were calculated from the equation $K_i = IC_{50}/[1 + (L/K_d)]$, where L is the concentration of radioligand and K_d is its dissociation constant.[23] Values represent the mean ± SE of 3–6 experiments in duplicate.

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Registry No. 1, 103616-11-1; 2, 103616-12-2; 3, 97168-80-4; 4, 103616-13-3; 5, 103617-34-1; 6, 103616-14-4; 7, 103616-15-5; 8, 103616-16-6; 9, 103499-93-0; 10, 103499-94-1; 11, 97134-60-6; 12, 103499-95-2; 13, 103499-96-3; 14, 97073-83-1; 15, 103499-97-4; 16, 97073-85-3; 17, 98634-03-8; 18, 72782-04-8; 19, 67025-97-2; 20 (n = 0), 110-15-6; 20a, 102817-61-8; 20b, 97073-86-4; 20c, 103499-98-5; 20d, 103499-99-6; 21a, 56-40-6; 21b, 556-50-3; 21c, 556-33-2; 21d, 637-84-3; 22, 5687-48-9; $ClCO(CH_2)_2COCl$, 543-20-4.

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Synthesis and Biological Activity of Analogues of β-Chlornaltrexamine and β-Funaltrexamine at Opioid Receptors

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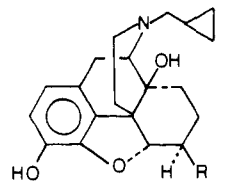
β-Chlornaltrexamine and β-funaltrexamine analogues **4–7** with different length "arms" to which an electrophilic moiety is attached were synthesized in an effort to obtain affinity labels that would selectively and irreversibly block specific opioid receptor types and subtypes. One of the compounds, **4**, was a potent, irreversible blocker of opioid receptors in the guinea pig ileum and mouse vas deferens preparations. The results of this study suggest that nucleophiles that are remote from the recognition locus are capable of alkylation by reactive electrophiles.

The nonequilibrium opioid receptor antagonists, β-chlornaltrexamine[1] (β-CNA) **1** and β-funaltrexamine[2] (β-FNA) **2**, have found wide use as tools in opioid research.[3] β-CNA is capable of alkylating at least three types of opioid receptors, and β-FNA is known to be highly selective for μ receptors. These ligands are effective both in vitro and in vivo.

Extensive structure–activity relationship studies of ligands related to **1** and **2** have revealed that the selectivity of covalent binding of opioid receptors is dependent on two consecutive recognition steps.[4] Thus, the ligand must have sufficient receptor affinity (primary recognition), and its electrophile must also be capable of reacting with a proximal receptor-based nucleophile in a selective fashion (secondary recognition).

This concept led to the development of ligands (e.g., **3**)



1. $N(CH_2CH_2Cl)_2$

2. $NHCO-C(H)=C(H)-CO_2CH_3$

3. $NHCO-CH_2N$ (succinimide)

that could irreversibly and selectively block one μ receptor subtype in the presence of other μ subtypes.[5] Our working hypothesis in this approach was that very closely related recognition sites (e.g., μ opioid receptor subtypes) possess a high degree of homology at their recognition locus, but considerably less homology as the distance from this locus is increased. The present study describes our effort to explore this approach further.

Design Rationale and Chemistry. In order to orient the electrophilic moiety in the ligand with receptor-based nucleophiles that may be distal to the recognition locus of the opiate pharmacophore, an oligoglycine "arm" was employed. Thus, the nitrogen mustard compound **4** is a β-CNA (**1**) analogue with a glycyl spacer, and the fumaramate esters (**5–7**) similarly can be viewed as being related to β-FNA.

Compound **4** was prepared by condensing the bromoacetamide **9**[5] with diethanolamine to yield **10**, which was treated with trioctylphosphine and CCl_4 to effect the replacement of OH by Cl.

