Alkylating Partial Muscarinic Agonists Related to Oxotremorine. *N*-[4-[(2-Haloethyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidones

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N-[4-[(2-Chloroethyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone (**3**) and *N*-[4-[(2-bromoethyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone (**4**) were synthesized. Compounds **3** and **4** cyclized in neutral aqueous solution to an aziridinium ion (**4A**). The rate constants for the cyclization of **3** and **4** at 37 °C were 0.025 and 0.89 min^{-1}, respectively. The aziridinium ion was equipotent with carbachol as a muscarinic agonist on the isolated guinea pig ileum. It was more potent than the corresponding 2-pyrrolidone derivative (**2A**) in alkylating muscarinic receptors in homogenates of the rat cerebral cortex. This higher potency was due to greater receptor affinity of **4A** as compared to **2A** rather than to greater rate constant for alkylation of muscarinic receptors. These properties of **3** and **4** and their low toxicity should make them valuable tools for receptor inactivation studies in vivo and in vitro.

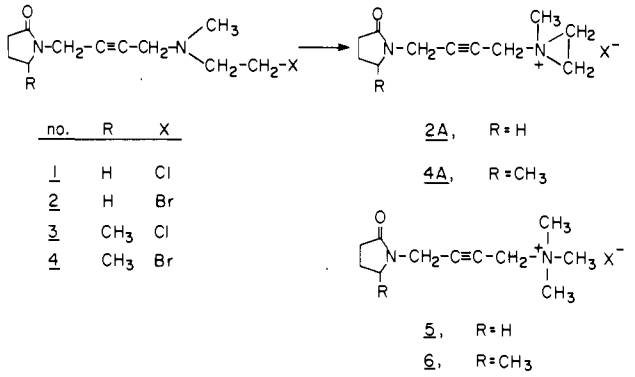
Irreversibly acting 2-chloroethylamine derivatives of muscarinic antagonists have wide application in studies of muscarinic receptors. For example, [^{3}H]propylbenzilylcholine mustard (PrBCM)[1,2] has been used in various receptor binding and labeling experiments[2-4] and in autoradiographic localization of muscarinic receptors.[5] More recently, [^{3}H]PrBCM has been employed in peptide mapping studies on muscarinic receptors[6] whose amino acid sequences have been established recently.[7] The latter studies are aimed at defining the ligand binding site on the receptor protein, in particular the residue that interacts with the positively charged nitrogen present in most muscarinic agonists and antagonists.[6]

Irreversibly acting agonists may have even broader application than antagonists to the study of muscarinic receptors and their function. For example, they may be more suited for elucidation of mechanisms of receptor activation and desensitization. We have described previously some alkylating analogues of the muscarinic agent oxotremorine, *N*-(4-pyrrolidino-2-butynyl)-2-pyrrolidone. The 2-chloroethylamine **1** (BM 123)[8] and the 2-bromoethylamine **2** (BR 401)[9] (Scheme I) cyclized spontaneously at neutral pH to an aziridinium ion (**2A**). This ion produced profound muscarinic effects in vitro and in vivo.[8-11] It also caused irreversible reduction in the binding of ^{3}H-labeled antagonists to muscarinic receptors in various neuronal and muscular tissues.[8,12,13] These properties of **1** and **2** and their ability to enter the central nervous system after systemic administration have made them valuable affinity labels for muscarinic receptors.[14,15] We report here the synthesis of two new haloethylamines, **3** (EK 17) and **4** (BR 402), which in being partial agonists lie between PrBCM and **1** in pharmacological profile. We also show that **3** and **4** are more potent than **1** and **2** in alkylating muscarinic receptors in the rat cerebral cortex. The higher potency of **3** and **4** is due to greater receptor affinity of the aziridinium ion (**4A**) rather than to greater rate of alkylation after formation of the reversible drug–receptor complex.

Results

Synthesis of 3 and 4. The amino alcohol **7**, used as a precursor of **3** and **4**, was synthesized in a Mannich reaction from 5-methyl-*N*-(2-propynyl)-2-pyrrolidone, paraformaldehyde, and *N*-methyl-2-hydroxyethylamine. The 2-chloroethylamine **3** was obtained as the hydrochloride salt by treatment of **7** with triphenylphosphine and carbon tetrachloride in dichloromethane. The 2-bromoethylamine **4** was prepared as the oxalate salt in an analogous reaction using carbon tetrabromide in place of carbon tetrachloride.

