Muscarinic Ganglionic Stimulants: Conformationally Restrained Analogues Related to [4-[[*N*-(3-Chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium Chloride

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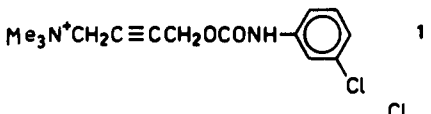
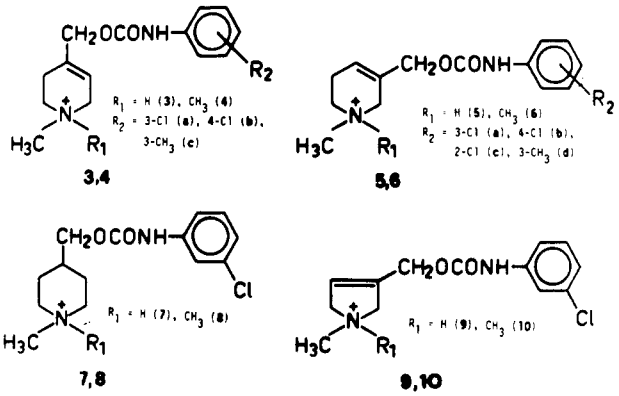
The synthesis of a series of tertiary and quaternary cyclic analogues (isoarecolinol, dihydroisoarecolinol, arecolinol, and 3-pyrroline-3-carbinol derivatives) of [4-[[*N*-(3-chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium chloride (McN-A-343) (**1**), a selective stimulant of muscarinic receptors in sympathetic ganglia (so-called M_1 receptors), is reported. The compounds **3–10** were tested for muscarinic ganglion-stimulating activity by recording blood pressure responses in pithed rats. All tertiary compounds tested had no ganglion-stimulating activity. Among the series of quaternary derivatives, only the isoarecolinol analogues **4a** and **4b** showed considerable ganglion-stimulating effects, whereas the dihydroisoarecolinol (**8**), the arecolinol (**6a, 6b**), and the 3-pyrroline-3-carbinol derivatives (**10**) were much less potent. Our experiments therefore demonstrate that in this series a quaternary nitrogen atom, unsaturation at C_2 of the ammonium side chain, and a certain spatial arrangement of the ammonium and the phenylcarbamate groups are essential for potent M_1-receptor stimulating activity.

The concept of muscarinic receptor subclassification into M_1- and M_2-receptor subtypes is mainly based on the discriminative properties of the selective muscarinic agonist [4-[[*N*-(3-chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium chloride (McN-A-343) (**1**) and the antimuscarinic agent pirenzepine.[1-10] In anesthetized and pithed animals, **1** causes a prominent rise in arterial blood pressure by selective stimulation of excitatory muscarinic receptors in sympathetic ganglia (so-called M_1 receptors).[1,4,9] This M_1 receptor mediated pressor response can be selectively antagonized by low doses of pirenzepine that have little or no effect on muscarinic receptors in effector organs such as heart or vascular endothelium (so-called M_2 receptors).[4,9]



On the basis of the finding that the *trans*-dihydro derivative of **1** (**2a**) and its 4-chlorophenyl analogue **2b** still retain powerful ganglion-stimulating M_1-receptor activity[11-14] (Figure 1), we have synthesized a series of cyclic analogues of **2a** and **2b**, in which the conformational flexibility of the cationic head has been reduced by incorporation into a ring system. All compounds (tertiary and quaternary phenyl carbamates of isoarecolinol (**3, 4**), arecolinol (**5, 6**), dihydroisoarecolinol (**7, 8**), and 3-pyrroline-3-carbinol (**9, 10**)) were tested for M_1-receptor stimulating activity by recording blood pressure responses in pithed rats. The structural requirements necessary for the activation of ganglionic muscarinic receptors in this series of compounds are discussed.

Chemistry. The starting materials (1-methyl-4-(hydroxymethyl)-1,2,5,6-tetrahydropyridine (isoarecolinol),[15] 1-methyl-3-(hydroxymethyl)-1,2,5,6-tetrahydropyridine (arecolinol),[16] and 1-methyl-4-(hydroxymethyl)piperidine (dihydroisoarecolinol))[17] were prepared according to reported methods. 1-Methyl-3-(hydroxymethyl)-3-pyrroline (**11**) was obtained by $LiAlH_4$ reduction of 1-methyl-



pyrroline-3-carboxylic acid methyl ester[18] at low temperature to avoid hydrogenation of the ring double bond. The

