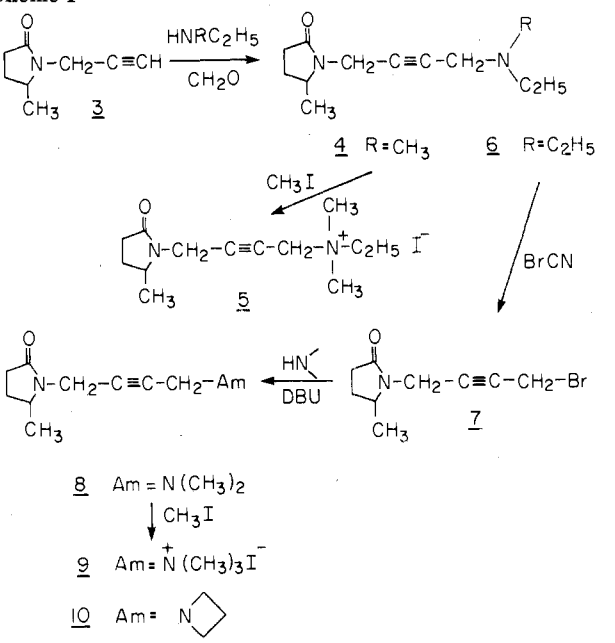


1 R = H
11 R = CH_3

2

We have recently resolved the muscarinic potency of numerous analogues of **1** into receptor affinity and efficacy components.[13-18] Compound **2** was found to have higher affinity for muscarinic receptors than **1** but substantially lower intrinsic efficacy, i.e., it may be considered a partial agonist.[14,18] As such, it may behave like an agonist or an antagonist, depending on receptor density, efficiency of receptor–effector coupling, and endogenous levels of acetylcholine at a particular site of action.[12,18] The partial agonist properties of **2** probably account for much of its selectivity.[11,12] We have suggested[12] that in AD, where the release of acetylcholine at certain central synapses is impaired, partial muscarinic agonists may be particularly useful as they would act on a virtually empty and pre-

Scheme I



3 **4** R = CH_3 **6** R = C_2H_5 **5** **7** **8** Am = $N(CH_3)_2$ **9** Am = $\overset{+}{N}(CH_3)_3\,I^-$ **10** Am = N

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Table I. Muscarinic and Antimuscarinic Activity of Some Oxotremorine Analogues in the Isolated Guinea Pig Ileum (I) and Urinary Bladder (B)[a]

compd	tissue	n^b	EC_{50}, μM	$E_{max}{}^c$	K_D,[d] μM	relative efficacy
carbachol	I	6	0.11 ± 0.01	100	14.1 ± 3.9	1.00
	B	9	0.90 ± 0.14	100	17.0 ± 2.1[e]	1.00
2[f]	I	4	0.19 ± 0.03	82.6 ± 0.3	0.24 ± 0.07	0.012 ± 0.002
	B	6		<5	0.14 ± 0.02	
4	I	7		0	1.8 ± 0.16	
	B	4		0	2.5 ± 0.4	
5	I	6	3.2 ± 0.6	28.7 ± 3.8	4.9 ± 0.9	0.0036 ± 0.0005
	B	4		0	3.5 ± 0.2	
6	I	5		0	1.1 ± 0.07	
	B	4		0	2.1 ± 0.3	
8	I	5	0.91 ± 0.06	102 ± 2.0	4.3 ± 0.7	0.040 ± 0.01
	B	4	15.0 ± 3.5	58.1 ± 3.4	11.8 ± 2.1	0.064 ± 0.005
9	I	4	0.18 ± 0.03	100 ± 1.4	1.7 ± 0.13	0.057 ± 0.007
	B	5	2.0 ± 0.6	71.4 ± 5.2	2.8 ± 0.4	0.062 ± 0.005
10	I	4	0.23 ± 0.03	90.0 ± 1.3	0.52 ± 0.13	0.020 ± 0.003
	B	6	4.6 ± 1.1	21.4 ± 2.2	0.58 ± 0.08	0.022 ± 0.002

[a] Values are means ± standard errors. [b] Number of test preparations used. [c] Maximal contractile response as a percentage of that elicited by carbachol. [d] Dissociation constant of the drug-receptor complex. [e] K_D value from ref 18. [f] Data for **2** are from ref 18.

sumably supersensitive postsynaptic receptor system and thus behave as agonists. At other synapses where acetylcholine levels are normal and postsynaptic receptors normosensitive, they may be inactive or act as antagonists. Partial agonists also may be less inclined than more efficacious agonists to cause receptor down-regulation upon prolonged exposure[19] and may actually cause receptor up-regulation.[9]

