(t, 3 H, J = 7.03 Hz). The oil was converted to the HCl salt form by dissolving it in EtOH and adding ethanolic HCl dropwise to pH 3. The EtOH was removed in vacuo, leaving a solid residue that was dissolved in a hot EtOH/ether solution and slowly reprecipitated to give 71 mg of **5** (62%): mp 240–243 °C; IR (KBr) 3700–2500 (v br s, NH, OH), 1720 (s, ester), 1655 (s, amide I) cm^{-1}; EIMS, m/e 525 (M^+). Anal. ($C_{28}H_{35}N_3O_7 \cdot HCl \cdot 0.5H_2O$) C, H, N.

(*E*)-17-(Cyclopropylmethyl)-4,5α-epoxy-6β-[*N*-(4-ethoxy-4-oxo-2-butenoyl)glycylglycinamido]-3,14-dihydroxymorphinan Hydrochloride (6). A procedure similar to that described for the synthesis of **5** was employed using **12b** (0.10 g, 0.189 mmol). The product was purified by recrystallization from EtOAc/THF/petroleum ether to give 0.74 g of 6 (64%): mp 210–231 °C dec; R_f = 0.60 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); ^1H NMR (CD_3OD, 99.8% D) δ 7.02 (AB_q, 2 H, J = 15.5 Hz, CH=CH, trans), 6.76 (s, 2 H, ArH), 4.71 (d, 1 H, C_5-H), 4.28 (q, 2 H, J = 7.03 Hz, CH_2CH_3), 3.99 (s, 2 H, $NDCH_2CO$), 3.90 (s, 2 H), 1.34 (t, 3 H J = 7.03 Hz, CH_2CH_3), 3.6–0 (m, 18 H); IR (KBr) 1725 (s), 1665 (s), 1305, 1175, 980 cm^{-1}; CIMS (NH_3), m/e 583 (MH^+). A sample of 6 was converted to the HCl salt for analysis. Anal. ($C_{30}H_{38}N_4O_8 \cdot HCl \cdot 1.5H_2O$) C, H, N.

(*E*)-17-(Cyclopropylmethyl)-4,5α-epoxy-6β-[*N*-(4-ethoxy-4-oxo-2-butenoyl)glycylglycylglycinamido]-3,14-dihydroxymorphinan Hydrochloride (7). Compound **12c** (150 mg, 0.250 mmol) was employed, using the synthesis described for **5**. The product **7** was purified by repetitive slow precipitation from ethanol (absolute) and ether: yield, 61 mg (35%); mp 220–225 °C dec; R_f 0.45 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); IR (KBr) 3700–2500 (br, NH, OH), 1720 (s, ester), 1660 (s, amide I), 1540 (m, amide II) cm^{-1}; ^1H NMR (D_2O) δ 6.88 (AB_q, 2 H, J = 15.6 Hz, CH=CH), 6.71 (s, 2 H, ArH), 4.52 (d, 1 H, J = 7 Hz, C_5-H), 4.22–3.98 (q, 2 H, J = 7.03 Hz, OCH_2CH_3), 4.01 (s, 2 H, $NDCH_2CO$), 3.87 (s, 2 H, $NDCH_2CO$), 3.83 (s, 2 H, $NDCH_2CO$), 1.12 (t, 3 H, J = 7.03 Hz, CH_2CH_3), 3.3–0.3 (m, 29 H); FABMS, m/e 640 (M^+). Anal. ($C_{32}H_{41}N_5O_9 \cdot HCl \cdot H_2O$) C, H, N.

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Registry No. **4**, 103500-50-1; **4**·2HCl, 103500-41-0; **5**, 103500-45-4; **5**·HCl, 103500-42-1; **6**, 103500-46-5; **6**·HCl, 103500-43-2; **7**, 103500-47-6; **7**·HCl, 103500-44-3; **8**·2HCl, 63463-07-0; **9**, 91409-49-3; **10**, 103500-48-7; **10**·2HCl, 103500-34-1; **11a**, 103500-49-8; **11a**·HCl, 103500-35-2; **11b**·HCl, 103500-36-3; **11c**·HCl, 103500-37-4; **12a**·2HCl, 103500-38-5; **12b**·2HCl, 103500-39-6; **12c**·2HCl, 103500-40-9; diethanolamine, 111-42-2; Z-glycylglycine, 2566-19-0; Z-glycylglycylglycine, 2566-20-3; Z-glycine, 1138-80-3; fumaric acid monoethyl ester, 2459-05-4.