Scheme I



Further details are given in the Experimental Section.

Kinetics of Solvolysis of 3 and 4. Rate constants for the cyclization (k_1) of **3** and **4** were estimated from halide ion release data and are summarized in Table I. The cyclization reaction followed first-order kinetics (Figure 1). Compound **4** cyclized about 35-fold more rapidly than **3**. These results agree with those obtained previously with **1** and **2**.[8,9] Because of its slow cyclization, **3** gave rise to lower peak concentrations of the aziridinium ion than did **4** (Figure 1). As expected, the rate constant for the decay

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Table I. Kinetic Parameters for the Solvolysis of 2-Haloethylamine Analogues of Oxotremorine in 50 mM Phosphate Buffer (pH 7.4) at 37 °C[a]

2-haloethylamine $\xrightarrow{k_1}$ aziridinium ion $\xrightarrow{k_2}$ hydrolysis products

no.	k_1,[b] min^{-1}	$t_{1/2}$, min	k_2,[c] min^{-1}	$t_{1/2}$, min	peak level of aziridinium ion,[d] %
1[e]	0.019	36.5	0.033	21.0	28
2[f]	0.85 ± 0.04	0.8	0.040 ± 0.001	17.5	87
3	0.025 ± 0.010	27.7	0.045 ± 0.002	15.3	27
4	0.89 ± 0.04	0.8	0.060 ± 0.004	11.6	75

[a] Values for k_1 and k_2 are means ± standard error from at least three estimates. [b] Apparent first-order rate constants for the cyclization reaction. [c] Rate constant for the decay of the aziridinium ion. [d] Peak levels of aziridinium ion are given as a percentage of the initial concentration of the parent 2-haloethylamine. [e] Values for 1 are from ref 8 and were obtained in 30 mM phosphate buffer at pH 7.0. [f] Values for 2 are from ref 9.

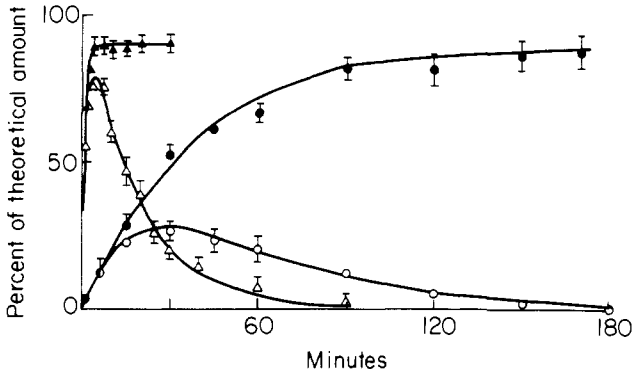

Figure 1. Halide ion release from 3 (●) and 4 (▲) and formation and decay of the aziridinium ion (4A) from 3 (○) and 4 (△) in 50 mM phosphate buffer (pH 7.4) at 37 °C. The ordinate shows concentrations of halide and aziridinium ions as a percentage of the maximum of 1 equiv/mol of the parent compounds. Values are means ± standard errors from three to five determinations. The theoretical curves are the best fit to the relevant rate equations.

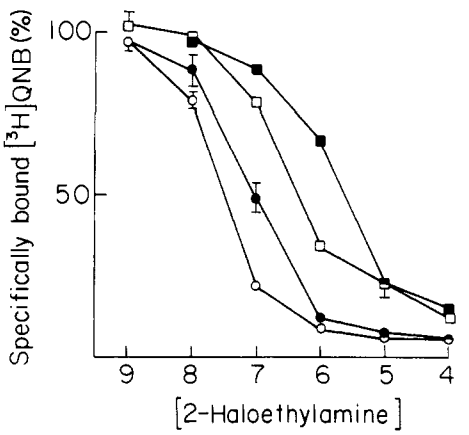

Figure 2. Specific binding of (−)-[^{3}H]QNB to the rat cerebral cortex treated with 1 (■), 2 (□), 3 (●), and 4 (○). Cortical homogenates were treated at 37 °C with the indicated concentrations of precyclized 1, 2, 3, and 4 for 30 min and then washed extensively. Each data point represents the mean binding value of five to six experiments, each done in triplicate. Vertical bars show standard errors. Data for 1 were obtained from ref 14.

