β-Lactam Analogues of Oxotremorine. 3- and 4-Methyl-Substituted 2-Azetidinones

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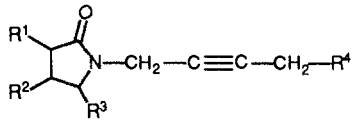
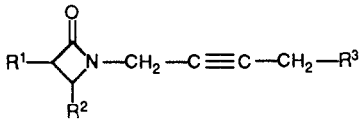
Four β-lactam analogues (8–11) of oxotremorine were synthesized and assayed for muscarinic and antimuscarinic activity on the isolated guinea pig ileum. The pharmacological results were compared with those obtained previously with the β-lactam analogue 7 and the 3-, 4-, and 5-methyl-substituted 2-pyrrolidones 2–6. The new compounds were less potent than the corresponding 2-pyrrolidones, regardless of whether they showed agonist (10 and 11), partial agonist (8), or antagonist properties (9) in the ileum assay. The agonists 10 and 11 were about 200-fold less potent than 7. Compounds 8–11 also were less potent than the similarly substituted 2-pyrrolidones in inhibiting the binding of the muscarinic antagonist (–)-[^{3}H]-*N*-methylscopolamine in homogenates of the rat cerebral cortex.

Oxotremorine (1) is a potent and centrally active muscarinic agonist.[1] Most structural modifications of 1 produce compounds that possess decreased intrinsic efficacy at muscarinic receptors and that are partial agonists or antagonists. Many of these have increased receptor affinity compared to 1.[2] However, β-lactam analogue 7 is a potent muscarinic agonist, being 6-fold less potent than 1.[3,4] In the present investigation, we have introduced methyl groups in the C3- and C4-positions of the β-lactam ring of 7. The new compounds are structurally related to 2–4, all being methyl-substituted derivatives of 1. Methyl substitution in the pyrrolidone ring of 1 produced interesting effects. It abolished efficacy but increased affinity (4), abolished efficacy and reduced affinity (3), or reduced affinity without affecting efficacy (2).[6]



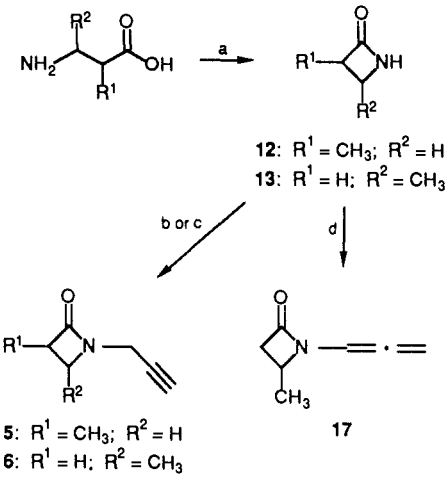
1: $R^1 = R^2 = R^3 = H$; $R^4 = NC_4H_8$
2: $R^1 = CH_3$; $R^2 = R^3 = H$; $R^4 = NC_4H_8$
3: $R^1 = H$; $R^2 = CH_3$; $R^3 = H$; $R^4 = NC_4H_8$
4: $R^1 = R^2 = H$; $R^3 = CH_3$; $R^4 = NC_4H_8$
5: $R^1 = R^2 = H$; $R^3 = CH_3$; $R^4 = N(CH_3)_2$
6: $R^1 = R^2 = H$; $R^3 = CH_3$; $R^4 = N^+(CH_3)_3$



7: $R^1 = R^2 = H$; $R^3 = NC_4H_8$
8: $R^1 = CH_3$; $R^2 = H$; $R^3 = NC_4H_8$
9: $R^1 = H$; $R^2 = CH_3$; $R^3 = NC_4H_8$
10: $R^1 = H$; $R^2 = CH_3$; $R^3 = N(CH_3)_2$
11: $R^1 = H$; $R^2 = CH_3$; $R^3 = N^+(CH_3)_3$

The new derivatives were evaluated for muscarinic and antimuscarinic activity on the isolated guinea pig ileum and for ability to inhibit binding of the muscarinic antagonist (–)-[^{3}H]-*N*-methylscopolamine ([^{3}H]NMS) to homogenates of rat cerebral cortex. Pharmacological results are presented in Table I. A C3-methyl substituent introduced in β-lactam derivative 7 decreased affinity and efficacy at muscarinic receptors whereas C4-methyl substitution in 7 abolished efficacy without having any effect on affinity. The lower potency of β-lactams 8–11 as compared to 2-pyrrolidones 2–4 may be rationalized on the basis of the different spatial positions of the methyl groups in the two series of compounds.

