45 (CH_2), 57 [$CH(CH_3)_2$], 64 (CHNH), 138 (C3-thienyl), 143 (C4-thienyl), 152 ppm (C2-thienyl); MS m/z 284.15 (M+, 100).

***N*-Isopropyl-2-(2(*RS*)-aminopropyl)-5-iodothiophene (14). Method A.** Boronic acid **13** (0.454 g, 2 mmol) was dissolved in 20 mL of H_2O and the resulting solution was stirred at ambient temperature and shielded from light. After the addition of 2.0 mL of 1.0 M sodium iodide (2 mmol) followed by chloramine-T (0.455, 2 mmol), the reaction mixture was stirred for an additional 30 min. The mixture was extracted three times with 10 mL of ether. The combined ether extracts were dried over sodium sulfate and the solvent was removed in vacuo to yield 170 mg of **14** (30% yield) as a colorless liquid. Pure *N*-isopropyl-2-(2(*RS*)-aminopropyl)-5-iodothiophene (**14**, 478 mg, 1.54 mmol) was obtained by treatment with 1 mL of 4 N HCl in dioxane as described for **5**. The dioxane solution was concentrated in vacuo and the residue crystallized from ethanol–ethyl ether to give 454 mg of hydrochloride salt (85%) as a white solid: mp 165–166 °C, ^{13}C NMR ($CDCl_3$) 20 (CH_3), 23 (CH_3), 38 (CH_2), 45 [$CH(CH_3)_2$], 50 (CHNH), 71 (C5-thienyl), 127 (C3-thienyl), 136 (C4-thienyl), 148 ppm (C2-thienyl); ^1H NMR ($CDCl_3$) 1.0 (m, 9 H, CH_3), 1.8 (s, 1 H, NH), 2.7 (t, J = 8 Hz, 2 H, CH_2), 3.0 (m, 2 H, CH), 6.5 (d, J = 4 Hz, 1 H, aromatic), 7.1 ppm (d, J = 4 Hz, 1 H, aromatic); MS m/z 310.81 (M+, 100).

Method B. Stannane **10** (173 mg, 0.5 mmol) in 5 mL of CH_2Cl_2 was reacted with iodine (127 mg, 0.5 mmol) as described for **8**. The dried CH_2Cl_2 was removed in vacuo to give 152 mg (98% yield) of **14** as a colorless oil.

2-(2(*RS*)-Aminopropyl)-5-[^{125}I]iodothiophene ([^{125}I]-8). A 15-mL round-bottomed flask containing *N*-chlorosuccinimide (1.6 mg, 0.012 mmol) was fitted with a septum inlet and equipped with a magnetic stirring bar and a gas outlet connected to a charcoal trap. Five milliliters of CH_2Cl_2 was introduced via a syringe and the solution cooled to 0–5 °C (ice bath) and stirred. Sodium [^{125}I]iodide (1.5 mg, 0.01 mmol, 5.5 mCi) was introduced via a syringe. The mixture initially turned pink from the formation of ICl. Stannylthiophene **7b** (3.04 mg, 0.01 mmol) in 1 mL of $CHCl_2$ was introduced via a syringe. The mixture initially turned pink from the formation of ICl. The resulting reaction mixture was removed from the ice bath and stirred for 30 min. The solution rapidly became lighter until a colorless solution resulted. The mixture was poured into 20 mL of 5% $NaHSO_3$, basified to pH = 10 with 1 N NaOH, and extracted several times with Et_2O. The combined Et_2O extracts were extracted two times with 15 mL of 1 N HCl. The combined 1 N HCl extracts were basified to pH = 10 with 6 N NaOH and the resulting solution extracted several times with Et_2O. The combined Et_2O extracts were washed with H_2O and dried over anhydrous Na_2SO_4. The Et_2O was evaporated by a stream of argon to give ^{125}I-labeled **8** (1.61 mCi, 29%). TLC (Al_2O_3-GF) ($CHCl_3$–CH_3OH, 98:2) showed one radioactive component (R_f = 0.5) which cochromatographed with the authentic standard.

***N*-Isopropyl-2-(2(*RS*)-aminopropyl)-5-[^{131}I]iodothiophene ([^{131}I]-14). Method A.** A 15-mL round-bottomed flask containing boronic acid **13** (5.6 mg, 0.02 mmol) dissolved in 1 mL of 1 N HCl was fitted with a septum inlet and equipped with a magnetic stirring bar, and a gas outlet was connected to a charcoal trap. The resulting solution was stirred at ambient temperature and shielded from light. Twenty microliters of 1.0 M sodium [^{131}I]-iodide (0.8 mCi, 0.02 mmol) was introduced via a syringe followed by 20 μL of 1.0 M chloramine-T (0.02 mmol), the reaction mixture was stirred for an additional 40 min. The mixture was basified to pH = 10 with 0.3 mL of 5 M KOH extracted with 5 mL of ether. A second 0.5 mL of 5 M KOH was added to the reaction mixture and the resulting solution extracted with 5 mL of ether. The combined ether extracts were dried over sodium sulfate, and the solvent was removed by a stream of nitrogen to yield 0.69 mCi (86% yield). The [^{131}I]-**14** showed a single radioactive component (98%) on thin-layer radiochromatographic analysis (SiO_2-GF, CH_2Cl_2–CH_3OH, 8.5:1) (R_f = 0.32) that cochromatographed with an authentic unlabeled standard (R_f = 0.32).

Method B. Stannane **10** (3.45 mg, 0.01 mmol) in 1 mL of CH_2Cl_2 was reacted with sodium [^{131}I]iodide (0.715 mCi, 0.01 mmol) and *N*-chlorosuccinimide (1.8 mg, 0.01 mmol) dissolved in a mixture of CH_2Cl_2–H_2O (4:1, 5 mL) as described for [^{125}I]-**8**. The dried ether extracts were removed by a stream of nitrogen to yield 0.67 mCi (74% yield). TLC (SiO_2-GF, CH_2Cl_2–CH_3OH, 8.5:1) (R_f = 0.32) of [^{131}I]-**14** showed one radioactive component (R_f = 0.32) which cochromatographed with the authentic standard.

Acknowledgment. Research sponsored by the National Institutes of Health (Grant GM-39081), Grant DDEFG-86 ER 60434 from the Environmental Research Department of Energy, and the Office of Health and Environmental Research Department of Energy under Contract DE-AC05-840 R21400 with Martin Marietta Energy Systems, Inc. The authors thank Valerie Hendrix for assistance in preparing the manuscript.

Phenyl-Substituted Analogues of Oxotremorine as Muscarinic Antagonists

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A series of phenyl-substituted analogues of the muscarinic agent oxotremorine (**1**) have been prepared. The new compounds (**3b–11b** and **9c**) were assayed for antimuscarinic activity on the isolated guinea pig ileum and in intact mice. They were also evaluated for ability to inhibit the binding of the muscarinic antagonist (−)-[^{3}H]-*N*-methylscopolamine to homogenates of the rat cerebral cortex. The phenyl-substituted derivatives were devoid of intrinsic muscarinic activity. Instead, they behaved as competitive muscarinic antagonists in these assays with similar or lower affinity for muscarinic receptors than the corresponding methyl-substituted analogues. The succinimide (**8b**) and the pyrrolidone (**3b**) derivatives of **1** substituted with a phenyl group at position 1 of the butynyl chain showed the highest antimuscarinic potency with dissociation constants (K_D) of 0.10 and 0.20 μM, respectively, in the ileum assay. The phenyl-substituted analogues showed an approximately 10-fold lower in vivo antimuscarinic potency than their corresponding methyl-substituted positional isomers. A correlation was observed between in vitro and in vivo potency within subsets consisting of methyl- and phenyl-substituted derivatives.

Introduction

In general, one or two aryl substituents are present in potent muscarinic antagonists. This situation may be exemplified with antagonists derived from 1,3-dioxolane,[1] 1,3-oxathiolane,[2] esters of ethanolamine,[3] oxadiazole,[4] es-

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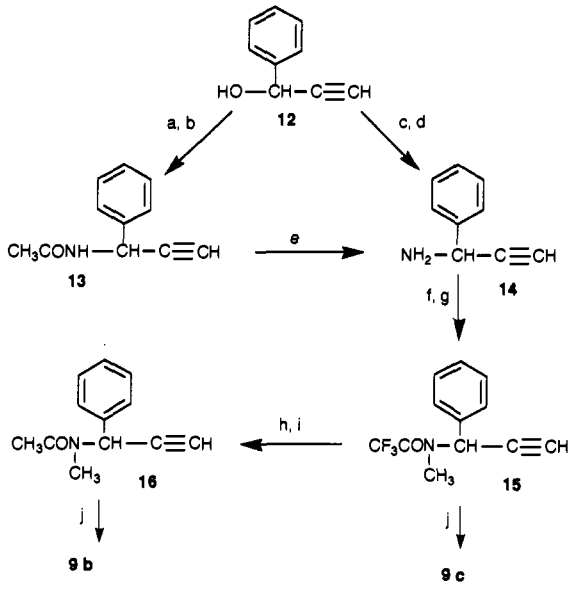
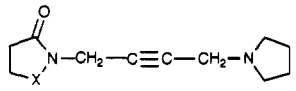
Scheme I[a]



[a] Reagents: (a) CH_3CN, 96% H_2SO_4, 0 → 20 °C; (b) H_2O; (c) MsCl, Et_3N, -78 °C; (d) $NaNH_2$, NH_3 (liq); (e) 3.5 M HCl, 90 °C; (f) $(CF_3CO)_2O$, pyridine, 0 °C; (g) pulverized K_2CO_3, CH_3CN, CH_3I, 20 °C; (h) $NaBH_4$, EtOH; (i) AcCl, pyridine, 0 °C; (j) pyrrolidine, $(HCHO)_n$, HOAc, CuCl, dioxane.

ters of 4-hydroxybut-2-ynylamines,[5] arecaidine esters,[6] etc. We have a current interest in agonists and antagonists derived from the muscarinic agent oxotremorine (1). Several structure–activity relationship (SAR) studies of methyl-substituted analogues of 1 have been performed;[7] whereas 1 is a potent muscarinic agonist, several of the methyl-substituted analogues are potent muscarinic antagonists. However, only a few amide-based derivatives of 1 containing phenyl groups have been reported.[8]



1: X = CH_2

2: X = CO

In the present study we have synthesized and tested pharmacologically a series of phenyl-substituted analogues of 1 in order to further explore the SAR of derivatives related to 1 and with the hope to arrive at analogues with increased affinity for muscarinic receptors. The new compounds (**3b–11b** and **9c**) were investigated for antimuscarinic activity on the isolated guinea pig ileum and for their ability to inhibit the binding of the muscarinic antagonist (-)-[^{3}H]-*N*-methylscopolamine ((-)-[^{3}H]NMS) to homogenates of the rat cerebral cortex. In vivo potencies were assayed as the ability of the compounds to inhibit oxotremorine-induced tremor in mice.