(k_2) of the aziridinium was similar whether the ion was formed from 3 or 4 (Table I). The kinetics of solvolysis of 4 was also studied at 24 °C. At this temperature 4 had a k_1 of 0.18 ± 0.02 min^{-1} and a k_2 of 0.0096 ± 0.0007 min^{-1}.

Muscarinic Activity in Vitro. Compounds 3 and 4 were assayed as muscarinic agonists on the guinea pig ileum. The potency of solutions of 3 and 4 in phosphate buffer varied greatly with time after dissolution. At the time of peak activity which coincided with the time of peak aziridinium ion concentration, 3 was 3.6-fold less potent than 4, which was 1.33 ± 0.07 (N = 9) times less potent than carbachol. Both compounds produced the same maximum response as carbachol. Compound 4, after cyclization, exceeded the trimethylammonium derivative 6 in muscarinic activity by (1.6 ± 0.09)-fold (N = 7). With the intermittent dose–response technique used, the tissue was exposed to each agonist concentration for about 15 s. Under these conditions, no alkylation of muscarinic receptors by 3 and 4 was detected. In contrast, when 3 and 4 were assayed by the cumulative dose–response technique (exposure time 1.5–2 min), there was a decrease (about 2–3-fold) in the sensitivity of the ileum to carbachol following exposure to 3 and 4. N-Methylatropine (0.1 μM) inhibited or abolished the actions of 3 and 4 whereas hexamethonium (0.3 mM) was without effect. These observations confirm the muscarinic nature of the contractile responses to 3 and 4. Treatment of solutions of 4 with sodium thiosulfate (0.3 mM for 15 min) abolished the response. The amino alcohol 7 elicited no contractions of the ileum but was a relatively weak antagonist.

On strips of the guinea pig urinary bladder, 2 produced the same maximum response as carbachol but was (9.6 ± 1.6)-fold (N = 4) more potent than carbachol. No receptor alkylation by 2 was readily apparent after the 3-min exposure required to record responses. Compound 4 (1 μM) also contracted bladder strips but the initial response was not maintained. Instead, it gradually declined and eventually disappeared. When the tissue, after extensive washing, was reexposed to 4 (3 μM), there was no response. Carbachol and 2, however, were able to produce maximal responses in tissues treated with 4 at 1 μM.

Acute Toxicity and Muscarinic Actions in Mice. The LD_{50} values of 3 and 4, injected iv as the parent compounds, were 396 ± 55 and 81 ± 11 μmol/kg, respectively. Neither compound elicited tremor. Compound 4 produced weak, short-lasting salivation at doses above 15 μmol/kg. With 3, salivation appeared only at doses near the LD_{50} value.

Alkylation of Muscarinic Receptors. Homogenates of the rat cerebral cortex were incubated for 30 min at 37 °C with 2, 3, or 4 which had been precyclized to contain maximum amount of aziridinium ion. The homogenates were then washed extensively and assayed for specific (−)-[^{3}H]-3-quinuclidinyl benzilate ([^{3}H]QNB) binding. As shown in Figure 2, there was a concentration-dependent inhibition of binding. In Figure 2, initial concentrations of the parent haloethylamines have been plotted on the abscissa in order to illustrate the data actually collected. Based on initial concentrations of haloalkylamines, the rank order of alkylating potency was 4 > 3 > 2 > 1. The data have been replotted in Figure 3 with calculated initial concentrations (A_0) of the aziridinium ion. As expected, the aziridinium ion 4A alkylated muscarinic receptors at

Table II. Dissociation Constants and Rate Constants for Alkylation at Muscarinic Receptors in the Rat Cerebral Cortex[a]

	equation 1		equilibrium binding parameters			
no.[b]	K_A, μM	k_3, min^{-1}	K_H, μM	K_L, μM	K_L/K_H	% high affinity sites
1	0.79 ± 0.18	0.064 ± 0.007				
2	0.35 ± 0.05	0.062 ± 0.007	0.0010 ± 0.0006	0.47 ± 0.04	466	16.5
3	0.058 ± 0.016	0.091 ± 0.009				
4	0.035 ± 0.004	0.096 ± 0.010	0.0010 ± 0.0004	0.071 ± 0.004	71	14.2

[a] Values are means ± standard errors of five to six separate experiments, each performed in triplicate. [b] Data for 1 and 2 refer to the aziridinium ion **2A** and those for 3 and 4 to the ion **4A**.