Scheme I[a]



12: $R^1 = CH_3$; $R^2 = H$
13: $R^1 = H$; $R^2 = CH_3$
15: $R^1 = CH_3$; $R^2 = H$
16: $R^1 = H$; $R^2 = CH_3$
17

[a] Reagents: (a) 2-chloro-1-methylpyridinium iodide, $(C_2H_5)_3N$, CH_3CN; (b) propargyl bromide, pulverized K_2CO_3, CH_3CN; (c) propargyl bromide, pulverized KOH, *n*-$Bu_4N^+Br^-$, THF; (d) propargyl bromide, NaH, THF.

Chemistry

Synthesis. 4-Methyl-2-azetidinone (13) was prepared from 3-aminobutanoic acid as described by Huang et al.[7] (see Scheme I). 3-Methyl-2-azetidinone (12) was prepared similarly from 3-amino-2-methylpropanoic acid by use of a minor modification; the yield of 12 was increased to 57%

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(1) Muscarinic agonists and partial agonists are of current interest because of their potential therapeutic benefits in the treatment of disorders associated with cholinergic hypofunction. See, e.g.: (a) Nordström, Ö.; Alberts, P.; Westlind, A.; Unden, A.; Bartfai, T. *Mol. Pharmacol.* **1983**, *24*, 1–5; (b) DeFeudis, F. V. *Drugs Today* **1988**, *24*, 473–490; (c) Hershenson, F.; Moos, W. H. *J. Med. Chem.* **1986**, *29*, 1125–1130; (d) Moos, W. H.; Davis, R. E.; Schwarz, R. C.; Gamzu, E. R. *Med. Res. Rev.* **1988**, *8*, 353–391.
(2) For a recent review, see: Ringdahl, B. In *The Muscarinic Receptors*; Brown, J. H., Ed.; Humana Press Inc.: Clifton NJ. In press.
(3) Resul, B.; Ringdahl, B.; Dahlbom, R. *Eur. J. Med. Chem.* **1981**, *16*, 379–381.
(4) The lower potency of 7 as compared to 1 may be due to the inhibition of resonance in the amide group of the β-lactam; in IR spectroscopy, the C=O absorption frequency of β-lactams is higher than that of 2-pyrrolidones (compare ref 5).
(5) Bebbington, A.; Brimblecombe, R. W. *Adv. Drug. Res.* **1965**, *2*, 143–172.
(6) Ringdahl, B.; Jenden, D. J. *Mol. Pharmacol.* **1983**, *23*, 17–25.
(7) Huang, H.; Iwasawa, N.; Mukaiyama, T. *Chem. Lett.* **1984**, 1465–1466.

0022-2623/90/1833-0580$02.50/0

Table I. In Vitro Muscarinic and Antimuscarinic Activity and Receptor Binding Affinity of Some Oxotremorine Analogues[a]

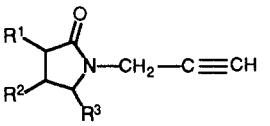
	guinea pig ileum[b]				rat cerebral cortex (-)-[^{3}H]NMS displacement[f]	
compd	N[c]	EC_{50}, μM	E_{max}[d]	K_D,[e] μM	K_i, μM	n_H[g]
1	6	0.035 ± 0.002	100	0.93 ± 0.08	0.83[h]	0.79 ± 0.04[h]
2	6	0.50 ± 0.03	100	11.5 ± 2.6	1.2 ± 0.1	0.75 ± 0.04
3	4		0	10.0 ± 1.1	5.0 ± 0.2	0.86 ± 0.01
4	4		0	0.093 ± 0.01	0.056 ± 0.002	0.75 ± 0.02
5	5	0.91 ± 0.06	102 ± 2	4.3 ± 0.7	2.2 ± 0.1	0.70 ± 0.07
6	4	0.18 ± 0.03	100 ± 1	1.7 ± 0.1	0.61 ± 0.05	0.66 ± 0.04
7	6	0.29 ± 0.02	100		0.94 ± 0.05	0.73 ± 0.01
8	5	24 ± 4	23 ± 4		5.6 ± 0.3	0.77 ± 0.03
9	6		0	1.7 ± 0.1	0.99 ± 0.11	0.80 ± 0.03
10	6	88 ± 9	95 ± 4		43 ± 8	0.77 ± 0.01
11	6	55 ± 8	97 ± 4		19.6 ± 2.0	0.74 ± 0.05

[a] Values are means ± standard errors. [b] Values given for 1–7 on the ileum are from refs 2, 3, 6, and 23. [c] Number of test preparations used. [d] Maximal contractile response as a percentage of that elicited by carbachol. [e] Dissociation constant of the drug–receptor complex. [f] The K_i and n_H values are based on three separate experiments, each performed in triplicate. [g] Apparent Hill coefficient. [h] Values for 1 are from ref 34.