Synthesis of the fumaramate esters **5–7** involved the mixed anhydride-mediated coupling of *N*-carbobenzyloxyglycine or its oligomer to β-naltrexamine[6] (**8**) to afford intermediates **11a–c**. Catalytic hydrogenolysis to oligomers **12a–c**, followed by coupling with fumaric acid monomethyl ester, afforded the desired compounds **5–7**.

Pharmacology. Compounds **4–7** were tested on the electrically stimulated longitudinal muscle of the guinea pig ileum[7] (GPI) and on the mouse vas deferens[8] (MVD) (Tables I and II, respectively).

In the GPI, compound **4** was a full, reversible agonist and possessed 8-fold greater agonist activity than morphine. All of the other members (**5–7**) in the series were partial agonists and exhibited a maximal response of about 45%. They appear to have varying degrees of reversible antagonist activity at a concentration of 20 nM. The ir-

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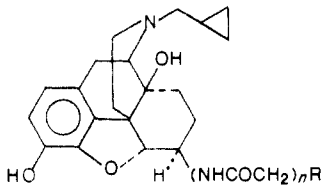

4. n = 1; R = $N(CH_2CH_2Cl)_2$
5. n = 1; R = NHCO-C(H)=C(H)$CO_2C_2H_5$
6. n = 2; R = NHCO-C(H)=C(H)$CO_2C_2H_5$
7. n = 3; R = NHCO-C(H)=C(H)$CO_2C_2H_5$
8. n = 0; R = NH_2
9. n = 1; R = Br
10. n = 1; R = $N(CH_2CH_2OH)_2$
11a. n = 1; R = $NHCO_2CH_2C_6H_5$
b. n = 2; R = $NHCO_2CH_2C_6H_5$
c. n = 3; R = $NHCO_2CH_2C_6H_5$
12a. n = 1; R = NH_2
b. n = 2; R = NH_2
c. n = 3; R = NH_2

reversible antagonism of morphine and ethylketazocine (EK) at a concentration of 200 nM was significant for 4, but not for 5–7.

None of the compounds behaved as full agonists in the MVD preparation, and maximal responses were seen between 55 and 60%. The nitrogen mustard 4 (200 nM) irreversibly blocked the agonist effect of morphine and [D-Ala^2,D-Leu^5]enkephalin (DADLE), while the Michael acceptors 5–7 afforded no significant change in the response to the agonists.

Discussion

The fact that the β-CNA analogue 4 irreversibly blocked agonist ligands that are selective for μ, κ, or δ receptors is in harmony with the high reactivity of the nitrogen mustard group via its aziridinium ion. This is consistent with the activity profile reported[9] for β-CNA and with the presence of nucleophiles in the vicinity of the recognition locus in these different opioid receptor types. In view of the reactivity of the nitrogen mustard group, the rank order irreversible antagonistic activities of 4 probably reflect its relative affinity for these receptor types.

None of the Michael acceptor ligands 5–7 irreversibly antagonized the selective agonists. The fact that these compounds behaved as reversible antagonists toward morphine and EK suggests that this apparent lack of irreversibility is due to a deficient second recognition step rather than to low affinity for the opioid receptor. Thus, the electrophiles in 4 and 5 are attached to the same arm (i.e., one glycyl group) and presumably are capable of projecting their electrophilic centers into similar nucleophilic environments that are remote from the primary recognition locus. It therefore seems reasonable that the great difference in irreversible antagonistic activity of 4 and 5 is related to (a) the large difference in reactivity between a nitrogen mustard and a Michael acceptor and (b) possibly the absence of a sulfhydryl group in that location.