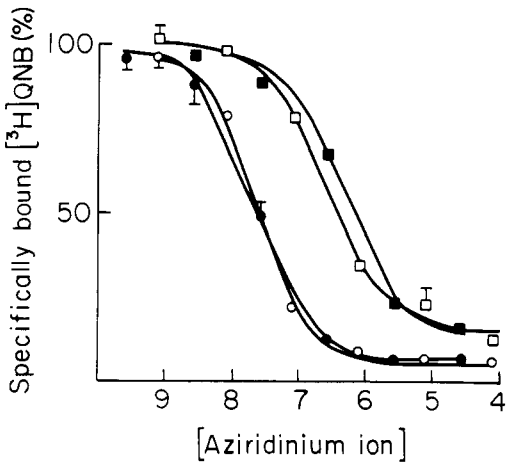

Figure 3. Specific binding of (−)-[^{3}H]QNB to the rat cerebral cortex treated with precyclized 1 (■), 2 (□), 3 (●), and 4 (○). Data are from Figure 2 but expressed in terms of aziridinium ion concentration. The theoretical curves represent the best fit to eq 1.

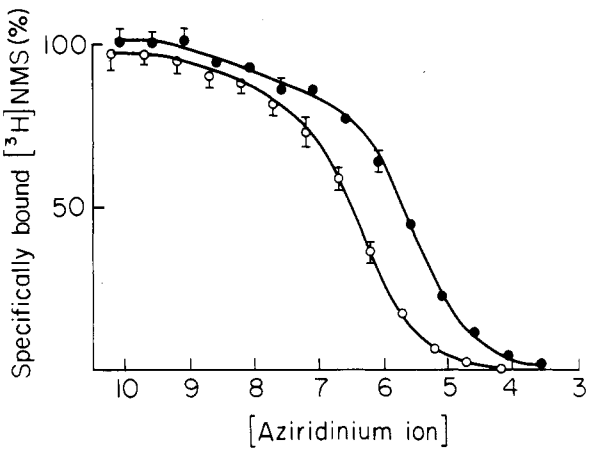

Figure 4. Competitive inhibition of (−)-[^{3}H]NMS binding to the rat cerebral cortex by **2A** (●) and **4A** (○). The specific binding of (−)-[^{3}H]NMS was measured at 0 °C in the presence of the indicated concentrations of **2A** and **4A**. Each data point represents the mean binding value of five experiments, each done in triplicate. Vertical bars show standard errors. The theoretical curves are the best fit to a two-site binding equation.

similar rates whether it was derived from **3** or **4**. It was more potent than the aziridinium ion (**2A**) derived from **1** and **2**. A single-site model (eq 1 in the Experimental Section) provided a satisfactory fit to the data in Figure 3. Apparent dissociation constants (K_A) and rate constants for alkylation (k_3) obtained from eq 1 are summarized in Table II. The K_A values obtained for **3** and **4** were similar but differed significantly ($P < 5 \times 10^{-4}$) from those of **1** and **2**. The difference among the four compounds in rate constants for alkylation was only marginal. No receptor alkylation was detected after 1 h of incubation at 0 °C of cortical homogenates with **4** at concentrations below 0.1 μM. At higher concentrations, receptor alkylation became apparent as evidenced by a loss (<20%) of (−)-[^{3}H]QNB binding sites. Compound **2** caused no significant alkylation under these conditions.