In previous studies we noted that compound **8** (Scheme I) had pharmacological properties quite similar to those of **2** although **8** was consistently less potent.[12] The lower potency of **8** appeared to be due primarily to lower affinity for muscarinic receptors as **8** had greater intrinsic efficacy than **2**.[18] Replacement of a dimethylamino group in analogues of oxotremorine by cyclic amines such as pyrrolidine increases affinity and decreases intrinsic efficacy.[16,17,20] For example, the pyrrolidine analogue of **8**, i.e., **11**, is a potent antagonist at muscarinic receptors.[12,13,20] It is also known that efficacy decreases in a regular fashion with increasing size of the amino group.[16,17] We therefore reasoned that the azetidine derivative **10**, BR 370 (Scheme I), should have higher affinity and lower intrinsic efficacy than **8** yet be more efficacious than the antagonist **11**. In other words, **10** should resemble **2** in its affinity–efficacy profile at muscarinic receptors. This report describes the synthesis and pharmacological properties of **10** and some closely related analogues.

Chemistry. The synthesis of compounds **4–10** is outlined in Scheme I. The analogues **4** and **6** were obtained from 5-methyl-*N*-(2-propynyl)-2-pyrrolidone (**3**) in a Mannich reaction. Treatment of **6** with cyanogen bromide (von Braun reaction) yielded the bromide **7**. Alkylation of dimethylamine and azetidine by **7** in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave **8** and **10** in 52–65% yield. Compound **8**[21] and its enantiomers[20] have been synthesized previously by a Mannich reaction between **3** or its enantiomers and dimethylamine.

Pharmacological Results

Guinea Pig Ileum. Compounds **4** and **6** elicited no contractile response on the isolated guinea pig ileum.

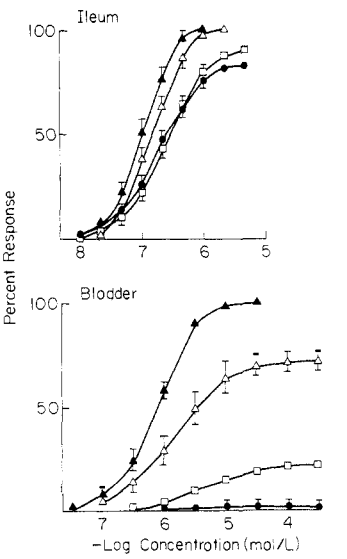

Figure 1. Concentration–response curves of carbachol (▲), **2** (●), **9** (△), and **10** (□) in strips of the guinea pig ileum and urinary bladder. Responses are expressed relative to the maximum response elicited by carbachol. The vertical bars show standard errors. The number of preparations used is given in Table I.

Instead, they were competitive antagonists to carbachol, whereas **5** was a partial agonist (Table I). The concentration–response curves of **8** and **9** were similar in shape and maximum response to that of carbachol (Table I), indicating full agonist activity. These results agree with those reported previously[20] for the enantiomers of **8** and **9** on the guinea pig ileum. However, both compounds differed from carbachol in having substantially greater affinity (lower K_D) and lower efficacy. Compound **10** displayed a concentration–response curve that was virtually superimposable on that of **2** (Figure 1). It had 2-fold greater efficacy and 2-fold lower affinity than **2** at ileal muscarinic receptors, resulting in the almost identical EC_{50} values.

Figure 2 shows the relationship between negative logarithms of the K_D values estimated on the guinea pig ileum for the 5-methyl-2-pyrrolidones **4–6** and **8–11** and those estimated previously for the corresponding 2-pyrrolidones[16] and succinimides.[22] There were significant linear relationships between K_D values in the three series of compounds. Furthermore, the slopes of the regression lines

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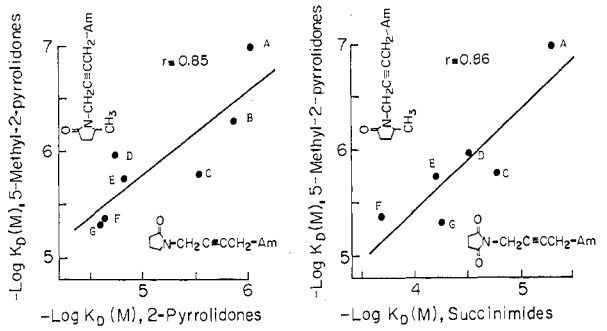

Figure 2. Relationships between negative logarithms of the dissociation constants (K_D) at muscarinic receptors in the guinea pig ileum of 5-methyl-2-pyrrolidones and those of the corresponding 2-pyrrolidones and succinimides. The letters refer to the amino substituent (Am). A, pyrrolidino; B, azetidino; C, trimethylammonium; D, diethylamino; E, methylethylamino; F, dimethylamino; and G, ethyldimethylammonium. K_D values of the 5-methyl-2-pyrrolidones are from Table I and those of the 2-pyrrolidones and succinimides are from ref 16 and 22, respectively.