by slow addition (3 h) of the β-amino acid. When all of the 3-amino-2-methylpropanoic acid was added in one portion, the yield of **12** was only 25%. Attempts to prepare **16** by reaction of β-lactam **13** with propargyl bromide and NaH in THF (0 °C → room temperature, 20 h; method III) yielded only allenic isomer **17**. When the reaction was interrupted after 2 h, 70% of the starting material (**13**) was recovered and a 9:1 mixture of **16** and **17** was isolated. Thus, it is apparent that the desired **16** isomerizes to **17** under these reaction conditions. It has been reported that allenic byproducts may be formed in the N-propargylation of 2-pyrrolidones and 2-azetidinones under various conditions.[8]

To investigate if the acetylene–allene isomerization could be minimized, we explored some other reaction conditions: N-alkylation of **12** and **13** by use of propargyl bromide and a large excess of K_2CO_3 in refluxing acetonitrile (method I) afforded the desired N-propargylated products and small amounts (5–10%) of allenic isomers. In addition, unidentified byproducts were formed. Following column chromatography, **15** and **16** were isolated in 38% and 51% yields, respectively. However, the method of choice for N-propargylation of lactams appears to be use of phase-transfer catalysis (method II).[13,14] Treatment of **12** and **13** with propargyl bromide, 0.2 equiv of $(n\text{-Bu})_4N^+Br^-$, and powdered KOH in THF afforded good yields of **15** (93%) and **16** (81%),[15] respectively, free from allenic isomers.

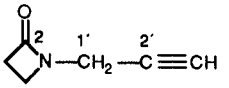
Similarly, when 2-pyrrolidone or 5-methyl-2-pyrrolidone was used as starting materials, method II cleanly produced *N*-propargyl lactams **18** or **21** in good yields (89% and 87%, respectively).[17]



18: $R^1 = R^2 = R^3 = H$
19: $R^1 = CH_3$; $R^2 = R^3 = H$
20: $R^1 = H$; $R^2 = CH_3$; $R^3 = H$
21: $R^1 = R^2 = H$; $R^3 = CH_3$

Target compounds **8–10** were obtained from the corresponding terminal acetylenic precursors **15** and **16**, respectively, by Mannich reactions. Treatment of Mannich base **10** with iodomethane afforded quaternary ammonium salt **11**.

Molecular Mechanics Calculations. In order to investigate differences in geometries between previously prepared pyrrolidone derivatives and the β-lactam derivatives, we studied the conformational distribution of model compounds by molecular mechanics calculations using the MMX-1986 force field.[18] For convenience, calculations were done on model compounds lacking the pyrrolidinomethyl moiety (**14–16** and **18–21**); it is known that, e.g., in oxotremorine,[19] the conformational preferences of the lactam and the pyrrolidine rings are affected very little by the other ring moiety. Therefore, it is not likely that the geometries obtained for the *N*-propargyl lactam moieties of the model compounds would differ considerably from that of the Mannich bases.



14

(8) Allenic byproducts form in the N-propargylation of 2-pyrrolidone by use of NaH,[9] and of methyl-substituted 2-pyrrolidones by use of Na/C_6H_5,[10] NaH,[9,11] or BuLi.[11] The same phenomenon has been reported by Davies et al.[12] in the N-propargylation of 4-(methylthio)-2-azetidinone by using *t*-BuO^-K^+ in DMF.

(9) Dickinson, W. B.; Lang, P. C. *Tetrahedron Lett.* **1967**, 3035–3040.

(10) Ringdahl, B.; Muhi-Eldeen, Z.; Ljunggren, C.;f Karlēn, B.; Resul, B.; Dahlbom, R.; Jenden, D. J. *Acta Pharm. Suec.* **1979**, *16*, 89–94.

(11) Amstutz, R.; Ringdahl, B.; Karlēn, B.; Roch, M.; Jenden, D. J. *J. Med. Chem.* **1985**, *28*, 1760–1765.

(12) Davies, D.; Pearson, M. *J. Chem. Soc., Perkin Trans 1* **1981**, 2539–2543.

(13) Reuschling, D.; Pietsch, H.; Linkies, A. *Tetrahedron Lett.* **1978**, 615–618.

(14) Takahata, H.; Hashizume, T.; Yamazaki, T. *Heterocycles* **1979**, *12*, 1449–1451.

(15) Alkylation of 2-azetidinones with phase-transfer catalysis by use of 18-crown-6, and KOH as a base has also been reported.[16] By this procedure we prepared **16** in 77% yield.