Affinity for Muscarinic Receptors. Although higher concentrations of **4A** caused receptor alkylation at 0 °C (see above), such alkylation can be shown theoretically to be negligible in the presence of 0.3 nM of (−)-[^{3}H]-*N*-methylscopolamine ([^{3}H]NMS). Thus the affinity of the aziridinium ions (**2A** and **4A**) could be determined in competition experiments with (−)-[^{3}H]NMS at 0 °C (Figure 4). Previous experiments performed in the rat cerebral cortex have shown that [^{3}H]NMS reaches equilibrium within 1 h at 0 °C.[14] Nonlinear regression and analysis of variance revealed a significant reduction in residual error when the competition curves of **2** ($F_{2,10} = 27.0$; $P = 9.3 \times 10^{-5}$) and **4** ($F_{2,9} = 43.6$; $P = 2.3 \times 10^{-5}$) were fitted to a two-site model as compared to a one-site model. The high-affinity dissociation constants (K_H) of **2A** and **4A** were similar whereas **4A** had 6.6-fold greater affinity than **2A** for the low-affinity binding site. Analysis of variance showed a significant increase in residual error when the **2A** and **4A** competition data were fitted by nonlinear regression analysis sharing the estimate of K_L between the data ($F_{1,19} = 15.8$; $P = 8.1 \times 10^{-4}$). When the competition data were fitted to a one-site model, the K_i values obtained for **2A** and **4A** were 0.33 and 0.053 μM, respectively.

Discussion

The kinetic parameters for the cyclization of **3** and **4** to the aziridinium ion **4A** and for the decay of the ion were very similar to those observed previously for **1**, **2**, and **2A**.[8,9] The observation that for **3** the rate constant (k_1) for formation of the ion was smaller than the rate constant (k_2) for its decay readily explains the low peak levels of the ion, the late appearance of the peak and the relatively long persistence of the ion in solution (Figure 1). Compound **4**, because of its rapid cyclization ($k_1 > k_2$) gave rise to almost 3-fold greater peak concentrations of the aziridinium ion.

The aziridinium salt **4A** was about 10-fold less potent than **2A** as a muscarinic agonist on the guinea pig ileum. A similar difference in potency was observed for the corresponding trimethylammonium salts (**6** and **5**). The lower potentcy of **6** as compared to **5** was due to 20-fold lower intrinsic efficacy of **6**. The affinity of **6** for muscarinic receptors was actually greater than that of **5**.[16,17] Determination of the binding affinities of **2A** and **4A** by inhibition of (−)-[^{3}H]NMS binding at 0 °C clearly showed **4A** to have greater affinity than **2A** for low-affinity (−)-[^{3}H]NMS binding sites in the rat cerebral cortex. The affinity of **2A** and **4A** for the relatively small population of high-affinity (−)-[^{3}H]NMS binding sites was similar. The receptor inactivation experiments, performed at 37

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°C, confirmed the greater affinity of the aziridinium ion **4A** derived from **3** and **4** as compared to the ion (**2A**) derived from **1** and **2**.

Several observations suggest that **4A** has lower intrinsic efficacy than **2A**. For example, on the guinea pig urinary bladder, which has a small receptor reserve for muscarinic agonists,[17-19] the response of **4A**, in contrast to that of **2A**, was very sensitive to receptor alkylation. Thus **4A** abolished its own response on the bladder, while leaving the maximum reponse to efficacious compounds such as carbachol and **2A** unaltered. Furthermore, **4** showed only very weak muscarinic actions in vivo. In contrast, **2** is an extremely potent muscarinic agent that surpasses oxotremorine in both central and peripheral muscarinic activity.[9,10] Since the lower muscarinic activity of **4** as compared to **2** does not appear to be due to distributional factors or to lower affinity for muscarinic receptors, it can be best explained by lower intrinsic efficacy of **4A**. In agreement with this suggestion, **4A** had a smaller ratio of low- and high-affinity dissociation constants (K_L/K_H) than **2A**. This ratio has been shown previously to reflect agonist efficacy.[20] Thus **4A** may be considered a partial agonist with regard to **2A**.

The aziridinium salt **4A** was more potent than **2A** in alkylating cortical muscarinic receptors. The interaction of **2A** with the receptor was shown previously to be described adequately by a quasi-equilibrium.[14] Thus **2A** appeared to equilibrate rapidly with the receptor forming a reversible complex which was transformed more slowly into a covalent complex. Assuming similar kinetics for the receptor interaction of **4A**, it appears that the greater potency of **4A** was not due to greater intrinsic alkylating ability but rather to its higher affinity for muscarinic receptors.