Preparation and Antitumor Activity of 7-Substituted 1,2-Aziridinomitosenes

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7-Methoxy-1,2-aziridinomitosenes were prepared from mitomycin A and its *N*-methyl homologue by catalytic reduction followed by air oxidation. Treatment of these products with amines, including ammonia, ethylenimine, 2-methylethylenimine, propargylamine, and furfurylamine gave the corresponding 7-(substituted amino) derivatives. Screening of these compounds against P-388 leukemia in mice revealed some good activities. The more easily reduced compounds gave prolongation of life span comparable to that of mitomycin C, but their optimal doses were higher. Among these compounds, a methyl group on the aziridine nitrogen increased potency. The 7-amino derivatives, which were difficult to reduce to hydroquinones, were essentially inactive. The aziridinomitosenes were subjected to a Hansch-type analysis, but no statistically significant correlation was found.

1,2-Aziridinomitosenes were prepared first by Patrick and co-workers, who reduced 7-methoxymitosanes such as mitomycin A (**1**), *N*-methylmitomycin A (**2**), and mitomycin B (**3**) catalytically and reoxidized the resulting intermediate hydroquinones with air.[1] The resulting aziridinomitosene structures (**6** and **7**, respectively) had 9,9a double bonds, because of the loss of methanol or water from these positions. This loss presumably occurs at the hydroquinone stage as shown by the transformation of **4** into **5** (Scheme I).[2] from the outset it was recognized that aziridinomitosenes had potent antibacterial activity,[1] and this was readily rationalized in terms of their forming the same reactive intermediates (**5**) as the natural mitomycins. These intermediates are able to dialkylate and cross-link double-helical DNA, probably as shown in structure **8**.[2] In contrast to the numerous mitosanes, only a few aziridinomitosenes have been prepared. This situation probably reflects the difficulty in obtaining them in good yields from the natural mitomycins. A previous study involved the preparation of 7-methoxy-1,2-(*N*-methylaziridino)mitosene (**7**) from mitomycin B and its conversion into 7-substituted derivatives including amino, ethylamino, and dimethylamino.[3] These derivatives were tested against representative species of bacteria, and their potencies as measured by minimal inhibitory concentration were compared with their quinone reduction potentials. It was concluded that no correlation existed, although it was noted that difficult-to-reduce compounds such as the amino and ethylamino derivatives had poor activity.[3] Only one aziridinomitosene, **7**, has been tested against experimental tumors. It was active against the solid tumor of sarcoma 180[4] and L-120 leukemia[5] but not superior to mitomycin C in either system.

Because our recent studies on 7-substituted mitosane analogues of mitomycin C indicated that the antitumor activity and toxicity could be modified and improved in some cases by varying the substituents on the quinone ring,[6] we thought it important to extend these studies to