Table II. Muscarinic Activity of Some Oxotremorine Analogues Administered Intraperitoneally to Mice[a]

compd	ED_{50}, μmol/kg		
	salivation	analgesia	hypothermia (Δt, °C)[b]
1[c]	0.29 ± 0.04	0.050 ± 0.009	0.22 ± 0.008 (9.1 ± 1.0)
2[c]	0.65 ± 0.09	0.37 ± 0.08	0.75 ± 0.07 (4.5 ± 0.5)
8	4.0 ± 0.5	1.5 ± 0.2	13.1 ± 1.9 (9.2 ± 0.8)
9	12.0 ± 1.7	*d*	*d*
10	1.3 ± 0.2	0.71 ± 0.09	4.9 ± 1.2 (8.2 ± 0.9)

[a] Values are means ± standard errors. [b] Maximum decrease in body temperature is given in parentheses. [c] Data for 1 and 2 are from ref 12. [d] No effect was observed at doses below the LD_{50}.

were not significantly ($P > 0.05$) different from one.

Guinea Pig Urinary Bladder. None of the analogues studied produced a full contractile response compared to carbachol on the urinary bladder (Table I). Compounds **4–6** were competitive antagonists, while **8–10** were partial agonists eliciting only a fraction of the maximum response of carbachol (Figure 1). The response to **10** was sufficiently low to allow its K_D to be estimated by the method used for competitive antagonists (Schild analysis).[23] As noted previously, **2** produced an insignificant response and may be regarded as an antagonist on the bladder.[11,18] The EC_{50} values of **8–10** were 10- to 20-fold greater than those observed on the ileum. In spite of the large differences in agonist potency and relative maximal responses between the ileum and bladder, the K_D of each compound showed good agreement (within 3-fold) in the two tissues. A plot of K_D values estimated on the ileum versus those obtained on the bladder gave a linear regression ($r = 0.95$, $P < 0.0005$) with a slope (1.13) that was not significantly ($P > 0.05$) different from one. Furthermore, the data points tended to fall on the line of equivalence.

Muscarinic and Antimuscarinic Activity in Intact Mice. Salivation was used as a measure of peripheral muscarinic activity. Analgesia and hypothermia served as measures of central muscarinic activity. Compounds **4–6** showed none of these effects at doses below their LD_{50} values. Compound **10** was somewhat less potent than **2** but more potent than **8** in producing salivation and analgesia (Table II). Although less potent than **2**, **10** elicited a maximal hypothermic response that was significantly greater than that produced by **2**. The quaternary derivative **9** caused salivation only.

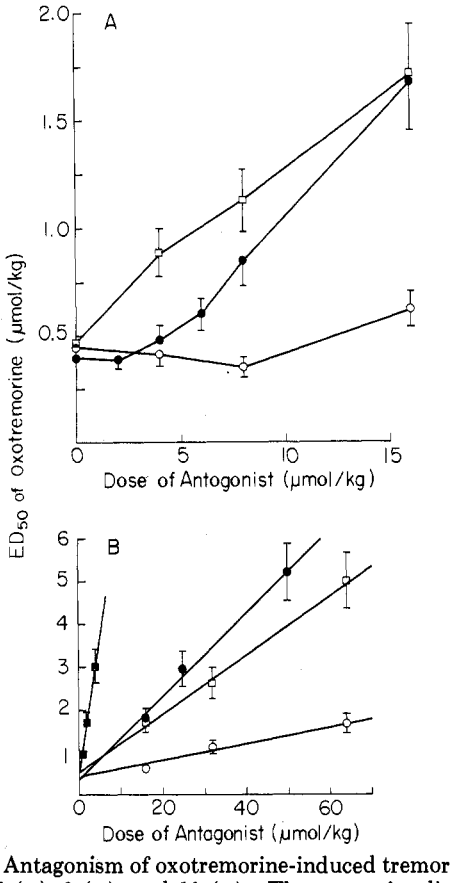

Figure 3. Antagonism of oxotremorine-induced tremor in mice by **2** (■), **6** (□), **8** (○), and **10** (●). The regression lines in B included the ED_{50} value of oxotremorine in the absence of antagonist but excluded the two lowest and four lowest doses of **8** and **10**, respectively, given in A (see Results). The data for **2** are from ref 12. At least six animals were used per dose. The vertical bars show standard errors.