Compound **2**, because of its rapid cyclization, readily enters the central nervous system only after intravenous administration.[10] However, its high acute toxicity by this route (LD_{50} = 0.7 μmol/kg in mice) limits its usefulness for inactivation of central receptors in vivo. Compound **4** not only is more potent than **2** as an alkylating agent but also is 100-fold less toxic. Compounds **3** and **4** should become valuable alternatives to **1** and **2** for receptor-alkylation studies in vivo and in vitro.

Experimental Section

Melting points were determined in a heated metal block using glass capillaries and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and agreed with theoretical values within ±0.4%. Mass spectra were recorded on a Hewlett-Packard 5981A mass spectrometer at 70 eV. ^{1}H NMR spectra were obtained at 23 °C on a Bruker AM360/Wb spectrometer at 360 MHz. Chemical shifts are reported in parts per million (δ) downfield from internal $(CH_3)_4Si$ standard.

***N*-[4-[(2-Hydroxyethyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone (7)** was synthesized from 5-methyl-*N*-(2-propynyl)-2-pyrrolidone,[16] *N*-methyl-*N*-(2-hydroxyethyl)amine, and paraformaldehyde in a Mannich reaction as described previously for similar compounds.[16] The product was purified by chromatography on a silica gel column using CH_2Cl_2/MeOH (9:1) as eluent. Compound **7** was obtained in 83% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 4.53 (app d of t, 1 H, J_1 = 17.6 Hz, J_2 = 1.7–2.0 Hz, C*H*C≡), 3.75–3.95 (m, 1 H, C*H*CH_3), 3.71 (app d, 1 H, J = 17.3 Hz, C*H*C≡), 3.60 (t, 2 H, J = 5.4 Hz, CH_2OH), 3.36 (t, 2 H, J = 2.0 Hz, CH_2C≡), 2.7 (br, 1 H, O*H*), 2.59 (t, 2 H, J = 5.4 Hz, NCH_2CH_2), 2.32 (s, 3 H, NCH_3), 2.1–2.5 (m, 3 H, CH_2CO and C*H*CH_2CO), 1.5–1.7 (m, 1 H, C*H*CH_2CO), 1.28 (d, 3 H, J = 6.3 Hz, CCH_3).

***N*-[4-[(2-Chloroethyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone (3) hydrochloride** was synthesized from **7**, CCl_4, and Ph_3P in CH_2Cl_2 according to a method described previously.[21] The yield of **2**·HCl was 41%: mp 112–114 °C (from ethanol/ether); ^{1}H NMR (CD_3OD) δ 4.42 (app d, 1 H, J = 18.4 Hz), 4.20 (app s, 2 H), 3.99 (app d, 1 H, J ~ 18 Hz), 3.99 (t, 2 H, J = 5.8 Hz), 3.8–3.95 (m, 1 H), 3.63 (t, 2 H, J = 5.8 Hz), 2.99 (s, 3 H), 2.2–2.5 (m, 3 H), 1.6–1.75 (m, 1 H), 1.31 (d, 3 H, J = 6.3 Hz); MS m/e (relative intensity) 193 (9.7), 150 (55.5), 112 (13.7), 108 (29.8), 107 (85.2), 94 (48). Anal. ($C_{12}H_{20}Cl_2N_2O$) C, H, N, Cl.

***N*-[4-[(2-Bromoethyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone (4) oxalate** was synthesized from **7**, CBr_4, and Ph_3P in CH_2Cl_2 as described previously for **2**:[9] mp 107–109 °C (from ethanol/ether); yield 30%; ^{1}H NMR (CD_3OD) δ 4.40 (app d, 1 H, J = 17.4 Hz), 4.05 (app s, 2 H), 3.98 (app d, 1 H, J = 17.5 Hz), 3.8–3.95 (m, 1 H), 3.72 (t, 2 H, J = 6.7 Hz), 3.54 (t, 2 H, J = 6.7 Hz), 2.87 (s, 3 H), 2.2–2.5 (m, 3 H), 1.6–1.7 (m, 1 H), 1.31 (d, 3 H, J = 6.1 Hz); MS m/e (relative intensity) 207 (6.8), 193 (6.1), 150 (57.2), 112 (15.0), 108 (36.0), 107 (100), 94 (40.8). Anal. ($C_{14}H_{21}N_2O_5Br$) C, H, N.

