case of cyclic lactam analogue 6, HPLC analysis of the crude product revealed the presence of two major components. Separation was easily achieved and analysis by FAB mass spectrometry indicated that the faster and the slower eluting components had MH^+ values of 852 and 1703, respectively. Obviously, the slower eluting peak corresponded to the side chain linked, antiparallel dimer that had been formed through interchain cyclization. Cyclodimerization was favored over cyclic monomer formation (66% dimer, 34% monomer). Final products were obtained as lyophilisates. Homogeneity was established by TLC and HPLC. All peptides were at least 95% pure, as judged from the HPLC elution profiles. Analytical parameters are presented in Table IV.

The synthesis of analogue **6a** has been described elsewhere.[20] DAGO was purchased from IAF Biochem International.

Binding Assays and Bioassays. Receptor binding studies with rat brain membrane preparations were performed as reported in detail elsewhere.[32] [^{3}H]DAGO and [^{3}H]DSLET at respective concentrations of 0.72 and 0.78 nM were used as radioligands, and incubations were performed at 0 °C for 2 h. IC50 values were determined from log dose–displacement curves and K_i values were calculated from the obtained IC50 values by means of the equation of Cheng and Prusoff,[33] using values of 1.3 and 2.6 nM for the dissociation constants of [^{3}H]DAGO and [^{3}H]DSLET, respectively.[4,34]

The GPI[35] and MVD[36] bioassays were carried out as reported in detail elsewhere.[32,37] A log dose–response curve was determined with [Leu^5]enkephalin as standard for each ileum and vas preparation, and IC50 values of the compounds being tested were normalized according to a published procedure.[38] K_e values for naloxone as antagonist were determined from the ratio of IC50 values obtained in the presence and absence of a fixed naloxone concentration.[39]

Acknowledgment. This work was supported by operating grants from the Medical Research Council of Canada (Grant MT-5655), the Quebec Heart Foundation, and the National Institute on Drug Abuse (Grant DA-04443-01). Naloxone hydrochloride was a gift from Endo Laboratories, Inc. We thank Drs. M. Evans and M. Bertand, Department of Chemistry, University of Montreal, for the performance of the FAB mass spectroscopic determinations. Thanks ara also due to H. Zalatan for typing the manuscript.

Registry No. 1, 118476-81-6; **1a**, 118476-88-3; **2**, 118476-82-7; **3**, 118476-83-8; **4**, 118476-84-9; **5**, 118476-85-0; **6**, 118476-86-1; **6a**, 96382-72-8; **7**, 118494-42-1; **7a**, 118476-87-2; BOC-Tyr(BOC)-OH, 20866-48-2; BOC-Arg(Tos)-OH, 13836-37-8; Fmoc-D-Orn-(BOC)-OH, 118476-89-4.

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Preparation and Antitumor Activity of Additional Mitomycin A Analogues

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On the basis of qualitative structure–activity relationships developed in the preceding article,[2] a series of 32 new mitomycin A analogues were prepared and tested in antitumor screens. Seven of them gave greater prolongation of life (ILS) than mitomycin C in the mouse P388 leukemia assay. They included examples with 7-O substituents such as cyclic ethers and nitrogen heterocycles. A Hansch analysis was attempted with log P and MR as the independent variables, but no statistically significant correlation could be made. Seven compounds, chosen mainly for their good potency (MED), were tested in the subcutaneous B16 melanoma assay in mice and four of them showed greater ILS than mitomycin C.

The synthesis and antitumor activity of mitomycin C analogues have been extensively investigated, resulting in data on about 500 new compounds. In contrast, relatively few mitomycin A analogues (7-methoxymitosanes) have been prepared, despite the high potency (in terms of minimal effective dose, MED) of mitomycin A against P388 leukemia and subcutaneous B16 melanoma in mice.[1] We recently addressed the question of mitomycin A analogues by preparing and testing a group of 26 compounds in which the 7-methoxy group was replaced by a wide variety of substituted alkoxy groups.[2] Many of these analogues were superior to mitomycin C against the two mouse tumors noted above. Although statistically significant QSAR could not be established for this set of analogues, we suggested that, on the basis of qualitative guidelines, future analogues might emphasize 7-O substituents including hydrophilic straight chains, cyclic ethers, and tertiary amines. In the present research, these guidelines are explored further. As described below, we have prepared and screened a series of 32 new analogues, most of which contain the type of 7-O substituents named above. A few new types of analogues, containing cyclopropyl, aryl, or silyl groups, also have been studied.

Chemistry

Two different methods were used for the preparation of mitomycin A analogues. One involved an alkoxide exchange in which mitomycin A was treated with alkali in

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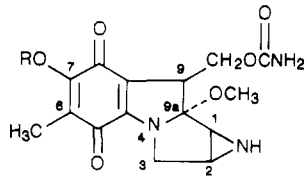
0022-2623/89/1832-0703$01.50/0

Table I. Preparation and Properties of O^7-Substituted Mitomycin A Analogues[a]