Chemistry

Synthesis. The synthetic routes to the major synthetic intermediates are outlined in Schemes I–V. Test compounds **3b**, **8b**, **9b**, and **9c** were prepared from 1-phenyl-2-propynylamine (**14**), which was synthesized by two alternative routes (Scheme I). Compound **14** could be obtained from the corresponding mesylate by treatment with sodium amide in liquid ammonia.[9] However, this route turned out to be unreliable because of the instability of the intermediate mesylate[10] and other related precursors of **14**.[9] Preferably, **14** was prepared, in 60% overall yield, via a Ritter reaction between acetonitrile and the commercially available alcohol **12**, followed by hydrolysis of the resulting amide **13**.[9] Attempts to prepare the N-methylated acetamide **16** by N-alkylation (NaH, iodomethane, THF) of **13** failed since we were only able to isolate the oxazole **17** or a mixture of **17** and the N-methylated allene **18** from the reaction mixture.[9] This is noteworthy, since N-methylation of *N*-(2-propynyl)acetamide[11] and *N*-(3-butyn-2-yl)acetamide[12] produces the expected N-methylated derivatives exclusively under similar conditions. However, **16** could be produced by cleavage of the trifluoroacetamide **15** by use of $NaBH_4$ in ethanol, to give the corresponding secondary amine **31**, followed by acetylation (Scheme I).[9]

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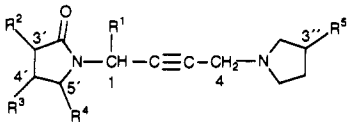


3a: $R^1 = CH_3$, $R^2 = R^3 = R^4 = R^5 = H$

3b: $R^1 = C_6H_5$, $R^2 = R^3 = R^4 = R^5 = H$

4a: $R^1 = H$, $R^2 = CH_3$, $R^3 = R^4 = R^5 = H$

4b: $R^1 = H$, $R^2 = C_6H_5$, $R^3 = R^4 = R^5 = H$

5a: $R^1 = R^2 = H$, $R^3 = CH_3$, $R^4 = R^5 = H$

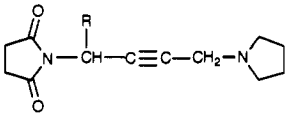
5b: $R^1 = R^2 = H$, $R^3 = C_6H_5$, $R^4 = R^5 = H$

6a: $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$, $R^5 = H$

6b: $R^1 = R^2 = R^3 = H$, $R^4 = C_6H_5$, $R^5 = H$

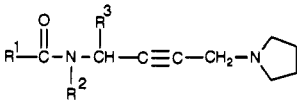
7a: $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = CH_3$

7b: $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = C_6H_5$



8a: $R = CH_3$

8b: $R = C_6H_5$



9a: $R^1 = R^2 = R^3 = CH_3$

9b: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = C_6H_5$

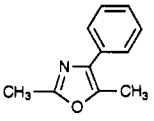
9c: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = C_6H_5$

10a: $R^1 = R^2 = CH_3$, $R^3 = H$

10b: $R^1 = C_6H_5CH_2$, $R^2 = CH_3$, $R^3 = H$

11a: $R^1 = CH_3$, $R^2 = C_2H_5$, $R^3 = H$

11b: $R^1 = CH_3$, $R^2 = C_6H_5CH_2$, $R^3 = H$



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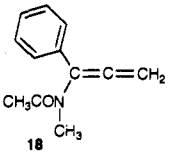
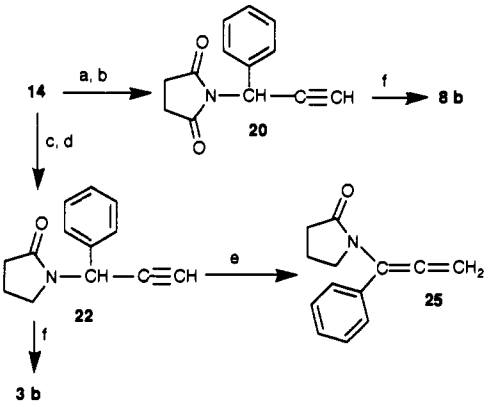


18

The succinimide derivative **20** was prepared by acylation of **14** with succinic anhydride in acetone followed by cyclization in the presence of acetic anhydride/sodium acetate (Scheme II). N-Alkylation of **14** with ethyl 4-bromobutyrate and ring closure of the resulting amino ester **21** afforded the lactam **22**. An alternative route to **22** involving cyclization of the 4-chlorobutyramide **23** (prepared by acylation of **14**), using powdered KOH in the presence of Bu_4NBr in THF was less useful, since the oxazole **24** and the allene **25** were produced as major products. The allene **25** was unstable and decomposed at room temperature in isolated form, but its structure was unambiguously determined by use of IR and NMR spectroscopy. The facile formation of allene **25** from **22** was demonstrated in an experiment using a catalytic amount of powdered KOH in THF (Scheme II).

An improved method for the preparation of 5-phenyl-2-pyrrolidone (**26**)[13] from 3-benzoylpropionic acid in 76%

Scheme II[a]



[a] Reagents: (a) succinic anhydride, acetone; (b) $(CH_3CO)_2O$, NaOAc; (c) $Br(CH_2)_3COOEt$, pulverized K_2CO_3, CH_3CN, 20 °C; (d) heat 90 °C neat; (e) pulverized KOH (0.2 equiv), THF, 0 → 20 °C; (f) pyrrolidine, $(HCHO)_n$, HOAc, CuCl, dioxane.

Table I. Physical Data of the New Compounds Tested[a]

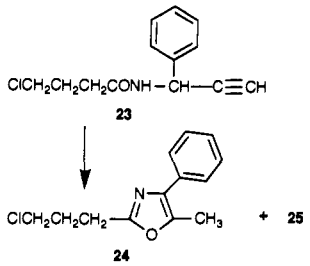
compd	yield, %	mp, °C	formula[b,c]
3b	86[d]	148.5–150	$C_{18}H_{22}N_2O{\cdot}C_2H_2O_4$
4b	82	133–134	$C_{18}H_{22}N_2O{\cdot}C_2H_2O_4$
5b	97[d]	114.5–115.5	$C_{18}H_{22}N_2O{\cdot}C_2H_2O_4$
6b	91[d]	115–117	$C_{18}H_{22}N_2O{\cdot}1.5C_2H_2O_4$
7b	50	90.5–93	$C_{18}H_{22}N_2O{\cdot}C_2H_2O_4{\cdot}H_2O$
8b	61	157–158	$C_{18}H_{20}N_2O_2{\cdot}C_2H_2O_4$
9b	93[d]	144–145	$C_{17}H_{22}N_2O{\cdot}C_2H_2O_4$
9c	68[d,e]	141–141.5	$C_{17}H_{19}F_3N_2O{\cdot}C_2H_2O_4$
10b	80	84.5–87	$C_{17}H_{22}N_2O{\cdot}C_2H_2O_4{\cdot}0.25H_2O$
11b	89	82–84	$C_{17}H_{22}N_2O{\cdot}C_2H_2O_4$

[a] For their preparation see the Experimental Section. [b] All compounds were analyzed for C, H, and N. The analytical results obtained were within ±0.4% except for C in compound **11b**. Anal. Calcd for $C_{17}H_{22}N_2O{\cdot}C_2H_2O_4$: C, 63.32%. Found: C, 62.9%. [c] The oxalate salts were recrystallized from acetone–methanol–ether. [d] No extractive workup after the Mannich reaction. The Mannich base was directly chromatographed on alumina after the dioxane was evaporated. [e] Ether was used as the chromatographic eluent.

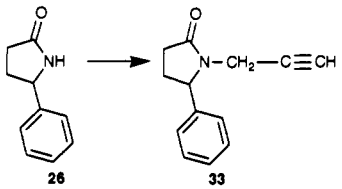
Table II. Antimuscarinic Activities and Receptor Binding Affinities of Some Oxotremorine Analogues[a]

compd	guinea pig ileum N[d]	guinea pig ileum K_D[e,f], μM	rat cerebral cortex (−)-[^{3}H]NMS displacement:[b] K_i, μM	intact mice: tremorolytic dose,[c] μmol/kg
3a		0.091 ± 0.01[g]		0.5[h]
3b	5	0.20 ± 0.02	0.11 ± 0.01	8.928 ± 2.78
4b	4	11.21 ± 3.58	6.91 ± 0.81	>200
5b	4	15.22 ± 2.12	5.77 ± 0.53	>200
6a	4	0.093 ± 0.01[i]	0.056 ± 0.002[j]	0.4[i]
6b	5	0.85 ± 0.14	0.54 ± 0.03	55.6 ± 8.6
7b	4	8.10 ± 0.53	2.40 ± 0.15	122 ± 34
8a	4	0.15 ± 0.01[k]		1.2[k]
8b	6	0.10 ± 0.03	0.12 ± 0.01	7.29 ± 1.11
9a	4	0.24 ± 0.07[l]	0.064 ± 0.007[m]	0.6[n]
9b	5	0.62 ± 0.05	0.31 ± 0.02	17.4 ± 4.1
9c	4	0.50 ± 0.08	0.23 ± 0.02	26.5 ± 1.4
10b	4	20.5 ± 0.6	5.20 ± 0.38	>200
11b	4	5.89 ± 0.51	1.29 ± 0.11	154 ± 37
atropine	4	0.0009 ± 0.0001[m]		0.9

[a] Values are means ± standard errors. [b] The K_i values are based on three separate experiments each performed in triplicate. [c] Dose required to double the dose of oxotremorine inducing a predetermined (grade 2) tremor intensity. [d] Number of test preparations used. [e] Dissociation constant of the drug–receptor complex. [f] The reported K_D values are 0.93, 11.5, 10.0, 2.1, 2.2, and 3.6 μM for **1** (agonist), **4a** (agonist), **5a**, **7a**, **10a** (agonist), and **11a**, respectively (ref 7). [g] Value is from ref 48. [h] Value is from ref 31. [i] Values are from ref 35. [j] Value is from ref 15. [k] Values are from ref 22. [l] Partial agonist. Value is from ref 49. [m] Values are from ref 50. [n] Value is from ref 51.