Table III. Tremorolytic Activity and Acute Toxicity of Some Oxotremorine Analogues Administered Intraperitoneally to Mice[a]

compd	tremor blockade ED_{50}, μmol/kg	acute toxicity LD_{50}, μmol/kg
2[b]	0.63 ± 0.02	665 ± 90
4	6.7 ± 1.4	636 ± 87
5	*c*	176 ± 24
6	5.1 ± 0.5	720 ± 99
8	22.8 ± 1.1[d]	402 ± 54
9	*c*	33.6 ± 4.6
10	4.6 ± 0.6[d]	192 ± 26
11[b]	0.36 ± 0.03[e]	107 ± 14
atropine	0.57 ± 0.06	104 ± 14

[a] Values are means ± standard errors. [b] Data for 2 and 11 are from ref 12. [c] No significant blockade was observed at doses below the LD_{50}. [d] These values, which were calculated from the linear portion of the antagonism isoboles (Figure 3b), overestimate potency at low antagonist doses (Figure 3a). [e] Value is for the *R* enantiomer.

None of the compounds studied produced tremor in mice. Instead, the tertiary amines inhibited tremor induced by **1** and therefore showed central antimuscarinic activity. With **4** and **6**, this antagonism appeared to be competitive over the whole dose range studied. Compounds **8** and **10** showed no dose-dependent antagonism of **1** at doses below 32 and 8 μmol/kg, respectively. At these and higher doses, they blocked the actions of **1** in an apparently competitive manner. Compound **10** was less potent than **6** at low doses but somewhat more potent at higher doses (Figure 3). Antagonist potencies were cal-

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culated from the linear portions of the antagonism isoboles and are summarized in Table III. There was a significant ($r = 0.94$, $P < 0.02$) linear relationship between the affinity of **2**, **4**, **6**, **8**, and **10** for muscarinic receptors in the ileum and their ability to block oxotremorine-induced tremor. The slope of the regression line was 1.10. The calculations were made on negative logarithms of K_D and ED_{50} values with use of molar concentrations and doses, respectively.

Discussion

Although analogues of oxotremorine having low intrinsic efficacy appear to have greater potential for selective actions than more efficacious agonists,[12] there are presently no general methods of estimating agonist efficacy (or affinity) at muscarinic receptors in vivo. In the present study, the isolated ileum and urinary bladder of the guinea pig were employed as simpler experimental systems to characterize the affinity and efficacy components of muscarinic agonist activity. Part of the utility of the ileum in this respect stems from its large receptor reserve for muscarinic agonists[14-16] and the resulting ability to detect stimulatory actions of compounds, such as **5**, having only residual intrinsic efficacy. However, the sensitivity of the ileum may give rise to potentially misleading results as the ileum produces full contractile responses also to agonists having relatively low efficacy. Thus carbachol and **9** had similar agonist profiles on the ileum (Figure 1), yet **9** had 18-fold lower intrinsic efficacy than carbachol (Table I). Agonists of low efficacy may therefore be indistinguishable from more efficacious agonists in a simple assay on the ileum. Such pitfalls were avoided in the present study by assaying new compounds also on the isolated guinea pig urinary bladder. This tissue, because of its small receptor reserve for muscarinic agonists, produces full contractile responses only to highly efficacious agonists, whereas agonists of lower efficacy produce submaximal or no responses.[11,18] The four compounds in Figure 1, for example, elicited similar maximal responses on the ileum, but differed markedly in their effects on the bladder.

Accurate estimates of agonist efficacy require determination of the dissociation constant (K_D) of the agonist–receptor complex.[24] The pharmacological methods used here to determine K_D values were shown previously to provide consistent and reliable estimates of agonist affinity and efficacy at muscarinic receptors in the guinea pig ileum and urinary bladder.[11,14,18,25] The results obtained with **4–6** and **8–10** agreed with earlier findings, which showed that the structural requirements underlying affinity and efficacy at muscarinic receptors are different.[14-17,22] The observation that the acyclic amines (**4–6**, **8**, and **9**) had quite similar affinities, whereas their relative efficacies decreased with increasing substitution on the nitrogen, confirms previous suggestions that efficacy, but not affinity, is critically dependent on the size of the amino or ammonium group.[16,17] In agreement with the original predictions, substitution of azetidine for dimethylamine in **8** increased affinity 10- to 20-fold and decreased intrinsic efficacy 2- to 3-fold. The relatively small decrease in efficacy was expected since azetidine is only slightly larger than dimethylamine and considerably smaller than the methylethylamino group of the antagonist **4**, which more resembles pyrrolidine in size as estimated from increments in apparent molal volumes at infinite dilution.[16] The 5-methyl-2-pyrrolidones **4–6** and **8–10** had higher affinity but lower efficacy at ileal muscarinic receptors than the corresponding 2-pyrrolidones[16] and succinimides.[22] Therefore, selective partial agonists such as **2**, having high affinity, are more likely to be found among the 5-methyl-2-pyrrolidones. Plots of dissociation constants of **4–6** and **8–11** versus those of the corresponding 2-pyrrolidones and succinimides afforded linear regressions with slopes of unity (Figure 2). Thus, the incremental changes in affinity caused by identical modification of the amino group in the three series were the same. These observations suggest that a given amino group contributes to affinity by the same mechanism in all series of compounds,[26,27] presumably because it binds identically with the anionic site of the muscarinic receptor.