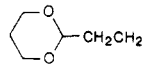
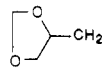
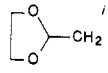
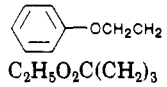
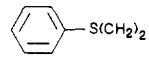
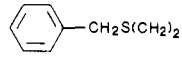
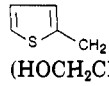
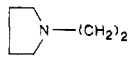
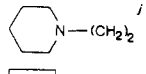
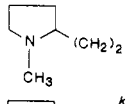
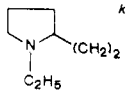
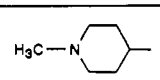
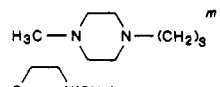
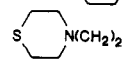
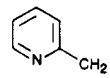
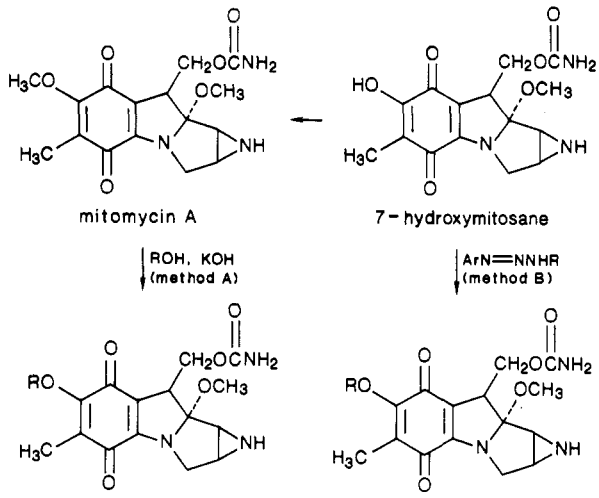
no.	R	method	yield, %	solvent impurity	melt. dec temp, °C	^{1}H NMR signals for the 7-substitutent,[b] δ
1		B	20	$0.5H_2O$	151–153	5.0–5.55 (m, 1 CH), 1.1–1.65 (m, 2, α-CH_2), 0.5–1.0 (m, 2, β-CH_2 overlapped with aziridine NH)[c]
2		A	63		68–92	4.08–4.18 (d, 2, CH_2O), 1.0–1.3 (m, 1 CH overlapped with aziridine NH), 0.4–0.7 (m, 2, α-CH_2), 0.25–0.40 (m, 2, β-CH_2)[c]
3	$Cl(CH_2)_2O(CH_2)_2$	A	58		101–104	4.35–4.55 (t, 2, OCH_2CH_2), 3.5–3.58 (m, 6, $ClCH_2CH_2OCH_2$[d])
4	$HOCH_2CH{=}CHCH_2$	A	39	$0.5H_2O$	85–105	5.6–5.9 (m, 2, CH=), 4.85–5.0 (m, 2, CH_2O), 4.1–4.3 (d, 2, CH_2OH)
5	$CH_3OCH_2CH(CH_3)$	A	39	$0.5H_2O$	indef.	4.4–5.0 (m, 1, CH[d]), 3.43–3.73 (m, 2, CH_2[d]), 3.3 (s, 3, CH_3O), 1.25–1.4 (d, 3, $CHCH_3$)
6	$CH_3OCH_2CH(OH)CH_2$	A	45	$0.5H_2O$	58–80	4.3–5.3 (m, 2, $CHCH_2O$[d]), 3.2–4.3 (m, 7, CH_3OCH_2CHOH[d])
7	$C_2H_5O(CH_2)_2$	A	46	$0.5H_2O$	68–78	4.22–4.5 (t, 2, CH_2O-quinone), 3.4–3.7 (m, 4, CH_2OCH2[d]), 1.8–2.1 (m, 2, CH_2CH_3 overlapped with 6-CH_3), 1.05–1.35 (t, 3, CH_2CH_3)
8		A	21	$0.5H_2O$	115–120	4.4–5.0 (m, 1, CH[d]), 3.75–4.2 (t, 2, equatorial H of CH_2O), 3.3–3.75 (m, 2, axial H of CH_2O[d]), 1.4–2.2 (m, 4, CH_2CH[d])
9		B	53	$0.5H_2O$	55–63	4.55–4.85 (m, 1, OCH_3 overlapped with 10-CH_2), 4.22–4.4 (t, 2, CH_2O-quinone), 3.8–4.22 (m, 4, CH_2O), 1.6–2.2 (m, 3, $CHCH_2$ + equatorial H of $CH_2CH_2CH_2$ overlapped with 6-CH_3), 1.15–1.47 (d, 1, axial H of $CH_2CH_2CH_2$)
10		A	15	$0.25H_2O$	>300	4.6–5.1 (m, 3, OCH_2O + CH overlapped with 10-CH_2 and NH_2), 3.95–4.2 (m, 4, CH_2O overlapped with one C-3 H)
11		A	16		161–163	5.07 (s, 1, one H of OCH_2O overlapped with NH_2), 4.95 (s, 1, one H of OCH_2O), 4.38–4.42 (d over m, 3, CH_2O-quinone + CHO), 3.82–4.15 (m, 2, CH_2O[d])
12		B	36	$0.25H_2O$	48–50	4.6–5.0 (m, 1, CH overlapped with 10-CH_2), 4.2–4.6 (d, 2, CH_2O-quinone), 3.8–4.2 (s, 4, CH_2O)
13		A	71		153–155	7.17–7.4 (d, 2, o-phenyl), 6.8-7.05 (t, 3, *m*- + *p*-phenyl), 4.6–4.75 (t, 2, CH_2O-quinone), 4.1–4.3 (t, 2, CH_2O-phenyl)
14	$C_2H_5O_2C(CH_2)_3$	B	47	$0.25H_2O$	85–88	4.25–4.41 (t, 2, CH_2O-quinone), 4.02–4.28 (q, 2, CH_2-ester), 2.35–2.6 (t, 2, CH_2CH_3), 1.85–2.2 (quintet, 2, $CH_2CH_2CH_2$), 1.15–1.35 (t, 3, CH_3CH_2)
15	$CH_3S(CH_2)_2$	A	72	$0.5H_2O$	72–86	4.35–4.55 (t, 2, CH_2O), 2.7–3.0 (t, 2, CH_2S overlapped with aziridine CH), 2.15 (s, 3, CH_3)
16	$HO(CH_2)_3S(CH_2)_3$	A	41	$1.25H_2O$	68–87	4.35–4.60 (t, 2, CH_2O overlapped with 10-CH_2), 3.57–3.87 (m, 2, CH_2OH[d]), 2.33–3.1 (m, 5, CH_2S + OH overlapped with aziridine CH), 1.73–2.3 (m, 4, $CH_2CH_2CH_2$)
17	$C_2H_5SCH_2CH(OH)CH_2$	A	15	$0.5H_2O$	110–115[f]	4.0–4.5 (m, 2, CH_2O), 3.4–3.8 (m, 1, CH[d]), 2.4–2.7 (m, 4, CH_2S), 1.0–1.4 (t, 3, CH_3 overlapped with 5-CH_3)
18	$HO(CH_2)_2S(CH_2)_2S(CH_2)_2$	A	38		70–95	4.32–4.51 (t, 2, CH_2O-quinone), 3.67–3.82 (t, 2, CH_2OH), 2.65–2.95 (m, 8, CH_2S overlapped with aziridine CH)
19		A	61	$0.25H_2O$	96–106	7.15–7.5 (m, 5, phenyl), 4.35–4.55 (t, 2, CH_2O), 3.15–3.3 (t, 2, CH_2S overlapped with 9a-CH_3O)
20		B	35		40–43	7.15–7.5 (m, 5, phenyl), 4.3–4.55 (m, 2, CH_2O, overlapped with 10-CH_2), 3.75 (s, 2-$CH_2C_6H_5$), 2.61–3.1 (m, 2, CH_2S overlapped with aziridine CH)
21		A	56	H_2O	70–75	7.25–7.4 (dd, 1, thiophene α-H), 6.9–7.1 (m, 2, thiophene β-H), 5.53 (s, 2, CH_2O)
22	$(HOCH_2CH_2)_2N(CH_2)_3$	B	29	$0.75H_2O$	73–88	4.8–5.3 (br, 2, OH), 4.35–4.7 (m, 6, CH_2O[d]), 2.3–3.9 (m, 6, CH_2N overlapped with aziridine CH), 1.5–2.0 (m, 2, $CH_2CH_2CH_2$ overlapped with 6-CH_3)
23		A	32	$0.5H_2O$	indef.	4.2–4.6 (m, 2, CH_2O overlapped with 10-CH_2), 2.68–3.0 (t, 2, CH_2N exocyclic overlapped with aziridine CH), 2.4–2.68 (t, 4, CH_2N of pyrrolidine ring), 1.5–2.0 (m, 4, $CH_2CH_2CH_2CH_2$ overlapped with 6-CH_3)
24		A	17	$0.75H_2O$	65–80	4.1–5.5 (m, 2, CH_2O overlapped with 10-CH_2 and NH_2), 2.1–2.75 (m, 6, CH_2N), 1.05–1.6 (m, 6, CH_2)
25		B	11	$0.5H_2O$	76–96	3.8–4.05 (m, 2, CH_2O overlapped with 10-CH_2), 2.2–2.4 (m, 3, CH_2N + CHN), 1.95–2.1 (m, 3, CH_3 overlapped with 6-CH_3), 1.5–1.95 (m, 6, CH_2)
26		B	13		60–65	4.2–4.45 (m, 2, CH_2O), 2.0–2.7 (m, 5, CH_2N + CHN), 1.4–2.0 (m, 6, CH_2), 1.0–1.2 (t, 3, CH_3)

