Amide, Urea, and Carbamate Analogues of the Muscarinic Agent [4-[[N-(3-Chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium Chloride

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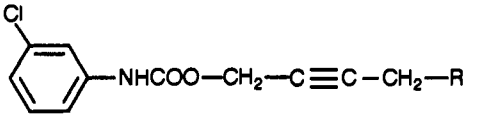
A series of amide, urea, and carbamate analogues of the muscarinic (M_1) ganglionic stimulant [4-[[N-(3-chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium chloride (McN-A-343; 1) was prepared. The C1-methyl-substituted carbamates 8–11 were resolved into the enantiomers. In order to investigate the ganglionic stimulant activity and affinity of the new compounds we studied their ability to increase mean arterial blood pressure (MAP) in the pithed rat and their ability to displace the M_1 receptor selective antagonist [^{3}H]pirenzepine from rabbit sympathetic ganglia. The quaternary ammonium derivatives of 1, but not their corresponding tertiary amines, displayed ganglionic stimulant properties. The urea derivative 14 and the acetamide derivative 18 were almost equipotent to 1 as ganglionic agonists. In addition, 14 and 18 showed only 2- to 3-fold less affinity to ganglionic muscarinic receptors than 1. Introduction of a methyl group in the 1 position of the butynyl chain of 1 and its 4-chlorophenyl analogue increased ganglionic stimulant potency. The resulting (±)-9 and (±)-11 were the most potent analogues in this study. They were found to be partial agonists and showed 5- and 16-fold higher potency than 1, respectively, in increasing the MAP. They also displayed 6- and 18-fold higher affinity than 1 for ganglionic M_1 receptors. The (S)-enantiomers of 9 and 11 were 1.5- and 4.9-fold more potent, respectively, than their antipodes as ganglionic muscarinic stimulants. The C1-methyl-substituted urea and acetamide derivatives (15 and 19) were 1.5- and 3-fold less potent than 1 and displayed several-fold lower affinity for ganglionic M_1 receptors. The new quaternary analogues retained the selectivity for ganglionic muscarinic receptors since they produced weak partial agonist effects on the guinea pig ileum and showed several-fold lower nicotinic activity than 1 in the frog rectus abdominis assay.

Introduction

Muscarinic receptors have been divided into M_1, M_2, and M_3 subtypes on the basis of pharmacological studies.[1,2] This classification was initially proposed on the basis of data derived from experiments with the M_1-selective agonist [4-[[N-(3-chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium chloride (McN-A-343; 1), and the M_1-selective antagonist pirenzepine.[1] M_1 muscarinic receptors are present in sympathetic ganglia, and in central nervous system areas such as the cerebral cortex and hippocampus.[3] Compound 1 is potent in stimulating M_1 muscarinic receptors in sympathetic ganglia, and produces a dose-dependent rise in arterial blood pressure. Although 1 has little or no agonist activity at ileal M_3 or atrial M_2 muscarinic receptors,[4] it is not selective in terms of affinity, since its dissociation constants at M_1 and M_2 binding sites in the rat cerebral cortex and myocardium, respectively, are very similar.[5,6]

Several analogues of 1 have been prepared and evaluated for ganglionic muscarinic activity.[7–10] Some of these analogues have similar or slightly higher potency as ganglionic stimulants as compared to 1; e.g., the 4-chlorophenyl[7,11] and the recently prepared 4-bromophenyl[10] analogues of 1 showed 3-fold higher potency than 1 in increasing the blood pressure, whereas the corresponding 3-bromophenyl analogue[10] was equipotent to 1. However, a shift of the chloro substituent to the 2-position in the phenyl ring of 1 decreased ganglionic stimulant activity 10-fold.[7] Replacement of the trimethylammonium group in 1 with a dimethylsulfonium group resulted in a partial agonist (3) with 2.5-fold lower pressor activity as compared to that of 1.[10]

Muscarinic agonists with selectivity for M_1 muscarinic receptors might be useful in the therapy of Alzheimer's disease.[12] Unfortunately, 1 and related quaternary ammonium salts scarcely penetrate the blood–brain barrier,[13] and tertiary amino analogues of 1 (e.g., dimethylamino derivative 2)[4] are inactive as ganglionic stimulants.[10]



1: R = $\overset{+}{N}(CH_3)_3$
2: R = $N(CH_3)_2$
3: R = $\overset{+}{S}(CH_3)_2$

However, the ability to stimulate ganglionic M_1 receptors is not confined to quaternary ammonium compounds, since

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(1) (a) Hammer, R.; Giachetti, A. Muscarinic Receptor Subtypes: M1 and M2. Biochemical and Functional Characterization. *Life Sci.* **1982**, *31*, 2991–2998. (b) Birdsall, N. J. M.; Hulme, E. C.; Stockton, J. M. Muscarinic Receptor Heterogeneity. *Trends Pharmacol. Sci.* **1984**, *5* (Suppl.), 4–8. (c) Mutschler, E.; Lambrecht, G. Selective Muscarinic Agonists and Antagonists in Functional Tests. *Trends Pharmacol. Sci.* **1984**, *5* (Suppl.), 39–44.

(2) For more recent subclassifications of muscarinic receptors based on both pharmacological characterization and molecular cloning studies; see: Levine, R. R.; Birdsall, N. J. M. Subtypes of Muscarinic Receptors IV. Nomenclature for Muscarinic Receptor Subtypes Recommended by Symposium. *Trends Pharmacol. Sci.* **1989**, *10* (Suppl.), VII.

(3) Wess, J.; Buhl, T.; Lambrecht, G.; Mutschler, E. Cholinergic Receptors. In *Comprehensive Medicinal Chemistry*; Emmett, J. C., Ed.; Pergamon Press: Oxford, 1990; Vol. 3, pp 423–491 and references cited therein.

(4) Roszkowski, A. P. An Unusual Type of Sympathetic Ganglionic Stimulant. *J. Pharmacol. Exp. Ther.* **1961**, *132*, 156–170.

(5) Eglen, R. M.; Kenny, B. A.; Michel, A. D.; Whiting, R. L. Muscarinic Activity of McN-A-343 and its Value in Muscarinic Receptor Classification. *Br. J. Pharmacol.* **1987**, *90*, 693–700.

(6) It has been proposed that the selectivity of 1 may be the result of differences in intrinsic efficacy and/or receptor reserve; see ref 5.

(7) Roszkowski, A. P.; Yelnosky, J. Structure–Activity Relationships Among a Series of Acetylenic Carbamates Related to McN-A-343. *J. Pharmacol. Exp. Ther.* **1967**, *156*, 238–245.

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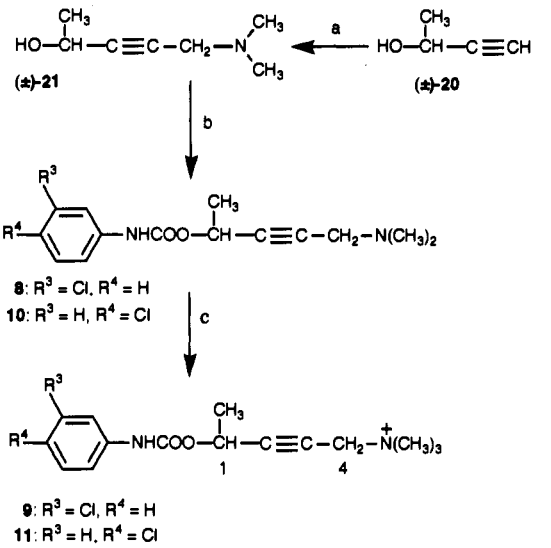
certain tertiary amines not directly related to 1 have ganglionic stimulant properties (e.g., arecaidine propargyl ester).[14] We speculated that it might be possible to generate centrally acting muscarinic agents[15] with preference for the M_1 subtype by isosteric replacement of the carbamoyl group in 1 with an ureido or an acetamido function. In the oxotremorine series such replacements provide increased potency at ileal muscarinic receptors (M_3)[16] and at central muscarinic receptors.[17] Thus, the *N*-methyl-substituted urea derivative 4 was 6-fold more potent in stimulating ileal muscarinic receptors than the carbamate analogue 5.[18] Compound 4 was also about 8-fold more

Scheme I[a]



[a] Reagents: (a) $(CH_3)_2NH$, $(HCHO)_n$, HOAc, CuCl, H_2O; (b) 3- or 4-chlorophenyl isocyanate, cat. Et_3N, THF; (c) MeI, acetone/ether.

Table I. Yields and Physical Data[a] of Intermediates and Compounds Tested

compd	method[b]	% yield	mp, °C	recryst solvent[c]	formula
(±)-8	A	95	77–78.5	A	$C_{14}H_{17}ClN_2O_2$
(*R*)-8	A	89	125–126	B	$C_{14}H_{17}ClN_2O_2 \cdot (COOH)_2$
(*S*)-8	A	92	125–126	B	$C_{14}H_{17}ClN_2O_2 \cdot (COOH)_2$
(±)-9	F	97	199–200	C	$C_{15}H_{20}BrClN_2O_2$
(*R*)-9	F	90	222–223	D	$C_{15}H_{20}BrClN_2O_2$
(*S*)-9	F	92	223–223.5	D	$C_{15}H_{20}BrClN_2O_2$
(±)-10	A	87	86–87.5	*d*	$C_{14}H_{17}ClN_2O_2$
(*R*)-10	A	93	161–161.5	B	$C_{14}H_{17}ClN_2O_2 \cdot (COOH)_2$
(*S*)-10	A	85	161–162	B	$C_{14}H_{17}ClN_2O_2 \cdot (COOH)_2$
(±)-11	F	89	142–144	C	$C_{15}H_{20}BrClN_2O_2$
(*R*)-11	F	89	192.5–194	C	$C_{15}H_{20}BrClN_2O_2$
(*S*)-11	F	87	195–197	C	$C_{15}H_{20}BrClN_2O_2$
12	D	85	74.5–75.5	A	$C_{14}H_{18}ClN_3O$
13	D	95	106–107	A	$C_{15}H_{20}ClN_3O$
14	F	98	189–190.5	C	$C_{15}H_{21}BrClN_3O$
15	F	96	159.5–162	C	$C_{15}H_{23}BrClN_3O$
16	E	72	90.5–92	E	$C_{15}H_{19}ClN_2O \cdot (COOH)_2$
17	E	94	88–90	B	$C_{15}H_{21}ClN_2O \cdot (COOH)_2$
18	F	91	130–132	C	$C_{15}H_{22}BrClN_2O$
19	F	81	*e*	C	$C_{17}H_{24}BrClN_2O$
(±)-21	*f*	65	87–89	E	$C_7H_{13}NO \cdot (COOH)_2$
(*R*)-21	*f*	88	75–78	C	$C_7H_{13}NO \cdot (COOH)_2 \cdot 0.5H_2O$
(*S*)-21	*f*	92	86.5–87.5	D	$C_7H_{13}NO \cdot (COOH)_2$
22	B	84	129.5–131	E	$C_6H_{13}F_3NO \cdot (COOH)_2$
23	B	86[g]			
24	C	77	154–156	C	$C_7H_{14}N_2 \cdot 2.5(COOH)_2$
25	C	80	144–146	F	$C_5H_{16}N_2 \cdot 1.5(COOH)_2$
27	E	87	107–109	*d*	$C_{12}H_{12}ClNO$
28	*f*	48	oil		$C_{13}H_{14}ClNO$
(*R*,*R*')-31	*f*	25	140–141.5[h]	G	$C_{20}H_{21}NO_4$
(*S*,*S*')-31	*f*	30	139.5–141.5[i]	G	$C_{20}H_{21}NO_4$

[a] $[\alpha]_D$ values of resolved compounds are presented in the Experimental Section. [b] Letters refer to methods of preparation in the Experimental Section. [c] A, *n*-hexane–ether; B, acetone–ether; C, trituration with ether; D, methanol–ether; E, acetone–methanol–ether; F, acetone–methanol–H_2O; G, acetone. [d] No recrystallization. [e] No melting point; 19 is very hygroscopic. [f] See the Experimental Section. [g] Previously prepared in 28% yield.[28] [h] Previously reported; lit.[35] mp 138.5–139 °C; lit.[36] mp 145 °C. [i] Previously reported; lit.[35] mp 138.5–139 °C.