Experimental Section

Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. IR spectra were obtained on a Perkin-Elmer 281 spectrometer. NMR spectra were obtained on a JEOL FT-90 spectrometer, with tetramethylsilane (Me_4Si) or sodium 2,2-dimethyl-2-sila-5-pentanesulfonate (DSS) as internal standards. Mass spectral analyses were performed on an AEI MS-30 or Finnegan 4000 for EI, or a Kratos MS 50 using a DS55 computer system for FAB mass spectrometry. EI mass spectra were conducted at 10–70 eV; CI mass spectra were obtained by using ammonia, methane, or isobutane as the reagent gas. FAB mass spectra were obtained by Ian Jardine at the Mayo Clinic/Foundation, Rochester, MN. A glycerol matrix was used for molecular weight determinations using sodium ions. Normal-phase TLC plates were Silica gel GF, 0.25 mm or 2000 μm thick, obtained from Analtech, Inc., Newark, DE. Reversed-phase TLC plates were KC(18)F: octadecylsilane bonded to silica gel, or LKC(18)F with a preadsorbant area, 200-μm layer, available from Whatman Chemical Separation, Inc., Clifton, NJ. Visualization of TLC plates was accomplished by using iodine crystals in a chamber, or the spray reagent *p*-nitrobenzylpyridine (NBP), or short-wave UV light. HPLC separations were performed on a Beckman 320 microprocessor-controlled two-pump system, using a RSil silica gel (12 μm; preparative; 250 × 10 mm) column from Alltech Associates, Deerfield, IL. All chemicals and solvents were of reagent grade. Chemicals were obtained from Aldrich Chemical Company, Milwaukee, WI; Sigma Chemical Company, St. Louis, MO; or Alfa Products, Thiokol/Ventron Division, Danvers, MA. Anhydrous THF was prepared by distillation over lithium aluminum hydride. Anhydrous DMF was obtained from spectral grade DMF by use of 4-Å molecular sieves.

6β-[2-[*N*,*N*-Bis(2-hydroxyethyl)amino]acetamido]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Dihydrochloride (10). Bromoacetamide 9[5] (0.10 g, 0.184 mmol) was added to a stirred solution of previously distilled diethanolamine (0.077 g, 0.737 mmol) in dry DMF (1 mL) at room temperature. Stirring was continued for 5 h and the solvent removed in vacuo overnight. To the residue was added H_2O (10 mL) and NaCl to saturation. The aqueous mixture (pH 10) was extracted with EtOAc (6 × 25 mL). The extract was dried and the solvent evaporated, leaving 0.445 g of 10 (98%) as a glass. The crude product was converted to the dihydrochloride salt and crystallized from a mixture of MeOH/EtOAc (5:20 mL)/H_2O (1 drop) to afford 0.358 g of 10 in three crops (80%): mp 200 °C dec; R_f = 0.39 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); IR (KBr) 1685 (CONH), 1300 (CH_2OH) cm^{-1}; CIMS (NH_3), m/e 486 (M^+ - 1), 341 ($M^+ - C_6H_{11}NO_3$), 144 ($C_6H_{11}NO_3^+$ - 1). Anal. ($C_{26}H_{37}N_3O_6 \cdot 2HCl \cdot H_2O$) C, H, N.

6β-[2-[*N*-(Benzyloxycarbonyl)amino]acetamido]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Hydrochloride (11a). β-Naltrexamine·2HCl·0.75H_2O (8;[6] 2.145 g, 5 mmol) was added with stirring to a mixture of *N*-methylmorpholine (1.4 mL, 12.7 mmol), water (2 mL), and DMF (2 mL), under nitrogen. To form the mixed anhydride solution, Z-glycine (1.048 g, 5 mmol) was stirred in dry THF (15 mL) at −30 °C, and *N*-methylmorpholine (0.55 mL, 5 mmol) and isobutyl chloroformate (0.65 mL, 5 mmol) were added. The above mixture containing 8 was cooled to −30 °C and added dropwise to the mixed anhydride solution over a 15-min period. The mixture was stirred for 45 min at −30 °C and allowed to rise to room temperature over an additional 2-h period. The solvents were evaporated in vacuo. Aqueous 50% $NaHCO_3$ solution (30 mL) was added to the gummy residue, and the mixture was extracted with ethyl acetate (4 × 50 mL). The organic extracts were dried and filtered and the solvent removed in vacuo, leaving an impure oil. The residue was dissolved in a minimum of methanol; ethyl acetate was added to dilute the solution, and the solution was purified by column chromatography (silica gel; EtOAc/MeOH/NH_4OH, 93:5:2) to give 1.44 g (54%) of pure product 11a, which crystallized upon standing (base form): mp 170 °C; R_f = 0.68 (EtOAc/MeOH/NH_4OH, 80:20:7:3), 0.37 (EtOAc/MeOH/NH_4OH, 93:5:2). The compound was converted to the hydrochloride salt by adding an equivalent amount of an HCl/MeOH solution to a solution of the compound in MeOH, followed by evaporation of the solvent: mp 210 °C dec; IR (KBr) 1715 (br s, OCO*N*H), 1670 (br s, *N*HCO) cm^{-1}; EIMS, *m/e* 533 (M^+), 425 ($M^+ - C_7H_8O$). Anal. ($C_{30}H_{36}N_3O_6Cl \cdot HCl \cdot 1.5H_2O$). C: calcd, 60.35; found, 60.80.