Table I (Continued)

no.	R	method	yield, %	solvent impurity	melt. dec temp, °C	^{1}H NMR signals for the 7-substitutent,[b] δ
27		A	44	H_2O	47–65	4.6–4.9 (m, 1, CHO[d]), 2.5–2.7 (t, 2, CH_2N equatorial H), 2.1–2.5 (s over t, 5, CH_3 + CH_2N axial H), 1.7–2.1 (m, 4, CH_2CH_2CH overlapped with 6-CH_3)
28		A	35	$1.75H_2O$	81–83	3.4–3.7 (m, 2, CH_2O[d]), 2.4–2.8 (m, 10, CH_2N), 2.3 (s, 3, CH_3), 1.77–2.0 (m, 2, $CH_2CH_2CH_2$)
29		B	29	$1.25H_2O$	132–136	4.25–5.0 (m, 2, CH_2O[d]), 2.5–3.05 (m, 10, CH_2N + CH_2S overlapped with aziridine CH)
30	$H_3CCONH(CH_2)_2$	B	32	$0.5H_2O$	>350	6–6.45 (br, 1, NH), 4.05–4.25 (t, 2, CH_2O), 3.3–3.8 (m, 2, CH_2[d]), 2.0 (s, 3, CH_3)
31		A	27	$0.5H_2O$	100–105[g]	8.5–8.63 (d, 1, pyridine α-H), 7.45–7.85 (m, 2, pyridine β- + γ-H), 7.13–7.33 (t, 1, pyridine β′-H), 5.4 (s, 2, CH_2O)
32	$(C_2H_5O)_3Si(CH_2)_3$	B	14	H_2O	73–88	4.15–4.5 (t, 2, CH_2O), 3.8–4.15 (m, 6, CH_3CH_2O[d]), 1.6–2.4 (m, 2, $CH_2CH_2CH_2$ overlapped with 6-CH_3), 1.1–1.15 (t, 9, CH_3), 0.5–1.0 (m, 2, CH_2Si)