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(1) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W.; Webb, J. S. *J. Am. Chem. Soc.* **1964**, *86*, 1889.
(2) Iyer, V. N.; Szybalski, W. *Science (Washington, D.C.)* **1964**, *145*, 55.
(3) Kinoshita, S.; Uzu, K.; Nakano, K.; Shimizu, M.; Takahashi, T. *J. Med. Chem.* **1971**, *14*, 103.
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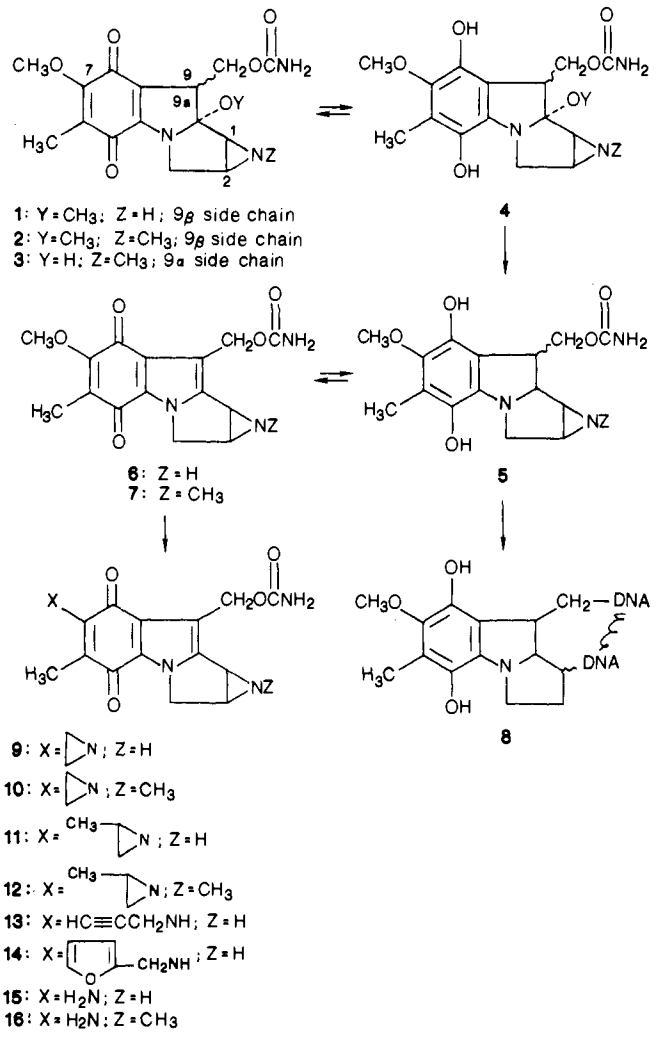
Scheme I



the aziridinomitosenes. A series of substituents at the 7-position, which would give some variation in the quinone reduction potential and in the octanol/water partition coefficient, was outlined for the aziridinomitosenes, with emphasis on those amines that gave good activity in the mitosane series. It should be noted that mitosenes have different reduction potentials than mitosanes, because the chromophore is indoloquinone rather than benzoquinone.[3] The mitosenes are more difficult to reduce than the corresponding mitosanes.

The first priority in preparing a series of aziridinomitosenes was to improve the yield for the mitosane to mitosene conversion, especially for mitomycin A (**1**). We were able to do this for 7-methoxymitosanes by the simple measure of using ethyl acetate as the solvent[3,7] (Experimental Section). In this manner, yields of about 55% were obtained for the purified aziridinomitosenes. It was not possible for us to convert 7-aminomitosanes such as mitomycin C directly into the corresponding aziridinomitosenes in significant yields. The formation of **15** from mitomycin C had been reported,[8] but that product was not characterized thoroughly. Furthermore, it was said to have a quinone reduction potential equal to mitomycin C, whereas Kinoshita showed that aziridinomitosenes have the same reduction potentials as other mitosenes because of their indoloquinone chromophores.[3] We were able to prepare **15** and **16** by treating **6** and **7** with ammonia in methanol, following the earlier reports of Kinoshita and Patrick. In a similar way, the 7-(1-aziridinyl) analogues **9** and **10**, the 7-(2-methyl-1-aziridinyl) analogues **11** and **12**, and the 7-propargylamino analogue **13** and 7-furfurylamino analogue **14** were prepared. The yields and properties of these 7-substituted aziridinomitosenes are given in Table I. The NMR signals for new 7-substituents also are listed. Structures also were supported by their IR spectra (not listed), which showed strong bands at 8.34 μm characteristic for fused aziridine rings.[1] Their UV–visible spectra were identical with those of related 1,2-disubstituted mitosenes (apomitomycins)[1] as indicated in the Experimental Section.