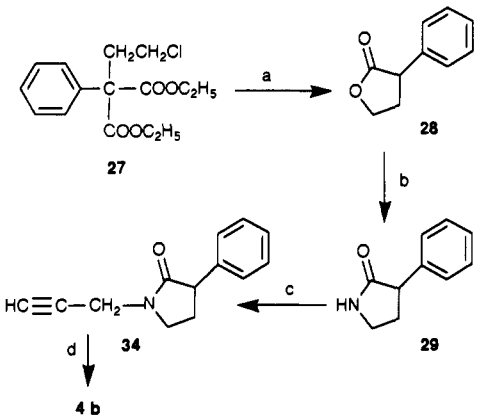


yield is given in the Experimental Section. N-Alkylation of **26** with propargyl bromide in the presence of pulverized KOH and Bu_4NBr in THF produced **33** in excellent yield.[14]



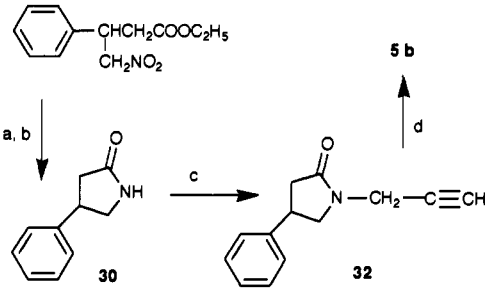
3-Phenyl-γ-butyrolactone (**28**)[16] (Scheme III) has been prepared in 50% yield by sequential treatment of the bromo analogue of **27** with 30% aqueous KOH and concentrated aqueous HCl.[16c] We prepared **28** in 78% yield (from diethyl phenylmalonate) by heating a mixture of the intermediate chloro compound **27** and concentrated aqueous HCl for 24 h. 3-Phenyl-2-pyrrolidone **29**[17] was prepared (in 76% yield) by heating a mixture of **28**, 25% aqueous NH_4OH, and ethanol in a sealed steel cylinder at 230 °C (Scheme III). The N-alkylation of **29** with propargyl bromide gave a moderate yield (50%) of product (**34**) under reaction conditions similar to those used for the preparation of **33** from **26**. This was mainly due to competitive propargylation in the 3-position of the lactam ring, producing the N,C-3-dipropargylated byproduct **35** in 22% yield (Scheme III). However, 4-phenyl-2-pyrrolidone (**30**), which was prepared according to a literature procedure[18] from ethyl 4-nitro-3-phenylbutyrate,[19] gave only the expected N-propargylated product (**32**) (in 97% yield) under similar alkylation conditions (Scheme IV).

Scheme III[a]



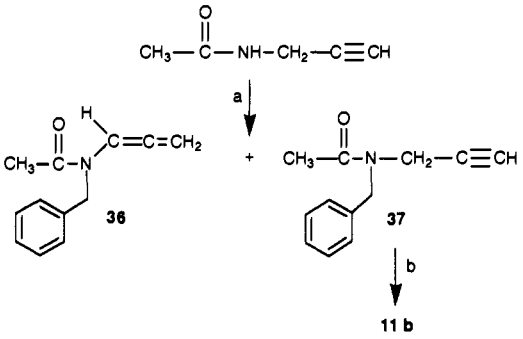
[a] Reagents: (a) concentrated aqueous HCl; (b) 25% aqueous NH_4OH, EtOH, 230 °C; (c) propargyl bromide, pulverized KOH, Bu_4NBr (0.2 equiv), THF, 0 → 20 °C; (d) pyrrolidine, $(HCHO)_n$, HOAc, CuCl, dioxane.

Scheme IV[a]



[a] Reagents: (a) H_2–Pd/C; (b) toluene, reflux; (c) propargyl bromide, pulverized KOH, Bu_4NBr (0.2 equiv), THF, 0 → 20 °C; (d) pyrrolidine, $(HCHO)_n$, HOAc, CuCl, dioxane.

Scheme V[a]



[a] Reagents: (a) NaH, THF, benzyl bromide, 0 → 20 °C; (b) pyrrolidine, $(HCHO)_n$, HOAc, CuCl, dioxane.

(13) (a) Rosenmund, K. W.; Engels, P. Über die Darstellung von in 5-Stellung Phenylierten Butyrolactamen. *Arch. Pharm. (Weinhem, Ger.)* **1951**, *284*, 209–216. (b) Reppe, W. et al. Äthinylierung. *Liebigs Ann. Chem.* **1955**, *596*, 1–224. (c) Miller, R. D.; Goelitz, P. An Efficient and General Synthesis of 5-Substituted Pyrrolidones. *J. Org. Chem.* **1981**, *46*, 1616–1618. (d) Rigo, B.; Fasseur, N.; Cherepy, N.; Couturier, D. Decarboxylation of Pyroglutamic Acids with P_2O_5/CH_3SO_3H: A General Synthesis of 5-Aryl-2-Pyrrolidones. *Tetrahedron Lett.* **1989**, *30*, 7057–7060.

(14) For N-propargylations of other lactams under phase-transfer conditions: see ref 15 and references cited therein.

(15) 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.

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(17) (a) Severin, T.; Poehlmann, H. Synthesen von 4-Oxocarbonsäuren und deren Estern mit Hilfe von α-Hydrazonoaldehyden. *Chem. Ber.* **1978**, *111*, 1564–1577. (b) Stamm, H.; Woderer, A.; Wiesert, W. Einstufensynthese von Pyrrolidonen durch Amidoethylierung Einfacher Ester mit N-Acylaziridinen. *Chem. Ber.* **1981**, *114*, 32–48. (c) Woderer, A.; Assithianakis, P.; Wiesert, W.; Speth, D.; Stamm, H. 2-(Acylimino)pyrrolidine und N-acylierte γ-Aminobutyronitrile durch Amidoethylierung von Einfachen Nitrilen mit N-acylaziridinen. *Chem. Ber.* **1984**, *117*, 3348–3364. (d) Pagliarini, G.; Cignarella, G.; Testa, E. Indagine Chimica sul Comportamento dei Fenilbutirrolattoni. *Farmaco, Ed. Sci.* **1966**, *21*, 355–369.

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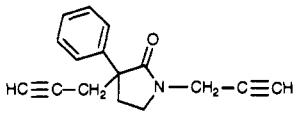
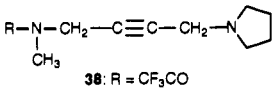


35

N-Benzylation of *N*-(2-propynyl)acetamide[20] afforded **37** together with minor amounts of its allenic isomer **36** (Scheme V). Use of a weaker base (KOH instead of NaH) also led to the formation of the allenic byproduct.[21] Basic hydrolysis (5 M NaOH) of the trifluoroacetamide derivative **38**[22] gave the acetylenic diamine **39**[23] which was converted into **10b** by acylation.



38: R = CF_3CO

39: R = H

The test compounds **3b–6b**, **8b**, **9b**, **9c**, and **11b** were obtained by cuprous-catalyzed Mannich reactions of the appropriate acetylenic precursor and pyrrolidine. The Mannich base **7b** was prepared similarly from *N*-propargyl-2-pyrrolidone[15] and 3-phenylpyrrolidine.[24] Physical data of the new acetylenic test compounds are presented in Table I.

NMR Spectroscopy. It has been suggested that the *Z*-rotational isomer of **9a** would correspond to the bioactive conformation.[12] Therefore, we determined the *Z*/*E* ratios of the novel acyclic amides presented herein. From NMR spectra[25] of the oxalate salts of the acetamide analogue **9b** and its trifluoroacetamide analogue **9c**, in CD_3OD solutions (at 23 °C), an equilibrium mixture of *Z*- and *E*-rotational isomers in a ratio of approximately 9:1 was observed.[26] A ^{19}F NMR spectrum of **9c** recorded under the same conditions gave the same *Z*/*E* ratio.[27] *Z*/*E* ratios around 7:3 and 6:5[28] were observed for the oxalate salts of compounds **10b** and **11b** in CD_3OD solutions, respectively.[29] The relative proportions of the two conformations were determined by integration of the *N*-Me resonances due to each rotational isomer in compounds **9b**, **9c**, and **10b**. The *Z*/*E* ratio for compounds **36** and **37** (around 1:1 in $CDCl_3$) and the oxalate salt of **11b** was determined by integration over signals due to the benzylic protons of each rotational isomer. In summary, the NMR spectral analysis indicates that the *Z*-rotational isomers of the phenyl-substituted derivatives are energetically accessible.