[a] Analytical results were within ±0.40% of theoretical values for all elements (C, H, N, S) except as shown in subsequent footnotes. In some examples, solvent impurities were added to reconcile the calculated and found values for these elements. Some products were hygroscopic. They were dried under vacuum, but they could not be heated because of instability. [b] The solvent was $CDCl_3$ unless indicated otherwise. [c] In the cyclopropane ring, β refers to H on the same side as the substituents and α refers to H on the opposite side. [d] This signal is overlapped by other signals. [e] Both **11** and **12** were isolated from the same reaction because the starting material, glycerol formal, was a mixture of 1,3-dioxan-5-ol and 1,3-dioxolane-4-methanol as supplied by Aldrich Chemical CO. [f] Partial melting at 65–88 °C. [g] Changed to semisolid at 55–70 °C. [h] N: calcd, 9.81; found, 9.03. [i] N: calcd, 9.87; found, 9.42. [j] H: calcd, 6.85; found, 6.40. [k] C: calcd, 60.00; found, 60.57. [l] N: calcd, 12.44; found, 11.82. [m] H: calcd, 7.20; found, 6.57.

Scheme I. Preparation of Mitomycin A Analogues



a solution of the alcohol (method A),[2,3] whereas the other involved treatment of 7-hydroxymitosane with an appropriate triazene (method B).[2,4] These methods are outlined in Scheme I. The new analogues and their physical properties are listed in Table I. The choice of a particular route was based mainly on the availability of the necessary alcohol or amine. The alkoxide-exchange method usually does not work with solid alcohols because dilution with solvents decreases the mass effect. However, the high solubility of 3,6-dithia-1,8-octanediol in tetrahydrofuran (ca. 50%) allowed the preparation of **18** in 38% yield.

Antitumor Activity

Table II gives the activity of mitomycin A analogues against P388 leukemia in mice. This system is the principal database for mitomycins. The compounds in Table II were not all screened at the same time, but each experiment contained a mitomycin C control group as well as an untreated group. Consequently, compounds should be compared on the basis of how each one related to its mitomycin C control, rather than directly. The maximum effect of the mitomycin C control at its optimal dose is given in parentheses after the maximum effect of each mitomycin A analogue. Among the 32 new compounds in Table II, seven gave greater prolongation of life (ILS) than mitomycin C. Compounds **12**, **17**, and **24** were the best ones. We reported previously that mitomycin A and its analogues are especially potent in terms of the minimal dose needed for an ILS ≥ 25% (MED).[1] Ten of the compounds in Table II, plus mitomycin A, gave lower MED values than mitomycin C and six more were equipotent with it (0.2 mg/kg). Despite these good activities and potencies, no compound showed the kind of unusual potency given by a few analogues in our previous article.

Although the P388 murine leukemia assay is relatively sensitive to mitomycin analogues, the subcutaneous B16 melanoma assay is stringent and insensitive to many compounds active in the former assay. Our previous article noted that some mitomycin A analogues were highly active in this melanoma assay, showing as many as 6/6 long-term survivors.[2] A group of seven compounds in Table II, selected mainly on the basis of low MED values against P388 leukemia, were tested against subcutaneous B16 melanoma. This selection followed from the consideration that any analogue of potential clinical interest would have to be highly potent because of the expense of preparation. As shown in Table II, two of these compounds gave distinctly greater ILS than mitomycin C and three of them had lower MED values. The best compound, **6**, gave 2/6 long-term survivors.

Structure–Activity Relationships

Previous attempts at quantitative correlations between the physical properties of mitomycins and their antitumor activity have usually been unrewarding. Our only success was limited to a series of N^7-phenylmitomycin C analogues for which it was found that log P accounted for 70% of the variance.[5] No statistically significant correlation was obtained with the previous set of mitomycin A analogues,

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Table II. Antitumor Activity of Mitomycin A Analogues[a]