Determination of pK_a values for the conjugate acids of aziridinomitosenes has not been reported, although they are of some theoretical and practical interest. Natural mitomycins such as mitomycin C and porfiromycin have very weakly basic aziridine nitrogens (pK_a = 1.19 and 0.91, respectively)[9] because of high steric hindrance and interference between the fourth valence pair of the aziridine nitrogen and the free p orbital of N-4.[10] These effects should be less pronounced in aziridinomitosenes because they are flatter molecules (because of the 9,9a double bond) and the orbitals on the aziridine nitrogen and N-4 are more remote from each other. The pK_a of analogues **6** and **7** in 0.1 M KCl solution containing 10% methanol (for solubility) were found to be 4.9 and 5.2, respectively, which shows that they are considerably more basic than the natural mitomycins, as anticipated. These pK_a values are 2–3 units below those of apomitomycins (pK_a = 7–8),[12] which have the aziridine ring opened. This difference is about the same as the one between ethylenimine (pK_a = 8.0) and ethylamine (pK_a = 10.8). Although **6** and **7** are more basic than natural mitomycins, they would not be protonated appreciably at physiological pH. Therefore, the log *P* values used for aziridinomitosenes in Table II do not need to be corrected for ionization.

Biological Activity. The activities of 7-substituted aziridinomitosenes against P-388 lymphocytic leukemia in mice are given in Table II. The activity of the mitomycin C control at its optimal dose is given in parentheses beside each compound. Assays were not all run concurrently, and the therapeutic effects vary from one experiment to another (depending on the tumor inoculum). Therefore, compounds should not be compared directly with each other, but compared on the basis of how each one relates to mitomycin C. It is evident from Table II that two compounds, **7** and **10**, provide greater prolongation of life than mitomycin C and that compound **9** also is highly active, especially in giving 30-day survivors. However, these beneficial effects occur at much higher doses than those of mitomycin C. Compounds **11** and **12** also have substantial activity at high doses. Another obvious effect in Table II is that the 7-amino derivatives **15** and **16**, which are the aziridinomitosene equivalents of mitomycin C and porfiromycin, have little or no activity. This result probably reflects the difficulty of reducing such compounds to their hydroquinones in biological systems, and it is consistent with the observations of Kinoshita and co-workers

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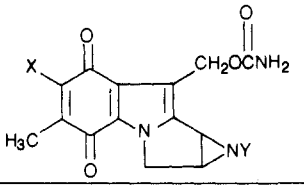
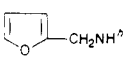
Table I. Properties of the Aziridinomitosenes[a]



no.	X	Y	yield, %	recryst solv	solv impurity	mp, °C	^{1}H NMR, δ:[b] 7-subst; solv impurity
6	CH_3O	H	20–55	ether	0.5 ether	265–267 dec	3.96 (s, 3); 1.3 (t), 3.6 (q)
7	CH_3O	CH_3	ref 1				
9		H	55	DMF–ether	1 DMF, 0.5 H_2O	250 dec	2.33 (s, 4)
10		CH_3	35	CH_2Cl_2–ether	CH_2Cl_2, 0.5 ether	220 dec	2.30 (s, 4); 1.3 (t), 3.6 (q)
11		H	50–55	CH_2Cl_2–C-H_3OH	1.25 CH_3OH	250 dec	1.45 (d, 3), 2.25 (m, 3); 4.05 (br s)
12		CH_3	31	CH_2Cl_2–C-H_3OH	2 CH_3OH	200 dec	1.45 (d, 3), 2.1–2.3 (m, 1); 2.5 (d, 2); 4.05 (br s)
13	$HC{\equiv}CCH_2NH$[g]	H	56	CH_2Cl_2–C-H_3OH	1 CH_3OH	250 dec	2.10 (s, 1), 2.75–3.43 (br s, 2); 4.05 (br s)
14		H	58	CH_2Cl_2–C-H_3OH	1 ether	228–230 dec	3.93 (br, 2), 5.47–6.77 (m, 3), 7.33 (br s, 1); 4.05 (br s)
15	H_2N[i]	H	15–20	CH_2Cl_2–C-H_3OH	4 H_2O	250 dec	6.9 (br s, 2)
16	H_2N	CH_3	ref 1				