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Table I. Aromatic Substituted Carbamates of Cyclic Amino Alcohols Related to 1 (McN-A-343)

compd	salt	mp,[a,b] °C	recrystn solvent	yield[c]	formula	anal.
3a	HCl	224–227	acetone	63	$C_{14}H_{18}Cl_2N_2O_2$	C, H, N
3b	HCl	197–199	ethanol	88	$C_{14}H_{18}Cl_2N_2O_2$	C, H, N, Cl
3c	HCl	204–206	ethanol	79	$C_{15}H_{21}ClN_2O_2$	C, H, N, Cl
4a	tosylate	152–154	acetone/ether	50	$C_{22}H_{27}ClN_2O_5S$	C, H, N
4b	bromide	217–219	acetonitrile	42	$C_{15}H_{20}ClBrN_2O_2$	C, H, N, Cl
5a	HBr	157–159	acetone/ether	88	$C_{14}H_{18}ClBrN_2O_2$	C, H, N
5b	HCl	208–210	ethanol	40	$C_{14}H_{18}Cl_2N_2O_2$	C, H, N, Cl
5c	HCl	158–160	acetonitrile	46	$C_{14}H_{18}Cl_2N_2O_2$	C, H, N, Cl
5d	HCl	209–211	ethanol	68	$C_{15}H_{21}ClN_2O_2$	C, H, N, Cl
6a	iodide	162–164	acetone/ether	64	$C_{15}H_{20}ClJN_2O_2$	C, H, N
6b	iodide	192–194	methanol	58	$C_{15}H_{20}ClJN_2O_2$	C, H, N, Cl, J
7	HBr	227–229	methanol/ether	87	$C_{14}H_{20}ClBrN_2O_2$	C, H, N
8	iodide	176–178	acetone/ether	88	$C_{15}H_{22}ClJN_2O_2$	C, H, N
9	HBr	175–177	acetone/ether	78	$C_{13}H_{16}ClBrN_2O_2$	C, H, N
10	tosylate	139–140	methanol/ether	78	$C_{21}H_{25}ClBrN_2O_2$	C, H, N

[a] Decomposition. [b] Colorless crystals. [c] Related to bases.

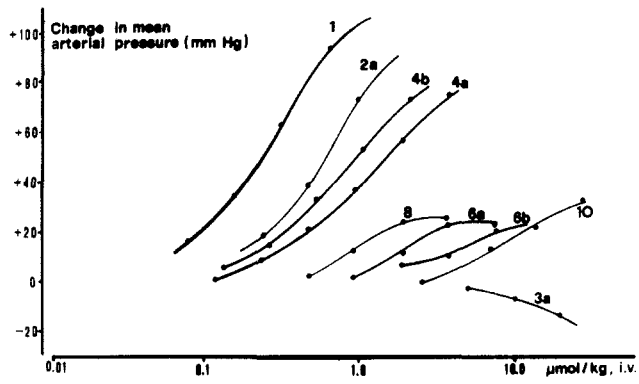

Figure 1. Blood pressure effects of **1**, **2a**, and some cyclic analogues in the pithed rat (n = 2–7, $\bar{x}$ values are shown). For reasons of clearness, no bars indicating statistical limits are shown. The average SEM amounted to about 10–15%.

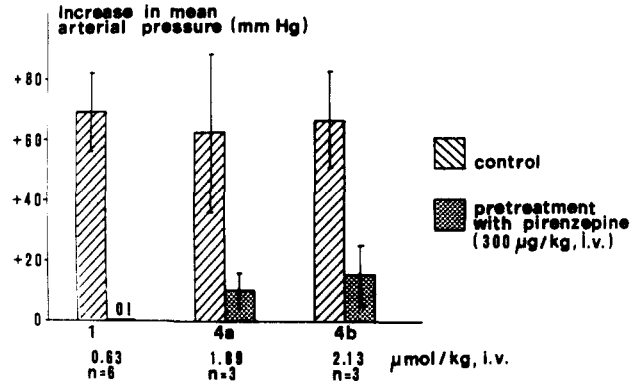

Figure 2. Effect of pirenzepine (300 μg/kg iv) on the pressor effects of **1**, **4a**, and **4b** in the pithed rat ($\bar{x}$ ± SD are shown, n = number of experiments).

alcohols were esterified with chloro- or methyl-substituted phenylisocyanates (Experimental Section and Table I), and the tertiary carbamates were converted to the quaternary ammonium derivatives by standard methods.

Results and Discussion

The most striking finding of our experiments was the observed difference in blood pressure responses to the tertiary and quaternary compounds. All tertiary cyclic analogues of **1** (**3a–c**, **5a–d**, **7**, **9**) were devoid of ganglion-stimulating M_1-receptor activity (i.e., no pressor response was observed). On the contrary, doses higher than 2 μmol/kg iv produced dose-dependent depressor effects, which were only partly blocked by atropine (0.5 mg/kg iv). The dose–response curves of all tertiary analogues (**3a–c**, **5a–d**, **7**, **9**) were nearly identical with that of **3a**, which is shown as an example for the whole series in Figure 1.