	intraperitoneal P388 leukemia[b]							subcutaneous B16 melanoma[c]		
			MED							
no.	max effect, % T/C	opt dose, mg/kg	mg/kg	mol/kg × 10^{-6} [d]	log (1/C)	log P[e]	MR[f]	max effect, % T/C	opt dose, mg/kg	MED, mg/kg
1	172 (211)	3.2	0.05	0.130	6.89	1.17	16.38	138 (135)	0.8	0.8
2	144 (189)	1.6	0.4	1.028	5.97	1.67	20.72			
3	183 (189)	3.2	<0.05	0.062	7.21	1.18	30.97	169 (154)	0.8	0.4
4	150 (172)	3.2	0.2	0.483	6.21	0.46	26.05			
5	206 (278)	0.8	0.2	0.481	6.21	0.79	25.99			
6	222 (278)	0.8	<0.1	0.116	6.94	−0.59	23.19	211 (170) [2]	0.8	0.2
7	133 (278)	1.6	0.2	0.465	7.33	0.79	24.94			
8	212 (278)	3.2	<0.1	0.117	6.93	1.29	30.59	135 (159)	1.6	1.6
9	117 (172)	0.2	-	-	-	0.82	42.41	154 (159)	0.4	<0.3
10	210 (200)	1.6	<0.1	0.118	6.93	0.31	33.86	222 (159)	1.2	<0.3
11	140 (200)	0.4	0.2	0.475	6.32	0.31	33.86			
12	267 (172)	0.4	<0.05	0.588	7.23	−0.18	28.21			
13	130 (200)	3.2	1.6	3.516	5.45	2.39	40.15			
14	171 (276)	12.8	6.4	14.10	4.85	1.49	36.24			
15	165 (200)	1.6	0.4	0.957	6.02	1.37	26.29			
16	153 (276)	6.4	<0.1	0.102	6.98	3.49	51.95			
17	>322 (278)	3.2	0.2	0.433	6.37	1.25	34.79			
18	140 (200)	0.8	0.8	1.603	5.84	1.86	51.95			
19	130 (200)	12.8	3.2	6.73	5.17	3.12	47.05			
20	156 (306)	6.4	0.2	0.425	6.37	3.58	51.65			
21	159 (132)	3.2	3.2	7.127	5.15	2.07	31.23			
22	106 (211)	0.2	-	-	-	−0.28	48.05			
23	217 (278)	6.4	<0.1	0.113	6.94	1.46	38.32	107 (159)	0.4	
24	191 (132)	12.8	0.4	0.871	6.06	1.96	42.98			
25	156 (>344)	12.8	3.2	7.033	5.21	1.46	38.32			
26	211 (278)	12.8	<0.1	0.109	6.96	1.96	48.62			
27	155 (132)	3.2	3.2	7.10	5.15	1.46	38.32			
28	167 (>344)	12.8	6.4	12.65	4.90	1.16	51.44			
29	172 (>344)	6.4	0.1	0.206	6.67	2.07	47.49			
30	150 (200)	12.8	0.4	0.932	6.03	−0.21	27.04			
31	164 (132)	0.8	0.4	0.920	6.04	0.91	30.22			
32	167 (211)	12.8	0.2	0.359	6.44	-	-			
mit A	180 (270)	3.2	0.05	0.143	6.84	0.26	7.87	177 (170)	0.3	0.05

[a] Determined at Bristol-Myers Co., Syracuse, NY. [b] A tumor inoculum of 10^6 ascites cells was implanted ip in CDF_1 female mice. Six mice were used at each dose of the mistosane, given ip once on day 1, and 10 control mice were injected with saline. A control group of six mice at each dose received mitomycin C in the same experiment: MST = median survival time; max effect (% T/C) = (MST treated/MST control) × 100 at the optimal dose (opt dose). MED = minimum effective dose (% T/C ≥ 125). The maximum effect of the mitomycin C control at its optimal dose (usually 3.2 or 4.8 mg/kg) in the same experiment is given in parentheses. [c] A tumor inoculum is implanted subcutaneously in BDF_1 female mice. Ten mice were used at each dose of the mitosane, given in three equal parts on days 1, 4, and 7, by intravenous administration in the tail vein. Ten control mice received intravenous saline. Definitions of the test result expressions are given above. The number of 61-day survivors at the optimal dose are given in brackets beside the maximum effect and the maximum effect of the mitomycin C control at its optimal dose (1–3 mg/kg) in the same experiment is given in parentheses. [d] For compounds where the MED was not reached, the next lower multiple of two was used in computing the moles/kilogram value (expressed as C in column 6). [e] log P for mitomycin A was determined by the method of Hansch et al. (Hansch, C.; Muir, R. M.; Fujita, T.; Malongy, P. P.; Geiger, F.; Struch, M. *J. Am. Chem. Soc.* **1963**, *85*, 2817) and other log P values were estimated from its value (0.26) and values of the 7-substituents (Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1969). [f] The MR values represent only the 7-substituent part of the molecule. They are taken from the preceding reference.

partly because they gave flat dose–response curves and there was much scatter in the data. We were not optimistic that the mitomycin A analogues in table II would afford useful QSAR. However, it did not seem proper to ignore this possibility, because there was reasonable data on 29 compounds, including mitomycin A, in the P388 assay. The values of log (1/C), log P, and MR (molar refractivity) for each compound are given in Table II, except for those compounds too inactive to have a MED. The dependent variable C is the MED expressed in moles/kilogram. When these data were subjected to a Hansch analysis, using the program SPSSX for multiple linear regression,[6] we found that there was no statistically significant correlation between any two variables. The R^2 values were 0.105 and 0.088 for attempted correlation of log (1/C) with log P and MR, respectively.

Although quantitative correlation failed, it is possible to make some crude qualitative comparisons between structure and activity against P388 leukemia. One surprising observation is that there is very little overlap between the analogues that give ILS greater than that of mitomycin C (**10**, **12**, **17**, **21**, **24**, **27**, and **31**) and those that have a lower MED than mitomycin C (**1**, **3**, **6**, **8**, **10**, **12**, **16**, **23**, **26**, **29**, and mitomycin A). Only the 1,3-dioxane and 1,3-dioxolane derivatives, **10** and **12**, were superior in both assays. Two other analogues with greater ILS had substituents based on aromatic heterocycles. Analogues with lower MED values included a variety of substituents such as straight-chain or cyclic ethers and tertiary amines.