(8) (a) Nelson, W. L.; Freeman, D. S.; Wilkinson, P. D.; Vincenzi, F. F. Stereochemical Analogs of a Muscarinic, Ganglionic Stimulant. cis- and trans-4-[*N*-(3-Chlorophenyl)carbamoyloxy]-2-butenyltrimethylammonium Iodides. *J. Med. Chem.* **1973**, *16*, 502–509. (b) Nelson, W. L.; Freeman, D. S.; Vincenzi, F. F. Stereochemical Analogs of a Muscarinic, Ganglionic Stimulant. 2. Cis and Trans Olefinic, Epoxide, and Cyclopropane Analogs Related to 4-[*N*-(3-Chlorophenyl)carbamoyloxy]-2-butynyltrimethylammonium Chloride(McN-A-343). *J. Med. Chem.* **1976**, *19*, 153–158. (c) Nelson, W. L.; Freeman, D. S.; Vincenzi, F. F. Stereochemical Analogs of a Muscarinic, Ganglionic Stimulant. 3. 2,3-Substituted Bicyclo[2.2.1]hept-5-enes and -heptanes Related to 4-[*N*-(3-Chlorophenyl)carbamoyloxy]-2-butynyltrimethylammonium Chloride (McN-A-343). *J. Med. Chem.* **1976**, *19*, 159–160.

(9) Lambrecht, G.; Moser, U.; Mutschler, E.; Walther, G.; Wess, J. Muscarinic Ganglionic Stimulants: Conformationally Restrained Analogues Related to [4-[[*N*-(3-Chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium Chloride. *J. Med. Chem.* **1986**, *29*, 1309–1311. See also: Mutschler, E.; Moser, U.; Wess, J.; Lambrecht, G. New Approaches to the Subclassification of Muscarinic Receptors. In *Recent Advances in Receptor Chemistry*; Melchiorre, C., Giannella, M., Eds.; Elsevier: Amsterdam, 1988; pp 195–217.

(10) Mellin, C.; Vargas, H. M.; Ringdahl, B. Dimethylsulfonium Analogues of the Muscarinic Agent McN-A-343: [4-[[*N*-(3- or 4-Halophenyl)carbamoyl]oxy]-2-butynyl]dimethylsulfonium Perchlorates. *J. Med. Chem.* **1989**, *32*, 1590–1593.

(11) Eltze, M.; Gmelin, G.; Wess, J.; Strohmann, C.; Tacke, R.; Mutschler, E.; Lambrecht, G. Presynaptic Muscarinic Receptors Mediating Inhibition of Neurogenic Contractions in Rabbit Vas Deferens are of the Ganglionic M_1-type. *Eur. J. Pharmacol.* **1988**, *158*, 233–242.

(12) (a) Iversen, L. L. The Cholinergic Hypothesis of Dementia. *Trends Pharmacol. Sci.* **1986**, *7* (Suppl.), 44–45. (b) Iversen, S. The Chemistry of Dementia. *Chem. Br.* **1988**, 338–342 and 364. (c) Krogsgaard-Larsen, P.; Jensen, B.; Falch, E.; Jörgensen, F. S. Heterocyclic Muscarinic Agonists: Structural and Therapeutic Aspects. *Drugs Future* **1989**, *14*, 541–561.

(13) However, an indirectly acting analogue of 1 is known; 4-[(2-bromoethyl)methylamino]-2-butynyl *N*-(3-chlorophenyl)carbamate enters into the brain and interacts covalently with muscarinic receptors after being cyclized to an aziridinium ion: Tertiary 2-Haloethylamine Derivatives of the Muscarinic Agent McN-A-343, [4-[[*N*-(3-Chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium Chloride. Ringdahl, B.; Mellin, C.; Ehlert, F. J.; Roch, M.; Rice, K. M.; Jenden, D. J. *J. Med. Chem.* **1990**, *33*, 281–286.

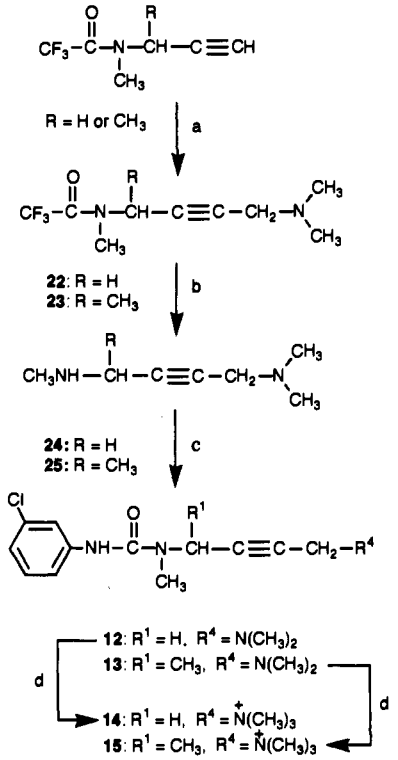
(14) Moser, U.; Lambrecht, G.; Wagner, M.; Mutschler, E. Structure–Activity Relationships of New Analogues of Arecaidine Propargyl Ester at Muscarinic M_1 and M_2 Receptor Subtypes. *Br. J. Pharmacol.* **1989**, *96*, 319–324.

(15) The pyrrolidine analogue of 1 has been reported to be a weak tremorogenic agent, about 430-fold less potent than oxotremorine. However, no data on muscarinic ganglionic properties were reported: Neumeyer, J. L.; Moyer, U. V.; Richman, J. A.; Rosenberg, F. J.; Teiger, D. G. Pharmacologically Active Compounds. I. Structural Modifications of Oxotremorine. *J. Med. Chem.* **1967**, *10*, 615–620.

(16) This nomenclature is proposed for the M_2 "glandular/smooth muscle" subtype in ref 2 and by Doods, H. N.; Mathy, M.-J.; Davidesko, D.; van Charldorp, K. J.; de Jonge, A.; van Zwieten, P. A. Selectivity of Muscarinic Antagonists in Radioligand and in Vivo Experiments for the Putative M_1, M_2 and M_3 Receptors. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 257–262.

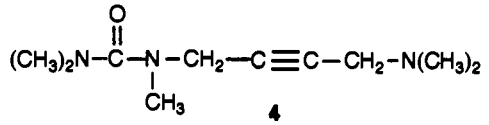
(17) It has been suggested that central muscarinic receptors mediating tremor are similar to the muscarinic receptors in the ileum that mediate contraction: Ringdahl, B. 5-Methyl-2-pyrrolidinone Analogues of Oxotremorine as Selective Muscarinic Agonists. *J. Med. Chem.* **1988**, *31*, 683–688.

Scheme II[a]



[a] Reagents: (a) $(CH_3)_2NCH_2N(CH_3)_2$, $(HCHO)_n$, CuCl, HOAc, dioxane; (b) $NaBH_4$, EtOH, or 5 M NaOH; (c) 3-chlorophenyl isocyanate, ether; (d) MeI, acetone/ether.

potent in stimulating central muscarinic receptors than **5** as seen from their tremorogenic effect in mice. A similar relationship was observed between the tertiary amide **6** and the ester **7** since **6** was more potent at ileal muscarinic receptors.[18-20]



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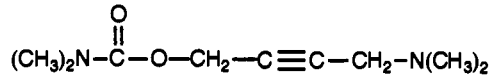

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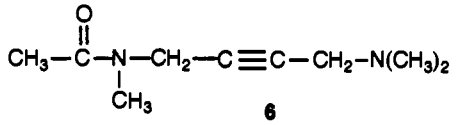

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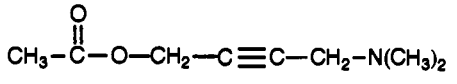

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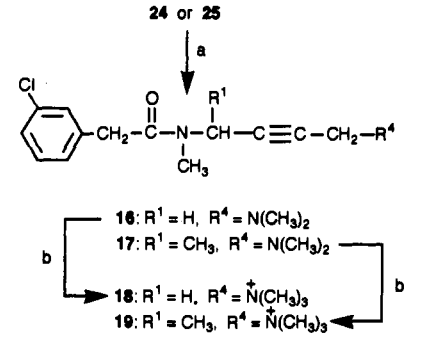
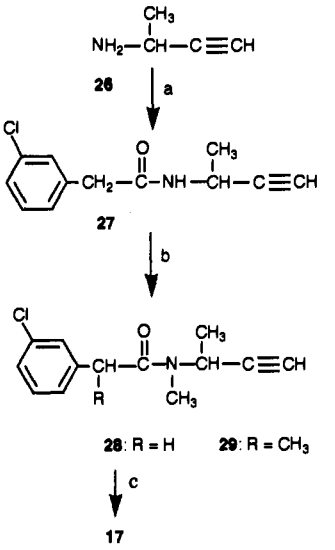
Scheme III[a]



[a] Reagents: (a) 3-chlorophenylacetyl chloride, NaOH, H_2O, dichloromethane; (b) MeI, acetone/ether.

Scheme IV[a]



[a] Reagents: (a) 3-chlorophenylacetyl chloride, NaOH, H_2O, dichloromethane; (b) NaH, MeI, THF; (c) $(CH_3)_2NH$, $(HCHO)_n$, CuCl, HOAc, dioxane.

Consequently, we prepared the *N*-methyl-substituted urea and amide derivatives **12** and **16**, respectively.[21] These derivatives would also help in assessing the importance of the carbamate "ether" oxygen for the observed ganglionic stimulant activity/selectivity profile of **1** and its carbamate analogues. The quaternary ammonium analogues **14** and **18** were prepared for comparative purposes.

In the oxotremorine series, methyl substitution in position 1 of the butynyl chain has produced compounds with interesting pharmacological profiles, ranging from partial muscarinic agonists [e.g., *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (BM-5)][23] to potent muscarinic antagonists.[24] Hence, we synthesized the

(18) Bebbington, A.; Brimblecombe, R. W.; Shakeshaft, D. The Central and Peripheral Activity of Acetylenic Amines Related to Oxotremorine. *Br. J. Pharmacol.* **1966**, *26*, 56–67.

(19) Compare also: Barlow, R. B.; Bremner, J. B.; Soh, K. S. The Effects of Replacing Ester by Amide on the Biological Properties of Compounds Related to Acetylcholine. *Br. J. Pharmacol.* **1978**, *62*, 39–50.

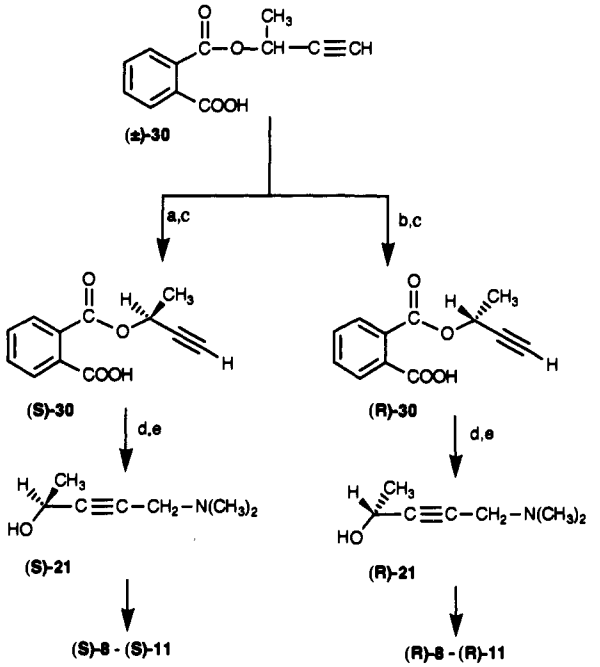
(20) It should be pointed out, however, that differences in muscarinic activity between **4** and **5** and between **6** and **7** might be due to differences in receptor subtype selectivity.

(21) Since replacement of the *N*-methyl group in the pyrrolidine analogue of **6** by a hydrogen atom abolished efficacy and caused a 15-fold reduction in ileal muscarinic affinity,[22,24] we considered it more interesting to prepare the *N*-methyl-substituted amide and urea analogues of **1** than the corresponding *N*-desmethyl analogues.

(22) Ringdahl, B.; Resul, B.; Jenden, D. J.; Dahlbom, R. Muscarinic Activity in the Isolated Guinea Pig Ileum of some Carboxamides Related to Oxotremorine. *Eur. J. Pharmacol.* **1982**, *85*, 79–83.

(23) Resul, B.; Dahlbom, R.; Ringdahl, B.; Jenden, D. J. *N*-Alkyl-*N*-(4-tert-amino-1-methyl-2-butynyl)carboxamides, a New Class of Potent Oxotremorine Antagonists. *Eur. J. Med. Chem.-Chim. Ther.* **1982**, *17*, 317–322.

Scheme V[a]



[a] Reagents: (a) (*S*)-α-methylbenzylamine, recrystallization; (b) (*R*)-α-methylbenzylamine, recrystallization; (c) 5 M HCl; (d) $(CH_3)_2NCH_2N(CH_3)_2$, $(HCHO)_n$, CuCl, HOAc, dioxane; (e) 5 M NaOH.

C1-methyl-substituted urea and acetamide derivatives **13** and **17**. Methyl groups were also introduced in **2** and its more potent 4-chlorophenyl regioisomer (giving **8** and **10**, respectively).[25] In addition, the quaternary trimethylammonium analogues **9**, **11**, **15**, and **19** were prepared and the racemic carbamate derivatives **8–11** were resolved into the enantiomers.