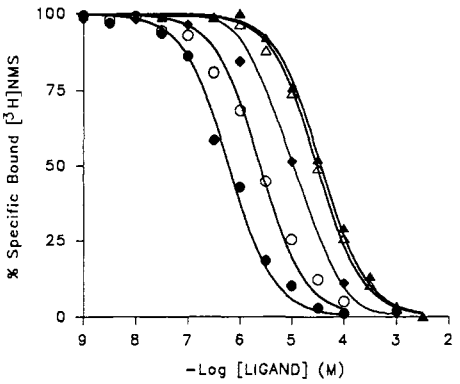


Figure 1. Competitive inhibition of (−)-[^{3}H]NMS specific binding to rat cerebral cortical muscarinic receptors by some phenyl-substituted oxotremorine analogues. The ligands are **3b** (●), **4b** (▲), **5b** (△), **6b** (○), and **7b** (◆). Each point represents the mean value of three experiments, each performed in triplicate. The standard errors (not shown) for each point varied from 1 to 4% of the mean value. The solid lines are the best-fit curve to a one-site binding model.

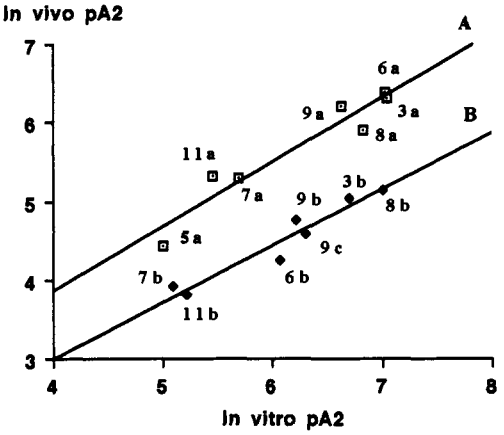


Figure 2. Relationship between in vitro and in vivo pA_2 values for some methyl- and phenyl-substituted analogues of oxotremorine. In vitro pA_2: antagonism of carbachol-induced contractions of the isolated guinea-pig ileum; in vivo pA_2: antagonism of oxotremorine-induced tremor in mice (Table II). Regression line A (which consists of methyl-substituted analogues) is described by: in vivo pA_2 = 0.816 × in vitro pA_2 + 0.605 (r^2 = 0.91) and regression line B (which consists of phenyl-substituted analogues) by: in vivo pA_2 = 0.717 × in vitro pA_2 + 0.140 (r^2 = 0.93). Values for the methyl-substituted analogues are from ref 7.

Pharmacology

Compounds **3b–11b** and **9c** were tested for antimuscarinic activity in the guinea pig ileum and in intact mice. The results are presented in Table II. For comparison, relevant data for compounds **3a**, **6a**, **8a**, **9a**, and atropine are included as well. K_D values for the antagonists were obtained from their ability to antagonize carbachol-induced contractions in the guinea pig ileum (spasmolytic activity). In all cases, the phenyl-substituted agents appeared to be competitive antagonists since their respective slopes in the Schild plot[30] were not significantly different from that of 1. In corroboration of the ileal assay findings, the com-

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(21) The formation of an allenic isomer has been noted previously in the N-propargylation of lactams: see ref 15 and references cited therein.

(22) Nilsson, B. M.; Ringdahl, B.; Hacksell, U. Derivatives of the Muscarinic Agent N-Methyl-N-(1-methyl-4-pyrrolidino-2-butynyl)acetamide. *J. Med. Chem.* **1988**, *31*, 577–582.

(23) For alternative methods for the preparation of **39** see: (a) Corbel, B.; Paugam, J.-P.; Sturtz, G. Amino-methylation of N-Propargylphosphoramides. Synthesis of Unsymmetrical Acetylenic Diamines. *Can. J. Chem.* **1980**, *58*, 2183–2188. (b) See ref 8c.

(24) Bettoni, G.; Cellucci, C.; Tortorella, V. The Absolute Configuration of 3-Phenylpyrrolidine. *J. Heterocycl. Chem.* **1976**, *13*, 1053–1055.

(25) For a review on NMR studies of amides, see: Stewart, W. E.; Siddall, T. H. Nuclear Magnetic Resonance Studies of Amides. *Chem. Rev.* **1970**, *70*, 517–551.

(26) A *Z*/*E* ratio of approximately 4:1 has been reported[22] for the oxalate salt of **9a** and its corresponding trifluoroacetamide analogue under similar conditions.

(27) For ^{19}F NMR spectral properties of some related trifluoroacetamides, see ref 22.

(28) The assignment of rotational isomers is ambiguous.

(29) A similar *Z*/*E* ratio was determined for the oxalate salt of **11b** in D_2O by integration over the signals due to the acetyl methyl group.

(30) Arunlakshana, O.; Schild, H. O. Some Quantitative Uses of Drug Antagonists. *Br. J. Pharmacol.* **1959**, *14*, 48–58.

pounds inhibited the specific binding of (−)-[^{3}H]NMS to rat cerebral cortex muscarinic receptors in an apparently competitive manner (Figure 1). The competition binding data were found to fit a one-site binding equation and the corresponding K_i values are shown in Table II. The potency of the phenyl-substituted derivatives in the receptor binding assay also correlated well with their affinity for ileal muscarinic receptors (Table II; $r^2 = 0.95$).

The central antimuscarinic activity of the new compounds was obtained from their ability to antagonize oxotremorine-induced tremor in mice (tremorolytic activity). When compared to methyl-substituted reference compounds **3a**, **6a**, **8a**, and **9a**, all of the new analogues exhibited low in vivo potency. Tremorolytic doses for compounds **4b**, **5b**, and **10b** were not determined because of their very low potency. In general, it could be stated that the tremorolytic potency of the phenyl-substituted analogues was directly correlated with antimuscarinic potency in the ileal assay ($r^2 = 0.93$; Figure 2, line B).

Discussion

Previous SAR studies of alkyl-substituted derivatives of **1** and related compounds are of relevance to the present investigation. Introduction of a methyl group at C1 or C5′ of **1** gave potent muscarinic antagonists (**3a** and **6a**, respectively) with higher affinity for muscarinic receptors in the guinea pig ileum than the parent agonist.[7] Derivatives of **1** substituted with an ethyl or a *n*-propyl group at C1 in the butynyl chain were also found to be potent muscarinic antagonists.[31] However, the affinity for ileal muscarinic receptors decreases slightly with increasing size of the alkyl substituent. Introduction of a methyl group at C1 in the succinimide analogue (**2**) of **1**, resulting in **8a**, also led to abolished efficacy and increased affinity as compared to **2**.[7] Analogous methyl substitution in the butynyl chain of open carboxamide analogues of **1** (e.g. **10a**)[7] may yield partial muscarinic agonists such as BM 5 (**9a**),[12] a compound which shows unusual selectivity in its muscarinic actions.[32] Selected pharmacological data for some of these compounds are presented in Table II.

In the present series of phenyl-substituted derivatives, all compounds lacked intrinsic activity and behaved as pure competitive antimuscarinic agents in the guinea pig ileum assay. Two compounds, the C1-phenyl-substituted pyrrolidone and succinimide derivatives **3b** and **8b**, respectively, were of similar potency to the methyl-substituted analogues **3a** and **8a** in this assay. The new compounds were also able to displace (−)-[^{3}H]NMS from cortical muscarinic binding sites in vitro and to counteract oxotremorine-induced tremor. However, they do not appear to be very potent antimuscarinic agents in vivo. Introduction of a phenyl group at C1 or C5′ in **1** produced muscarinic antagonists with 5-fold higher (**3b**) or almost similar affinity (**6b**) for ileal muscarinic receptors, as compared to **1**. However, a phenyl group at C3′, C4′, or C3″ (giving **4b**, **5b**, and **7b**, respectively) gave antagonists with 12-, 16-, and 9-fold lower affinity for the same receptors as compared to **1**. These findings corroborate an earlier report[31] which showed that positions C1 and C5′, when monomethylated, represent sites that contribute to high-affinity attachment of the ligand to the active site of the muscarinic receptor. In support of this observation are also the current findings that phenyl derivatives **4b** and **5b** had K_D values similar to those of their corresponding methyl analogues (Table II), despite the increase in steric bulk. Thus, it appears that phenyl or methyl substituents at C3′ and C4′ of the lactam ring produce similar effects on binding affinity. In contrast, the C1-, C5′-, and C3″-phenyl-substituted derivatives **3b**, **6b**, and **7b** had approximately 2-, 9-, and 4-fold larger K_D values, respectively, compared to their similarly substituted methyl analogues (Table II).

Focusing on the C1 position, it should be noted that phenyl substitution at this position produces antagonists regardless of whether the amide moiety is part of a pyrrolidone (**3b**) or a succinimide ring (**8b**), or an acetamide (**9b**) or a trifluoroacetamide moiety (**9c**). Together, these and earlier findings suggest (i) that the C1- and C5′-positions approximate specific, possibly hydrophobic, binding domains associated with the muscarinic receptor active site and (ii) that the introduction of sizeable alkyl (i.e. larger than methyl) or aryl groups at these positions induces steric hindrance, thus lowering ligand affinity for the receptor.

The affinity difference between the 2-phenylacetamide derivative **10b** and its acetamide homologue **10a** agreed well with the observed difference between their related lactam analogues **4b** and **1**, respectively. Thus, **10b** lacked intrinsic activity and had about 10-fold lower affinity for ileal muscarinic receptors as compared to **10a**.

Taken together, the pharmacological data show that the phenyl group does not confer greater affinity to the ligand than a methyl group in any one of the compounds studied. This is probably not due to conformational restraints imposed by the phenyl group since (a) NMR-studies (vide supra) demonstrate that the *Z*-rotational isomers of the phenyl-substituted acyclic amides are energetically accessible and (b) preliminary molecular mechanics (MMX) calculations indicate that the conformational preferences of, e.g. **6a** and **6b**, are identical. Therefore, in contrast with other classes of muscarinic ligands containing aromatic groups (vide supra), it does not appear that the phenyl group binds strongly to an accessory receptor binding site in any of the novel oxotremorine derivatives used in this study.