(16) Hirai, Y.; Kamide, I.; Yamazaki, T. *Heterocycles* **1981**, *15*, 1101–1103.

(17) The yield was the same but the reaction was slower when carried out without the phase-transfer catalyst.

(18) MMX is a molecular mechanics program that uses the QSPE 395 and the π-VESCF routines with all of Allinger's options implemented. The MMX program is part of the molecular modeling package PCMODEL (Serena Software, P.O. Box 3076, Bloomington, IN 47402-3076).

(19) Kier, L. M. *J. Pharm. Sci.* **1970**, *59*, 112–114. For the X-ray structure determination of oxotremorine sesquioxalate, see: Dahlbom, R.; Jönsson, P.-G. *J. Pharm. Pharmacol.* **1975**, *27*, 544–546.

The following strategy was used for the calculations: First, possible ring conformations of the lactam rings were constructed and minimized. Second, methyl groups were added to the minimized ring geometries, and the resulting starting geometries were minimized. Third, an *N*-propargyl substituent was added to the minimized rings in 11 different ways so that $\tau_x = \tau$(C2',Cl',N,C2) 0°, 30°, 60°, 90°, 120°, 180°, 210°, 240°, 270°, 300°, and 330° (for numbering of the propargylic lactam system, see formula 14). Fourth, these new starting geometries were minimized. The resulting geometries and related steric energies are presented in the supplementary material. The β-lactam derivatives appeared to have only four distinctly different energy minima whereas the pyrrolidone derivatives had six to eight. This observation is related to the fact that the β-lactam ring is almost planar[20] whereas the pyrrolidone ring preferentially adopts two envelope conformations. Most of the compounds preferred to adopt conformations with τ_x values around 90°, 160°, −160°, and −90°. However, we were able to identify only six local minima of the C5-methyl-substituted pyrrolidone derivative (*R*)-**21**. Apparently, the C5-methyl of (*R*)-**21** destabilizes conformations in which τ_x values are around −160°. The energy-minimized conformations had relative steric energies equal to or less than 1.0 kcal/mol. Thus, all identified conformations may be considered as energetically favored. The geometry of **18** agreed well with that of the same structural moiety in the crystal structure of trimethyl[4-(2-oxopyrrolidin-1-yl)but-2-ynyl]ammonium iodide.[21] In fact, when the pyrrolidone moiety from the X-ray structure was superimposed on the corresponding energy-minimized conformation of **18**, the average distance between the respective ring atoms was 0.045 Å. In addition, our results agree fairly well with those of Kier who performed extended Hückel calculations on oxotremorine in its protonated form.[19] Thus, we believe that the calculations presented here are reliable.

Pharmacology

Compounds **8–11** were tested for muscarinic and antimuscarinic activity in the isolated guinea pig ileum. The C3-methyl-substituted β-lactam **8** was a weak partial agonist producing only a fraction of the maximal contractile response of carbachol (Table I). The corresponding C4-methyl-substituted regioisomer (**9**) elicited no response but was an antagonist to carbachol. The slope of its Schild plot (0.99 ± 0.08) was not significantly different from **1**, suggesting competitive antagonism.[22] The dimethylamino (**10**) and trimethylammonium (**11**) derivatives of **8** were full agonists although of low potency. Their responses, like that of **8**, were antagonized or abolished by *N*-methylatropine.

Compounds **2–11** inhibited the specific binding of (−)-[^{3}H]-NMS in the rat cerebral cortex in vitro (Table I). Apparent Hill coefficients (n_H) were significantly ($P < 0.025$ by Student's *t* test) smaller than **1**, suggesting the presence of more than one binding site for the compounds. However, because of the limited number of inhibitor concentrations used (7–9), the competition data were analyzed by a simple one-site model, which provided a satisfactory fit to the experimental data (Figure 1). The concentrations of the various inhibitors that saturate half of the binding sites (K_i) are given in Table I.

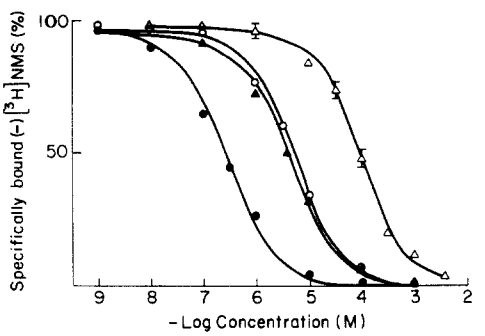


Figure 1. Competitive inhibition of (−)-[^{3}H]NMS binding in rat cerebral cortex by **4** (●), **7** (▲), **9** (○), and **11** (△). Values are means ± standard errors from three experiments each performed in triplicate. The concentration of (−)-[^{3}H]NMS used was 0.3 nM. The theoretical curves are the best fit to a one-site binding equation.