Compounds **8–19** were assayed for effects on arterial blood pressure in the pithed rat, for muscarinic activity on the isolated guinea pig ileum, and for nicotinic activity on the frog rectus abdominis muscle. In addition, the M_1-receptor affinity of the quaternary ammonium derivatives was studied in a binding assay.

Chemistry

The syntheses of the compounds are outlined in Schemes I–V. Physical data of the intermediates **21–25**, **27**, **28**, and **31** and the test compounds **8–19** are presented in Table I. The intermediate amino alcohol (±)-**21**[26] was prepared in 65% yield by a Mannich condensation[27] of (±)-3-butyn-2-ol [(±)-**20**] with dimethylamine (Scheme I). The carbamates (±)-**8** and (±)-**10** were obtained from (±)-**21** by reactions with 3- and 4-chlorophenyl isocyanate, respectively (method A).

The trifluoroacetamides **22** and **23**[28] were obtained from their corresponding terminal acetylenic precursor by reaction with *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane in the presence of cuprous chloride (method B, Scheme II). The intermediate acetylenic diamines **24** and **25** were prepared by $NaBH_4$ cleavage[29] of **22** and **23**, respectively (method C). Treatment of **24** and **25** with 3-chlorophenyl isocyanate (method D) or 3-chlorophenylacetyl chloride (method E) gave the urea derivatives **12** and **13** (Scheme II) and the amides **16** and **17** (Scheme III), respectively. An alternative route to **17** starting from 1-methyl-2-propynylamine (**26**)[30] and involving sequential N-acylation, N-methylation, and a Mannich condensation with dimethylamine was less useful, mainly due to competitive methylation of the benzylic carbon during the N-methylation (NaH, THF, iodomethane) of the secondary acetamide **27**. This produced the N,C1′-dimethylated byproduct **29** in addition to the desired N-methylated amide **28** (Scheme IV). The quaternary ammonium salts (**9**, **11**, **14**, **15**, **18**, and **19**; Schemes I–III) were prepared by methylation of the corresponding tertiary amines with an excess of bromomethane in a mixture of acetone–ether (method F). The corresponding methiodides of **16** and **17** were very hygroscopic and turned yellow upon storage at room temperature.

(*R*)- and (*S*)-**21**, which served as intermediates to the enantiomers of **8–11**, were prepared from resolved 3-butyn-2-ol hydrogen phthalate, (*R*)- and (*S*)-**30**, respectively (Scheme V). Racemic **30** has been resolved into the enantiomers previously via fractional crystallization of the diastereomeric brucine[31–33] or (α-methylbenzyl)ammonium[34–36] salts. By use of the latter method we obtained the enantiomeric (α-methylbenzyl)ammonium salts of **30**, i.e., (*R*,*R*′)- and (*S*,*S*′)-**31**, which upon treatment with 5 M aqueous HCl furnished (*R*)- and (*S*)-**30**, respectively. The enantiomers of 3-butyn-2-ol (**20**) have been obtained

(24) Ringdahl, B. Structural Determinants of Muscarinic Agonist Activity. In *The Muscarinic Receptors*; Brown, J. H., Ed.; Humana Press Inc.: Clifton, NJ, 1989; pp 151–218 and references cited therein.
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(26) For an alternative preparation of (±)-**21**, see: Reppe, W.; et al. Äthinylierung. *Liebigs Ann. Chem.* **1955**, *596*, 1–224.
(27) The amino alcohol (±)-**21** has been prepared previously in low yield by a Mannich reaction from (±)-**20**: Marszak, I.; Marszak-Fleury, A.; Epsztein, R.; Guermont, J. P.; Jacob, J.; Montezin, G. Recherches sur les Relations entre la Structure et le Comportement Physiologique des Composés Aminés non Saturés et Saturés. I. Synthèse et Propriétés de Certains Esters et Alcools à Fonction Ammonium Quaternaire. *Mem. Serv. Chim. Etat. (Paris)* **1951**, *36*, 411–419. Compare: *Chem. Abstr.* **1954**, *48*, 1954f.
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(36) Grattan, T. J.; Whitehurst, J. S. Chiral 2,2-Disubstituted Cyclohexanones; Annulation via Claisen Rearrangement Products. *J. Chem. Soc., Perkin Trans. 1* **1990**, 11–18.

previously from (*R*,*R*')- and (*S*,*S*')-**31**, respectively, by a method involving a tedious extraction–distillation procedure.[35] To circumvent this step, a Mannich condensation was carried out directly on the enantiomers of **30** followed by basic ester hydrolysis in situ. By this procedure we obtained (*R*)- and (*S*)-**21** in 88 and 92% yield from (*R*,*R*')- and (*S*,*S*')-**31**, respectively (Scheme V).

Since we did not isolate the enantiomers of **20**, the absolute configuration of which is known,[31,34] we established the absolute configuration of the enantiomers of **21** indirectly by performing a base-promoted ester hydrolysis of the resolved 3-butyn-2-ol hydrogen phthalate that was derived from the (*R*)-(+)-(α-methylbenzyl)ammonium salt after the recrystallization process[37] (i.e., what was supposed to be (*R*,*R*')-**31**). An ether solution of the liberated 3-butyn-2-ol was dextrorotatory[38] at the sodium D line. Since (+)-**20** is known to have (*R*) configuration, this established the absolute configuration of the enantiomers of **21**. The enantiomeric purity of the enantiomers of **21** was estimated by preparing and analyzing the (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters of (*R*)- and (*S*)-**21**, respectively (giving **32a** and **32b**). The diastereomeric excess (% de) of **32a** and **32b** was 99.0 and 99.2%, respectively (capillary GLC analysis). In addition, the (*S*)-MTPA esters were analyzed by NMR (^{1}H, ^{13}C, and ^{19}F) after extractive workup. In each ^{19}F NMR spectrum of the (*S*)-MTPA esters, only one diastereomer was observed. Use of (*R*)-(−)-α-methoxyphenylacetyl chloride in the presence of pyridine to estimate the enantiomeric excess of (*R*)- and (*S*)-**21** was not useful, since GLC analysis of the (*R*)-*O*-methylmandelic acid ester of (*R*)-**21** indicated that racemization of the *O*-methylmandelic moiety occurred during the derivatization process. This phenomenon has been reported previously in the preparation of *O*-mandelic esters from optically active α-methoxyphenylacetyl chloride.[39]

Analysis of NMR spectra of oxalate salts of the amides **16**, **17**, and **22** and the quaternary ammonium compounds **18** and **19**, in CD_3OD solutions (at 23 °C), indicated the presence of an equilibrium mixture of *Z* and *E* rotational isomers in a ratio of approximately 3:1. A ^{1}H NMR spectrum of **28** in $CDCl_3$ revealed a similar *Z*/*E* ratio. The relative proportions of the two conformations were determined by integration over signals due to the benzylic protons of each rotational isomer in compounds **16**–**19** and **28**. The *Z*/*E* ratio of **22** was determined from its ^{19}F NMR spectrum as previously described for related trifluoroacetamides.[28,40]

Pharmacological Results and Discussion

Pressor Activity in the Pithed Rat and Receptor Binding Affinity in Vitro. The ability of the novel analogues to increase mean arterial blood pressure (MAP) in the pithed rat served as an indicator of agonist potency at ganglionic muscarinic receptors, whereas M_1 receptor affinity was determined from competition binding assays using [^{3}H]pirenzepine as the label (Table II). The basal MAP of the pithed rats used in these studies was 58 ± 3 mmHg (N = 30). With the exception of the dimethylamino analogues (i.e., **8**, **10**, **12**, **13**, **16**, and **17**) the new compounds dose-dependently increased MAP (Figure 1). The reference compound **1** (i.e., McN-A-343) maximally raised MAP to 130 ± 3 mmHg after iv administration (Figure 1). Qualitatively, **1** and its analogues produced similar cardiovascular effects: (1) the rise in MAP was preceded by a small hypotensive effect (5–12 mmHg); (2) the subsequent hypertensive effect was transient, with the peak reached within 2 min, and then returning to baseline by 5 min after infusion. Being similar to the action of **1**, the rise in MAP induced by the new analogues was mediated by ganglionic muscarinic receptors since pretreatment with either iv *N*-methylatropine (1.3 μmol/kg) or pirenzepine (1.6 μmol/kg) completely abolished the effect. This dose of pirenzepine has been used previously to selectively block ganglionic M_1 receptors in the rat.[41]

Table II. Intravenous Doses of **1** and Its Analogues Required To Produce a Half Maximal Pressor Response in the Pithed Rat and the Corresponding Affinities for Ganglionic M_1-Receptors

compd	ED_{50},[a] iv (μmol/kg)	IPA[b]	K_A, μM[c]
1	0.45 ± 0.03	1.0	8.75 ± 1.08
(±)-**9**	0.09 ± 0.01	0.76	1.46 ± 0.18
(*R*)-**9**	0.18 ± 0.01	1.0	0.78 ± 0.26
(*S*)-**9**	0.12 ± 0.01	0.70	2.12 ± 0.63
(±)-**11**	0.029 ± 0.004	0.75	0.48 ± 0.04
(*R*)-**11**	0.039 ± 0.006	0.80	0.49 ± 0.22
(*S*)-**11**	0.008 ± 0.001	0.66	0.18 ± 0.05
14	0.55 ± 0.09	1.0	24.8 ± 5.0
15	0.69 ± 0.09	1.0	83.3 ± 3.4
18	0.92 ± 0.21	1.0	20.2 ± 4.5
19	1.48 ± 0.27	1.0	416 ± 10
pirenzepine			0.011 ± 0.001
4-DAMP			0.021 ± 0.002
AF-DX 116			6.81 ± 1.5

[a] Doses are the mean ± SEM. [b] Intrinsic pressor activity of the analogues (relative to McN-A-343) indicates the ability of the agents to maximally elevate arterial pressure. [c] Equilibrium dissociation constant of the agonists as determined by competition binding assays using [^{3}H]pirenzepine to label muscarinic receptors in rabbit sympathetic ganglia homogenates.

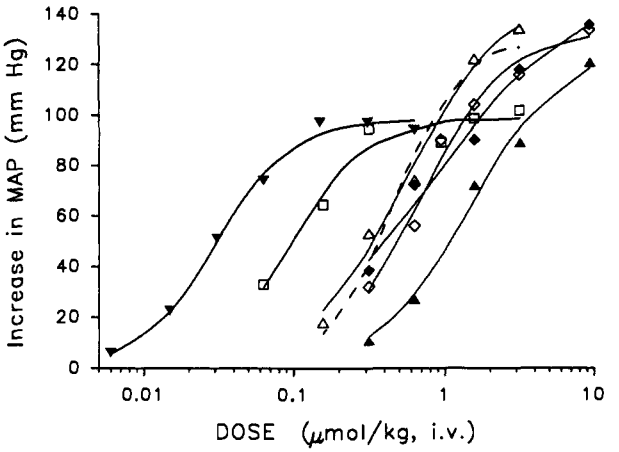

Figure 1. Dose–pressor response curves of (±)-**11** (▼), (±)-**9** (□), **1** (dashed line), **14** (△), **18** (◆), **15** (◇), and **19** (▲) obtained from arterial pressure determinations in the pithed rat. Data points are mean values. Five to eight rats were used for each compound. The standard error about the means ranged between 2 and 9 mmHg.

(37) See the Experimental Section.

(38) One group (ref 33) have reported that they obtained (−)-3-butyn-2-ol from (+)-3-butyn-2-ol hydrogen phthalate after resolution via the brucine salt. In contrast, in refs 31, 32 (resolution via brucine salt) and ref 34 (resolution via α-methylbenzylammonium salt) they obtained (+)-3-butyn-2-ol from (+)-3-butyn-2-ol hydrogen phthalate.

(39) Haller, R.; Schneider, H. J. Bestimmung der Enantiomerenreinheit Optisch Aktiver 1,1-Diphenylalkan-2-ole durch NMR-Spektroskopie. *Arch. Pharm.* **1974**, *307*, 31–38.

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(41) Vargas, H. M.; Ringdahl, B. Antimuscarinic Potency and Functional Selectivity of Oxotremorine Analogs at Muscarinic Receptor Subtypes in the Rat. *Life Sci.* **1990**, *47*, 2065–2073.