[a] Analytical results were within ±0.40% of theoretical values for all elements (C, H, N), except as shown in subsequent footnotes. In some examples, the solvent impurities indicated in the table had to be added to reconcile the calculated and found values for these elements. NMR signals for the solvent impurities are given in the table. It was not possible to obtain exact ratios for protons in the solvent impurities with respect to those in the compound by integrating the spectra. [b] The solvent was $CDCl_3$ unless specified otherwise. [c] H: calcd, 5.89; found, 7.26. [d] N: calcd, 12.38; found, 10.36. [e] H: calcd, 5.93; found, 6.56. N: calcd, 14.64; found, 13.84. [f] H: calcd, 6.71, found, 6.12. [g] H: calcd, 5.41; found, 4.98. [h] N: calcd, 12.27; found, 11.07. [i] N: calcd, 14.97; found, 13.41.

on the poor antibacterial activity of **16**.[3] Compounds **13** and **14**, which have quinone reduction potentials between those of the 7-amino analogues and highly active compounds such as **10** (Table III), are active, but they do not show the increases in life span that characterized the related propargylamino and furfurylamino derivatives in the mitosane series.[6]

Structure–activity relationships among the aziridinomitosenes are given in Table III. These data were subjected to a Hansch analysis using the BMDP program for multiple linear regression. An indicator variable (*I*) was included to determine whether methylation of the aziridine nitrogen had any effect other than its contribution to log *P*. This analysis showed that there was no statistically significant correlation between antitumor potency and the chosen set of variables. The only variable of any importance was *I*, which accounted for 50% of the variance. It is interesting to note that methylation of the aziridine nitrogen of aziridinomitosenes tends to increase potency as measured by MED. (Compare the pairs **6** and **7**, **9** and **10**, and **11** and **12** in Table III). This observation is in contrast to the corresponding mitosanes (for example, mitomycin C and porfiromycin), for which the *N*-methyl analogues tend to be less potent.[6] A possible explanation for this phenomenon might be found in the change in relative basicity of the unmethylated and methylated aziridine nitrogens that occurs on going from a mitosane to an aziridinomitosene. Thus, as mentioned above, mitomycin C is slightly more basic than porfiromycin, probab' because of the very low accessibility of the nitrogen ne pair in the latter. However, conversion to an aziridinomitosene makes this lone pair accessible, which correlates with the slightly greater basicity of **7** over **6**. Greater basicity would be important to antitumor activity because protonation of nitrogen initiates aziridine ring opening and DNA alkylation.

Three of the aziridinomitosenes were tested against L-1210 lymphocyte leukemia in mice according to the NCI protocol (Table IV). This tumor system is not very sensitive to mitomycins, as shown by the percent T/C of 146 at 6.0 mg/kg for mitomycin C. Compound **11** gave a comparable prolongation of life, but at an 8-fold higher dose. Compound **9** also was active at 48 mg/kg, but **15** had no activity.

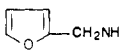
Experimental Section

General Procedures. Melting points were determined on a Laboratory Instruments Mel-Temp apparatus and are uncorrected. UV and visible spectra were taken on a Beckman DU-8 spectrometer. NMR spectra were taken on a Varian EM-360L 60-MHz spectrometer, and absorptions are reported (as ppm) downfield from Me_4Si. Adsorbents were purchased from Brinkmann Instruments, Inc. Elemental analyses were performed by Chemalytics, Inc., Tempe, AZ, or the Analytical Center, University of Arizona. Analytical results were within ±0.4% of theoretical values unless stated otherwise in Table I.

7-Methoxy-1,2-aziridinomitosene (6). Mitomycin A (**1**) was prepared in 57% yield from mitomycin C by a procedure based on the reported one,[12] except that 0.05 N sodium hydroxide was used for the hydrolysis and a nitrogen atmosphere was maintained throughout this reaction. The crude product was purified by chromatography on silica gel with ethyl acetate as solvent.

A solution of mitomycin A (100 mg, 0.29 mmol) in 25 mL of freshly distilled ethyl acetate was treated with platinum oxide and shaken with hydrogen for 2 h. The mixture was filtered under nitrogen and exposed to air for 30 min to regenerate a quinone. The resulting orange-red solution was concentrated under reduced pressure at room temperature, and the residue was purified by chromatography on a silica gel plate (20 × 20 cm, 2 mm thick) with methanol–chloroform (3:7) as solvent. From the orange band was obtained 50 mg (55%) of **6** as orange-red crystals that decomposed above 250 °C: NMR ($CDCl_3$) indicated loss of the 9a-methoxy group protons at 3.17 ppm; UV (CH_3OH) λ_{max} 2.34 nm (ε 21 700), 286 (ε 15 000), 340 (ε 4500), 446 (ε 1300); MS (EI) *m/z* 317.