6β-[*N*-(Benzyloxycarbonyl)glycylglycinamido]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Hydrochloride (11b). Z-Glycylglycine (0.666 g, 2.5 mmol) and

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Table I. Activities of Electrophilic Oligoglycinamide Derivatives of Naltrexamine in the GPI

compd no.	agonism[b]	antagonism (IC_{50} ratio)[a] reversible[c] morphine	reversible[c] EK	irreversible[d] morphine	irreversible[d] EK
4	IC_{50} = 3.8 ± 1.0 nM (3)[e]			27.4 (2)	12.3 (2)
5	IC_{30} = 10 nM (1), 42% at 1 μM[f]	2.5 ± 1.3	2.5 (2)	0.6 (2)	2.0 (2)
6	IC_{20} = 10 nM (2), 45% at 100 nM[f]	16.8 ± 4.0	4.8 ± 2.2	3.9 ± 1.6 (3)	1.3 (2)
7	IC_{20} = 10 nM (3), 45% at 100 nM[f]	12.3 ± 0.9	3.3 ± 0.7	1.3 (2)	0.8 (2)

[a] IC_{50} of morphine or ethylketazocine (EK) in treated GPI divided by control IC_{50} in same preparation. [b] Morphine IC_{50} = 29 ± 7 nM (17); EK IC_{50} = 0.07 ± 0.08 nM (21). [c] Testing conduct in presence of 4–6 (10 nM) without washing. [d] The preparation was washed thoroughly (30 times) after exposure to the ligand (200 nM) for 30 min, and then tested with morphine or EK. [e] Numbers in parentheses represent the number of determinations. [f] Maximum percent inhibition at the concentration specified.

Table II. Activities of Electrophilic Oligoglycinamide Derivatives of Naltrexamine in the MVD

compd no.	agonism[a] (IC_{30})	irreversible antagonism (IC_{50} ratio)[b] morphine	DADLE
4	20 nM (2),[c] 55% at 1 μM	54% at 3 μM[d] (2)	4.0 ± 1.2 (4)
5	7 nm (2), 56% at 3 μM	1.8 (2)	0.8 (2)
6	10 nM (3), 59% at 1 μM	4.3 ± 3.1 (3)	1.2 ± 0.5 (3)
7	20 nM (3), 61% at 1 μM	1.2 (2)	1.2 (2)

[a] Morphine IC_{50} = 1.4 ± 0.3 μM (30); DADLE IC_{50} = 0.36 ± 0.05 nM (30). [b] IC_{50} of morphine or [D-Ala^2,D-Leu^5]enkephalin (DADLE) in the treated (incubate 200 nM of 4–7 for 30 min followed by 30 washes) GPI divided by the control IC_{50} in the same preparation. [c] Number of replicate experiments is in parentheses. [d] The maximum percent inhibition at the specified concentration.