Conclusions

The two main methods for synthesis of mitomycin A analogues, alkoxide exchange on mitomycin A and reaction of triazenes with 7-hydroxymitosane, provided 32 new compounds. Certain of these compounds gave greater ILS than mitomycin C against P388 leukemia and/or subcutaneous B16 melanoma, and one-third of them had lower MED values than mitomycin C. These low MEDs are consistent with those of previous mitomycin A analogues. Attempted QSAR derivation was unsuccessful and even qualitative correlations were not simple. The most active

(6) SPSSx is available from the Vogelback Computer Laboratory, Northwestern University, Evanston, IL.

Table III. Chromatographic Solvent Systems for Isolating the Products

	solvent system		
		preparative TLC	
compd	column	1st isolation	2nd isolation
1		$CHCl_3$–MeOH (9:1)	
2		Et_2O	$CHCl_3$–acetone (1:1)
3	$CHCl_3$ then acetone[a]	$CHCl_3$	$CHCl_3$–acetone (1:1)
4		Et_2O–acetone (9:1)[a]	Et_2O–acetone (6:4)
5		Et_2O	$CHCl_3$–MeOH (9:1)
6	Et_2O–acetone (8:2) then acetone	Et_2O–acetone (8:2)	
7		Et_2O[a]	$CHCl_3$–MeOH (9:1)
8		Et_2O–THF (4:1)	Et_2O–acetone (17:3)
9		$CHCl_3$–MeOH (9:1)	
10 + 11	Et_2O then $CHCl_3$–MeOH (8:2)	Et_2O–acetone (13:7)	
12		$CHCl_3$–acetone (1:1)	
13	Et_3N then $CHCl_3$–MeOH (9:1)	$CHCl_3$–acetone (6:4)	
14		$CHCl_3$–MeOH (9:1)	$CHCl_3$–MeOH (9:1)
15	Et_2O then $CHCl_3$–MeOH (9:1 or 8:2)	$CHCl_3$–MeOH (9:1)	
16	$CHCl_3$–acetone (1:1)	$CHCl_3$–acetone (1:1)	Et_2O–acetone (7:3)
17	Et_2O then acetone	Et_2O–acetone (8:2)	
18	Et_2O then $CHCl_3$–MeOH (9:1)	$CHCl_3$–MeOH (9:1)	
19	Et_2O then $CHCl_3$–MeOH (9:1 or 8:2)	$CHCl_3$–MeOH (9:1)	
20		$CHCl_3$–MeOH (19:1)	
21	Et_2O then $CHCl_3$–MeOH (9:1 or 8:2)	$CHCl_3$–MeOH (9:1)	
23		Et_3N[b]	acetone[b]
24	Et_3N then acetone[c]	$CHCl_3$–acetone (1:1)	
25		Et_3N	acetone then Et_3N–acetone (99:1)
26		Et_3N	Et_3N–acetone (24:1)
27	Et_3N then acetone–Et_3N (2:1)	$CHCl_3$–MeOH (7:3)	
28	Et_3N then acetone–Et_3N (19:1)[d]		
29		$CHCl_3$–MeOH (9:1) then $CHCl_3$–acetone (4:6)	$CHCl_3$–acetone (4:6)
30		$CHCl_3$–acetone (4:6)	$CHCl_3$–MeOH (9:1)
31	$CHCl_3$–MeOH (19:1) then $CHCl_3$–MeOH (9:1)	$CHCl_3$–MeOH (9:1)	
32		$CHCl_3$–MeOH (9:1)	

[a] In these isolations, the product (pink) remains on the base line and the excess alcohol and byproducts move from it. [b] The product was extracted from silica gel by Et_3N–acetone (1:3 or 1:4). [c] The oily product from this isolation was treated with Et_2O and the insoluble material was filtered off and discarded. The red filtrate was concentrated under reduced pressure and the residue was purified by preparative TLC as indicated in the table. [d] The oily product from this isolation was crystallized from ether.

and potent analogues were those with cyclic diether (**10** and **12**), linear hydroxy ether, and thioether (**17** and **6**) functionalities. In our previous article, linear diethers, linear hydroxy ethers, and thioethers were found to be highly active especially against subcutaneous B16 melanoma in mice.[2]

These results suggest that further studies on diethers and hydroxy ethers might be valuable. We are attempting to gain more information on the DNA binding of these compounds through computer modeling.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL FX90Q (90 MHz) spectrometer and absorptions are reported as downfield from Me_4Si. Elemental analysis were performed by Mic Anal, Inc., Tucson, AZ. Analytical values were within ±0.4% of theoretical values unless specified otherwise.

Preparation of Mitomycin A Analogues. Method A. In the standard procedure, mitomycin A (100 mg) in 2 mL of the alcohol was stirred at room temperature under nitrogen for 45 min with 500 mg of a 1.6% solution of KOH in the alcohol. The mixture was neutralized with solid CO_2 while the reaction vessel was immersed into a water bath at room temperature. With high freezing point alcohols as in examples **8**, **13**, **15**, **16**, **19**, **21**, **24**, **25**, **27**, **28**, and **31**, the mixture was dissolved first in Et_2O and then decomposed with solid CO_2 and filtered from insoluble material, if any. The product was separated from excess alcohol and byproducts by column chromatography on silica gel and/or preparative TLC on silica gel (except that neutral Al_2O_3 was used for **24**) using the solvent systems given in Table III. Yields, melting points (decomposition ranges), and NMR data for the products are given in Table I. The following compounds were prepared by the indicated changes in the standard procedure: **2**, 240 mg of 1.6% KOH; **3**, 300 mg of 1.6% KOH; **4**, 148 mg of mitomycin A and 600 mg of 1.6% KOH; **10** + **11**, 216 mg of mitomycin A in 4 mL of the alcohol and 1.0 g of 1.6% KOH; **16**, 120 mg of mitomycin A and 300 mg of 1.6% KOH; **17**, 300 mg of 1.6% KOH; **23**, 4 mL of the alcohol and 240 mg of 1.6% KOH; **28**, 1.0 g of 1.6% KOH.