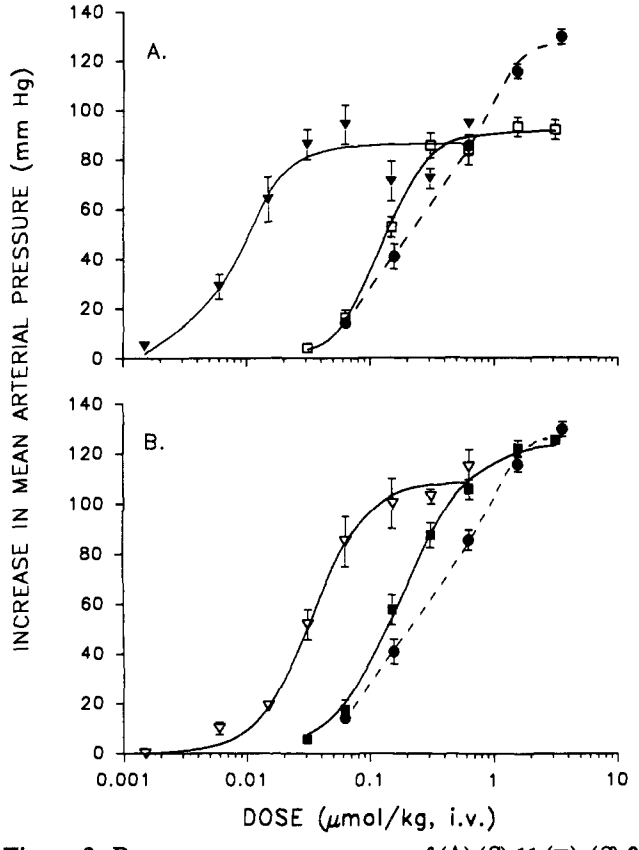


Figure 2. Dose–pressor response curves of (A) (*S*)-11 (▼), (*S*)-9 (□) and (B) (*R*)-11 (▽), (*R*)-9 (■) obtained from arterial pressure determinations in the pithed rat. The reference compound 1 (dashed line) is included for comparative purposes. Data points are mean values from five to eight rats.

Analysis of the dose–response curves for the carbamate analogues (±)-9 and (±)-11 indicated that these compounds produced submaximal pressor responses when compared to 1 (Figure 1). Despite the reduced efficacy of these compounds, the ED_{50} values (dose needed to produce a half-maximal pressor response) indicated that (±)-9 and (±)-11 were 5- and 16-fold more potent, respectively, than 1 (Table II). Of the two racemates, the 4-chlorophenyl analogue (±)-11 was 3-fold more potent than the 3-chlorophenyl analogue (±)-9. In addition to the increased pressor potency, (±)-9 and (±)-11 also exhibited higher affinity for the ganglionic M_1 muscarinic receptor (Table II). In fact, (±)-11 exhibited an 18-fold higher receptor affinity, an increase of the same order of magnitude as the 15-fold lower shift in ED_{50} relative to 1.

Since the racemates of 9 and 11 were the most potent ganglionic stimulants, these were resolved into the enantiomers. Compound (*S*)-11 was the most potent enantiomer of the resolved compounds with an ED_{50} value of 0.008 ± 0.001 μmol/kg. The rank order of pressor potency in this series was (*S*)-11 > (*R*)-11 > (*S*)-9 > (*R*)-9 (Figure 2). It was observed that the (*S*) configuration conferred the greater potency (Table II); the $ED_{50\text{-}R}/ED_{50\text{-}S}$ ratios for 9 and 11 were 1.5 and 4.9, respectively. However, the affinity was differentially affected by the (*R*) and (*S*) forms; the $K_{A\text{-}R}/K_{A\text{-}S}$ ratios for 9 and 11 were 0.4 and 2.7, respectively. Despite the increased pressor potency of the resolved analogues, only (*R*)-9 showed an ability equivalent to 1 to maximally elevate arterial pressure (Figure 2B); (*S*)-9, (*R*)-11, and (*S*)-11 were partial agonists (Table II).

Dose–response comparisons of the urea (14 and 15) and acetamide (18 and 19) analogues (Figure 1) showed that these agents remained full agonists in the pithed rat (Figure 1). The urea derivative 14 was equipotent with 1, which implied that the carbamoyl group could be isosterically replaced by an *N*-methyl-substituted ureido functionality without loss of intrinsic activity. However, this substitution lowered binding affinity 3-fold. In contrast, introduction of a C1-methyl group in 14, to yield 15, reduced in vivo potency and in vitro affinity 1.5- and 10-fold relative to 1. The acetamide derivative 18 and its C1-methyl analogue 19 were 2- to 3-fold less potent than 1 in vivo, and their in vitro affinity was 2.3- and 48-fold lower, respectively.

It should be noted that for quaternary compounds containing a C1-methyl group (i.e., 9, 11, 15, and 19), differential effects on potency, efficacy, and affinity were observed, depending on whether the analogues were carbamate, urea, or acetamide derivatives. For example, C1-methyl-substituted carbamates (9 and 11) were consistently more potent in the pithed rat and binding assays than their corresponding urea and acetamide analogues. In contrast, 15 and 19 resembled full agonists in vivo (i.e. higher efficacy), while the carbamates, with the exception of (*R*)-9, consistently behaved as partial agonists.

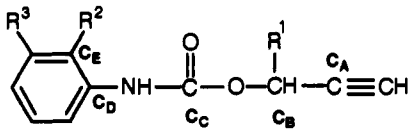
Guinea Pig Ileum. Over the concentration range of 1 × 10^{-6} to 1 × 10^{-4} M, the quaternary ammonium salts produced a concentration-dependent contraction of the ileum. At the highest concentrations (±)-9, (±)-11, 14, 15, 18, 19, and 1 elicited contractions which averaged only 58, 50, 31, 30, 48, 28, and 35%, respectively, of the maximal effect produced with carbachol. The tertiary amino derivatives were completely devoid of intrinsic activity in the ileum, but significantly antagonized the carbachol-induced contraction at bath concentrations greater than 100 μM. The nature of this antagonism was not investigated further.

Frog Rectus Abdominis. The potency of the new analogues at nicotinic receptors was investigated in the frog rectus preparation and the results are expressed as the equipotent molar ratios (EPMR) relative to carbachol. In this assay, carbachol had an EC_{50} value of 4.9 ± 0.6 μM (N = 8). This agrees with earlier estimates of its potency.[42] The newly synthesized quaternary ammonium analogues of 1, but not the corresponding tertiary amines, displayed an ability to contract the rectus abdominis. A comparison of the EPMR value of 1 (5.3 ± 0.6; N = 6) with those of analogues (±)-9, (±)-11, 14, 15, 18, and 19 (116.6 ± 27.0, 103.2 ± 5.0, 51.0 ± 2.4, 467.3 ± 39.0, 141.4 ± 24.6, and 475 ± 23, respectively; N = 5–8 per compound) indicated that the new analogues were several-fold less potent than 1.

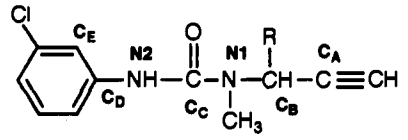
Structure–Activity Relationships. Ganglionic stimulant activity was retained in the quaternary urea and acetamide derivatives of 1 studied herein. Apparently, the carbamoyl group can be isosterically replaced in 1 without a large loss of the ability to stimulate ganglionic muscarinic receptors. This indicates that the carbonyl oxygen may be of more importance for ganglionic muscarinic stimulation than the carbamate "ether" oxygen in 1. It may be noted that it has been suggested that the distance between the "ether" oxygen and the charged nitrogen of 1 should be of importance for M_1 agonist activity.[8a,9]

The absence of any ganglionic M_1 agonist activity of the new tertiary amino analogues indicates that ganglionic muscarinic activity among structurally related analogues to 1 is confined to quaternary ammonium salts [or sulfonium salts (vide supra)] regardless of the structure of the carbonyl group containing moiety, that is, it may be part

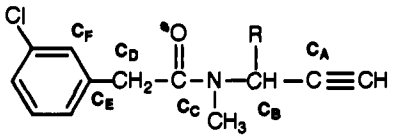
(42) Ringdahl, B. A Comparison of the Stimulant Activities of Oxotremorine Analogues on the Frog Rectus Abdominis and the Guinea Pig Ileum. *Eur. J. Pharmacol.* 1984, *99*, 177–184.



33: $R^1 = H$; $R^2 = H$; $R^3 = Cl$
34: $R^1 = CH_3$; $R^2 = H$; $R^3 = Cl$
35: $R^1 = H$; $R^2 = Cl$; $R^3 = H$



36: R = H
37: R = CH_3



38: R = H
28: R = CH_3

Figure 3. Definition of dihedral angles: **33–35**, $\tau_1 = \tau(C_E\text{–}C_D\text{–}N\text{–}C_C)$, $\tau_2 = \tau(C_A\text{–}C_B\text{–}O\text{–}C_C)$; **36** and **37**, $\tau_1 = \tau(C_E\text{–}C_D\text{–}N2\text{–}C_C)$, $\tau_2 = \tau(C_A\text{–}C_B\text{–}N1\text{–}C_C)$; **38** and **28**, $\tau_1 = \tau(C_F\text{–}C_E\text{–}C_D\text{–}C_C)$, $\tau_2 = \tau(C_A\text{–}C_B\text{–}N\text{–}C_C)$.

of an urea, amide, or a carbamate function. This contrasts to results in previous studies of ileal muscarinic stimulant activity (M_3) in the oxotremorine series where certain tertiary amino analogues of *N*-methyl-substituted ureas and amides (e.g., compounds **4** and **6** and their pyrrolidine analogues) showed similar potencies as their quaternary trimethylammonium salts.[18] However, it has been observed previously in a series of tertiary heterocyclic amines (e.g., arecolinol and isoarecolinol derivatives) related to **1**, which were inactive as M_1 agonists in the pithed rat, that structure–activity relationships found at M_1 receptors may be different from those found at M_3[16] receptors.[9]

The slightly lower (3- and 2-fold, respectively) M_1 receptor binding affinity observed for the urea and acetamide derivatives **14** and **18** when compared to that of **1** might be related to the extra steric bulk produced by the introduction of a *N*-methyl group in a position corresponding to the "ether" oxygen of **1**. Alternatively, these observations might be related to differences in electronic properties. Introduction of a methyl group at the C1 position in **1** and its 4-chlorophenyl analogue (giving **9** and **11**) led to increased affinity for the ganglionic M_1 receptor (6- and 18-fold, respectively). In contrast, there was a substantial loss (20-fold) in M_1 receptor affinity when a methyl group was introduced at the C1 position in the acetamide derivative **18** (giving **19**). The same structural modification in the urea derivative **14** (giving **15**) resulted only in a 3-fold decrease in M_1 receptor affinity.

In an attempt to rationalize similarities and differences in intrinsic activity and M_1 receptor binding affinity among the analogues of **1**, we investigated the conformational preferences of model compounds lacking the (trimethylammonio)methyl or (dimethylamino)methyl moieties.[43]

Table III. Geometrical Parameters for Low-Energy (MMX) Conformations of *Z* Conformers of Model Compounds **28**, **33–38**[a,b]

model compound	conformational characteristics[c] τ_2,[d] deg	ΔE_s[e]
33 (1)	176 ± 4	0.0
	−176 ± 4	0.0
	77 ± 2	0.3
	−77 ± 1	0.3
34[f] (9, 11)	151	0.0
	84 ± 2	0.4
	−50	1.2
35[g]	177 ± 3	0.0
	−172	0.0
	76 ± 2	0.3
	−76 ± 2	0.3
36 (14)	144 ± 3	0.4
	−144 ± 3	0.4
	91 ± 3	0.0
	−91 ± 3	0.0
37[f] (15)	137 ± 3	0.0
	−53 ± 2	2.1
38 (18)	138 ± 2	0.5
	−139 ± 2	0.5
	89 ± 4	0.0
	−88 ± 4	0.0
28[f] (19)	136 ± 1	0.0
	−55 ± 3	1.9

[a] The dihedral angles are defined in Figure 3. [b] The derivative corresponding to the model compound is given within parentheses. [c] These τ_1 values (deg) were adopted by conformers of the various model compounds: **33**, 165 ± 7, −166 ± 7, 21 ± 4, and −17 ± 8; **34**, 167 ± 9, −170 ± 10, 22 ± 4, and −21 ± 5; **35**, 150 ± 2 and −151 ± 2; **36**, 152 ± 2, −152 ± 2, 29 ± 2, and −29 ± 2; **37**, 152 ± 2, −152 ± 2, 29 ± 3, and −29 ± 3; **38**, 89 ± 18 and −87 ± 16; **28**, 87 ± 18 and −91 ± 19. [d] These τ_2 values were observed at all τ_1 values shown in footnote c. [e] Relative steric energy in kilocalories/mole. [f] Signs are for the (*R*) enantiomer. [g] Compare ref 51.

Thus, low-energy conformers of **28** and **33–38** (Figure 3) were identified[45] by molecular mechanics calculations using the MMX-89.0 force field[47] (see the Experimental Section). The dihedral angles of the model compounds are defined in Figure 3, and the results are shown in Table III. The similarities in potency (ED_{50} values) and binding affinities of carbamate **1**, urea **14**, and acetamide **18** might be related to their common ability to adopt τ_2 values around 85° and −85°. Alternatively, an extensive variation in preferred

(43) It is known that, e.g. in oxotremorine, the conformational preferences of the lactam and the pyrrolidine ring are affected very little by each other.[44] Therefore, we assumed that the geometries and relative steric energies obtained for conformations of the model compounds would be similar to the corresponding tertiary amines/quaternary ammonium derivatives.