Compound **10** resembled **2** and **8** in its sialagogic and analgesic effects. These responses appear to have a large effective receptor reserve[28] for muscarinic agonists[12] and therefore are stimulated by agonists having low intrinsic efficacy. The observation that **10**, in contrast to **2**, elicited a full hypothermic response suggests a fairly large receptor reserve also for this response. Compound **10**, like **2** and **8**, failed to produce tremor, confirming that the tremor response is associated with a small receptor reserve[12,17] and therefore is stimulated only by more efficacious agonists such as **1**. The alternative explanation that the receptors mediating tremor differ from those mediating the other in vivo responses seems less likely as **2**, **8**, and **10** antagonized tremor induced by **1** and therefore had affinity for those central receptors involved in the expression of tremor. Furthermore, tremorolytic potency was highly correlated with affinity for ileal muscarinic receptors. This correlation shows that the tertiary amines did not differ substantially in their ability to penetrate the blood–brain barrier. The correlation also indicates that central muscarinic receptors mediating tremor are similar to those muscarinic receptors in the ileum that mediate contraction.

Although **8** and **10** had pharmacological properties in vivo that were qualitatively similar to those of **2**, they differed from **2** in one important aspect. Compounds **8** and **10** caused significant blockade of tremor-mediating receptors only at doses well above those that produced salivation, analgesia, and hypothermia. In contrast, **2** blocked tremor at doses that also produced the above effects. The relative inactivity of low doses of **8** and **10** in blocking tremor induced by **1** (Figure 3) may be explained if one assumes that **8** and **10** have residual efficacy at tremor-mediating receptors and/or if there is a biological threshold for the tremor response. Classical drug–receptor theory then predicts that they may be virtually inactive when tested in the presence of certain doses of another agonist.[29] Compound **2** because of its lower intrinsic efficacy will behave like a competitive antagonist also at low doses (Figure 3). The situation is somewhat similar to that observed in vitro where **2**, **8**, and **10** were full or nearly full agonists on the ileum but antagonists (**2**) or weak partial agonists (**8** and **10**) on the bladder. It can be shown on the latter tissue that **8** and **10** are more or less inactive when combined with concentrations of carbachol that produce effects similar to the maximal effects of **8** and **10** (B. Ringdahl, unpublished results). These observations suggest that the effects of partial agonists are very sensitive to small changes in intrinsic efficacy and to variations in the concentration of competing agonist, e.g., endogenous

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acetylcholine, at the receptor.[12]

Compounds **4** and **6** showed no evidence of agonism in any of the assays. They had about $^1/_{10}$ of the tremorolytic activity of atropine, but less than $^1/_{1000}$ of its in vitro parasympatholytic activity. Similar observations have been made with other analogues of **1**[30-32] and have been ascribed in part to their favorable distribution into the brain.[33]

The results obtained here support previous suggestions that regional differences in receptor reserve (a function of agonist efficacy) may be important in achieving selective muscarinic actions.[12,18] Selectivity based on this mechanism should manifest itself also in the absence of distinct subtypes of muscarinic receptors. Further studies of partial agonists maintaining the high affinity of **2** and **10** for muscarinic receptors, but having a wider range of intrinsic efficacies, seem likely to uncover more selective muscarinic stimulants. As shown in this study, the detailed knowledge acquired about the structural requirements for affinity and intrinsic efficacy among analogues of **1**[12-18,20,22] greatly facilitates the design of such analogues. As these structural requirements are quite different, intrinsic efficacy can be altered independently of affinity by relatively minor changes in chemical structure.