The preparation of compound **18** involved a considerable variation in the procedure. It was prepared as follows: A saturated solution of 3,6-dithia-1,8-octanediol (solid) in the least amount of THF was added to a solution of 100 mg of mitomycin A in 1 mL of THF. To this mixture was added a solution of 8 mg of KOH and 0.5 g of 3,6-dithia-1,8-octanediol in 0.5 mL of THF. The resulting mixture was stirred at 35 °C for 15 min. After decomposition with solid CO_2, the THF was evaporated under reduced pressure and the product was isolated from the residue by chromatography (cf. Table III).

All the alcohols used in the preparation of mitomycin A analogues by method A were available commercially from Aldrich Chemical Co. Compounds **10** and **11** were isolated from the same reaction, because the starting alcohol, glycerol formal, was supplied as a 98% mixture of 1,3-dioxan-5-ol and 1,3-dioxolane-4-methanol.

Method B. A solution of 7-hydroxymitosane, obtained from the hydrolysis of 200 mg of mitomycin C,[7] in 15–40 mL of CH_2Cl_2 was stirred at room temperature and under N_2 with a solution of the triazene, prepared as described below, in CH_2Cl_2 for the appropriate time. The solvent was removed under reduced pressure and the product was isolated by chromatography on silica gel as described in Table III. Yields, melting points (decomposition ranges), and NMR data are given in Table I. The following amounts of the triazenes and reaction times were used: **1**, 500 mg in 5 mL, 1h; **9**, 600 mg in 10 mL, 8h; **12**, 430 mg in 20 mL, 24h; **14**, 500 mg in 5 mL, 24h; **20**, 500 mg in 20 mL, 16h; **22**, 840 mg in 20 mL, 60h; **25**, 800 mg in 5 mL, 17h; **26**, 800 mg in 25 mL,

(7) Matsui, M.; Yamada, Y.; Uzu K.; Hirata, T. *J. Antibiot.* **1968**, *21*, 189.

24h; **29**, 500 mg in 20 mL, 18h; **30**, 473 mg in 20 mL, 15h; **32**, 1.0 g in no solvent, 2h.

Product **22** was insoluble in the reaction media. It was filtered off, washed well with Et_2O and $CHCl_3$, and then characterized directly.

Preparation of 3-Substituted 1-Phenyltriazenes. A cold (0 °C) solution of 1 equiv of the appropriate amine free base in *N,N*-dimethylformamide containing excess K_2CO_3 was stirred and treated with a cold solution of 1.1 equiv of benzenediazonium hexafluorophosphate in *N, N*-dimethylformamide, added in portions. After the addition was complete, the mixture was stirred 2h at 0–5 °C and then poured into ice water. An ether extract (hexane in case of 3-cyclopropyl) was washed several times with water, dried, and concentrated under reduced pressure to give the desired triazene, which in most cases was used without purification for preparation of the corresponding mitomycin A analogue. The ^1H NMR spectra were measured for all triazenes. They showed signals for the phenyl protons at δ 7.0–7.5, and the NH at δ 7.7–8.3 [except for the derivatives with 2-methylene-1,3-dioxolane (δ 8.3–8.9), cyclopropyl (δ 8.7–9.4), and 3-(triethoxysilyl)propyl (δ 8.9–9.8)]. Signals for each of the 3-substituents were essentially the same as given in Table I. Complete ^1H NMR assignments are given below. The following triazenes were prepared.

3-Cyclopropyl-1-phenyltriazene: yield 40%; red oil; ^1H NMR ($CDCl_3$) δ 8.7–9.4 (br, 1, NH), 6.8–7.7 (m, 5, Ar-H), 3.2–3.6 (m, 1, CH), 0.55–1.15 (m, 4, CH_2).

3-[2-(1,3-Dioxan-2-yl)ethyl]-1-phenyltriazene: yield 53%; yellow crystals (hexane); mp 79–80 °C; ^1H NMR ($CDCl_3$) δ 7.8–8.4 (br, 1, NH), 7.0–7.6 (m, 5, Ar-H), 4.5–4.9 (t, 1, OCHO), 3.45–4.35 (m, 6, CH_2O + CH_2N), 1.7–2.3 (m, 3, $OCHCH_2$ + eq H of $CH_2CH_2CH_2$), 1.1–1.5 (m, 1, ax. H of $CH_2CH_2CH_2$). Anal. ($C_{12}H_{17}N_3O_2$) C, H, N.

3-(2-Methylene-1,3-dioxolanyl)-1-phenyltriazene: yield 57%; yellow oil; ^1H NMR ($CDCl_3$) δ 8.3–8.9 (br, 1, NH), 7–7.8 (s, 5, Ar-H), 5.0–5.35 (t, 1, OCHO), 3.7–4.25 (s, 6, CH_2O + CH_2NH). Anal. ($C_{10}H_{13}N_3O_2$) C, H, N.