(44) Kier, L. B. Molecular Orbital Conformation of Oxotremorine and a Comparison With the Muscarinic Pattern. *J. Pharm. Sci.* **1970**, *59*, 112–114.

(45) Only *Z* conformers[46] of the model compounds were considered but also *E* conformers may be energetically accessible as indicated by NMR spectroscopy (**18**, **19**, and **28**) and preliminary MMX calculations. However, the carbamates **33** and **34** seem to prefer the *Z* conformation (preliminary MMX calculations). This is supported by a literature report on methyl phenylcarbamate; see: Remko, M.; Frecer, V.; Cizmárik, J. Conformational Analysis of Methyl Phenylcarbamate and its Methoxy Derivatives by MO Calculations. *Arch. Pharm.* **1983**, *316*, 9–15.

(46) It has been suggested that the biological activity in a series of acyclic amides related to oxotremorine (e.g. BM-5) should reside in the *Z* conformer; see ref 23.

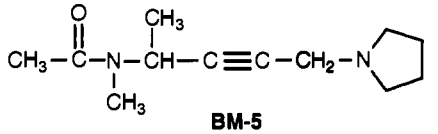
(47) MMX is a molecular mechanics program that is an enhanced version of Allinger's MMP2 program. The MMX program is part of the molecular modeling package PCMODEL (Serena Software, P.O. Box 3076, Bloomington, IN 47402-3076).

τ_2 values might be tolerated for potent ganglionic stimulant activity.[48] The value of the dihedral angle τ_1, which defines the spatial orientation of the phenyl ring in relation to the carbamate, urea, or acetamide moiety, is similar in low-energy conformations of model compounds **33**, **34**, **36**, and **37**. In these compounds the carbamate group and the urea moiety are twisted by about 25° out of the plane of the phenyl ring. This is in reasonable agreement with literature data obtained from the crystal structure of phenylurea[49] and from MO calculations performed on methyl phenylcarbamate.[45] The dihedral angle τ_1 adopts values of 90 ± 15° and −90 ± 15° in the acetamido model compounds **28** and **38**. This is due to a difference in hybridization between the benzylic carbon (sp^3 carbon) in **28**, **38** and nitrogens N and N2 (nitrogen close to sp^2) in **33–37**, respectively.[50] The observation that **38** prefers to adopt other τ_1 values than those of **33** and **36** is not reflected in the efficacy or binding affinity of the corresponding test compounds, since **18** and **14** are almost equipotent in increasing the MAP and exhibit similar binding affinity.[51]

Larger differences in preferred τ_2 values were observed for low-energy conformations of the C1-methyl-substituted compounds. The carbamate model compound **34** (Figure 3) adopted low-energy conformations ($\Delta E_s \leq 1.5$ kcal/mol) with τ_2 values around 150°, 80°, and −50°. In contrast, only τ_2 values around 135° and −50° were represented in low-energy conformations of the urea and acetamido model compounds **37** and **28**, respectively. The absence of τ_2 values around 80° in low-energy conformations of **37** and **28** is probably due to unfavorable interactions between the C1- and the *N*-methyl group in such conformations.

The C1-methyl-substituted carbamate analogue (±)-**9** showed 8- and 16-fold higher ganglionic stimulant activity (ED_{50} values) and 57- and 284-fold higher affinity than the corresponding urea and acetamide analogues **15** and **19**, respectively. Thus, a C1-methyl group might increase efficacy and M_1 receptor affinity in derivatives in which τ_2 values around 80° are energetically accessible. It is also possible that the *N*-methyl group in **15** and **19** prevents an efficient receptor interaction by sterical means in conformations in which τ_2 adopts values different from those preferred in the analogues lacking the C1-methyl group (**14** and **18**). The 5-fold difference in receptor binding affinity between the C1-methyl-substituted urea and acetamide analogues **15** and **19**, respectively, might be related to differences in the spatial position of the phenyl ring. *Z* conformations of **15** and **19** readily adopt similar τ_2 values (vide supra), but a less well tolerated deviation in the spatial position of the phenyl ring (i.e. τ_1) of **19** might result when τ_2 adopt values different from those observed in low-energy conformations of the analogues lacking the C1-methyl substituent.

The pronounced difference (20-fold) in receptor binding affinity between **18** and **19** is probably not related to their abilities to adopt a *Z* or *E* conformation around the amide bond since the observed *Z*/*E* ratio (^{1}H NMR in CD_3OD solutions; vide supra) was similar in **18** and **19**.



BM-5

The low stereoselectivity exhibited by **9** and **11** in terms of M_1 agonist potency (ED_{50} values) and the absence of any correlation between the configuration at the C1 position and ganglionic M_1 receptor affinity are in contrast to the structure–activity relationships observed at ileal M_3 receptors for a series of resolved analogues of oxotremorine. For example the (*R*) enantiomers of BM-5 and its dimethylamino and trimethylammonium analogues are 20- to 30-fold more potent in inducing contractions of the ileum, and in addition, they have higher affinity (8- to 27-fold) for ileal M_3 receptors than the (*S*) enantiomers.[24,52]

Experimental Section

Chemistry. General Comments. Melting points were determined in open glass capillaries on a Thomas-Hoover apparatus. IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a JEOL FX 90Q spectrometer at 89.55, 22.5, and 84.3 MHz, respectively, unless otherwise noted. ^{1}H and ^{13}C NMR spectra were referenced to internal tetramethylsilane. Dioxane (3.6 and 68.0 ppm, respectively) was used as internal reference for the ^{1}H and ^{13}C NMR spectra of **24** and **25**. ^{19}F NMR spectra were referenced to internal $CFCl_3$. Assignments of ^{13}C NMR resonances in **12**, **24**, **25**, and **27** are based on off-resonance spectra. All spectra were in accordance with the assigned structures. Capillary GLC analyses for determination of diastereomeric excess (% de) were performed on a Carlo Erba 6000 Vega instrument equipped with a FID-40 flame ionization detector and a Milton Roy CI-10B integrator; GLC column: DB-5 fused silica (30 m, i.d. = 0.32 mm); carrier helium (60 kPa). Thin-layer chromatography was carried out on aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm) or on aluminum oxide 60 F_{254} neutral (type E) (E. Merck). Column chromatography was performed on silica using Kieselgel 60 (230–400 mesh, E. Merck) or on alumina using aluminum oxide 90 (E. Merck). Chromatographic spots were visualized by UV and/or aqueous $KMnO_4$ spraying. Reactions were carried out under N_2. The elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden, or Analytische Laboratorien, Gummersbach, Germany, and were within ±0.4% of the calculated values.

(±)-4-(Dimethylamino)-1-methyl-2-butyn-1-ol [(±)-21].[53] A solution of glacial acetic acid (4.07 g, 67.8 mmol) in water (10

(48) Compare MMX calculations performed on model compounds to oxotremorine and analogues of oxotremorine: Nilsson, B. M.; Ringdahl, B.; Hacksell, U. β-Lactam Analogues of Oxotremorine. 3- and 4-Methyl Substituted 2-Azetidinones. *J. Med. Chem.* **1990**, *33*, 580–584.

(49) Kashino, S.; Haisa, M. The Crystal and Molecular Structure of Phenylurea. *Acta Crystallogr.* **1977**, *B33*, 855–860.

(50) A different relative spatial orientation of phenyl groups has previously been noted for the *N*,*N*-diphenylcarbamoyl analogue of 4-DAMP. The study was based on X-ray data and attempted to rationalize the low activity and poor selectivity of the former compound; see: Barlow, R. B.; Johnson, O. The X-ray Crystal Structure of 4-diphenylcarbamyl-*N*-methyl-piperidine methobromide (the Carbamate Analogue of 4-DAMP methiodide). *Br. J. Pharmacol.* **1989**, *98*, 425–428.

(51) However, **35** (a model for the 2-chlorophenyl analogue of **1**) showed a limited rotational flexibility about the N–C_D bond when compared to **33**. Only τ_1 values of about 150° and −150° were observed (Table III). This might explain the reported[7] 10-fold lowered ganglionic stimulant activity when the chlorine atom was shifted from the 3- to the 2-position in the phenyl ring of **1**. Alternatively, the 2-chloro substituent per se might prevent an efficient receptor interaction by sterical means.

(52) Dahlbom, R.; Jenden, D. J.; Resul, B.; Ringdahl, B. Stereochemical Requirements for Central and Peripheral Muscarinic and Antimuscarinic Activity of some Acetylenic Compounds Related to Oxotremorine. *Br. J. Pharmacol.* **1982**, *76*, 299–304.

(53) Difficulties were encountered in the purification step when the Mannich reaction was conducted under the conditions described for **22** (method B), since unidentified side products (TLC) were formed in addition to **21**. This may be related to the pH of the solution since it has been reported to strongly influence the yield of Mannich reactions of for example propargyl alcohol; see: Salvador, R. L.; Simon, D. A Study of the Mannich Reaction with Propargyl Alcohol. *Can. J. Chem.* **1966**, *44*, 2570–2575. Compare also: Harrow, T. A.; Harison, C. E.; Williamson, W. R. N. The Preparation of Derivatives of Dibenz[b,d]oxepin. *J. Chem. Soc. C* **1971**, 2098–2104.

mL) was added dropwise to a stirred ice-cooled solution of dimethylamine in water (7.52 g of a 40% solution, 67 mmol). The resulting mixture had a pH of about 8 (pH paper). Paraformaldehyde (1.47 g, 49 mmol) and cuprous chloride (325 mg, 3.3 mmol) were successively added, and after 15 min a solution of (±)-3-butyn-2-ol [(±)-**20**] (2.29 g, 32.6 mmol) in water (15 mL) was added to the mixture. The flask was sealed, and the ice bath was replaced with an oil bath which was heated to 70 °C for 2 h. The dark green solution was acidified with 2 M aqueous hydrochloric acid (60 mL), and the mixture was washed with ether (3 × 130 mL). The aqueous layer was alkalinized by addition of solid K_2CO_3 to pH 10 with ice-cooling. The resulting solution was extracted with ether (4 × 200 mL). Drying (K_2CO_3) of the combined organic layers, filtration, and concentration in vacuo (30 °C water bath)[54] gave an oil which was purified by column chromatography on silica using chloroform–methanol (5%) as eluent. This gave 2.7 g (65%) of pure (±)-**21** as an oil. Part of the base was converted into the oxalate salt and recrystallized: TLC R_f (free base on silica) = 0.24 [chloroform–methanol (10%)]; IR (free base, neat liquid) 3700–3000 cm^{-1}; ^1H NMR (oxalate, CD_3OD) δ 4.55 (qt, J = 6.6 and 1.7 Hz, C1-H), 4.07 (d, J = 1.7 Hz, C4-H's), 2.93 (s, NCH_3's), 1.42 (d, J = 6.6 Hz, C1-CH_3); ^{13}C NMR (oxalate, CD_3OD) δ 166.40 (oxalate C=O's), 94.04 (C2), 72.64 (C3), 58.31 (C1), 47.78 (C4), 42.59 (NCH_3's), 24.31 (C1-CH_3).

Method A. (*S*)-4-(Dimethylamino)-1-methyl-2-butynyl *N*-(3-Chlorophenyl)carbamate [(*S*)-8]. A solution of 3-chlorophenyl isocyanate (726 mg, 4.73 mmol) in THF (10 mL) was added to an ice-cooled stirred solution of (*S*)-**21** (vide infra) (99.2% ee, 523 mg, 4.11 mmol) in THF (20 mL). Triethylamine (180 mg, 1.8 mmol) was added to the reaction mixture. The ice bath was removed after 1 h, and the stirring was continued at room temperature. After 7.5 h, more 3-chlorophenyl isocyanate (190 mg, 1.24 mmol) and triethylamine (73 mg, 0.7 mmol) were added. After being stirred for 19 h, the mixture was concentrated in vacuo. Aqueous hydrochloric acid (1 M, 10 mL) was added to the semisolid residue, and the mixture was extracted with ether (4 × 100 mL). The aqueous layer was alkalinized by addition of solid $NaHCO_3$ to pH 8 and then extracted with ether (5 × 150 mL). Drying (K_2CO_3) of the combined organic layers, filtration, and concentration in vacuo afforded crude (*S*)-**8** as a viscous oil. This material was chromatographed on a silica column using chloroform–methanol (5%) as eluent to give 1.06 g (92%) of pure (*S*)-**8**. Part of the colorless oily base was converted into the oxalate salt and recrystallized: TLC R_f (free base on silica) = 0.31 [chloroform–methanol (10%)]; IR (free base, neat liquid) 1735, 1715 (shoulder) cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 7.53–7.49 (m, 1 Ar H), 7.27–6.96 (m, NH and 3 Ar H's), 5.52 (qt, J = 6.8 and 1.7 Hz, C1-H), 3.29 (d, J = 1.8 Hz, C4-H's), 2.30 (s, NCH_3's), 1.55 (d, J = 6.6 Hz, C1-CH_3); ^{13}C NMR (free base, $CDCl_3$) δ 152.48 (C=O), 139.35, 134.56, 129.87, 123.23, 118.84, 116.74 (Ar C's), 83.79 (C2), 80.12 (C3), 61.56 (C1), 47.84 (C4), 44.01 (NCH_3's), 21.87 (C1-CH_3).