The correlation between in vitro parasympatholytic potency (i.e. affinity for ileal muscarinic receptors) and in vivo potency (tremorolytic dose in mice) seems to be good within the series of phenyl-substituted oxotremorine analogues presented here.[33] However, the correlation between these two parameters is less good ($r^2 = 0.47$) in a set consisting of both phenyl- and methyl-substituted derivatives with similar K_D values; e.g. compound **8b** has an affinity similar to that of **3a**, **6a**, and **8a** but is approximately 10-fold lower in vivo antimuscarinic potency. In contrast, when analyzing the correlation between in vitro and in vivo affinity within subsets consisting of methyl- (regression line A) and phenyl-substituted (regression line B) derivatives, respectively, fairly good correlations are obtained ($r^2 = 0.91$ and 0.93, respectively; Figure 2). The subsets produce lines with similar slopes but with different

(31) Ringdahl, B.; Jenden, D. J. Affinity, Efficacy and Stereoselectivity of Oxotremorine Analogues for Muscarinic Receptors in the Isolated Guinea Pig Ileum. *Mol. Pharmacol.* **1983**, *23*, 17–25.

(32) (a) Nordström, Ö.; Alberts, P.; Westlind, A.; Undén, A.; Bartfai, T. Presynaptic Antagonist-Postsynaptic Agonist at Cholinergic Synapses: N-Methyl-N-(1-methyl-4-pyrrolidino-2-butynyl)-acetamide. *Mol. Pharmacol.* **1983**, *24*, 1–5. (b) Engström, C.; Undén, A.; Ladinsky, H.; Consolo, S.; Bartfai, T. BM-5, a Centrally Active Partial Muscarinic Agonist with Low Tremorogenic Activity. *Psychopharmacology (Berlin)* **1987**, *91*, 161–167.

(33) Previous studies with antagonists related to oxotremorine have demonstrated a highly significant linear relationship between affinity for ileal muscarinic receptors and tremorolytic activity in mice: see ref 31.

intercepts. This difference is a consequence of the lower in vivo potencies of the compounds in the phenyl-substituted series.

The base strength of the tertiary amines should be similar in both series of compounds, and on the basis of the increased lipophilicity of the phenyl-substituted analogues, an increased in vivo potency was anticipated. The unexpectedly low in vivo potency of these novel analogues might be due to differences in the mode of binding to muscarinic receptors between methyl- and phenyl-substituted oxotremorine analogues,[34] to (unverified) differences in metabolic profiles or to other pharmacokinetic differences.[36]

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hoover apparatus. IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer. ^{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. ^{1}H and ^{13}C NMR spectra were referenced to internal tetramethylsilane. Dioxane (68.0 ppm) was used as internal reference for the ^{13}C NMR spectrum of compound **39** in D_2O. ^{19}F NMR spectra were referenced to internal $CFCl_3$. Samples for ^{1}H and ^{13}C NMR spectroscopy were dissolved in $CDCl_3$ unless otherwise noted. Some assignments of ^{13}C NMR resonances are based on off-resonance spectra. All spectra were in accordance with the assigned structures. Thin-layer chromatography (TLC) was carried out on aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm) of aluminum oxide 60 F_{254} neutral (type E, E. Merck). Flash chromatography was carried out on silica. Chromatographic spots were visualized by UV and/or spraying with aqueous $KMnO_4$. All reactions, except the preparation of compound **29**, were carried out in an atmosphere of dry nitrogen. 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.

1-Phenyl-2-propynylamine (14). A suspension of *N*-(1-phenyl-2-propynyl)acetamide (**13**)[9] (12.47 g, 72 mmol) and 3.5 M aqueous HCl (410 mL) was heated to 90 °C for 5 h. The resulting solution was extracted with ether (300 mL). The aqueous layer was alkalinized by addition of solid $NaHCO_3$ to pH 8.5 and extracted with ether (4 × 200 mL). The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo. The oily residue was subjected to flash chromatography using ether–light petroleum (1:1) followed by ether as eluants to yield 7.0 g (74%) of **14** as an oil: TLC R_f = 0.35 (ether). For spectroscopic data of **14** (free base), see ref 9. An analytical sample was prepared as the oxalate salt (double salt; from methanol–H_2O): mp 195 °C dec. Anal. ($C_9H_9N{\cdot}0.5C_2H_2O_4$) C, H, N.

***N*-(1-Phenyl-2-propynyl)succinamic Acid (19).** A mixture of **14** (1.92 g, 15 mmol) and succinic anhydride (1.45 g, 14.5 mmol) in THF (45 mL) was stirred at room temperature for 12 h and then at 45 °C for 7 h. Addition of 3 M aqueous HCl (120 mL) and extraction with CH_2Cl_2 (2 × 150 mL) followed by drying ($MgSO_4$), filtration, and concentration in vacuo of the combined organic layers afforded 2.75 g (81%) of **19** as a solid, which was pure according to ^{1}H NMR analysis. An analytical sample was obtained after recrystallization from acetone–ether–*n*-hexane: mp 121–123 °C; IR (KBr disk) 3290, 1710, 1655, 1530 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.60–7.20 (m, 5 H), 5.89 (d, *J* = 2.4 Hz, C1-H), 2.89 (d, *J* = 2.4 Hz, C3-H), 2.73–2.35 (m, 4 H); ^{13}C NMR (CD_3OD) δ 176.01 and 173.01 (C=O′ s), 139.59, 129.37, 128.75, 127.74 (Ar C′ s) 82.49 (C2), 74.12 (C3), 45.15 (benzylic CH), 31.04 and 29.86 (CH_2′ s). Anal. ($C_{13}H_{13}NO_3$) C, H, N.

***N*-(1-Phenyl-2-propynyl)succinimide (20).** A mixture of **19** (1.2 g, 5.2 mmol), anhydrous sodium acetate (0.43 g, 5.24 mmol), and acetic anhydride (2.65 g, 26 mmol) in THF (30 mL) was refluxed for 20 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between saturated aqueous $NaHCO_3$ (150 mL) and ether (100 mL) with stirring for 1 h. The aqueous layer was extracted with additional portions of ether (2 × 175 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography with ether as eluent, afforded 935 mg (84%) of **20**: mp 112–114 °C; TLC R_f = 0.65 (ether); IR (KBr disk) 3250, 2120, 1700 (br) cm^{-1}; ^{1}H NMR δ 7.62–7.18 (m, 5 H), 6.20 (d, *J* = 2.6 Hz, C1-H), 2.69 (s, 2 × CH_2), 2.56 (d, *J* = 2.8 Hz, C3-H); ^{13}C NMR δ 175.32 (C=O), 135.21, 128.47, 127.86 (Ar C′ s), 77.73 (C2), 74.21 (C3), 44.81 (benzylic CH), 28.01 (2 × CH_2). Anal. ($C_{13}H_{11}NO_2$) C, H, N.

Ethyl 4-(1-Phenyl-2-propynylamino)butyrate (21). A mixture of **14** (795 mg, 6.06 mmol), ethyl 4-bromobutyrate (1.30 g, 6.64 mmol), and powdered K_2CO_3 (2.85 g, 20.6 mmol) in acetonitrile (11 mL) was stirred at room temperature for 14.5 days. Solids were filtered off, and the resulting filter cake was washed several times with ether. The combined filtrates were concentrated in vacuo to give a reddish oil which was purified by column chromatography using gradient elution (ether–light petroleum (1:4) → ether). The first fractions contained 120 mg (5.5%) of the tertiary amine *N,N*-bis[3-(ethoxycarbonyl)propyl]-1-phenyl-2-propynylamine,[37] isolated as an oil. Further elution gave fractions containing 895 mg (60%) of pure **21** as a light yellow oil. The last fractions contained the starting amine **14**, isolated as its oxalate [double salt (vide supra); 154 mg, 7.2%]. An analytical sample of **21** was prepared as the oxalate salt (double salt; from acetone–methanol–ether): mp 130.5–132.5 °C; TLC R_f = 0.45 [free base, ether–light petroleum (1:1)]; IR (free base, neat liquid) 3290, 1730 cm^{-1}; ^{1}H NMR (free base) δ 7.62–7.21 (m, 5 H), 4.60 (d, *J* = 2.4 Hz, C1-H), 4.11 (q, *J* = 7.2 Hz, CH_3CH_2O), 3.0–2.6 (m, 2 H), 2.49 (d, partially obscured, *J* = 2.2 Hz, C3-H), 2.45–2.25 (m, 2 H), 2.00–1.62 (m, 3 H), 1.23 (t, *J* = 7.2 Hz, CH_3CH_2O); ^{13}C NMR (free base) δ 173.50 (C=O), 139.75, 128.41, 127.73, 127.39 (Ar C′ s), 83.72 (C2), 73.38 (C3), 60.22 (OCH_2), 53.86 (benzylic CH), 46.20, 32.12, 25.07 (CH_2′ s), 14.21 (CH_3). Anal. ($C_{15}H_{19}NO_2{\cdot}0.5C_2H_2O_4$) C, H, N.

***N*-(1-Phenyl-2-propynyl)-2-pyrrolidone (22).** Compound **21** (3.45 g, 14.1 mmol) was heated neat at 90 °C for 3 days. The resulting product was partitioned between CH_2Cl_2 (200 mL) and 2.5 M aqueous HCl (2 × 60 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The dark brown oily residue was chromatographed using ether–light petroleum (2:1) followed by ether as eluants to give 1.26 g (45%) of pure **22** as an oil. An analytical sample was obtained after distillation: bp 128–131 °C (0.4 mmHg). Upon refrigeration, **22** formed crystals: 36–38 °C; TLC R_f = 0.51 (ether); IR (neat liquid) 3280, 3220, 2120, 1680 (br) cm^{-1}; ^{1}H NMR δ 7.55–7.20 (m, 5 H), 6.31 (d, *J* = 2.4 Hz, C1-H), 3.70–3.37 (m, 1 H), 3.17–2.85 (m, 1 H), 2.55 (d, *J* = 2.6 Hz, C3-H), 2.60–2.20 (m, partially obscured, 2 H), 2.16–1.75 (m, 2 H); ^{13}C NMR δ 174.24 (C=O), 135.95, 128.60, 128.20, 127.27 (Ar C′ s), 78.94 (C2), 74.64 (C3), 46.65 (benzylic CH), 42.59, 30.91, 17.54. Anal. ($C_{13}H_{13}NO$) C, H, N.