Experimental Section

Melting points were determined in a heated metal block with use of glass capillaries and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and agreed with theoretical values within ±0.4%. Mass spectra were recorded on a Hewlett-Packard 5981A mass spectrometer at 70 eV. ^{1}H NMR spectra were obtained at 40 °C on a Bruker WP 200 spectrometer at 200 MHz. Chemical shifts are reported in parts per million (δ) downfield from internal $(CH_3)_4Si$ standard. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} analytical plates. Visualization was done with I_2.

***N*-[4-(Ethylmethylamino)-2-butynyl]-5-methyl-2-pyrrolidone (4) Sesquioxalate.** A mixture of 16 g (0.12 mol) of 5-methyl-*N*-(2-propynyl)-2-pyrrolidone (**3**),[20,21] paraformaldehyde (3.5 g, 0.12 mol), methylethylamine (7 g, 0.12 mol), and CuCl (0.2 g) in 200 mL of dioxane was stirred in a sealed flask at 30 °C for 24 h. The dioxane was evaporated under vacuum, and the residue was taken up in 1 N HCl. The water solution was extracted three times with CH_2Cl_2, and K_2CO_3 and NH_4OH were added to adjust the pH to 10. Extraction with CH_2Cl_2 (3 × 100 mL), drying (K_2CO_3), and evaporation yielded a brown oil that was purified by distillation to give 15.9 g (65%) of **4**: bp 118 °C (0.04 mmHg), R_f 0.46 in MeOH–$CHCl_3$ (3:17). Compound **4** was converted to the sesquioxalate salt, which was recrystallized from ethanol–ether: mp 96–97 °C. ^{1}H NMR (CD_3OD): δ 4.41 (d of t, 1 H, J_1 = 17.6 Hz, J_2 = 2.0 Hz), 4.10 (t, 2 H, J = 2.0 Hz), 3.97 (d, 1 H, J = 17.6 Hz), 3.87 (m, 1 H), 3.28 (q, 2 H, J = 7.3 Hz), 2.91 (s, 3 H), 2.36 (m, 3 H), 1.65 (m, 1 H), 1.33 (t, 3 H, J = 7.3 Hz), 1.29 (d, 3 H, J = 6.3 Hz). MS: m/e (relative intensity) 150 (21.3), 112 (21.8), 111 (16.9), 108 (19.3), 96 (40.4), 94 (26.2), 68 (29.0), 58 (100). Anal. ($C_{15}H_{23}N_2O_7$) C, H, N.

***N*-[4-(Ethylmethylamino)-2-butynyl]-5-methyl-2-pyrrolidone methiodide (5)** was prepared by the addition of an excess of MeI to a solution of **4** in acetone. Mp: 141–142 °C (from ethanol–ether). Anal. ($C_{13}H_{23}IN_2O$) C, H, N.

***N*-[4-(Diethylamino)-2-butynyl]-5-methyl-2-pyrrolidone (6) sesquioxalate** was synthesized similarly from **3**, paraformaldehyde, and diethylamine. The yield of **6** was 56%. Bp: 110 °C (0.18 mmHg). R_f 0.49 in MeOH–$CHCl_3$ (3:17). The sesquioxalate was recrystallized from ethanol–ether. Mp 75–76 °C. ^{1}H NMR (CD_3OD): δ 4.39 (d of t, 1 H, J_1 = 17.8 Hz, J_2 = 2.0 Hz), 4.12 (t, 2 H, J = 2.0 Hz), 3.98 (d, 1 H, J = 17.6 Hz), 3.86 (m, 1 H), 3.29 (q, 4 H, J = 7.3 Hz), 2.36 (m, 3 H), 1.66 (m, 1 H), 1.32 (t, 6 H, J = 7.3 Hz), 1.30 (d, 3 H, J = 6.3 Hz). MS: m/e (relative intensity) 150 (46.4), 112 (18.2), 110 (38.7), 108 (25.5), 94 (19.4), 72 (100). Anal. ($C_{16}H_{25}N_2O_7$) C, H, N.

***N*-(4-Bromo-2-butynyl)-5-methyl-2-pyrrolidone (7).** Compound **6** (25 g, 0.11 mol) in ether was added dropwise to 13.2 g (0.12 mol) of BrCN in ether. The solution was left at room temperature overnight. The ether solution was extracted twice with 0.5 N HCl, washed with water, and dried over $MgSO_4$. After evaporation of the ether, the residue was distilled to give a forrun at 45 °C (0.25 mmHg) and 14.2 g (56%) of **7**. Bp: 138 °C (0.25 mmHg). R_f 0.72 in MeOH–$CHCl_3$ (3:17). MS: m/e (relative intensity) 151 (11.7), 150 (100), 112 (16.7), 84 (50.8), 83 (27.5), 81 (10.2), 79 (10.9). Anal. ($C_9H_{12}BrNO$) C, H, N.