Discussion

The observations that **8** was a weak partial agonist whereas its desmethyl homologue **7** was a full agonist suggests that the C3-methyl substituent of **8** decreased efficacy at ileal muscarinic receptors. The affinity of **8** for cortical receptors, as reflected by its K_i value, was 6-fold lower than that of **7**. Compound **8** also had lower affinity and efficacy than the 3-methyl-2-pyrrolidone **2** whereas its affinity for receptors in the cortex was similar to that of the 4-methyl-2-pyrrolidone **3**.

A shift of the methyl group of **8** to the 4-position of the β-lactam ring (**9**) was accompanied by an additional decrease of efficacy since **9** was a competitive antagonist. As **9** and **7** had almost identical affinity for cortical receptors, it appears that the result of C4-methyl substitution in **7** was a selective reduction of efficacy. The affinity of **9** for muscarinic receptors in the ileum and cortex was almost 20-fold lower than that observed for the 5-methyl-2-pyrrolidone derivative **4**. Similarly, the agonists **10** and **11** were much less potent than the corresponding 5-methyl-2-pyrrolidones (**5** and **6**). The affinity difference between **10** and **5** and between **11** and **6** at cortical receptors agreed well with the difference observed between **9** and **4**. Thus, the incremental changes in affinity caused by identical modification of the amino group of 4-methyl-2-azetidinones and 5-methyl-2-pyrrolidones were similar. This observation confirms that the effects of structural changes in the amino group on affinity are virtually independent of the structure of the lactam ring. Similar observations have been made in other series of oxotremorine analogues.[23,24]

In an attempt to rationalize the low potency of **8** and **9**, we compared, by computer-aided fittings, low-energy (MMX) conformations of model compounds **19–21** with those of **15** and **16**. When the amide moiety and the methylene group of the side chain of the pyrrolidone derivatives were superimposed[25] on the same structural elements of the β-lactams, the methyl groups in the different ring systems assumed quite different spatial positions; for

(20) Yang, Q. C.; Seiler, P.; Dunitz, J. D.; *Acta Crystallogr., Sect. C* **1987**, *43*, 565–567.
(21) Baker, R. W.; Pauling, P. J. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1247–1249.
(22) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48–58.
(23) Ringdahl, B. *J. Med. Chem.* **1988**, *31*, 683–688.
(24) Ringdahl, B. *Eur. J. Pharmacol.* **1987**, *140*, 13–23.
(25) Since the amide functionality is considered to be one of the pharmacophore elements of oxotremorine (compare ref 5), we used it as a stationary point in the superpositions. The position of the methylene group is also of great importance since it will (indirectly) determine the relative position of the protonated basic nitrogen which is another key structural element in the compounds studied.

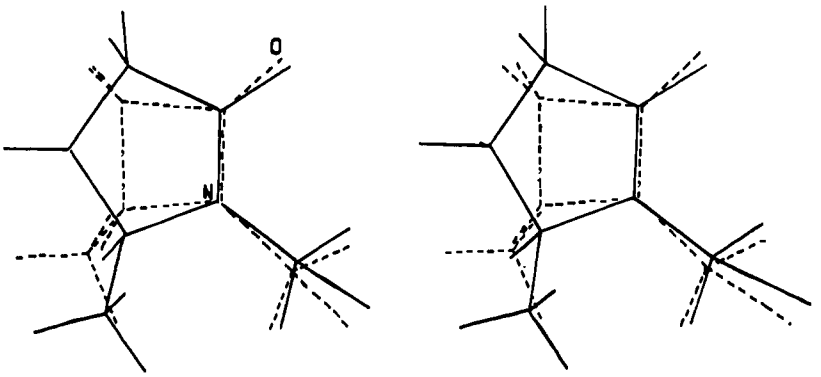


Figure 2. Computer-generated stereopair of low-energy conformations of (*R*)-**21** (solid lines) and (*R*)-**16** (dashed lines). The average distance between fitted atoms (C1′, N, C2, and O) was 0.12 Å. The distance between the two methyl groups was 1.0 Å. The fitting was made by use of the least-squares fitting procedure in MIMIC (methods for interactive modeling in chemistry).[35]

example, in fittings of (*R*)-**21** [a model for the potent antagonist (*R*)-**4**][11] with (*R*)-**16** (a model for the less potent antagonist **9**) the distance between the methyl groups varied from 0.4 to 1.0 Å (compare Figure 2). Similarly, fittings of (*S*)-**19** with (*S*)-**15**, and (*R*)-**20** with (*S*)-**15** or (*R*)-**16**, produced superpositions in which the distance between the methyl groups varied from 0.6 to 1.2 Å, from 1.6 to 2.3 Å, and from 1.7 to 2.4 Å, respectively. The molecular mechanics calculations did not indicate that the methyl groups in the β-lactam derivatives **8** and **9** considerably affected the conformational preferences of the rest of the molecules. Therefore, the most likely rationale for the different effects after methyl substitution in the β-lactam **7** as compared to the pyrrolidone **1** is that the methyl groups of **8** and **9** adopt relative spatial positions that interfere with an optimal receptor interaction.

