Development of New Mitomycin C and Porfiromycin Analogues

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New mitomycin C and porfiromycin analogues were prepared by treating mitomycin A and *N*-methylmitomycin A with a variety of amines, including aziridines, allylamines, propargylamines, chloroalkylamines, hydroxyalkylamines, glycine derivatives, aralkylamines, and heterocyclic amines. All analogues were evaluated against P-388 murine leukemia and selected ones were examined for their leukopenic properties. Certain analogues were found to be superior to mitomycin C in potency, efficacy, and therapeutic ratio in the P-388 assay. The most active substituents at the mitosane 7 position included aziridine, 2-methylaziridine, propargylamine, furfurylamine, methyl glycinate, and 3-aminopyridine. Mitomycin A and the 7-aziridino, 7-(2-methylaziridino), and 3-aminopyridine analogues were less leukopenic than mitomycin C. Certain other analogues, including propargylamino and methyl glycinate, were highly leukopenic. The three compounds tested against B-16 melanoma in mice were significantly more effective than mitomycin C in this assay. Previously established structure–activity relationships were found inadequate to account for all of the new data.

Mitomycin C is active against a relatively broad spectrum of experimental tumors, including both hematological and solid types. In clinical practice, it is limited to certain carcinomas.[1] The toxicity of mitomycin C, particularly its myelosuppressive effect, has prevented it from gaining wider and more rapid acceptance in cancer chemotherapy.[1] Numerous analogues (1) of mitomycin C have been prepared in the hope of obtaining compounds with improved therapeutic properties. The semisynthetic analogues have involved substituents (Y) on the aziridine ring (Scheme I),[2] new carbamoyl or acyl groups (Z) on the hydroxymethyl side chain,[3] and replacement of the 7-substituent (X) in the quinone ring with other functional groups, especially substituted amines.[2,4] None of these analogues has emerged as a clinical agent, although the 7-hydroxy analogue (1, X = OH, Y = H, Z = $CONH_2$) of mitomycin C has received intensive study recently in Japan. This analogue is stated to be less leukopenic than mitomycin C, although it is also much less potent.[1,5] Totally synthetic mitomycin analogues of the mitosene (mitomycins with 9,9a double bonds and no aziridine ring)[6] and indoloquinone[7] types have been prepared, but mainly for their antibacterial activity. The most active antitumor agent of the mitosene type is considerably less active than mitomycin C.[6]

During the course of these analogue studies, the mitomycin mode of action has been intensively investigated.[8-11] Although our knowledge of this complex process is incomplete, it appears to involve reduction of a mitomycin to the corresponding hydroquinone, with initial noncovalent bonding occurring between the intermediate radical anion and DNA. The elements of methanol are readily lost from positions 9 and 9a of the hydroquinone, affording an indolohydroquinone (2) that has two sites for alkylation of DNA. It is thought that cross-linking of DNA is the lethal event for cancer cells, although the formation of hydrogen peroxide as a consequence of successive redox cycles of DNA-bound mitomycin might be another factor.[11]

Based on our analysis of mode of action studies and structure–activity relationships for mitomycin analogues developed mainly by Kinoshita and co-workers,[2,3,12] we thought that additional analogues should be prepared and tested. In particular, the combination of a predictive antitumor screen, such as P-388 leukemia in mice, with an assay for the production of leukopenia in mice appeared to offer an advantageous approach to analogue development. Our goal became the preparation of compounds that were at least as potent and efficaceous as mitomycin C in P-388 leukemia but which caused significantly less leukopenia. For the initial group of analogues, we decided to concentrate on new 7-substituted compounds. The 7 position is especially important because it controls the reduction potential of the quinone ring, thus offering a chance to gain some selectivity between normal cells and certain cancer cells.[2,13] Furthermore, it is conceivable that a third alkylating function can be introduced at this position, for example, with aziridino derivatives such as **5–8**. Finally, variations in lipophilicity can be produced by different 7-substituents.[13]

The starting point for this investigation was the preparation of significant amounts of mitomycin A (**3**) and *N*-methylmitomycin A (**4**). Previously published methods for their synthesis from mitomycin C proved adequate,[14,15] with overall yields up to 60% realized. However, we were unable to obtain good yields when the scale was increased beyond 1 g.