Method B. *N*-Methyl-*N*-[4-(dimethylamino)-2-butynyl]trifluoroacetamide (22). Glacial acetic acid (1.45 g, 24.2 mmol) was added to a stirred ice-cooled mixture of *N*,*N*,*N*¹,*N*¹-tetramethyldiaminomethane (1.31 g, 12.8 mmol), paraformaldehyde (0.95 g, 31.5 mmol), and cuprous chloride (0.40 g, 4.0 mmol) in dioxane (20 mL). A solution of *N*-methyl-*N*-(2-propynyl)trifluoroacetamide[28] (4.0 g, 24.2 mmol) in dioxane (5 mL) was added to the mixture. The ice bath was removed, and the resulting mixture was stirred at room temperature for 46 h. The dioxane was evaporated under reduced pressure, and the oily residue was taken up in 1.5 M aqueous hydrochloric acid (350 mL) and extracted with ether (3 × 100 mL). The aqueous layer was alkalinized to pH 8 by addition of solid $NaHCO_3$ and extracted with dichloromethane (4 × 200 mL). The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo. This afforded 4.54 g (84%) of the title compound as an oil which was sufficiently pure (^1H NMR) to use as such in the next step (method C). An analytical sample was prepared as the oxalate salt: TLC R_f (free base on alumina) = 0.66 (ether); IR (free base, neat liquid) 1710 (shoulder), 1700 cm^{-1}; ^1H NMR (oxalate, CD_3OD) δ 4.40 (t, J = 1.9 Hz, C1-H's), 4.10 (t, J = 1.8 Hz, C4-H's), 3.24 (q, $J_{H,F}$ = 1.5 Hz, *Z*-$CONCH_3$), 3.11 (br s, *E*-$CONCH_3$), 2.92 (s, NCH_3's); ^{13}C NMR (oxalate; 67.5 MHz, CD_3OD) δ 166.54 (oxalate C=O's), 158.00 (q, $J_{C,F}$ = 35.4 Hz, CF_3C=O), 117.76 (q, $J_{C,F}$ = 287 Hz, CF_3), 85.26 (*Z*-C2), 85.05 (*E*-C2), 74.99 (*E*-C3), 74.41 (*Z*-C3), 47.74 (*Z*-C4), 47.67 (*E*-C4), 42.77 (NCH_3's), 40.06 (q, $J_{C,F}$ = 3.7 Hz, *E*-C1), 39.25 (br s, *Z*-C1), 35.21 (q, $J_{C,F}$ = 3.7 Hz, *Z*-$CONCH_3$), 34.88 (br s, *E*-$CONCH_3$); ^{19}F NMR (oxalate, CD_3OD) δ (*Z*/*E* ratio = 76:24) −68.81 (m, *E*-CF_3), −69.74 (q, $J_{F,H}$ = 1.5 Hz, *Z*-CF_3).

Method C. *N*-Methyl-4-(dimethylamino)-1-methyl-2-butynylamine (25). Sodium borohydride (2.20 g, 58 mmol) was added in portions to an ice-cooled stirred solution of **23** (3.91 g, 16.6 mmol) in dry ethanol (100 mL). The ice bath was removed, and the stirring was continued at room temperature for 1 h. The reaction mixture was chilled on an ice bath, and 1 M aqueous hydrochloric acid was added dropwise to destroy excess of sodium borohydride. Most of the ethanol was evaporated under reduced pressure, and the residue was taken up in 2.5 M aqueous hydrochloric acid (175 mL) and extracted with ether (3 × 100 mL). The aqueous layer was alkalinized by addition of solid K_2CO_3 to pH 10 and extracted with ether (4 × 175 mL). The combined organic layers were dried over K_2CO_3, filtered, and concentrated in vacuo. The oily residue was chromatographed on an alumina column with ether–methanol (5%) as eluent. This gave 1.87 g (80%) of pure **25**. An analytical sample was prepared as the oxalate salt: TLC R_f (free base on alumina) = 0.57 [ether–methanol (10%)]; ^1H NMR (oxalate, D_2O) δ 4.17 (qt, J = 7.0 and 1.7 Hz, C1-H), 3.99 (d, J = 1.7 Hz, C4-H's), 2.81 (s, CH_2NCH_3's), 2.64 (s, CH_3NH), 1.49 (d, J = 6.8 Hz, C1-CH_3); ^{13}C NMR (oxalate, D_2O) δ 169.36 (oxalate C=O's), 85.45 (C2), 78.32 (C3), 48.17 (C4), 48.02 (C1), 43.70 (CH_2NCH_3's), 31.77 (CH_3NH), 18.83 (C1-CH_3).

***N*-Methyl-4-(dimethylamino)-2-butynylamine (24).**[55] Compound **24** was prepared from **22** by the above procedure: TLC R_f (free base on alumina) = 0.39 [ether–methanol (10%)]. Alternatively, and in 70% yield, **24** was prepared by exposure of **22**·oxalate (338 mg, 1.08 mmol) to 5 M NaOH (20 mL) for 3 h at room temperature followed by standard workup:[40] ^1H NMR data (free base in $CDCl_3$) were in accordance with those reported;[55] ^{13}C NMR (oxalate, D_2O) δ 165.72 (oxalate C=O's), 81.65 and 78.56 (acetylenic carbons), 48.24 (C4), 43.70 (CH_2NCH_3's), 39.22 (C1), 33.52 (CH_3NH).

Method D. 1-(3-Chlorophenyl)-3-[4-(dimethylamino)-1-methyl-2-butynyl]urea (13). A solution of 3-chlorophenyl isocyanate (0.76 g, 4.94 mmol) in ether (15 mL) was added dropwise to a stirred solution of **25** (0.63 g, 4.49 mmol) in ether (10 mL) at room temperature. After 3.5 h, 3 M aqueous hydrochloric acid (300 mL) was added and the resulting mixture was washed with ether (3 × 100 mL). The aqueous layer was alkalinized by addition of solid K_2CO_3 to pH 10 and then extracted with ether (4 × 130 mL). Drying (K_2CO_3) of the combined organic layers, filtration, and concentration in vacuo afforded 1.26 g (95%) of pure **13** as a solid. Part of this material was recrystallized: TLC R_f = 0.66 [ether–methanol (3%)]; IR (KBr disk) 3300, 3270 (w), 1645, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.52–7.47 (m, 1 ArH), 7.35–7.16 (m, 2 ArH's), 7.10–6.92 (m, 1 ArH), 6.59 (br, NH), 5.33 (qt, J = 7.0 and 2.0 Hz, C1-H), 3.26 (d, J = 2.0 Hz, C4-H's), 3.01 (s, $CONCH_3$), 2.29 (s, CH_2NCH_3's), 1.38 (d, J = 6.8 Hz, C1-CH_3); ^{13}C NMR ($CDCl_3$) δ 154.57 (C=O), 140.31, 134.22, 129.62, 122.95, 120.20, 118.16 (Ar C's), 84.03 (C2), 78.94 (C3), 47.96 (C4), 44.16 (CH_2NCH_3's), 42.96 (C1), 29.56 ($CONCH_3$), 20.23 (C1-CH_3).

Method E. *N*-Methyl-*N*-[4-(dimethylamino)-1-methyl-2-butynyl]-2-(3-chlorophenyl)acetamide (17). A solution of 2-(3-chlorophenyl)acetyl chloride (1.34 g, 7.09 mmol) [prepared from 3-chlorophenylacetic acid and an excess of thionyl chloride, by stirring at room temperature for 15 h, followed by heating at 70 °C for 1 h. The excess of thionyl chloride was coevaporated with added dichloromethane] in dichloromethane (30 mL) was added to a mixture of **25** (0.71 g, 5.06 mmol) in dichloromethane (5 mL), NaOH (243 mg, 6.07 mmol), and water (35 mL). The mixture was stirred for 2.5 h at room temperature. Aqueous hydrochloric acid (3 M, 300 mL) was added, and the mixture was

(54) The boiling point reported for (±)-**21** is 95 °C at 2 mmHg (ref 26).

(55) For a previous preparation of **24**, see: Corbel, B.; Paugam, J.-P.; Sturtz, G. Aminomethylation of *N*-propargylphosphoramides. Synthesis of Unsymmetrical Acetylenic Diamines. *Can. J. Chem.* **1980**, *58*, 2183–2188.

washed with ether (3 × 100 mL). The aqueous layer was alkalinized by addition of solid K_2CO_3 to pH 10 and extracted with ether (4 × 130 mL). The combined ether layers were dried (K_2CO_3), filtered, and concentrated in vacuo to afford 1.40 g (94%) of pure **17** as a pale yellow oil. Part of the base was converted into the oxalate salt and recrystallized: TLC R_f (free base on alumina) = 0.73 [ether–methanol (3%)]; IR (free base, neat liquid) 1645 cm^{-1}; ^1H NMR (oxalate; 270 MHz, CD_3OD) δ 7.34–7.16 (m, Ar H's), 5.59 (qt, J = 7.1 and 1.8 Hz, *Z*-C1-H), 5.10 (m, *E*-C1-H), 4.06 (d, J = 2.0 Hz, *Z*-C4-H's), 4.03 (d, J = 2.0 Hz, *E*-C4-H's), 3.86 (s, *E*-benzylic CH'), 3.83 (s, *E*-benzylic CH''), 3.78 (s, *Z*-benzylic CH_2), 3.07 (s, *Z*-$CONCH_3$), 2.94 (s, *E*-$CONCH_3$), 2.89 (s, *Z*-NCH_3's), 2.86 (s, *E*-NCH_3's), 1.40 (d, partially obscured, *E*-C1-CH_3), 1.37 (d, J = 7.0 Hz, *Z*-C1-CH_3); ^{13}C NMR (oxalate; 67.5 MHz, CD_3OD) δ 172.49, (*Z*-C=O), 172.20 (*E*-C=O), 166.52 (oxalate C=O's), 138.56 (*E*), 138.50 (*Z*), 135.49 (*E*), 135.39 (*Z*), 131.31 (*E*), 131.16 (*Z*), 130.24 (*Z*), 130.08 (*E*), 128.71 (*Z*), 128.55 (*E*), 128.19 (*E*), 128.08 (*Z*) (Ar C's), 90.12 (*Z*-C2), 89.32 (*E*-C2), 74.37 (*E*-C3), 73.68 (*Z*-C3), 47.83 (*Z*-C4), 47.71 (*E*-C4), 47.07 (*E*-C1), 43.07 (*Z*-C1), 42.77 (NCH_3's), 41.05 (*Z*-benzylic C), 40.83 (*E*-benzylic C), 31.34 (*Z*-$CONCH_3$), 28.95 (*E*-$CONCH_3$), 20.40 (*E*-C1-CH_3), 19.11 (*Z*-C1-CH_3).

Method F. (±)-4-(Dimethylamino)-1-methyl-2-butynyl *N*-(3-Chlorophenyl)carbamate Methobromide [(±)-9]. An excess of bromomethane was added to an ice-cooled solution of (±)-**8** (410 mg, 1.43 mmol) in acetone (10 mL) and ether (3 mL). The flask was sealed, the ice bath was removed after 30 min, and the stirring was continued for 5 h at room temperature. The mixture was concentrated in vacuo, and the solid residue was triturated twice with ether to give 520 mg (97%) of pure (±)-**9**: IR (KBr disk) 1735, 1725 cm^{-1}; ^1H NMR (CD_3OD) δ 7.62–7.57 (m, 1 ArH), 7.37–7.24 (m, 2 ArH's), 7.16–6.96 (m, 1 ArH), 5.49 (qt, J = 6.8 and 1.5 Hz, C1-H), 4.46 (d, J = 1.5 Hz, C4-H's), 3.26 (s, NCH_3's), 1.61 (d, J = 6.8 Hz, C1-CH_3); ^{13}C NMR (CD_3OD) δ 154.29 (C=O), 141.42, 135.49, 131.16, 124.00, 119.40, 117.82 (ArC's), 92.43 (C2), 74.00 (C3), 62.04 (C1), 57.20 (C4), 53.33 (NCH_3's), 20.97 (C1-CH_3).