Experimental Section

Chemistry. 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 NMR and ^{13}C NMR were recorded on a JEOL FX 90Q spectrometer at 89.55 and 22.5 MHz, respectively, and were referenced to internal tetramethylsilane. Assignments of the ^{13}C NMR resonances are based on off-resonance spectra. Mass spectra were obtained on a LKB 9000 spectrometer using a direct insertion probe. All spectra were in accordance with the assigned structures. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} or on alumina plates 60 F_{245} neutral (type E), E. Merck. The elemental analyses, which were performed by Mikro Kemi AB, Uppsala, Sweden, and Dr. H. Malissa and G. Reuter, Engelskirchen, West Germany, were within 0.4% of the calculated values.

4-Methyl-2-azetidinone (13). This compound was prepared on a 16-mmol scale by a literature procedure[7] in 65% yield. The crude product was resuspended in chloroform and purified by column chromatography on silica gel with ether–methanol (19:1) as eluent: TLC R_f = 0.4 [SiO_2, ether–methanol (19:1)]. Spectroscopic data of **13** were in accordance with those reported (IR,[26] ^{1}H NMR,[27] ^{13}C NMR[28]).

3-Methyl-2-azetidinone (12).[26] A procedure similar to that described for **13** was used with a minor modification. A solution of 2-chloro-1-methylpyridinium iodide (4.11 g, 16.1 mmol) and triethylamine (2.96 g, 29.2 mmol) in CH_3CN (730 mL) was heated to reflux under nitrogen, and 3-amino-2-methyl propanoic acid (1.8 g, 14.6 mmol) was added in portions over 3 h. The reaction mixture was heated to reflux for an additional 2 h. The solvent was evaporated, and the residue was resuspended in CH_2Cl_2 and purified by column chromatography on silica gel with ether–methanol (19:1) as eluent. The yield of pure **12** was 0.71 g (57%): TLC R_f = 0.4 [SiO_2, ether–methanol (19:1)]; ^{1}H NMR ($CDCl_3$) δ 7.05–6.45 (br s, NH), 3.46 (app t, 1 H, J = 5 Hz), 3.35–3.05 (m, 1 H), 2.94 (dd, 1 H, J = 2 Hz, J = 5 Hz), 1.32 (d, J = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 172.83 (C=O), 45.65 (C3), 43.21 (C4), 13.29 (C3-CH_3).

General Procedure for the Propargylation of Lactams. Method I. Preparation of *N*-(2-Propynyl)-4-methyl-2-azetidinone (16). Propargyl bromide (2.9 g, 24.4 mmol) was added to a stirred mixture of **13** (1.89 g, 22.2 mmol) and anhydrous powdered K_2CO_3 (30.7 g, 222 mmol) in CH_3CN (80 mL). The mixture was heated to reflux under nitrogen. Additional propargyl bromide (3.16 g, 26.6 mmol) was added after 24 h. After reflux for 45 h the mixture was filtered and the volatiles were evaporated. Column chromatography on silica gel with ether–petroleum ether (4:1) as eluent afforded 1.42 g (51%) of **16**. An analytical sample was obtained after distillation: bp 48 °C/0.3 mmHg; TLC R_f = 0.5 (SiO_2, ether); IR (neat) 3240, 2120, 1745 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.18 (dd, 1 H, J = 2.6 Hz, J = 17.6 Hz), 3.81 (dd, 1 H, J = 2.6 Hz, J = 17.6 Hz), 4.0–3.7 (m, 1 H, partially obscured), 3.09 (dd, 1 H, J = 5 Hz, J = 14.5 Hz), 2.53 (dd, 1 H, J = 2 Hz, J = 14.5 Hz), 2.28 (t, J = 2.4 Hz, C≡C*H*), 1.39 (d, J = 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.13 (C=O), 76.88 (C2′), 72.06 (C3′), 47.38 (C4), 44.08 (C3), 29.35 (C1′), 18.35 (C4-CH_3); MS (20 eV) m/z 123 (M^+). Anal. (C_7H_9NO) C, H, N.