Table II. Activity of Aziridinomitosenes against P-388 Murine Leukemia[a]

compd	X	Y	dose, mg/kg per inj	effect: MST (% T/C)	av wt cge, g
6	CH_3O	H	25.6	167	−0.4
			12.8	139	−0.8
			6.4	122	0
			3.2	122 (250)	−0.4
7	CH_3O	CH_3	12.8	213	−1.7
			6.4	213	−0.3
			3.2	188	+0.5
			1.6	156 (181)	−0.2
			0.8	150	+0.8
9		H	51.2	206 [2]	−1.2
			25.6	189 [1]	−0.4
			12.8	172	+0.9
			6.4	156	+1.1
			3.2	144 (217)	+1.0
			1.6	144	+1.0
			0.8	111	+0.9
10		CH_3	51.2	139	−2.5
			25.6	239	−2.2
			12.8	172	−1.9
			6.4	172	−1.1
			3.2	117 (217)	−0.1
			1.6	122	0
11		H	51.2	219	−1.3
			25.6	181	−1.0
			12.8	156	−0.5
			6.4	144	−0.4
			3.2	138	0
			1.6	125 (244)	0
12		CH_3	12.8	238	−27
			6.4	194	−1.2
			3.2	163 (319)	−1.3
			1.6	156	−0.3
			0.8	125	−0.5
			0.4	138	0.3
			0.2	125	0.8
13	$HC{\equiv}CCH_2NH$	H	64	172	−0.9
			32	144	0
			16	128	−0.3
			8	122	−0.2
			3.2	(228)	
14		H	64	183	−1.8
			32	189	−1.2
			16	156	−1.7
			8	144	+0.1
			4	122	−0.7
			3.2	(228)	
15	H_2N	H	25.6	128	−0.8
			12.8	117	−0.8
			6.4	117	−1.2
			1.6	(211)	
16	H_2N	CH_3	25.6	100	−0.1
			12.8	100	−0.4
			1.6	(211)	−0.4

[a] Assay conducted at Bristol-Myers Co., Syracuse, NY. A tumor inoculum of 10^6 ascites cells was implanted ip into BDF_1 female mice. Six mice were used at each dose of the aciridinomitosene, which was given on day 1 only. Ten control mice received saline on day 1 only. MST = median survival time in days; % T/C = MST treated/MST control × 100; significant effect 125. The average weight change refers to the difference in weight between the treated and control groups. The maximum therapeutic effect of the mitomycin C control at its optimal dose is given in parentheses for each compound. At all doses recorded there was total survival of the mice on day 5. Thirty-day survivors are given in brackets for compound **9**. No other analogue had such survivors.

7-Methoxy-1,2-(*N*-methylaziridino)mitosene (7). *N*-Methylmitomycin A (**2**) was prepared in 60% yield from porfiromycin by the published procedure,[7] except that 0.05 N sodium hydroxide and a nitrogen atmosphere were used for the hydrolysis. Conversion of **2** into the corresponding mitosene **7** was accomplished in 50% yield by the previously described method.

General Method for the Preparation of 7-Substituted 1,2-Aziridinomitosenes (9–16). A solution of the 7-methoxy-1,2-aziridinomitosene (50 mg) in 5 mL of dry *N*,*N*-dimethylformamide was treated with 3 equiv of an amine, and the mixture was stirred at room temperature under nitrogen for 72–96 h. It was poured into 50 mL of water and extracted into ethyl acetate