In contrast to the tertiary compounds, all quaternary derivatives (**4a,b**, **6a,b**, **8**, **10**) produced pressor effects, but great quantitative differences were observed. The most potent agents among the quaternary cyclic analogues were the 3- and 4-chloro-substituted isoarecolinol derivatives (**4a**, **4b**). Both compounds were of similar potency (about one-fourth to one-sixth as potent as **1**), leading to dose-dependent increases in mean arterial pressure in a dose range from 0.1 to 3 μmol/kg iv (Figure 1). These pressor responses were mainly mediated by ganglionic muscarinic M_1 receptors, as pirenzepine (300 μg/kg iv) strongly antagonized these effects (80% block) (Figure 2). The remaining pressor activity, which was not blocked by pirenzepine, could also not be antagonized by atropine or hexamethonium. Hydrogenation of the ring double bond in **4a** led to the piperidine derivative **8**, which exhibited only weak pipenzepine-sensitive pressor activity ($^1/_{15}$ as potent as **1**, maximum increase: 20–30 mmHg only) (Figure 1). This finding is in accordance with the view expressed earlier by Nelson et al.[11,12] that in the series of derivatives of **1** C-2 unsaturation is essential for strong muscarinic ganglion-stimulating activity.

A dramatic decrease in ganglion-stimulating potency (about $^1/_{100}$ compared to that of **1**) was also observed for the quaternary pyrroline derivative **10**, which produced only small pirenzepine-sensitive pressore responses at a higher dose range (3–30 μmol/kg) (Figure 1). As a result of the poor conformational flexibility of the pyrroline ring system, **10** might not be able to exist in a conformation with a distance of about 5.7 Å[11,12] between the quaternary nitrogen and the carbamate ether oxygen, which has been shown to be necessary for an optimal fit to the receptor.

The same may be true for the quaternary arecolinol derivatives **6a** and **6b**, which do no longer possess a chain of four carbon atoms linking the carbamate group with the quaternary nitrogen atom. Both compounds were about 50 times less potent than **1** with a maximum increase in mean arterial pressure of about 20–30 mmHg only (Figure 1). However, it cannot be excluded that electronic parameters, such as an unfavorable electronic interaction between the carbamate group and the double bond, might be responsible for the weak activity of **6a** and **6b**.

In conclusion, our experiments on cyclic analogues of **1** confirm the assumption of Nelson et al.,[11,12] made on the basis of pharmacological data on open-chain derivatives like **2a,b**, that in this series of compounds a quaternary nitrogen, unsaturation at C-2 of the amino alcohol group, and a defined distance (5.7 Å) between the phenyl carbamate and the ammonium group are essential for strong muscarinic ganglion-stimulating activity. In our experiments, only compounds **4a** and **4b** fulfill these demands.

Experimental Section

Chemical Methods. Melting points were determined in open glass capillaries with a Büchi-Tottoli melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 spectrophotometer. ^1H NMR spectra were determined on a Perkin-Elmer R 32 spectrometer with Me_4Si as an internal standard. All spectral data for the products were consistent with the assigned structures. Microanalyses were performed by the Department of Organic Chemistry, University of Frankfurt, FRG, and were correct within ±0.4% of the theoretical values. TLC was performed on Merck silica gel plates 60 F 254 in toluene/chloroform/acetone (10:6:4). Spots were visualized under 254-nm illumination or with Dragendorff spraying reagent.

General Procedure for the Preparation of Carbamates 3–10. To a stirred solution of 15 mmol of the corresponding alcohol in 40 mL of anhydrous Et_2O was added dropwise a solution of 16 mmol of the substituted phenyl isocyanate in 25 mL of anhydrous Et_2O. The mixture was stirred for 24 h at room temperature. The insoluble solid (*N,N'*-bis(4- or 3-chlorophenyl)urea) was removed by filtration and the Et_2O removed by rotary evaporation. The residue was dissolved in dilute HCl and was extracted with Et_2O (3 × 30 mL). The aqueous layer was made alkaline with saturated sodium carbonate and extracted with Et_2O (3 × 30 mL). The extract was dried (Na_2SO_4), and the solvent was evaporated to yield yellow or colorless oils, which solidified upon standing. The products were used without further purification to form the desired tertiary and quaternary salts (Table I) in the usual manner; IR (KBr/Nujol) ν_{max} 1720–1740 (C=O), 1595–1612 (C=C), 1215–1230 (C–O–C); ^1H NMR (CD_3OD) δ 6.77–7.93 (m, 4 H, aromatic), 5.8–6.2 (m, 1 H, C=CH), 4.4–4.7 (s, 2 H, OCH_2), 2.7–2.97 (s, 3 H, NCH_3), 3.15–3.25 (s, 6 H, $^+N(CH_3)_2$).