Measurements of Formation and Decomposition of Aziridinium Ion. The reaction mixture, kept at constant temperature (24 or 37 °C), contained **3** or **4** (1.5 mM) in 50 mM sodium phosphate buffer. Halide ion release during the cyclization was measured by argentometric titration.[8,9] The method used to quantify the aziridinium ion was essentially identical with that described previously.[8,9] In short, the aziridinium ion was allowed to react quantitatively with sodium thiosulfate and excess thiosulfate was determined by back-titration with iodine. Rate constants for the cyclization reaction (k_1) and for the decay of the aziridinium ion (k_2) were estimated by fitting kinetic models to the data by an unweighted Gauss-Newton nonlinear regression routine as described previously.[8]

Guinea Pig Ileum and Urinary Bladder. Segments of the ileum and strips of the bladder were set up at 37 °C in Tyrode solution (pH 7.4) as described previously.[19] Contractions were recorded isotonically at 1 g of tension with an electromechanical displacement transducer and a potentiometric recorder. Relative potencies were obtained in three-point assays by comparing concentrations of agonist giving equal responses.[22] The tissue was exposed to each agonist concentration until the response reached a plateau, i.e. about 15 s on the ileum and about 3 min on the bladder. Carbachol was used as the standard agonist.

Acute Toxicity. LD_{50} values were determined in male Swiss–Webster mice (24–30 g body weight) by iv injection of **3** and **4** by using the up-and-down method.[23] Mortality counts were taken at 30 min.

Preparation of Brain Homogenates. Cerebral cortex from male Sprague–Dawley rats (300–350 g body weight) was homogenized in 50 volumes of 50 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 30000*g* for 10 min and resuspended in 50 mM phosphate buffer to a concentration of 25 mg of original wet tissue weight/mL of buffer. Receptor alkylation by **2**, **3** and **4** was studied essentially as described previously for **1**.[14] Thus aliquots (2 mL) of homogenate were incubated at 37 °C for 10 min and 0.1 mL of precyclized **2**, **3**, or **4** was added to give a final concentration of 0.1 mM to 1 nM. Control homogenates were treated identically except that 0.1 mL of phosphate buffer was added instead of drug. The homogenates were incubated for an additional 30 min at 37 °C. The reaction was terminated by the addition of sodium thiosulfate (1.0 mM) and atropine (0.1 mM). The tissues were washed by centrifugation at 30000*g* for 10 min followed by resuspension of the pellets in phosphate buffer. The washing was repeated four times. After the third resuspension, the homogenates were incubated at 37 °C for 20 min to allow sufficient time for the dissociation of atropine from the receptor.

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The final pellets were frozen at -20 °C and thawed the next day for [^{3}H]QNB binding experiments as described below. Other experiments in which receptor alkylation by **2A** and **4A** was studied at 0 °C were performed similarly except that the homogenates were kept on ice.

Muscarinic Receptor Binding Assays. All binding assays were run in triplicate. Nonspecific binding was estimated by carrying out incubations in the presence of 10 μM atropine. The pellets from the homogenates treated with **2**, **3**, and **4** were thawed and resuspended to a concentration of 10 mg of original wet tissue weight/mL of phosphate buffer. The binding of the specific muscarinic antagonist (-)-[^{3}H]QNB (34.7 Ci/mmol, New England Nuclear) was measured by the rapid filtration method of Yamamura and Snyder[24] with minor modifications. Homogenates (0.1 mL) were incubated with (-)-[^{3}H]QNB (0.4 nM) in a final volume of 2 mL of 50 mM phosphate buffer (pH 7.4). Incubations lasted 1 h at 37 °C. Tissue-bound (-)-[^{3}H]QNB was trapped by vacuum filtration of the incubation mixture over Whatman glass fiber filters (GF/B).