***N*-(1-Phenyl-2-propynyl)-4-chlorobutyramide (23).** 4-Chlorobutyryl chloride (2.62 g, 18.6 mmol) was added to a cooled (ice bath) mixture of **14** (1.63 g, 12.4 mmol), CH_2Cl_2 (35 mL), $NaHCO_3$ (3.65 g, 12.4 mmol), and water (30 mL). The reaction mixture was stirred for 3 h while the temperature was allowed

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(36) A similar trend has been observed previously in a series of acetylenic diamines related to oxotremorine; i.e. the tremorolytic potency decreased when the lipophilicity increased: Cho, A. K.; Jenden, D. J. The Mechanism of Tremorine Antagonism by Related Compounds. *Int. J. Neuropharmacol.* **1964**, *3*, 27–36. See also, Brimblecombe, R. W.; Inch, T. D.; Wetherell, J.; Williams, N. Structure–Activity Relations for Anticholinergic 2-[1-Aryl(or Cyclohexyl)-1-hydroxy-1-phenyl]methyl-1,3-Dioxolans. *J. Pharm. Pharmacol.* **1971**, *23*, 649–661.

(37) The spectral properties of this compound are given in the supplementary material.

to warm up to room temperature. The aqueous layer was extracted with CH_2Cl_2 (4 × 150 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude solid was dissolved in CH_2Cl_2–CH_3OH (2.5%) and purified by column chromatography using gradient elution [ether–light petroleum (1:3) → (1:1)]. This gave 2.76 g (94.5%) of pure **23** as a solid. An analytical sample was obtained after recrystallization from ether–*n*-hexane: mp 89.5–90.5 °C; TLC R_f = 0.25 [*n*-hexane–ether (1:1)]; IR (KBr disk) 3300, 3270, 1645, 1532 cm^{-1}; ^1H NMR δ 7.58–7.28 (m, 5 H), 6.30–6.00 (br m, partially obscured, NH), 6.00 (dd, *J* = 2.2 Hz, 8.3 Hz, C1-H), 3.58 (t, *J* = 6.0 Hz, $ClCH_2$), 2.49 (d, *J* = 2.4 Hz, C3-H), 2.46–1.94 (m′ s, 4 H); ^{13}C NMR δ 170.67 (C=O), 138.06, 128.64, 128.11, 126.63 (Ar C′ s), 81.57 (C2), 72.95 (C3), 44.30 ($ClCH_2$ and benzylic CH), 32.84, 27.87. Anal. ($C_{13}H_{14}ClNO$) C, H, N.

2-(3-Chloropropyl)-5-methyl-4-phenyloxazole (24) and *N*-(1-Phenyl-1,2-propadienyl)-2-pyrrolidone (25). Powdered KOH (312 mg, 5.56 mmol) and Bu_4NBr (208 mg, 0.65 mmol) were added to a precooled (ice bath) solution of compound **23** (1.34 g, 5.68 mmol) in THF (25 mL). The resulting mixture was stirred for 3 h while the temperature was allowed to warm up to room temperature. The mixture was concentrated in vacuo and the residue was resuspended in CH_2Cl_2 and chromatographed using gradient elution [ether–light petroleum (1:3) → (1:1)]. This gave 0.38 g (28%) of **24** as a colorless oil which crystallized from *n*-hexane when refrigerated: mp 33–35 °C; TLC R_f = 0.71 [*n*-hexane–ether (1:1)]; IR (KBr disk) 1600, 1590 cm^{-1}; ^1H NMR δ 7.70–7.25 (m, 5 H), 3.67 (t, *J* = 6.3 Hz, $ClCH_2$), 2.93 (t, *J* = 7.3 Hz, 2 H), 2.49 (s, CH_3), 2.27 (m, 2 H); ^{13}C NMR δ 160.82, 143.28, 134.14, 132.19, 128.36, 126.94, 126.38 (Ar C′ s), 43.80, 29.63, and 25.12 (CH_2′ s), 11.62 (CH_3). Anal. ($C_{13}H_{14}ClNO$).

Further elution with ether followed by ether–methanol (3%) afforded the allene **25**: TLC R_f = 0.20 (ether). When fractions containing **25** were concentrated in vacuo on a water bath at 20 °C, partial decomposition occurred. However, the following selected spectral properties were obtained: IR (neat liquid) 1945, 1690 cm^{-1}; ^1H NMR δ 5.48 (s, =CH_2); ^{13}C NMR δ 206.99 (=C=), 174.59 (C=O), 83.64 (=CH_2), 48.99, 30.95, 18.32.

The same spectral characteristics were observed when **25** was isolated by flash chromatography (by use of ether as eluant) from a reaction in which **22** was treated with powdered KOH (0.2 equiv) in THF (0 → 20 °C, 3 h).

5-Phenyl-2-pyrrolidone (26). A mixture of 3-benzoylpropionic acid (2.40 g, 13.5 mmol), ammonium acetate (30.0 g, 389 mmol), and $NaBH_3CN$ (0.85 g, 13.5 mmol) in methanol (95 mL) was stirred at room temperature for 48 h. Concentrated aqueous HCl (180 mL) was then added dropwise. The aqueous mixture was concentrated in vacuo, extracted with ether (3 × 100 mL) and evaporated to dryness. The crude amino acid was added in two portions (one hour between additions) to a stirred solution of 2-chloro-1-methylpyridinium iodide (3.8 g, 14.9 mmol) and triethylamine (5.3 g, 52.3 mmol) in acetonitrile (700 mL) heated to reflux. Heating was continued for 6 h, solids were filtered off, and the filtrate was concentrated in vacuo. The crude product was resuspended in $CHCl_3$ and purified by flash chromatography using ether followed by ether–methanol (19:1) as eluants. This gave 1.67 g (76% from 3-benzoylpropionic acid) of pure **26** as a solid: mp 104–106 °C (lit.[13a] mp 103–105 °C); TLC R_f = 0.35 [ether–methanol (19:1)]; ^{13}C NMR (CD_3OD) δ 180.92 (C=O), 144.10, 129.65, 128.60, 126.65 (Ar C′ s), 59.45 (benzylic CH), 31.97, 31.29. IR and ^1H NMR data were in accordance with those reported.[13c]

3-Phenyl-γ-butyrolactone (28). A modification of a literature method[16c] was used. A solution of diethyl phenylmalonate (5.0 g, 21.2 mmol) in THF (20 mL) was added to 80% NaH (1.22 g, 41 mmol, freed from mineral oil by *n*-hexane washings). 1-Bromo-2-chloroethane (15.17 g, 106 mmol) was added, and the mixture was stirred and heated to reflux. Additional portions of NaH (305 mg, 10.2 mmol) and 1-bromo-2-chloroethane (3.0 g, 21.4 mmol) were added after 36 h, and heating was continued for another 8 h. Addition of ethanol (30 mL) to the reaction mixture, concentration of the mixture in vacuo, and flash chromatography of the crude residue, using *n*-hexane–ether (9:1) as eluent, afforded 5.52 g (87%) of diethyl (2-chloroethyl)phenylmalonate (**27**) as an oil which was sufficiently pure (NMR) to use as such in the next step: TLC R_f = 0.25 [*n*-hexane–ether (9:1)]; IR (neat liquid) 1730 cm^{-1}; ^1H NMR δ 7.35 (br s, 5 H), 4.25 (q, *J* = 7.0 Hz, 2 × CH_3CH_2O), 3.58–3.30 (m, 2 H), 2.88–2.60 (m, 2 H), 1.23 (t, *J* = 7.0 Hz, 2 × CH_3CH_2O); ^{13}C NMR (C_6D_6) δ 169.80 (C=O′ s), 136.72, 128.57, 128.08, 127.86 (Ar C′ s), 62.20 (benzylic C), 61.67 (2 × OCH_2CH_3), 40.58, 39.84, 13.77 (2 × OCH_2CH_3). Anal. ($C_{15}H_{19}ClO_4 \cdot 0.25H_2O$) C, H.

The lactone **28** was obtained by heating to reflux a mixture of **27** (5.32 g, 17.8 mmol) and concentrated aqueous HCl (200 mL) from **24 h**. Dilution with water (150 mL) and extraction of the mixture with CH_2Cl_2 (3 × 200 mL) was followed by drying ($MgSO_4$), filtration, and concentration in vacuo of the combined organic layers to furnish the crude product. Flash chromatography with ether–light petroleum (1:3) as eluent afforded 2.61 g (78%, from diethyl phenylmalonate) of the title compound as an oil: TLC R_f = 0.33 [*n*-hexane–ether (1:2)]. Spectroscopic data[16d] of **28** were in accordance with those reported (IR,[38] ^1H NMR[39]).

3-Phenyl-2-pyrrolidone (29). Compound **28** (950 mg, 5.86 mmol), 25% aqueous NH_4OH (50 mL), and ethanol (50 mL) were heated in a sealed steel cylinder at 230 °C for 25 h. The mixture was concentrated at reduced pressure, and the residue was partitioned between 3 M aqueous HCl (200 mL) and CH_2Cl_2 (175 mL). The aqueous layer was extracted with an additional portion of CH_2Cl_2 (175 mL). Drying (K_2CO_3), filtration, and concentration of the organic layer afforded crude **29**. Flash chromatography with ether–methanol (19:1) as eluent yielded 700 mg (74%) of pure **29** as a white solid: mp 86–87 °C (lit.[17b] mp 87–88 °C); TLC R_f = 0.26 [ether–methanol (19:1)]; ^{13}C NMR δ 179.40 (C=O), 139.53, 128.66, 127.95, 126.93 (Ar C′ s), 47.87 (benzylic CH), 40.74, 30.64. IR[17b] and ^1H NMR[17a,b] data were in accordance with those previously reported.