Table III. Structure–Activity Relationships among Aziridinomitosenes

compd	MED,[a] mg/kg	*C*,[b] mol/kg	log (1/*C*)	$E_{1/2}$,[c] V	log *P*[d]	*I*[e]
6	12.8	3.61×10^{-5}	4.44	−0.40	0.87*	0
7	0.8	2.41×10^{-6}	5.62	−0.40	1.28	1
9	1.6	3.90×10^{-6}	5.41	−0.45	0.30	0
10	1.6	4.02×10^{-6}	5.40	−0.45	0.71	1
11	1.6	3.44×10^{-6}	5.46	−0.44	0.82	0
12	0.2	6.6×10^{-7}	6.18	−0.44	1.23	1
13	16	4.49×10^{-5}	4.35	−0.49	1.21	0
14	8	2.01×10^{-5}	4.70	−0.51	1.33	0
15	25.6	8.47×10^{-5}	4.07	−0.54	0.23	0

[a] Activity against P-388 murine leukemia from Table II. MED = minimum effective dose (% T/C = 125). [b] Obtained by dividing the MED by the molecular weight, including solvent, and by 1000. [c] Determined by differential-pulse polarography on a Model 174A EG&G polarographic analyzer. The electrolyte was 1.0 M KCl solution and the standard was 10^{-3} M $CdCl_2$ in 1.0 M KCl. Mitomycin analogues were 10^{-3} M in 1.0 M KCl. $E_{1/2}$ values are given relative to the saturated calomel electrode. The following conditions were used: potential scan, 0.1 V/in.; potential scan rate, 1 mV/s; voltage range, 1.5 V; initial potential, −0.1 or +0.2 V; modulation amplitude, 25; rate of mercury drops, 60/min. [d] Distribution between 1-octanol and water containing phosphate buffer at pH 7.4. Determination by the method of Hansch (Hansch, C.; Muir, R. M.; Fujita, T.; Malongy, P. P.; Geiger, F.; Struch, M. *J. Am. Chem. Soc.* **1963**, *85*, 2817) is indicated by an asterisk. Other values are estimated from log *P* for the corresponding mitosanes, based on the differences between 1 and mitomycin A and between mitomycin A and its *N*-methyl derivative. [e] An indicator variable designed to probe any effect, other than its contribution to lipophilicity, of the methyl group on the aziridine nitrogen.

Table IV. Activity of Aziridinomitosenes against L-1210 Murine Leukemia[a]

compd	dose, mg/kg per inj	effect: MST (% T/C)	av wt cge, g
15	48	94	0.2
	24	100	−0.3
NSC 335 653	12	100	0.0
	6	100	0.1
9	48	131	−4.2
	24	120	−2.6
NSC 335 654	12	122	−1.3
	6	118	−0.4
13	40	140	−2.8
	20	122	−1.8
NSC 355 655	10	111	−0.6
	5	101	−0.2
	12	118	−4.8
Mito C	6	146	−3.3
NSC 026980	3	118	−0.9
	1.5	116	−0.8

[a] Assay conducted at Arthur D. Little, Inc., Cambridge, MA. A tumor inoculum of 10^6 ascites cells was implated ip into BDF_1, female mice. Six mice were used at each dose of the aziridinomitosene, which was given on day 1 only. The control group consisted of 36 mice. MST = median survival time in days; % T/C = MST treated/MST control ×100; significant effect 125. The average weight change refers to the difference in weight between the treated and control groups. At all doses recorded there was total survival of the mice on day 5.

(50 mL × 5). This extract was dried over sodium sulfate and concentrated under pressure at room temperature. The crude product was purified on a silica gel plate (20 × 20 cm, 2 mm thick) with methanol-chloroform (3:7) as solvent. The red band gave the product in 50–58% yield after extraction with acetone and concentration. Table I gives the physical properties and NMR signals for the products. In all cases, the 7-methoxy group protons at δ 4.0 were absent. Compound **15** showed the following UV and visible absorption: (CH_3OH) λ_{max} 245 nm (ε 18000), 305 (ε 11600), 250 (ε 7200) sh, 518 (ε 1000).

Determination of the pK_a for 6 and 7. A solution of 2.4 mg (0.0076 mmol) of 6 or 7 in a solvent composed of 1 mL of CH_3OH and 90 mL of 0.1 M KCl was titrated with standardized 0.01 N HCl, added in 0.1-mL aliquots, and the pH was measured with a Chemcadet digital meter. After 1.3 mL had been added, the solution was titrated back with standardized 0.01 N NaOH. The pK_a, determined from inflection points, was 4.8 in the forward direction and 5.0 in the reverse direction for 6 and 5.25 and 5.20 for **7**.