Method II. Preparation of *N*-(2-Propynyl)-3-methyl-2-azetidinone (15). A solution of **12** (505 mg, 5.93 mmol) and propargyl bromide (0.78 g, 6.53 mmol) in THF (4 mL) was added to a stirred mixture of powdered KOH (400 mg, 7.12 mmol) and $(n\text{-Bu})_4N^+Br^-$ (383 mg, 1.19 mmol) in THF (3 mL) at 0 °C. The temperature was then allowed to rise to room temperature. The mixture was filtered after 6 h, and the solvent was evaporated. The residue was chromatographed on silica gel with ether as eluent to give 0.68 g (93%) of pure **15** as an oil. An analytical sample was obtained after distillation: bp 48–50 °C/0.35 mmHg; TLC R_f = 0.45 (SiO_2, ether); IR (neat) 3240, 2120, 1745 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.02 (dd, J = 0.4 Hz, J = 2.6 Hz, NC*H*$_2$C≡C), 3.51 (app t, 1 H, J = 5 Hz), 3.40–3.05 (m, 1 H), 2.98 (dd, 1 H, J = 2.4 Hz, J = 5 Hz), 2.29 (t, J = 2.6 Hz, C≡C*H*), 1.32 (d, J = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.60 (C=O), 76.78 (C2′), 72.48 (C3′), 46.67 (C4), 44.60 (C3), 31.10 (C1′), 13.37 (C3-CH_3); MS (20 eV) m/z 123 (M^+). Anal. (C_7H_9NO) C, H, N.

Method III. Preparation of *N*-Allenyl-4-methyl-2-azetidinone (17). A solution of propargyl bromide (0.29 g, 2.46 mmol) and **13** (0.19 g, 2.23 mmol) in THF (5 mL) was added to a suspension of NaH (80% dispersion in mineral oil, 65 mg, 2.16 mmol) in THF (2 mL) at 0 °C (ice bath) kept under nitrogen. The ice bath was removed after 0.5 h, and the stirring was continued for 20 h. The reaction mixture was filtered and concentrated. The residue was chromatographed on silica gel with ether–petroleum ether (2:1) as eluent, affording 100 mg (36%) of the title compound as an oil which decomposed on standing at room temperature: TLC R_f = 0.75 (SiO_2, ether); IR (neat) 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.68 (t, J = 6.6 Hz, C*H*=C=), 5.40 (d, J = 6.6 Hz, =C=CH_2), 4.0–3.6 (m, 1 H), 3.17 (dd, 1 H, J = 5.2 Hz, J = 14.9 Hz), 2.58 (dd, 1 H, J = 2.6 Hz, J = 14.9 Hz), 1.39 (d, J = 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 201.74 (=C=), 164.09 (C=O), 92.66 (=CH_2),

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86.64 (—CH=), 47.72 (C4), 44.51 (C3), 18.10 (C4-CH_3); MS (20 eV) m/z 123 (M^+).

***N*-(4-Pyrrolidinyl-2-butynyl)-3-methyl-2-azetidinone Sesquioxalate (8).** A solution of **16** (187 mg, 1.52 mmol) in dioxane (5 mL) was added to a stirred mixture of paraformaldehyde (59 mg, 1.97 mmol), CuCl (catalytic amount), glacial acetic acid (100 mg, 1.67 mmol), and pyrrolidine (108 mg, 1.52 mmol) in dioxane (20 mL). The mixture was kept at 45 °C for 7 h and was concentrated. The residue was purified by column chromatography on alumina with ether–methanol (24:1) as eluent to afford 300 mg (96%) of pure **8** as an oil. The base was converted to the sesquioxalate: mp 92.5–94 °C (from methanol–ether); TLC R_f (base) = 0.45 [Al_2O_3, ether–methanol (24:1)]; IR (neat, base) 1750 cm^{-1}; ^1H NMR (CD_3OD) δ 4.14 (m, $CH_2C{\equiv}CCH_2$), 3.6–3.1 (overlapping m's, 6 H), 3.02 (dd, 1 H, J = 2.2 Hz, J = 5.3 Hz), 2.10 (m, pyrrolidine β-H's), 1.26 (d, J = 7.2 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 173.16 (ring C=O), 164.89 (oxalate C=O), 83.73 (C2′), 74.65 (C3′), 54.39 (pyrrolidine, α-C's), 48.24, 45.55, 44.32, 32.32 (C1′), 24.46 (pyrrolidine, β-C's), 13.50 (C3-CH_3). Anal. ($C_{12}H_{18}N_2O{\cdot}1.5C_2H_2O_4$) C, H, N.