4-Phenyl-2-pyrrolidone (30). This compound was prepared from ethyl 4-nitro-3-phenylbutyrate[19] as described previously:[18] TLC R_f = 0.26 [ether–methanol (19:1)]; ^{13}C NMR δ 178.26 (C=O), 142.22, 128.78, 126.99, 126.75 (ArC′ s), 49.72, 40.18 (benzylic CH), 38.23. IR and ^1H NMR data were in agreement with those reported.[40]

***N*-Methyl-1-phenyl-2-propynylamine (31).** This compound was prepared from **14** on a 8.8-mmol scale according to the method described previously[9] in 79% yield after purification by flash chromatography using ether as eluent; TLC R_f = 0.55 (ether). An analytical sample was prepared as the oxalate salt (double salt) from acetone–methanol–ether: mp 176–179 °C dec; IR (free base, neat liquid) 3290, 1600 (w), 1450 cm^{-1}; ^1H NMR (free base) δ 7.60–7.20 (m, 5 H), 4.50 (d, *J* = 2.2 Hz, C1-H), 2.49 (d, partially obscured, *J* = 2.4 Hz, C3-H), 2.46 (s, CH_3), 1.61 (s, NH); ^{13}C NMR (free base) δ 139.56, 128.53, 127.92, 127.55 (Ar C′ s), 83.44 (C2), 73.62 (C3), 55.50 (benzylic CH), 33.63 (CH_3). Anal. ($C_{10}H_{11}N \cdot 0.5C_2H_2O_4$) C, H, N.

***N*-Propargyl-4-phenyl-2-pyrrolidone (32).** A solution of **30** (858 mg, 5.32 mmol) and propargyl bromide (728 mg, 6.12 mmol) in THF (15 mL) was added to a mixture of powdered KOH (358 mg, 6.39 mmol) and Bu_4NBr (343 mg, 1.06 mmol) in THF (10 mL). The reaction mixture was cooled externally with an ice bath. The temperature was allowed to warm up to room temperature. The mixture was filtered after 6 h, and the filtrate was concentrated in vacuo. Flash chromatography of the oily residue with ether as eluent afforded 1.03 g (97%) of pure **32**. An analytical sample was obtained after distillation: bp 125–128 °C (0.05–0.1 mmHg); TLC R_f = 0.44 (ether); IR (neat liquid) 3290, 3230, 2110, 1690 (br) cm^{-1}; ^1H NMR δ 7.48–7.10 (m, 5 H), 4.15 (d, *J* = 2.6 Hz, C1-H′ s), 4.05–3.35 (m, 3 H), 3.00–2.32 (m, 2 H), 2.28 (t, *J* = 2.4 Hz, C3-H); ^{13}C NMR δ 173.17 (C=O), 142.13, 128.82, 127.03, 126.65 (Ar C′ s), 77.46 (C2), 72.55 (C3), 53.31, 38.67, 36.91 (benzylic CH), 31.78. Anal. ($C_{13}H_{13}NO$) C, H, N.

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N-Propargyl-5-phenyl-2-pyrrolidone (33). Compound **33** was prepared from **26** by the above method in 100% yield: bp 124–126 °C (0.2 mmHg); TLC R_f = 0.44 (ether); IR (neat liquid) 3280, 2120, 1690 (br) cm^{-1}; ^1H NMR δ 7.47–7.15 (m, 5 H), 4.88–4.51 (overlapping m and dd, benzylic CH and C1-H′), 3.22 (dd, J_{gem} = 17.3 Hz, J = 2.4 Hz, C1-H″), 2.78–2.30 (m, 3 H), 2.16 (t, J = 2.4 Hz, C3-H), 2.10–1.72 (m, 1 H); ^{13}C NMR δ 174.77 (C=O), 140.12, 128.91, 128.17, 126.59 (Ar C′ s), 77.43 (C2), 71.96 (C3), 61.18 (benzylic CH), 30.11, 29.96, 27.98. Anal. ($C_{13}H_{13}NO$) C, H, N.

N-Propargyl-3-phenyl-2-pyrrolidone (34). Compound **34** was prepared from **29** by the above method in 50% yield after flash chromatography with gradient elution [ether–light petroleum (1:2) → (2:1)]. The product (**34**) was obtained as an oil which solidified after a few days in the freezer. In addition, *N*,3-dipropargyl-3-phenyl-2-pyrrolidone (**35**) was obtained in 22% yield as an oil.

34: mp 51–52 °C; TLC R_f = 0.62 (ether); IR (neat liquid) 3280, 3230, 2110, 1690 cm^{-1}; ^1H NMR δ 7.50–7.16 (m, 5 H), 4.19 (d, J = 2.6 Hz, C1-H′ s), 3.81–3.31 (m, 3 H), 2.76–1.91 (m, 2 H), 2.26 (t, J = 2.6 Hz, C3-H), ^{13}C NMR δ 174.27 (C=O), 139.50, 128.63, 127.79, 126.96 (Ar C′ s), 77.67 (C2), 72.48 (C3), 47.87 (benzylic CH), 44.53, 32.27, 27.73. Anal. ($C_{13}H_{13}NO$) C, H, N.

35: TLC R_f = 0.45 [ether–light petroleum (1:1)]; IR (neat liquid) 3290, 2110, 1690 cm^{-1}; ^1H NMR δ 7.55–7.20 (m, 5 H), 4.18 (d, J = 2.6 Hz, 2 H), 3.65–3.19 (m, 2 H), 2.81 (d, J = 2.4 Hz, 1 H), 2.79 (d, J = 2.6 Hz, 1 H), 2.70–2.40 (m, 2 H), 2.22 (t, J = 2.4 Hz, 1 H), 1.99 (t, J = 2.6 Hz, 1 H); ^{13}C NMR δ 174.62 (C=O), 140.74, 128.57, 127.27, 126.25 (Ar C′ s), 80.54, 77.43, 72.48, 70.91 (acetylenic C′ s), 51.76, 43.33, 32.40, 31.04, 28.54. Anal. ($C_{16}H_{15}NO$) C, H, N.

N-Benzyl-N-(1,2-propadienyl)acetamide (36) and N-Benzyl-N-(2-propynyl)acetamide (37). A solution of *N*-(2-propynyl)acetamide[20] (1.53 g, 15.8 mmol) in THF (35 mL) was added to sodium hydride (522 mg of a 80% dispersion in mineral oil, 17.4 mmol, freed from mineral oil by *n*-hexane washings) at 0 °C (ice bath). Benzyl bromide (2.84 g, 16.6 mmol) was added by use of a syringe, and the mixture was stirred for 4.5 h while the temperature was allowed to reach room temperature. The mixture was filtered, and the filter cake formed was washed with ether. The filtrate was concentrated in vacuo, and the oily residue was purified by flash chromatography using gradient elution [ether–light petroleum (1:3) → ether]. The first fractions gave 0.12 g (4%) of the allenic isomer **36** as an oil: TLC R_f = 0.65 (ether); IR (neat liquid) 1960, 1660 cm^{-1}; ^1H NMR [an asterisk indicates the presence of a pair of signals of almost equal intensity which represent the same proton(s) or carbon(s) of the amide rotamers] δ 7.67* and 6.71* (t, J = 6.6 and 6.1 Hz, respectively, —CH=), 7.41–7.08 (m, 5 H), 5.31* and 5.27* (d, J = 6.1 and 6.3 Hz, respectively, =CH_2), 4.71* and 4.65* (s, benzylic CH_2), 2.26* and 2.12* (s, CH_3); ^{13}C NMR δ 202.02* and 201.21* (=C=), 169.06* and 168.35* (C=O), 137.41, 136.67, 128.64, 128.14, 127.74, 127.19, 126.88, 125.63 (Ar C′ s), 100.78* and 99.21* (=CH_2), 87.38* and 86.88* (—CH=), 49.70* and 46.89* (benzylic CH_2), 22.00* and 21.69* (CH_3).

Further elution gave fractions containing 2.5 g (84%) of **37** as an oil. An analytical sample was obtained after distillation: bp 119–121 °C (0.4–0.5 mmHg); TLC R_f = 0.52 (ether); IR (neat liquid) 3290, 3240, 2110, 1655 cm^{-1}; ^1H NMR (CD_3OD) δ 7.42–7.19 (m, 5 H), 4.71* and 4.64* (s, benzylic CH_2), 4.15* and 4.04* (d, J = 2.4 Hz, C1-H′ s), 2.77* and 2.63* (t, J = 2.4 Hz, C3-H), 2.23* and 2.13* (s, CH_3); ^{13}C NMR δ 169.99 (C=O), 136.39, 135.65, 128.48, 128.17, 127.87, 127.34, 127.09, 126.20, (Ar C′ s), 78.45* and 77.80* (C2), 72.49* and 71.72* (C3), 50.44* and 47.60* (benzylic CH_2), 36.67* and 33.52* (C1), 21.16 (CH_3). Anal. ($C_{12}H_{13}NO$) C, H, N.

N-Methyl-4-(1-pyrrolidinyl)-2-butynylamine (39). A mixture of trifluoroacetamide derivative **38**[22] (1.96 g, 7.89 mmol) and 5 M aqueous NaOH (35 mL) was stirred at room temperature for 2.5 h. Extraction with ether (4 × 100 mL), drying (K_2CO_3), filtration, and concentration of the organic layer afforded 1.08 g (90%) of **39** as an oil which was pure according to ^1H NMR analysis. The ^1H NMR data obtained were in accordance with those reported.[23] TLC R_f (free base on alumina) = 0.2 [ether–methanol (10%)]. An analytical sample was prepared as its dioxalate salt from methanol–acetone–ether: mp 137–139 °C; ^{13}C NMR (dioxalate, D_2O) δ 166.74 (C=O′ s), 80.20 and 79.46 (acetylenic C′ s), 55.18 (pyrrolidine α-C′ s), 44.71 (C4), 39.15 (C1), 33.44 (CH_3), 24.52 (pyrrolidine β-C′ s). Anal. $C_9H_{16}N_2{\cdot}2C_2H_2O_4$) C, H, N.