***N*-Methyl-3-(hydroxymethyl)-3-pyrroline (11).** To a stirred suspension of 0.26 g (6.8 mmol) of $LiAlH_4$ in 26 mL of anhydrous THF, cooled at −45 °C, was added dropwise 1.7 g (12 mmol) of *N*-methyl-3-pyrroline-3-carboxylic acid methyl ester[18] in 17 mL of anhydrous THF over a period of 0.5 h. The mixture was stirred at −40 °C for 3 h. The excess of $LiAlH_4$ was carefully decomposed by dropwise treatment with acetone/H_2O (1:1). After addition of 20 mL of ether, the white solid was removed by suction filtration and was washed with acetone. The solvent was removed by rotary evaporation, and the oily residue was purified by vacuum distillation to yield 0.74 g (54%) of a colorless oil; bp 55–57 °C (0.1–0.2 mmHg); mp (HBr/acetone) 95 °C; IR (KBr) ν_{max} 3050 (OH); ^1H NMR ($CDCl_3$) δ 5.65 (m, 1 H, C=CH), 4.15 (s, 2 H, CH_2O), 3.48–3.67 (m, 4 H, CH_2NCH_2), 2.47 (s, 3 H, NCH_3). Anal. (picrate) ($C_{16}H_{20}N_4O_9$) C, H, N.

Pharmacology. Male Wistar rats weighing ca. 200–300 g were anesthetized with pentobarbitone sodium (60 mg/kg ip). The left jugular vein was cannulated for the administration of drugs. Arterial blood pressure was measured from the cannulated right common carotid artery. After catheterization of the trachea, the rats were pithed with a steel rod and artificial respiration with room air was provided (1 mL/100 g of body weight, 60 strokes/min). Body temperature was carefully monitored with a rectal thermometer and was maintained at 37 ± 1 °C by an overhead heating lamp. All drugs were dissolved in 0.9% saline and given iv.

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Molecular Basis for Anticancer Drug Amplification: Interaction of Phleomycin Amplifiers with DNA

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The interaction of two phleomycin amplifiers, *N,N*-dimethyl-2-[[4'-(thien-2''-yl)pyrimidin-2'-yl]thio]ethylamine (**1S**, high activity) and *N*-[2''-(dimethylamino)ethyl]-4-(thien-2'-yl)pyrimidin-2-amine (**1N**, low activity) with DNA has been evaluated. The visible absorption bands of both compounds shift to longer wavelengths, and both exhibit hypochromicity on titration with DNA. The effects for **1S** at low concentration are significantly greater than for **1N**. **1S** increases the DNA Tm by 2.5 °C while **1N** causes only a 1.0 °C increase under the same conditions. Spectrophotometric binding analysis of the interaction of **1S** and **1N** with calf thymus DNA indicates that **1S** binds over 4 times more strongly to this DNA than **1N**. Both compounds increase DNA viscosity, cause downfield shifts in DNA ^{31}P NMR spectra, and shift the DNA imino base pair protons upfield, conclusively demonstrating that they bind to DNA by intercalation. Signals for the aromatic protons of **1S** and **1N** are shifted upfield on addition of DNA as expected for intercalation. The shifts for all aromatic protons are similar on **1S** and on **1N**, indicating that both the pyrimidine and thiophene are inserted between the DNA base pairs in the complex. NOE experiments demonstrate that the compounds are in the s-cis conformation both free in solution and in the DNA intercalation complex. Semiempirical INDO/S calculations indicate greater polarization of the π-electron system of **1S** than **1N**. This greater polarization may account for the stronger interaction of **1S** with DNA base pairs than **1N**. The interaction of these compounds with DNA is strongly correlated with their biological amplification activity.

One method of enhancing the activity of currently available anticancer drugs is to find compounds that alone may have no activity but that amplify the action of the drugs. Considerable biological data are available on the activity amplification of phleomycin (PLM) and bleomycin (BLM) by a variety of compounds (amplifiers);[1] however, little is known about the mechanism(s) of amplification in these or any other systems. The best amplifiers of PLM and BLM are composed of at least two fused or unfused conjugated aromatic rings, and they are cationic or can acquire a positive charge by protonation of a nitrogen. As a rule, an anionic or potential anionic center in the molecule decreases activity. Since these features affect the

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