***N*-Methyl-*N*-(1-methyl-2-propynyl)-2-(3-chlorophenyl)-acetamide (28).** Sodium hydride (0.22 g of an 80% dispersion in mineral oil, 7.3 mmol) was added to a stirred solution of **27** (1.4 g, 6.3 mmol) in THF (25 mL) at room temperature. A solution of iodomethane (1.14 g, 8.0 mmol) in THF (10 mL) was added dropwise to the reaction mixture after 1 h, and the stirring was continued for 4 h at room temperature. The mixture was concentrated in vacuo and the residue was partitioned between water (75 mL) and ether (100 mL). The aqueous layer was extracted with additional portions of ether (2 × 100 mL). The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo. The oily residue was chromatographed on an alumina column using ether as eluent. First eluted was 0.3 g (19%) of a byproduct identified (^1H NMR) as *N*,2-dimethyl-*N*-(1-methyl-2-propynyl)-2-(3-chlorophenyl)acetamide (**29**) (tentatively assigned as a 1:1 mixture of diastereomers). Further elution gave 0.71 g (48%) of pure **28**.[56]

28: TLC R_f (alumina) = 0.16 [ether–*n*-hexane (1:3)]; IR (neat liquid) 3300, 3240, 2120, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37–6.97 (m, Ar H's), 5.63 (qd, J = 7.0 and 2.4 Hz, *Z*-C1-H), 4.72 (m, *E*-C1-H), 3.73 (br, *E*-benzylic CH_2), 3.67 (s, *Z*-benzylic CH_2), 2.99 (s, *Z*-$CONCH_3$), 2.95 (s, *E*-$CONCH_3$), 2.36 (d, J = 2.2 Hz, *E*-C3-H), 2.29 (d, J = 2.4 Hz, *Z*-C3-H), 1.32 (d, J = 7.0 Hz, C1-CH_3); ^{13}C NMR ($CDCl_3$) δ 169.43 (C=O), 136.60, 134.33, 129.84, 129.03, 127.06 (br) (ArC's), 82.46 (*Z*-C2), 81.35 (*E*-C2), 72.70 (*E*-C3), 71.99 (*Z*-C3), 46.26 and 45.24 (*E*-C1 and *E*-benzylic CH_2),[57] 41.23 and 40.58 (*Z*-C1 and *Z*-benzylic CH_2),[57] 30.33 (*Z*-$CONCH_3$), 28.04 (*E*-$CONCH_3$), 20.38 (*E*-C1-CH_3), 19.24 (*Z*-C1-CH_3).

29: TLC R_f (alumina) = 0.32 [ether–*n*-hexane (1:3)]; IR (neat liquid) 3300, 3240, 2120, 1640 cm^{-1}; ^1H NMR[58] ($CDCl_3$) δ 7.36–7.06 (m, ArH's), 5.67 (qd, J = 6.9 and 2.2 Hz, C1-H), 3.86 (q, J = 6.8 Hz, PhC*H*CH_3), 2.92 (br, NCH_3),[59] 2.88 (s, NCH_3),[60] 2.87 (s, NCH_3),[61] 2.44 (d, J = 2.4 Hz, C3-H),[59] 2.34 (d, J = 2.4 Hz, C3-H),[60] 2.24 (d, J = 2.4 Hz, C3-H),[61] 1.42 (d, J = 7.0 Hz, PhCHC*H*$_3$), 1.33 (d, partially obscured, J = 7.0 Hz, C1-CH_3),[60] 1.21 (d, J = 7.0 Hz, C1-CH_3),[61] 0.94 (d, J = 7.0 Hz, C1-CH_3).[59]

(*R*)-3-Butyn-2-ol Hydrogen Phthalate (*R*)-(α-Methylbenzyl)ammonium Salt [(*R*,*R*')-31]. Compound (*R*,*R*')-**31** was prepared as previously described by eight recrystallizations of the diastereomeric (*R*)-(α-methylbenzyl)ammonium salts [from (*R*)-α-methylbenzylamine (Merck-Schuchardt)] of (±)-3-butyn-2-ol hydrogen phthalate [(±)-**30**] from acetone:[35] ^1H NMR (CD_3OD) δ 7.75–7.27 (m, 10 ArH's), 5.58 (qd, J = 6.7 and 2.0 Hz, C1-H), 4.41 (q, J = 6.8 Hz, benzylic CH), 2.91 (d, J = 2.2 Hz, C3-H), 1.59 (d, J = 7.0 Hz, ArCHC*H*$_3$),[58] 1.57 (d, J = 6.8 Hz, C1-CH_3);[58] ^{13}C NMR (CD_3OD) δ 176.50 and 168.35 (C=O's), 143.18, 140.00, 132.55, 130.11, 129.84, 129.62, 128.91, 128.45, 127.64 (ArC's), 83.20 (C2), 74.58 (C3), 62.01 (C1), 52.13 (benzylic C), 21.53 (C1-CH_3),[62] 20.94 (ArCH*C*H$_3$).[62]

(*S*)-3-Butyn-2-ol Hydrogen Phthalate (*S*)-(α-Methylbenzyl)ammonium Salt [(*S*,*S*')-31]. Compound (*S*,*S*')-**31** was prepared by the above procedure using (*S*)-α-methylbenzylamine (Merck-Schuchardt).

(*R*)-4-(Dimethylamino)-1-methyl-2-butyn-1-ol [(*R*)-21]. Aqueous hydrochloric acid (5 M, 160 mL) was added to a solution of (*R*,*R*')-**31** (8.82 g, 26 mmol) in water (160 mL). The resulting solution was extracted with ether (4 × 175 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to give a viscous oil of (*R*)-3-butyn-2-ol hydrogen phthalate [(*R*)-**30**] (TLC R_f on silica = 0.22 [chloroform–methanol (10%)]. This oil was dissolved in dioxane (250 mL), and paraformaldehyde (827 mg, 28 mmol), cuprous chloride (205 mg, 2.1 mmol), glacial acetic acid (1.66 g, 28 mmol), and *N*,*N*,*N*^{1},*N*^{1}-tetramethyldiaminomethane (4.25 g, 41.6 mmol) were successively added to the solution. The reaction flask was sealed and the mixture was heated at 55 °C (oil-bath temperature) for 4 h. Most of the dioxane was evaporated, and water (90 mL) and 5 M NaOH (90 mL) were added to the residue. The resulting mixture was stirred at room temperature for 2.5 h. Ether (300 mL) was added to the alkaline solution, and the layers were separated. Additional extractions of the aqueous layer with ether (4 × 300 mL) followed by drying (K_2CO_3), filtration, and concentration in vacuo (30 °C water bath)[54] of the combined ether layers gave crude (*R*)-**21** as an oil. This material was chromatographed on a silica column using chloroform–methanol (5%) as eluent to give 2.91 g [88% from (*R*,*R*')-**31**)] of NMR-pure (*R*)-**21** as a pale yellow oil. Part of the base was converted into the oxalate salt and recrystallized.

(*S*)-4-(Dimethylamino)-1-methyl-2-butyn-1-ol [(*S*)-21]. Compound (*S*)-**21** was prepared from (*S*,*S*')-**31** (9.01 g, 26.6 mmol) by the above procedure. The yield of (*S*)-**21** after column chromatography was 3.38 g [92% from (*S*,*S*')-**31**)].

Estimation of the Enantiomeric Purity of the Amino Alcohols (*R*)- and (*S*)-21. (*R*)- and (*S*)-**21** were converted into the (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters (**32a** and **32b**, respectively) by a modified literature procedure.[63] Triethylamine (29 mg, 0.29 mmol) was added, by use of a syringe, to solutions of (*R*)- and (*S*)-**21** (32 mg, 0.25 mmol), respectively, dissolved in dichloromethane (1 mL), each placed in a vial with a screw-cap. A solution of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [prepared from (*S*)-(−)-MTPA (Fluka, Chiraselect) (545 mg, 2.33 mmol) and thionyl chloride (38 mL), by stirring at 70 °C (oil-bath temperature) for

(56) In one experiment, treatment of a solution of **27** in THF with 1.03 equiv of sodium hydride and iodomethane, respectively, at −10 °C, gave a mixture of **27**, **28**, and **29** according to TLC analysis of the reaction mixture.
(57) No attempt was made to assign these signals.
(58) The assignment of the benzylic α-methyl group and the C1-methyl group is based on proton decoupling experiments.
(59) Minor rotamer of one diastereomer.
(60) Major rotamer of one diastereomer.
(61) Major rotamer of other diastereomer.
(62) A ^{13}C–^1H correlation NMR experiment (recorded on a JEOL JNM-EX 270 spectrometer) supported this assignment.
(63) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J. Org. Chem.* **1969**, *34*, 2543–2549. (b) Dale, J. A.; Mosher, H. S. Nuclear Magnetic Resonance Enantiomer Reagents. Configurational Correlations via Nuclear Magnetic Resonance Chemical Shifts of Diastereomeric Mandelate, *O*-Methylmandelate, and α-Methoxy-α-trifluoromethylphenylacetate (MTPA) Esters. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

18 h and then at 80 °C for 11.5 h followed by evaporation of the excess of thionyl chloride. The oily residue was dissolved in dichloromethane (4 mL)] in dichloromethane (2 mL) was added to each vial. The mixtures were stirred at room temperature for 21.5 h and then directly analyzed by capillary GLC; temperature: 155 °C (oven)/300 °C (injector). The % de obtained was 99.0 and 99.2%, respectively, for the (*S*,*R*) and the (*S*,*S*) diastereomers **32a** and **32b**, respectively, with GLC retention times of 23.9 and 25.0 min, respectively. Prior to NMR[64] (^{1}H, ^{13}C, and ^{19}F) spectral analysis of each (*S*)-MTPA ester derivative, the mixture was processed as follows: 1 M aqueous hydrochloric acid (40 mL) was added and the mixture was extracted with ether (4 × 30 mL). The aqueous layer was alkalinized by addition of solid $NaHCO_3$ and extracted with ether (4 × 40 mL). The combined ether layers were dried twice over Na_2SO_4, filtered, and concentrated in vacuo. The oily residue was reanalyzed on GLC under the conditions given above. This gave values of % de in agreement with those obtained from the crude reaction mixtures. TLC R_f on silica = 0.65 [chloroform–methanol (10%)] for **32a** and **32b**, respectively.

(***S***)-[(***R***)-4-(**Dimethylamino**)-1-**methyl**-2-**butynyl**] α-**methoxy**-α-(**trifluoromethyl**)**phenylacetate** (**32a**): ^{1}H NMR ($CDCl_3$) δ 7.62–7.31 (m, 5 ArH's), 5.68 (qt, J = 6.8 and 1.7 Hz, C1-H), 3.56 (q, $J_{H,F}$ = 1.3 Hz, OCH_3), 3.26 (d, J = 1.5 Hz, C4-H's), 2.24 (s, NCH_3's), 1.59 (d, J = 6.8 Hz, C1-CH_3); ^{19}F NMR ($CDCl_3$) δ −72.34; ^{13}C NMR ($CDCl_3$) δ 165.48 (C=O), 132.09 (Ar C), 129.71 (q, $J_{C,F}$ = 288 Hz, CF_3), 129.62, 128.38, 127.46 (ArC's), 84.65 (q, $J_{C,F}$ = 27.8 Hz, benzylic C), 82.27 and 81.29 (acetylenic C's), 62.88 (C1), 55.44 (app q, $J_{C,F}$ = 1.4 Hz, OCH_3), 47.84 (C4), 43.98 (NCH_3's), 21.37 (C1-CH_3).

(***S***)-[(***S***)-4-(**Dimethylamino**)-1-**methyl**-2-**butynyl**] α-**methoxy**-α-(**trifluoromethyl**)**phenylacetate** (**32b**): ^{1}H NMR ($CDCl_3$) δ 7.62–7.31 (m, 5 ArH's), 5.69 (qt, J = 6.8 and 1.7 Hz, C1-H), 3.59 (q, $J_{H,F}$ = 1.3 Hz, OCH_3), 3.29 (d, J = 1.7 Hz, C4-H's), 2.27 (N-CH_3's), 1.53 (d, J = 6.8 Hz, C1-CH_3); ^{19}F NMR ($CDCl_3$) δ −72.11; ^{13}C NMR ($CDCl_3$) δ 165.57 (C=O), 132.37 (ArC), 129.68 (q, $J_{C,F}$ = 288 Hz, CF_3), 129.62, 128.38, 127.36 (ArC's), 84.50 (q, $J_{C,F}$ = 27.8 Hz, benzylic C), 82.43 and 81.41 (acetylenic C's), 62.69 (C1), 55.41 (app q, $J_{C,F}$ = 1.4 Hz, OCH_3), 47.84 (C4), 43.98 (NCH_3's), 21.22 (C1-CH_3).