8·2HCl (1.07 g, 2.5 mmol) were coupled and worked up by the procedure described for the preparation of **11a**. The product **11b** was purified by precipitation from a mixture of MeOH/EtOAc/ether: yield, 1.08 g (70%); mp 203–205 °C dec; R_f = 0.63 (EtOAc/MeOH/H_2O/NH_4OH, 80:30:7:3); IR (KBr) 3700–2500 (br, OH, NH), 1715, 1660 (urethane, amide I), 1530 (amide II) cm^{-1}; EIMS, *m/e* 590 (M^+), 482 ($M^+ - C_7H_8O$, 55% of base). Anal. ($C_{32}H_{41}N_4O_8Cl·H_2O$) C, H, N.

6β-[*N*-(Benzyloxycarbonyl)glycylglycylglycinamido]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Hydrochloride (11c). Z-Glycylglycylglycine (1.0 g, 3.1 mmol) and 8·2HCl (1.33 g, 3.1 mmol) were coupled and worked up as described for **11a** to afford 1.85 g (87%) of **11c**. An analytically pure sample was obtained by dissolving the compound in hot EtOH, adding ether, and cooling the clear solution to form a precipitate: mp 249–250 °C dec; R_f = 0.50 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); IR (KBr) 3700–2500 (v br, NH, OH) 1720 (s, urethane), 1655 (s, amide I), 1530 (m, amide II), 1270 (m, amide III) cm^{-1}; FABMS, *m/e* 648 (M^+). Anal. ($C_{34}H_{41}N_5O_8·HCl$) C, H, N.

6β-(2-Aminoacetamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Dihydrochloride (12a). Compound **11a** (1.50 g, 2.5 mmol) was dissolved in MeOH (75 mL); an equivalent of HCl gas in MeOH was added; and the mixture was hydrogenated over 10% Pd/C (0.30 g) at atmospheric pressure and room temperature for 2 h. The mixture was filtered of catalyst, which was washed with MeOH and water; the filtrate and washings were combined and evaporated in vacuo to give 1.21 g (97%) of **12a**: mp 250–270 °C dec. An analytical sample was purified by precipitation from glacial acetic acid: R_f = 0.2 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3), 0.56 (RP-Alltech, MeOH/H_2O/AcOH, 10:5:1); IR (KBr) 3700–2500 (v br, NH, OH), 1680 (m, NHCO) cm^{-1}; EIMS, *m/e* 339 (M^+). Anal. ($C_{22}H_{31}N_3O_4Cl_2·2HCl·1.5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-6β-(glycylglycinamido)-3,14-dihydroxymorphinan Dihydrochloride (12b). Compound **11b** (1.24 g, 1.98 mmol) was converted to **12b** by the procedure described for the preparation of **12a**: yield, 1.06 g (89%); mp 280 °C dec; R_f = 0.16 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); IR (KBr) 1670 (br, amide I), 1120 (s), 1030 (s) cm^{-1}; EIMS, *m/e* 456 (M^+), 399 (M^+ - glycine). Anal. ($C_{24}H_{32}N_4O_5·2HCl·3H_2O·0.5CH_3OH$) C, H, N, Cl.

17-(Cyclopropylmethyl)-4,5α-epoxy-6β-(glycylglycylglycinamido)-3,14-dihydroxymorphinan Dihydrochloride (12c). Compound **11c** (0.380 g, 0.555 mmol) was converted to **12c** as described for the preparation of **12a**: yield, 0.310 g (98%); mp 235 °C dec; R_f = 0.14 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); IR (KBr) 3700–2300 (v br, NH, OH), 1660 (s, amide I), 1540 (m, amide II) cm^{-1}. Anal. ($C_{26}H_{35}N_5O_6·2HCl·H_2O$) C, H, N.