Synthesis of Test Compounds 3b–9b, 9c, and 11b (Table I). Preparation of N-(4-Pyrrolidino-2-butynyl)-3-phenyl-2-pyrrolidone (4b). A solution of compound **34** (309 mg, 1.55 mmol) in dioxane (10 mL) was added to a mixture of paraformaldehyde (58 mg, 1.94 mmol), CuCl (58 mg, 0.59 mmol), pyrrolidine (127 mg, 1.78 mmol), and glacial acetic acid (93 mg, 1.55 mmol) in dioxane (5 mL). The mixture was stirred at 50 °C for 8 h. The dioxane was evaporated under reduced pressure, and the oily residue was taken up in 3 M aqueous HCl (200 mL) and extracted with ether (3 × 100 mL). The aqueous layer was alkalinized to pH 8 with solid $NaHCO_3$ and extracted with CH_2Cl_2 (4 × 125 mL). The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo. The residue was purified by column chromatography on alumina using ether–methanol (3%) as eluent, yielding **4b** as a light yellow oil. The base was converted into the oxalate salt by addition of a saturated solution of oxalic acid in ether. The oxalate of **4b** was obtained as a viscous oil which solidified after several days in the freezer: TLC R_f (free base on alumina) = 0.49 [ether–methanol (3%)]; IR (free base, neat liquid) 1690 cm^{-1}; ^1H NMR (oxalate, CD_3OD) δ 7.48–7.12 (m, 5 H), 4.30–4.18 (m, 2 H), 4.18–4.05 (m, 2 H), 3.85–3.25 (m′ s, 7 H), 2.80–1.90 (m′ s, 6 H); ^{13}C NMR (oxalate, CD_3OD) δ 177.18 (C=O), 166.49 (oxalate C=O′ s), 141.08, 129.68, 129.03, 128.11 (Ar C′ s), 84.44 (C2), 74.40 (C3), 54.32 (pyrrolidine α-C′ s), 49.45, 46.36, 44.26, 33.23, 29.03, 24.40 (pyrrolidine β-C′ s).

N-Methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-2-phenylacetamide (10b). 2-Phenylacetyl chloride (0.67 g, 4.33 mmol) was added, by use of a syringe, to a cooled (ice bath) mixture of **39** (527 mg, 3.46 mmol), $NaHCO_3$ (1.16 g, 13.84 mmol), CH_2Cl_2 (15 mL), and water (15 mL). The reaction mixture was stirred overnight while the temperature was allowed to reach room temperature. The layers were separated, and the aqueous layer was extracted with ether (4 × 150 mL). The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo. The crude oil was purified by flash chromatography using $CHCl_3$–CH_3OH (9:1) as eluent. The yield of pure **10b** was 745 mg (80%). The oily base was converted into the oxalate salt as described above for **4b** and recrystallized; TLC R_f (free base) = 0.42 [$CHCl_3$–CH_3OH (9:1)]; IR (free base, neat liquid) 1645 cm^{-1}; ^1H NMR (oxalate, CD_3OD) δ (*Z*/*E* ratio = 7:3) 7.34–7.09 (m, 5 H), 4.28 (br, 2 H), 4.08 (br, 2 H), 3.82 (s, *E*-benzylic CH_2), 3.77 (s, *Z*-benzylic CH_2), 3.50–3.21 (m, partially obscured, pyrrolidine α-H′ s), 3.12 (s, *Z*-*N*-CH_3), 2.99 (s, *E*-*N*-CH_3), 2.16–1.91 (m, pyrrolidine β-H′ s); ^{13}C NMR (oxalate, CD_3OD) δ 173.56 (C=O), 166.03 (oxalate C=O′ s), 136.04, 130.02, 129.74, 127.98 (Ar C′ s), 85.67 (*Z*-C2), 84.87 (*E*-C2), 75.11 (*E*-3), 73.78 (*Z*-C3), 54.32 (pyrrolidine α-C′ s), 44.35 (C4), 41.23 (C1), 40.83 (*E*-benzylic CH_2), 37.83 (*Z*-benzylic CH_2), 36.07 (*Z*-*N*-CH_3), 34.03 (*E*-*N*-CH_3), 24.52 (pyrrolidine β-C′ s).

Pharmacology. Guinea Pig Ileum. A standard guinea pig ileum preparation was set up in Tyrode solution (pH 7.4) at 37 °C as described previously.[41] The Tyrode solution contained hexamethonium (0.3 mM). Dissociation constants (K_D) of antagonists were estimated using carbachol as the agonist. The antagonist was allowed to equilibrate with the tissue for 15 min before the addition of carbachol. At least three concentrations of each antagonist were used.

Tremorolytic Activity in Mice. Male Swiss-Webster mice (24–32 g body weight) were used. The test compounds were administered intraperitoneally at a dose of 200 μmol/kg 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 intravenously was estimated by the up-and-down method[42] using intermittent spontaneous (grade 2) tremor[43] as the endpoint. Compounds that showed no significant

(41) 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.
(42) Dixon, W. The Up and Down Method for Small Samples. *J. Am. Stat. Assoc.* **1965**, *60*, 967–978.
(43) See: Cho and Jenden, ref 36.

antagonism of oxotremorine under these conditions were not tested further. Compounds that significantly blocked oxotremorine at a dose of 200 μmol/kg were tested at two additional doses. For the latter compounds, the ED_{50} value of oxotremorine was plotted against the dose of antagonist (including zero) used for premedication. The dose of antagonist which doubled the ED_{50} value of oxotremorine was estimated by linear regression analysis.[44] Under these conditions, atropine and **9a** had tremorolytic doses of 0.9 and 0.6 μmol/kg, respectively.

Muscarinic Receptor Binding Assay. Cerebral cortex from male Sprague-Dawley rats (200–300 g body weight) was homogenized in 50 volumes of 50 mM sodium–potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 30000*g* for 10 min and resuspended in phosphate buffer to a concentration of 10 mg of original wet tissue weight/mL of buffer. The binding of (–)-[^{3}H]NMS (80 Ci/mmol) was measured by the filtration assay of Yamamura and Snyder.[45] Homogenate of cortex (0.1 mL) was incubated with nonlabeled ligand and (–)-[^{3}H]NMS (0.3 nM) in a total volume of 2 mL of 50 mM phosphate buffer. Incubations lasted for 30 min at 30 °C. Binding in the presence of 10 μM atropine was defined as nonspecific. IC_{50} values (concentration of nonlabeled ligand that causes half-maximal inhibition of specific (–)-[^{3}H]NMS binding) were obtained by fitting a one-site inhibition equation to the ligand/(–)-[^{3}H]NMS competition data by nonlinear regression analysis.[46] The IC_{50} values were corrected for receptor occupancy by (–)-[^{3}H]NMS as described by Cheng and Prusoff[47] to give K_i values (concentration that causes half-maximal receptor occupancy in the absence of (–)-[^{3}H]NMS). The dissociation constant of (–)-[^{3}H]NMS (0.076 nM) was determined independently by nonlinear regression analysis of seven-point saturation curves.

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Registry No. **3b**, 137517-95-4; **3b**·oxalate (1:1), 137517-96-5; **4b**, 137517-97-6; **4b**·oxalate (1:1), 137517-98-7; **5b**, 137517-99-8; **5b**·oxalate (1:1), 137518-00-4; **6b**, 137518-01-5; **6b**·oxalate (2:3), 137518-02-6; **7b**, 137518-03-7; **7b**·oxalate (1:1), 137518-04-8; **8b**, 137518-05-9; **8b**·oxalate (1:1), 137518-06-0; **9b**, 137518-07-1; **9b**·oxalate (1:1), 137518-08-2; **9c**, 137518-09-3; **9c**·oxalate (1:1), 137518-10-6; **10b**, 137518-11-7; **10b**·oxalate (1:1), 137518-12-8; **11b**, 137518-13-9; **11b**·oxalate (1:1), 137518-14-0; **13**, 123772-66-7; **14**, 50874-15-2; **14**·oxalate (2:1), 137518-15-1; **15**, 123772-76-9; **16**, 123772-77-0; **19**, 137518-16-2; **20**, 137518-17-3; **21**, 137518-18-4; **21**·oxalate (2:1), 137518-19-5; **22**, 137518-20-8; **23**, 137518-21-9; **24**, 137518-22-0; **25**, 137518-23-1; **26**, 22050-10-8; **27**, 137518-24-2; **28**, 6836-98-2; **29**, 6836-97-1; **30**, 1198-97-6; **31**, 123772-78-1; **31**·oxalate (2:1), 137518-25-3; **32**, 137518-26-4; **33**, 137518-27-5; **34**, 137518-28-6; **35**, 137518-29-7; **36**, 137518-30-0; **37**, 137518-31-1; **38**, 111903-44-7; **39**, 75858-55-8; **39**·oxalate (1:2), 137518-32-2; succinic anhydride, 108-30-5; ethyl 4-bromobutyrate, 2969-81-5; *N,N*-bis[3-(ethoxycarbonyl)propyl]-1-phenyl-2-propynylamine, 137518-33-3; 4-chlorobutyryl chloride, 4635-59-0; 3-benzoylpropionic acid, 2051-95-8; γ-aminobenzenebutanoic acid, 1011-60-5; diethyl phenylalonate, 83-13-6; 1-bromo-2-chloroethane, 107-04-0; ethyl 4-nitro-3-phenylbutyrate, 41441-40-1; propargyl bromide, 106-96-7; *N*-(2-propynyl)acetamide, 65881-41-6; benzyl bromide, 100-39-0; pyrrolidine, 123-75-1; 2-phenylacetyl chloride, 103-80-0.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectral data for compounds **3b**, **5b–9b**, **9c**, **11b**, and *N,N*-bis[3-(ethoxycarbonyl)propyl]-1-phenyl-2-propynylamine and ^{19}F NMR spectral data for compound **9c** (4 pages). Ordering information is given on any current masthead page.

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