***N*-(4-Pyrrolidinyl-2-butynyl)-4-methyl-2-azetidinone Oxalate (9).** Compound **9** was prepared from **16** by the procedure described above. The crude Mannich base was purified by acid/base extraction (1 M HCl/ether and $NaHCO_3/CH_2Cl_2$) before being converted to the oxalate salt: yield 57%; mp 90–91.5 °C (from ethanol–acetone–ether); IR (neat, base) 1750 cm^{-1}; ^1H NMR (CD_3OD) δ 4.13 (m, $CH_2C{\equiv}CCH_2$), 4.0–3.7 (m, 1 H), 3.43 (m, pyrrolidine α-H's), 3.09 (dd, 1 H, J = 5 Hz, J = 14.7 Hz), 2.54 (dd, 1 H, J = 2.4 Hz, J = 14.7 Hz), 2.10 (m, pyrrolidine β-H's), 1.38 (d, J = 6.1 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 168.93 (C=O), 166.31 (C=O), 84.07 (C2′), 74.31 (C3′), 54.32 (pyrrolidine, α-C's), 49.51, 44.69, 44.26, 30.42 (C1′), 24.46 (pyrrolidine, β-C's), 18.65 (C4-CH_3). Anal. ($C_{12}H_{18}N_2O{\cdot}C_2H_2O_4$) C, H, N.

***N*-[4-(Dimethylamino)-2-butynyl]-4-methyl-2-azetidinone Oxalate (10).** Compound **10** was prepared in a sealed flask by a procedure similar to that described for **8**. An excess of dimethylamine was added at 0 °C. The yield of **10** was 69%: mp 106–107 °C (from acetone–methanol–ether); IR (neat, base) 1750 cm^{-1}; ^1H NMR (CD_3OD) δ 4.19–4.03 (m, $CH_2C{\equiv}CCH_2$), 4.0–3.7 (m, 1 H), 3.09 (dd, 1 H, J = 4.8 Hz, J = 14.7 Hz), 2.92 [s, $N(CH_3)_2$], 2.53 (dd, 1 H, J = 2.4 Hz, J = 14.7 Hz), 1.38 (d, J = 6.1 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 168.90 (C=O), 166.34 (C=O), 85.36 (C2′), 73.47 (C3′), 49.51, 47.68, 44.69, 42.71, 30.45 (C1′), 18.68 (C4-CH_3). Anal. ($C_{10}H_{16}N_2O{\cdot}C_2H_2O_4$) C, H, N.

***N*-[4-(Dimethylamino)-2-butynyl]-4-methyl-2-azetidinone Methiodide (11).** Iodomethane (530 mg, 3.76 mmol) was added to a solution of **10** (170 mg, 0.94 mmol) in acetone (10 mL). Ether was added after 12 h, and the precipitate formed was recrystallized from acetone–methanol–ether to give 287 mg (95%) of **11**: mp 127–128 °C; IR(KBr) 1755 cm^{-1}; ^1H NMR (CD_3OD) δ 4.52 (t, 2 H, J = 2 Hz), 4.22 (m, 2 H), 4.08–3.78 (m, 1 H), 3.30 [s, $N^+(CH_3)_3$], 3.13 (dd, 1 H, J = 5 Hz, J = 14.5 Hz), 2.56 (dd, 1 H, J = 2.2 Hz, J = 14.6 Hz), 1.42 (d, J = 5.9 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 168.96 (C=O), 87.71 (C2′), 72.61 (C3′), 57.51, 53.64, 49.72, 44.81, 31.01 (C1′), 18.93 (C4-CH_3). Anal. ($C_{11}H_{19}IN_2O$) C, H, N.

Guinea Pig Ileum. A standard guinea pig ileum preparation was set up in Tyrode solution (pH 7.4) at 37 °C as described previously.[29] 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. Spasmogenic activity (EC_{50} values) of **8**, **10**, and **11** was estimated by interpolation at the 50% response level of each compound. The dissociation constant of the antagonist **9** was estimated by using carbachol as the agonist.[22] Compound **9** was allowed to equilibrate with the tissue for 15 min before the addition of carbachol. Five different concentrations of **9** were used.

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 30000g 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.[30] 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.[31] The IC_{50} values were corrected for receptor occupancy by (–)-[^{3}H]NMS as described by Cheng and Prusoff,[32] 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. Apparent Hill coefficients (n_H) were calculated as described by Weiland and Molinoff.[33]

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Supplementary Material Available: Identified low-energy molecular mechanics (MMX) conformations of compounds **14**, (*R*)-**15**, (*R*)-**16**, **18**, (*R*)-**19**, (*R*)-**20**, and (*R*)-**21** (2 pages). Ordering information is given on any current masthead page.

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