***N*-[4-(Dimethylamino)-2-butynyl]-5-methyl-2-pyrrolidone (8) Sesquioxalate.** Compound **7** (2.0 g, 0.0087 mol) was added at 0 °C to ~0.5 g (~0.01 mol) of dimethylamine and 1.3 g (0.0087 mol) of DBU in anhydrous ether. The reaction vessel was sealed, and the mixture was left at room temperature for 20 h. After filtration, the solution was concentrated in vacuum. The oily residue was purified on a silica gel column with use of CH_2Cl_2–MeOH (9:1) as eluent to give 1.1 g (65%) of **8**. R_f 0.44 in MeOH–$CHCl_3$ (3:17). The sesquioxalate was recrystallized from ethanol–ether and had mp 125–126 °C (lit.[21] mp 124.5–125.5 °C).

***N*-[4-(Dimethylamino)-2-butynyl]-5-methyl-2-pyrrolidone methiodide (9)** was prepared by adding an excess of MeI to a solution of **8** in acetone. Mp: 150–152 °C (from methanol–ether). Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

***N*-(4-Azetidinyl-2-butynyl)-5-methyl-2-pyrrolidone (10) oxalate** was synthesized from **7** and azetidine in the presence of DBU as described above for **8**. The yield of **10** was 52%. R_f 0.38 in MeOH–$CHCl_3$ (3:17). The oxalate was recrystallized from methanol–ether. Mp: 127–128 °C. ^{1}H NMR (CD_3OD): δ 4.40 (d of t, 1 H, J_1 = 17.6 Hz, J_2 = 1.7–2.0 Hz), 4.19 (t, 4 H, J = 8.2 Hz), 4.05 (t, 2 H, J = 2.0 Hz), 3.96 (d, 1 H, J = 17.6 Hz), 3.86 (m, 1 H), 2.38 (m, 5 H), 1.66 (m, 1 H), 1.30 (d, 3 H, J = 6.3 Hz). MS: m/e (relative intensity) 151 (35.8), 150 (22.0), 112 (100), 109 (64.5), 108 (38.6), 107 (42.0), 106 (32.8), 94 (84.6). Anal. ($C_{14}H_{20}N_2O_5$) C, H, N.

Guinea Pig Ileum. A standard guinea pig ileum preparation was set up in Tyrode solution (pH 7.4) at 37 °C as described previously.[14] The Tyrode solution contained hexamethonium (0.3 mM). Contractions were recorded isotonically at 1 g of tension with an electromechanical displacement transducer and a potentiometric recorder. Concentration–response curves were constructed by the cumulative dose–response technique by increasing stepwise the concentration of agonist by a factor of 2.15.

The dissociation constants (K_D) and efficacies (relative to that of carbachol) of **8** and **9** at ileal muscarinic receptors were estimated after fractional receptor inactivation with propylbenzilylcholine mustard (30–50 nM for 15 min) according to a previously described method.[14,18] The K_D values and relative efficacies of **5** and **10** were determined by comparison of their concentration–response curves with that of carbachol as described for **2**.[18] Dissociation constants of the antagonists **4** and **6** were determined against carbachol. The antagonists were allowed to equilibrate with the tissue for 15 min before the addition of carbachol. Competitiveness of the antagonism was investigated as described by Arunlakshana and Schild.[23] Dissociation constants were calculated by dividing each concentration of antagonist by the dose ratio − 1, where the dose ratio is the ratio of the EC_{50} values of carbachol in the presence and absence of antagonist.

Guinea Pig Urinary Bladder. Strips of the bladder were prepared as described previously[11] and suspended at 37 °C in Krebs–Henseleit solution (pH 7.4) containing hexamethonium (0.3 mM). Contractions were recorded isotonically at 1 g of tension. Agonist doses were added cumulatively by increasing the concentration by a factor of 3.16.

Dissociation constants and relative efficacies of **8** and **9** at muscarinic receptors in the bladder were estimated by comparison of their concentration–response curves with that of carbachol as described previously for compounds acting as partial agonists on the bladder.[11,18] Dissociation constants of the antagonists **4–6** and

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of the partial agonist **10** were determined against carbachol as described above for antagonists on the ileum. The compounds were allowed to equilibrate with the tissue for 30 min.