6β-[2-[*N*,*N*-Bis(2-chloroethyl)amino]acetamido]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Dihydrochloride (4). Carbon tetrachloride (350 μL) was added to a stirred mixture of precursor **10** (138 mg, 0.239 mmol) and trioctylphosphine (350 μL) in distilled dry CH_3CN (1.4 mL) under nitrogen at room temperature. At 14-h intervals, the reaction supernatant was removed and fresh solvent and reagents added to the remaining unreacted starting material **10**. This procedure was repeated 3 times, or until all the starting material was reacted. The supernatant aliquots were dropped into a stirred EtOAc solution, and petroleum ether was added until a white precipitate formed. The precipitate was centrifuged into a pellet, resuspended in EtOAc, and recentrifuged. This procedure removed any unreacted trioctylphosphine and its oxide leaving 99 mg (67%) of **4** as a white solid. This crude material was purified of polar impurities by dry column chromatography on silica gel (Woelm TSC, preequilibrated with EtOAc/TEA, 100:1, eluted with EtOAc/TEA, 100:0.1). Fractions containing pure product were combined, and toluene was added and evaporated in vacuo to aid the removal of TEA. Care was taken not to concentrate the product in the free base form and to keep the solution as cold as possible. A solution of ethereal HCl was added dropwise to pH 3 and the solvent removed in vacuo, leaving 12 mg of **4** (8%): mp 193 °C dec; R_f = 0.66 (EtOAc/MeOH/NH_4OH, 80:20:2); IR (KBr) 3600–2300 (br, NH, OH), 1690 (s, amide I), 1560 (s, amide II), 1503 (d), 1320 (m), 1125 (s), 1030 (s) cm^{-1}; FABMS, *m/e* 524, 526, 528 (MH^+ + isotopes), 385 ($M^+ - N(CH_2CH_2Cl)_2$. Anal. ($C_{26}H_{35}N_3O_4Cl_2·2HCl·H_2O$). H: calcd, 6.40; found, 6.87.

(*E*)-17-(Cyclopropylmethyl)-4,5α-epoxy-6β-[2-[*N*-(4-ethoxy-4-oxo-2-butenoyl)amino]acetamido]-3,14-dihydroxymorphinan Hydrochloride (5). Fumaric acid monoethyl ester (30.27 mg, 0.21 mmol) was dissolved in freshly distilled dry THF (1 mL) and stirred over 4-Å molecular sieves at −30 °C. To the above solution were added *N*-methylmorpholine (25 μL, 0.21 mmol) and isobutyl chloroformate (26 μL, 0.20 mmol). A solution of **12a** (100 mg, 0.20 mmol) dissolved in dry DMF (1 mL) and *N*-methylmorpholine (50 μL, 0.44 mmol) at −30 °C was added dropwise after allowing the above mixture to stir for 2 min. Stirring was continued for 4 h, as the temperature was allowed to rise to room temperature. The molecular sieves were removed by filtration through a strainer that was rinsed with ethanol, and the combined filtrate was evaporated in vacuo. The residue was added to a half-saturated $NaHCO_3$ solution (10 mL) and extracted with EtOAc (5 × 10 mL). The combined organic extracts were washed with H_2O (20 mL), dried over Na_2SO_4, filtered, and evaporated, leaving **5** as an oil: R_f = 0.69 (EtOAc/MeOH/H_2O/NH_4OH, 80:20:7:3); ^{1}H NMR ($CDCl_3$) δ 8.048 (br s, 2 H, NHCO), 6.68 (AB_q, 2 H, *J* = 3.35 Hz, ArH), 6.89 (AB_q, 2 H, *J* = 15.28 Hz, CH=CH, trans), 4.53 (d, 1 H, *J* = 7.48 Hz), 4.16 (q, 2 H, CH_2CH_3), 4.21 (s, 2 H, $NHCH_2CO$), 1.28 (t, 3 H, CH_2CH_3); ^{1}H NMR (D_2O exchanged, CD_3OD 99.9%) δ 6.90 (AB_q, 2 H, *J* = 15.77 Hz, CH=CH), 6.65 (s, 2 H, ArH), 4.49 (d, 1 H, *J* = 7.7 Hz, C_5-H), 4.28 (q, 2 H, *J* = 7.03 Hz), 4.0 s, 2 H, $NHCH_2CO$), 1.34

(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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