3-(Carbethoxypropyl)-1-phenyltriazene; yield 47% yellow oil; ^1H NMR ($CDCl_3$) δ 8.0–8.4 (br, 1, NH), 7.0–7.65 (m, 5, Ar-H), 3.9–4.35 (q, 2, CH_2O), 3.55–3.85 (t, 2, CH_2N), 1.7–2.6 (m, 4, CH_2CO + $CH_2CH_2CH_2$), 1.0–1.4 (t, 3, CH_3).

3-[2-(Benzylthio)ethyl]-1-phenyltriazene: yield 51%; yellow needles (hexane); ^1H NMR ($CDCl_3$) δ 7.7–8.7 (br, 1, NH), 7.3 (s, 10, Ar-H), 3.7–3.9 (s over t, 4, CH_2N + $ArCH_2S$), 2.6–2.8 (t, 2, SCH_2).

3-[3-[Bis(2-hydroxyethyl)amino]propyl]-1-phenyltriazene: yield 11%; dark red oil; ^1H NMR ($CDCl_3$) δ 7.7–8.1 (br, 1, NH), 3.8–4.25 (br, 2, OH), 3.45–3.8 (t, 6, CH_2OH + CH_2NH), 2.3–2.8 (t, 6, CH_2N), 1.4–2.1 (m, 2, CCH_2C).

3-[2-(1-Methylpyrrolidin-2-yl)ethyl]-1-phenyltriazene: yield 38%; dark red oil; ^1H NMR ($CDCl_3$) δ 7.0–7.8 (m, 6, Ar-H + NH), 3.6–3.9 (t, 2, CH_2N), 2.95–3.25 (m, 2, CH_2N endocyclic), 2.5–2.75 (m, 1, CHN), 2.35 (s, 3, CH_3), 1.5–2.35 (m, 6, CH_2 in ring + CH_2 exocyclic).

3-[2-(1-Ethylpyrrolidin-2-yl)ethyl]-1-phenyltriazene: yield 30%; dark red oil; ^1H NMR ($CDCl_3$) δ 7.0–8.33 (m, 6, Ar-H + NH), 3.55–4.0 (m, 2, CH_2NH), 3.05–3.55 (t over m, 3, CH_2N endocyclic + CHN), 2.33–3.05 (m, 2, CH_2N exocyclic), 1.5–2.33 (m, 6, CH_2), 0.8–1.5 (t, 3, CH_3).

1-Phenyl-3-[2-(thiomorpholino)ethyl]triazene: yield 63%; brown oil; ^1H NMR ($CDCl_3$) δ 7.85–8.5 (br, 1, NH), 7.0–7.8 (m, 5, Ar-H), 3.60–4.05 (t, 2, CH_2NH), 2.45–3.00 (br, 10, CH_2N + CH_2S).

3-(2-Acetamidoethyl)-1-phenyltriazene: yield 8%; dark red oil; ^1H NMR ($CDCl_3$) δ 6.5–7.5 (m, 7, Ar-H + NHN + NHCO), 3.2–3.9 (m, 4, CH_2NH), 1.9 (s, 3, CH_3).

1-Phenyl-3-[3-(triethoxysilyl)propyl]triazene: yield 20%; yellow oil; ^1H NMR ($CDCl_3$) δ 8.9–9.8 (br, 1, NH), 6.9–7.6 (m, 5, Ar-H), 3.5–4.2 (m, 8, CH_2O + CH_2N), 1.53–2.6 (m, 2, $CH_2CH_2CH_2$), 1.05–1.53 (t, 9, CH_3), 0.5–10 (m, 2, CH_2 Si).

All the amines used for the preparation of the triazenes are known. Most of them were available from Aldrich. However, four of them were prepared by procedures different from those in the literature. Thus, 2-(benzylthio)ethanamine[8-10] and 4-2-aminoethyl)thiomorpholine[11,12] were prepared by the following general procedure.

A solution of 1 equiv of sodium hydroxide or sodium carbonate in a few milliters of water was added to a solution of 1 equiv of benzyl mercaptan or thiomorpholine in *N,N*-dimethylformamide. The resulting solution was treated with a solution of 1 equiv of *N*-(2-bromoethyl)phthalimide in *N,N*-dimethylformamide. After refluxing for 20 h, the mixture was poured into ice–water. The crude phthalimide derivative was crystallized from methanol.

***N*-[2-(Benzylthio)ethyl]phthalimide** was obtained in 92% yield, mp 78–79 °C (lit.[8] mp 81–82 °C); ***N*-(2-thiomorpholinoethyl)phthalimide** was obtained in 24% yield, mp 120–122 °C; ^1H NMR ($CDCl_3$) δ 7.6–8.1 (m, 4, Ar-H), 3.6–4.0 (t, 2, CH_2NCO), 2.4–3.0 (m, 10, CH_2N + CH_2S). Anal. ($C_{14}H_{16}N_2O_2S$) C, H, N. Hydrazinolysis of the phthalimide derivative with hydrazine hydrate in methylene chloride–methanol gave the corresponding amine, which was identical with that described in the literature.[8-12]

2-(Aminomethyl)-1,3-dioxolane and 2-(aminoethyl)-1,3-dioxane[13] were prepared from the corresponding halo compound and potassium phthalimide in *N,N*-dimethylformamide, followed by hydrazinolysis of the product with hydrazine hydrate in methylene chloride.

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