Muscarinic and Antimuscarinic Activity in Intact Mice. Male Swiss-Webster mice (24-32 g) were injected intraperitoneally. All experiments were carried out at 20.5 ± 1.0 °C. Threshold doses for salivation were estimated by the up-and-down method.[34] The presence or absence of salivation was determined by lightly pressing the mouth of the animal to an absorbent paper tissue. Effects on core body temperature were measured with a digital thermometer with the probe inserted about 25 mm in the rectum. The compounds were administered to groups of six mice at five to six dose levels and measurements were made every 20 min for 3 h. The difference between post- and pretreatment temperature were calculated and dose-response curves were constructed from the maximal hypothermic response. ED_{50} values were estimated by fitting a logistic function to the dose-response curves.[35] The tail-flick assay[36] was used to estimate effects on nociceptive thresholds. The compounds were administered to groups of ten mice at three to four dose levels. A cut-off time of 15 s was employed. Those animals that had posttreatment reaction times greater than the control mean reaction time plus 3 SD were considered as having significantly increased reaction times. ED_{50} values were estimated by probit analysis.

Antagonism of oxotremorine-induced tremor was studied by ip administration of antagonists to groups of six or more mice, while six control animals remained untreated. Twenty minutes after drug administration, the ED_{50} value of oxotremorine, injected iv, was estimated by the up-and-down method with intermittent spontaneous (grade 2) tremor[37] as the end point. The ED_{50} value of oxotremorine was plotted against the dose of antagonist used for premedication. That dose of antagonist that doubled the ED_{50} value of oxotremorine was estimated by linear regression analysis.

Acute Toxicity in Mice. LD_{50} values were estimated by the up-and-down method.[34] Compounds were administered intraperitoneally, and mortality counts were taken at 30 min.

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Registry No. **3**, 18327-34-9; **4**, 112483-23-5; **4**·$^3/_2$oxalate, 112483-27-9; **5**, 112483-24-6; **6**, 112483-19-9; **6**·$^3/_2$oxalate, 112483-20-2; **7**, 112483-25-7; **8**, 71970-74-6; **8**·$^3/_2$oxalate, 71970-75-7; **9**, 112483-26-8; **10**, 112483-21-3; **10**·oxalate, 112483-22-4; MeNHEt, 624-78-2; $HNEt_2$, 109-89-7; $HNMe_2$, 124-40-3; azetidine, 503-29-7.

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6- and 8-Hydroxy-3,4-dihydro-3-(dipropylamino)-2*H*-1-benzopyrans. Dopamine Agonists with Autoreceptor Selectivity

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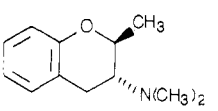
The dopamine agonist profiles of 3,4-dihydro-3-(3-dipropylamino)-2*H*-1-benzopyran-6- and -8-ol (**4** and **5**, respectively) were examined. Both **4** and **5** exhibited greater relative affinity for receptors labeled with the dopamine agonist ligand [^{3}H]propylnorapomorphine than for those labeled with the dopamine antagonist ligand [^{3}H]haloperidol. Both compounds attenuated the stimulation of brain dopamine synthesis caused by γ-butyrolactone (GBL) and decreased the firing rate of substantia nigra dopamine neurons in rats. This profile of activity, together with the ability of the dopamine antagonist haloperidol to reverse the inhibition of dopamine neuronal firing, indicate that both compounds are brain dopamine agonists.

Several years ago the preclinical and clinical profiles of *trans*-3,4-dihydro-2-methyl-3-(dimethylamino)-2*H*-1-benzopyran (CI-686) (**1**) were examined.[1,2] Although **1** displayed both stimulating and blocking effects on behaviours known to depend on brain dopamine (DA) in experimental animals, these effects did not appear to be mediated by direct actions at brain DA receptors. Since this time, there has been a rapid development of compounds that are potent and selective agonists or antagonists at DA receptors.[3,4] For example, several hydroxy-substituted aminotetralins related to **1** have been described as DA agonists.[5] In accordance with the model of McDermed et al., DA agonist activity is maximized when such compounds contain a hydroxyl group at a meta position on the aromatic ring of the incorporated phenethylamine DA pharmacophore and a dipropyl-substituted amino moiety (i.e., 5-or 7-hydroxy analogues **2** and **3**, respectively).[6]

Because of the structural similarity between **1** and these aminotetralins, we examined the pharmacological profiles



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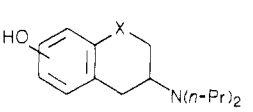

2: 5-OH; X = CH_2
3: 7-OH; X = CH_2
4: 6-OH; X = O
5: 8-OH; X = O

of analogues of **1**, namely the 6- and 8-hydroxy-3,4-dihydro-3-(dipropylamino)-2*H*-1-benzopyrans, **4** and **5**, respectively, that incorporate critical features for DA agonist

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