Establishment of the Absolute Configuration of the Enantiomers of 21. This experiment was carried out starting from a batch of (*R*,*R*')-**31** (100 mg, 0.29 mmol) obtained after eight recrystallizations. The material was partioned between 5 M aqueous HCl (3 mL) and ether (2.5 mL). The layers were separated, and the aqueous layer was extracted with ether (2 × 2.5 mL). TLC analysis of the organic layer showed one spot (corresponding to that of 3-butyn-2-ol hydrogen phthalate; vide supra). Most of the ether was evaporated in a stream of nitrogen, and the residue was treated with 5 M NaOH (1.5 mL) and stirred in a vial. The ester hydrolysis was complete after 2.5 h [TLC analysis [SiO_2, chloroform–methanol (10%)] showed one spot]. The layers were separated, and the aqueous layer was extracted with an additional portion of ether (1.5 mL). The organic layers were dried (K_2CO_3), and the optical rotation was then measured on the decanted ether solution. This gave an α value of +0.680.

Optical Rotations. The resolved compounds presented in Table I have the following optical rotations at 22 °C. $[\alpha]_D$: (*R*)-**8**·oxalate, +101.7° (*c* 1.0, CH_3OH); (*S*)-**8**·oxalate, −100.6° (*c* 1.0, CH_3OH); (*R*)-**9**, +98.5° (c 1.0, C_2H_5OH); (*S*)-**9**, −99.1° (*c* 0.98, C_2H_5OH); (*R*)-**10**·oxalate, +98.1° (*c* 0.97, CH_3OH); (*S*)-**10**·oxalate, −100.6° (*c* 0.97, CH_3OH); (*R*)-**11**, +94.7° (*c* 1.10, C_2H_5OH); (*S*)-**11**, −95.1° (*c* 1.01, C_2H_5OH); (*R*)-**21**·oxalate, +14.6° (*c* 1.14, C_2H_5OH); (*S*)-**21**·oxalate, −16.0° (*c* 1.09, C_2H_5OH); (*R*,*R*')-**31**, +5.2° (*c* 1.12, C_2H_5OH); (*S*,*S*')-**31**, −5.5° (*c* 0.88, C_2H_5OH).

Molecular Mechanics Calculations. Energetically accessible ($\Delta E_s \leq 3$ kcal/mol) conformations were generated of model compounds lacking the propargyl substituent. Nonrestricted energy minimizations were performed on starting geometries having τ_1 values (Figure 3) in 30° increments from 0 to 360°. Propargyl substituents were added (to give **33**, **35**, **36**, and **38**; Figure 3) to the energy-minimized conformations. The conformational preferences about the dihedral angle τ_2, which define the conformation of the propargyl groups, were explored by 30° incremental changes in τ_2 (0–360°). Starting geometries of model compounds **28**, **34**, and **37** (Figure 3) were constructed from low-energy conformations of **38**, **33**, and **36**, respectively, and energy minimized similarly after incremental changes in τ_2. A variation of τ_1 from 0 to 360° in 30° increments was carried out on identified low-energy conformations of **28**, **33–38** to identify possible discrepancies in τ_1 preferences as compared to the compounds lacking the propargyl substituent. Relative steric energies (≤3 kcal/mol) and geometries of identified low-energy conformations of **28**, **33–38** are presented in Table III.

Pharmacology. Blood Pressure Recordings in the Pithed Rat. Male Sprague-Dawley rats (250–300 g body weight) were used. Animals were anesthetized with sodium amytal (125 mg/kg, ip) and then intubated with a tracheal tube. Rats were pithed via insertion of a steel rod through the ocular orbit and subsequently respirated with room air (1 mL/100 g body weight 75 strokes/min). PE 50 catheters filled with heparinized saline (50 units/mL) were inserted into both the left common carotid and the left femoral vein for determination of arterial pressure and drug infusion, respectively. Blood pressure was measured with a Deseret disposable transducer and recorded with a Soltec polygraph. Body temperature was monitored with a rectal thermistor probe and maintained at 37 °C with a heating pad. After surgery, each animal was allowed to stabilize for 30 min before experiments commenced. All drugs were dissolved in 0.9% saline and injected in a volume of 0.1 mL/100 g body weight. Ganglionic nicotinic receptors were blocked by pretreatment with pentolinium tartrate (18.5 μmol/kg, iv) 20 min prior to agonist infusion. In some experiments, *N*-methylatropine bromide (1.3 μmol/kg, iv) or pirenzepine dihydrochloride (1.6 μmol/kg, iv) was administered 10 min before agonists were tested. Dose–response curves for two or three compounds were constructed using the same rat.

Guinea Pig Ileum. A standard guinea pig ileum preparation was set up in Tyrode solution (pH 7.4) at 37 °C as described previously.[65] 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.

Frog Rectus Abdominis. A standard frog rectus abdominis preparation was set up at 23 °C in aerated Clark-Ringer solution (pH 7.4) as described previously.[42] Contractions were recorded as described for the ileum. The preparation was exposed to each drug concentration for 5 min. Equipotent molar ratios relative to carbachol were determined in three-point assays.[66]

Pirenzepine Binding to Rabbit Sympathetic Ganglia. Approximately 100 isolated rabbit sympathetic ganglia (Pel-Freeze, Rogers, AR) were placed in 20 volumes of 50 mM sodium–potassium phosphate buffer (pH 7.4) and then minced with a pair of scissors and homogenized with a Polytron (setting 6; 3–15 s bursts). The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was subsequently centrifuged at 45000*g* for 30 min at 4 °C. The pellet was resuspended in fresh buffer to a final tissue concentration of 80 mg/mL.

Competition binding assays were performed using the filtration method described by Yamamura and Snyder.[67] Generally, the ganglia homogenate (0.1 mL; 80–100 μg of protein) was incubated with the novel ligands (0.1 mL) and 3.0 nM [^{3}H]pirenzepine (PZ; 72.9 Ci/mmol); New England Nuclear, Wilmington, DE) in a total volume of 1 mL of 50 mM phosphate buffer. Nonspecific binding was measured in the presence of 10 μM atropine. IC_{50} values

(64) The reported chemical shift differences for the diastereomers **32a** and **32b** were verified by recording ^{1}H, ^{13}C, and ^{19}F NMR spectra on mixtures of **32a** and **32b**.

(65) Ringdahl, B. Determination of Dissociation Constants and Relative Efficacies of Oxotremorine Analogs at Muscarinic Receptors in the Guinea-Pig Ileum by Pharmacological Procedures. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 199–206.

(66) Edinburgh Pharmacology Staff. *Pharmacological Experiments on Isolated Preparations*; Livingstone: Edinburgh, 1968; pp 13 and 28.

(67) Yamamura, H. I.; Snyder, S. H. Muscarinic Cholinergic Binding in Rat Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1725–1729.

(concentration of ligand which reduced maximal specific [^{3}H]PZ binding by 50%) were obtained by fitting the data to a one site binding model by nonlinear regression. The IC_{50} values were corrected for receptor occupancy by [^{3}H]PZ as described by Cheng and Prusoff[68] to give K_A values (concentration of ligand that causes half-maximal receptor occupancy in the absence of [^{3}H]PZ). For comparative purposes, unlabeled pirenzepine (M_1), AF-DX 116 (M_2) and 4-DAMP (M_3) were used to determine the rank order of potency of the subtype selective antagonists. All assays were performed in polyethylene tubes and equilibrated at 30 °C for 60 min. Bound radioactivity was trapped on Whatman GF/B glass fiber filters that were soaked in 50 mM sodium–potassium phosphate buffer containing 0.05% polyethylenimine (pH 7.4).

In separate studies, saturation experiments were conducted by incubating the tissue homogenate with [^{3}H]PZ (1–100 nM) in a total volume of 1 mL. Nonspecific binding was determined in the presence of 10 μM atropine. Protein was determined by the method of Lowry et al.[69] These studies showed that [^{3}H]PZ had a K_D = 15.5 ± 0.7 nM and a B_{max} = 580 ± 72 fmol/mg protein (N = 4). In addition, 11-point competition curves for pirenzepine (M_1), AF-DX 116 (M_2; 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one) and 4-DAMP (M_3; 4-(diphenylacetoxy)-*N*-methylpiperidine methiodide) were constructed to confirm the muscarinic subtype associated with the rabbit ganglia. The affinities of these compounds are shown in Table II.

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Registry No. (±)-**8**, 141063-74-3; (*S*)-**8**, 141194-08-3; (*S*)-**8** oxalate, 141194-09-4; (*R*)-**8** oxalate, 141194-07-2; (±)-**9**, 141063-75-4; (*S*)-**9**, 141194-11-8; (*R*)-**9**, 141194-10-7; (±)-**10**, 141063-76-5; (*R*)-**10** oxalate, 141194-13-0; (*S*)-**10** oxalate, 141194-15-2; (±)-**11**, 141063-77-6; (*R*)-**11**, 141194-16-3; (*S*)-**11**, 141194-17-4; **12**, 141063-78-7; (±)-**13**, 141063-79-8; **14**, 141063-80-1; (±)-**15**, 141063-81-2; **16**, 141063-83-4; (±)-**17**, 141063-84-5; (±)-**17** oxalate, 141063-85-6; **18**, 141063-86-7; (±)-**19**, 141063-87-8; (±)-**20**, 65337-13-5; (*R*)-**21**, 141194-18-5; (*S*)-**21**, 141194-19-6; (±)-**21** oxalate, 141063-89-0; (*R*)-**21** oxalate, 141269-21-8; (*S*)-**21** oxalate, 141269-22-9; **22**, 141063-90-3; **22** oxalate, 141063-91-4; (±)-**23**, 141063-92-5; **24**, 75858-50-3; **24** oxalate, 141063-93-6; (±)-**25**, 141063-94-7; (±)-**25** oxalate, 141063-95-8; (±)-**36**, 61489-97-2; (±)-**27**, 141063-96-9; (*R*)-**28**, 141063-97-0; (*S*)-**28**, 141064-08-6; (±)-**29** (isomer 1), 141063-98-1; (±)-**29** (isomer 2), 141064-04-2; (*R*)-**30**, 3113-93-7; (*S*)-**30**, 100837-07-8; (*R,R'*)-**31**, 114351-86-9; (*S,S'*)-**31**, 100837-08-9; **32a**, 141063-99-2; **32b**, 141064-05-3; **33**, 3004-45-3; (*R*)-**34**, 141064-00-8; (*S*)-**34**, 141064-06-4; **35**, 25217-01-0; **36**, 69921-30-8; (*R*)-**37**, 141064-01-9; (*S*)-**37**, 141064-07-5; **38**, 141064-02-0; (*S*)-(−)-MTPA, 17257-71-5; (*R*)-MTPA chloride, 39637-99-5; $NHMe_2$, 124-40-3; $CF_3CON(Me)CH_2C{\equiv}CH$, 111903-30-1; $Me_2NCH_2NMe_2$, 51-80-9; (±)-$CF_3CON(Me)CH(Me)C{\equiv}CH$, 141064-03-1; ClC_6H_4-*m*-CH_2COCl, 41904-39-6; 3-chlorophenyl isocyanate, 2909-38-8; 3-chlorophenylacetic acid, 1878-65-5.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectral data for compounds (±)-**10** (base), (±)-**11**, **12**, **14–16**, **18**, **19**, **27**, and ^{1}H NMR spectral data for (*R*)-**8**-oxalate, (*S*)-**10**-oxalate, (±)-**21** (base), and **24**-oxalate (2 pages). Ordering information is given on any current masthead page.

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Synthesis and Anticonvulsant Activity of Enaminones

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A new series of novel enaminones has been synthesized from cyclic β-dicarbonyl precursors which were condensed with morpholine, pyrrolidine, phenethylamine, hydrazines, substituted benzyl amines, and substituted anilines. These compounds were subsequently evaluated for anticonvulsant activity in a variety of anticonvulsant models by the National Institute of Neurological and Communicative Disorders and Stroke and in our laboratory. Several of these compounds exhibited potent anticonvulsant activity with a remarkable lack of neurotoxicity. The most active analog, methyl 4-[(*p*-chlorophenyl)amino]-6-methyl-2-oxo-cyclohex-3-en-1-oate (**27**), was protective in the maximal electroshock (MES) seizure test in the rat with an oral ED_{50} of 5.8 mg/kg with no toxicity noted at doses up to 380 mg/kg, thus providing a protective index (TD_{50}/ED_{50}) of >65.5. A similar protective index for **27** was noted upon intraperitoneal (ip) administration in mice. The anticonvulsant effect of **27** occurred within 15 min of administration and the compound remained active beyond 4 h. Compound **27** was also active in the rat corneal kindled model. The application of Free–Wilson analysis to structure–activity correlation in this series is discussed.

Enamines have been shown by previous workers[1–6] to be highly unstable in aqueous solution. Enaminones, enamines of β-dicarbonyl compounds, however are quite stable and have been employed as prodrugs with variable results.[7–12] In addition, two articles have reported the potential use of enaminones for biological purposes.[13,14] Scheone and co-workers[13] indicated that several enaminones prepared were evaluated for hypoglycemic effectiveness; however, they reported poor activity. Kase'

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