The binding of the specific muscarinic antagonist (-)-[^{3}H]NMS (87 Ci/mmol, New England Nuclear) was measured similarly. Measurements of the competitive inhibition of (-)-[^{3}H]NMS binding by **2A** and **4A** were carried out at 0 °C. Incubations lasted 1 h. Binding parameters were determined by unweighted nonlinear regression analysis. A two-site equation was fitted to the **2A** and **4A**/(-)-[^{3}H]NMS competition data to provide estimates of the apparent dissociation constants at the high (K_H') and low (K_L') affinity binding sites.[20] The apparent dissociation constants were corrected for receptor occupancy by (-)-[^{3}H]NMS according to the relationship[25] $K = K'/(1 + y/K_{NMS})$ were K is the true dissociation constant (K_H or K_L), y is the concentration of (-)-[^{3}H]NMS (0.3 nM), and K_{NMS} is the dissociation constant of (-)-[^{3}H]NMS (0.055 nM). The latter was determined independently by nonlinear regression analysis of seven-point (-)-[^{3}H]NMS binding isotherms.

Analysis of Receptor Inactivation Data. Alkylation of muscarinic receptors by **2**, **3**, and **4** was analyzed in terms of the model used earlier to describe the interaction of **1** with the receptor.[14] It was assumed that the reversible interaction of the aziridinium ion with the receptor was sufficiently fast to allow it to be essentially in equilibrium with the receptor and that the rate of the covalent reaction, described by the rate constant k_3, was proportional to the amount of reversibly bound ligand. The resulting equation, integrated over the time interval beginning at $t = 0$, is

$$Y = \left(\frac{A_0 e^{-k_0 t} + K_A}{A_0 + K_A} \right)^{k_3/k_0} \quad (1)$$

where Y is the proportion of receptors remaining unalkylated, t is time, A_0 is the concentration of the aziridinium ion at time zero, K_A is the apparent dissociation constant of the reversible drug-receptor complex, and k_0 is the rate constant for the exponential decay of the aziridinium ion. Values for k_0 were obtained from Table I ($k_0 \sim k_1$ for **1** and **3** and $k_0 = k_2$ for **2** and **4**). A_0 was calculated from the initial concentration of the parent haloethylamine and the peak level of the aziridinium ion (Table I). This equation (eq 1) was fitted to data from experiments in which residual (-)-[^{3}H]QNB binding was measured after 30 min of exposure to various concentrations of precyclized **2**, **3**, and **4** by unweighted nonlinear regression.

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New 4-(Heteroanilido)piperidines, Structurally Related to the Pure Opioid Agonist Fentanyl, with Agonist and/or Antagonist Properties

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A research program based on certain heterocyclic modifications (**12**-**50**) of the fentanyl (**1**) molecule has generated a novel class of opioids. In the mouse hot-plate test, these compounds were weaker analgesics than **1**. Two types of antagonists were observed in morphine-treated rabbits: those (e.g., **28**) that reversed both respiratory depression and analgesia and those (e.g. **32**) that selectively reversed respiratory depression. Evaluation of in vitro binding affinities to rat brain opioid receptors was inconclusive for a common locus of action for the agonist as well as the antagonist component. Further pharmacological evaluation of **32**, *N*-(2-pyrazinyl)-*N*-(1-phenethyl-4-piperidinyl)-2-furamide, in the rat showed it to be a potent analgesic (ED_{50} = 0.07 mg/kg, tail-flick test) with little cardiovascular and respiratory depression when compared to fentanyl.

Intravenous infusion of opioids with a skeletal muscle relaxant and an inhalation anesthetic is a widely accepted practice in surgical procedures. Among many anesthesiologists, fentanyl (**1**), the prototype of the 4-anilidopiperidine class of analgesics, has become the first-choice opioid for this regimen.[1] In recent years a number of new 4-anilidopiperidines exhibiting an array of analgesic profiles have been introduced,[2] and among these, the development of alfentanil (**2**) warrants special consideration.[2,3] While less potent than **1**, **2** has a more rapid onset and shorter duration of action. In addition, the respiratory depressant effect of **2** is less severe than that of **1**.[4,5] With the growing volume of outpatient surgical procedures, such

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