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Registry No. 1, 4055-39-4; **2**, 18209-14-8; 6, 5047-66-5; **7**, 15973-07-6; **9**, 103422-20-4; **10**, 103422-21-5; **11**, 103422-22-6; **12**, 103422-23-7; **13**, 103456-57-1; 14, 103422-24-8; **15**, 103422-25-9; 16, 32633-56-0; aziridine, 151-56-4; 2-methylaziridine, 75-55-8; propargylamine, 2450-71-7; 2-(aminomethyl)furan, 617-89-0.

Irreversible Blockage of Opioid Receptor Types by Ester Homologues of β-Funaltrexamine

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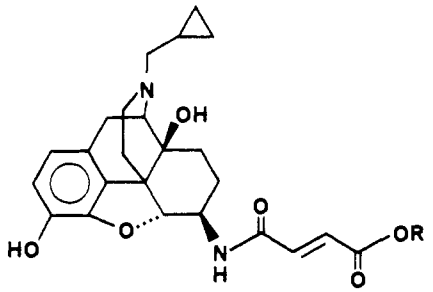
A series of ester homologues **2–5** of the μ receptor nonequilibrium antagonist β-funaltrexamine (1, β-FNA) was synthesized. These ligands were of interest in our investigation of the relationship between the structure of the ester function and the ability to irreversibly block μ opioid receptors. While all of the ligands were potent reversible agonists in the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations, most appeared to behave as irreversible antagonists of morphine. The benzyl **5** and phenethyl 6 esters possessed irreversible μ antagonist potency that was of similar magnitude to that of β-FNA in the GPI. In the MVD, all esters appeared to irreversibly block the agonist effect of morphine, but none of the compounds irreversibly antagonized [D-Ala^2,D-Leu^5]enkephalin to a significant degree. [^{3}H]Dihydromorphine displacement studies revealed no relationship between the affinity of the esters 1–6 and the irreversible blockage of μ receptors in the GPI or MVD. Possible reasons for the observed structure–activity relationship are discussed.

The nonequilibrium opioid antagonist β-funaltrexamine[1] (1, β-FNA) is employed widely as a pharmacologic probe.[2]



1, R = CH_3
2, R = CH_2CH_3
3, R = $(CH_2)_2CH_3$
4, R = $(CH_2)_3CH_3$
5, R = $CH_2C_6H_5$
6, R = $CH_2CH_2C_6H_5$

Its high selectivity for μ receptors both in vivo and in vitro has made β-FNA a useful ligand for investigating the involvement of μ receptors in opioid activity.

The high selectivity of β-FNA has been attributed to two recognition steps.[3] The first is manifested by the affinity of the ligand for the receptor, and the second involves the proper alignment of its electrophilic center with a proximal nucleophile on the receptor. This second recognition step appears to be particularly important when a selective electrophile is present, and in this regard it has been observed that the orientation and reactivity of a Michael acceptor group determines the effectiveness of irreversible blockage of μ receptors.

In an effort to reduce the number of variables in the structure–activity relationship of this series, we have examined β-FNA analogues **2–6** that differ only in the ester function. This series was of interest because it was anticipated that such modification should alter the steric and lipophilic parameters of the analogues without introducing significant changes in the intrinsic reactivity of the Michael acceptor moiety.

Chemistry. The β-FNA analogues **2–6** were synthesized by coupling 6β-naltrexamine[4] with the appropriate acid chlorides in aqueous THF containing K_2CO_3. Contaminants arising from acylation of the phenolic group were removed by a triethylamine-catalyzed ester interchange with the same alcohol as that contained in the fumaramate ester group. The acid chlorides for the above coupling were synthesized by first reacting maleic anhydride with an equimolar amount of the appropriate alcohol in a 10% molar excess of triethylamine. The resulting alkyl triethylammonium maleates were converted to the free carboxylates by extraction from aqueous HCl and heated with thionyl chloride to yield the acid chlorides.

The trans double bond in the acid chlorides was verified by NMR after they were reacted with 6β-naltrexamine to

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