Treatment of mitomycin A (**3**) and *N*-methylmitomycin A (**4**) with ethylenimine gave the known 7-aziridino derivative[12,14] **5** and the new 7-aziridino derivative **6**, respectively. In these preparations and subsequent ones, it was essential to purify the products by careful chroma-

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(3) Kinoshita, S.; Uzu, K.; Nakano, K.; Takahashi, T. *J. Med. Chem.* **1971**, *14*, 109.
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(5) Imai, R. "Abstracts of the Meeting", 24th Meeting of the Japan Chemotherapy Society, June, 1976.
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(7) Weiss, M. J.; Redin, G. G.; Allen, Jr., G. R.; Dornbush, A. C.; Lindsay, H. L.; Poletto, J. F.; Remers, W. A.; Roth, R. H.; Sloboda, A. *J. Med. Chem.* **1968**, *11*, 742.
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(10) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110.
(11) Tomasz, M. *Chem.-Biol. Interact.* **1976**, *13*, 89.
(12) Kojima, R.; Driscoll, J.; Mantel, N.; Goldin, A. *Cancer Chemother. Rep.* **1972**, *3*, 121.
(13) Remers, W. A.; Schepman, C. S. *J. Med. Chem.* **1974**, *17*, 729.
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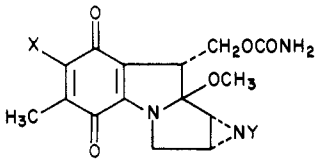
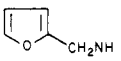
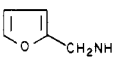
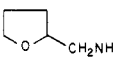
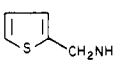
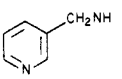
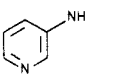
(15) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767.

0022-2623/81/1824-0975$01.25/0

Table I. Activity of Mitosanes against P-388 Murine Leukemia[a]



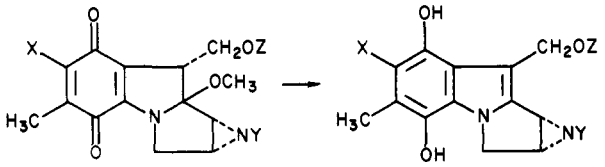
compd	X	Y	max effect MST and 30-day survivors	max effect mito C control	opt dose, mg/kg	MED, mg/kg	TR
3 (mit A)	CH_3O	H	180	270	3.2[b]	0.05	64
4	CH_3O	CH_3	180 (1)	270	3.2[b]	0.4	8
mit C	H_2N	H	178 (0)-306 (2)		3.2	0.2	16
porfiro	H_2N	CH_3	225	270	12.8[b]	0.8	16
5		H	266 (2)	300	1.6	0.025	64
6		CH_3	248 (2)	229	3.2	0.2	16
7		H	233	250	3.2	0.1	32
8		CH_3	163	247	3.2	0.4	8
9		CH_3	167	250	12.8	6.4	2
10	$H_2C{=}CHCH_2NH$	H	150	250	6.4	3.2	2
11	$H_2C{=}CHCH_2NH$	CH_3	189	247	12.8	3.2	4
12	$H_2C{=}C(CH_3)CH_2NH$	H	245 (2)	300	25.6	1.6	16
13	$H_2C{=}(Cl)CH_2NH$	H	178	242	25.6	3.2	8
14	$HC{\equiv}CCH_2NH$	H	358 (4)	200	12.8	0.2	64
15	$HC{\equiv}CCH_2NH$	CH_3	300 (2)	200	25.6	1.6	16
16	$HC{\equiv}CCH_2NCH_3$	H	210	300	12.8	0.8	16
17	$HC{\equiv}CC(CH_3)_2NH$	H	178	217	25.6	1.6	16
18	$ClCH_2CH_2NH$	H	190	245	12.8	1.6	8
19	$ClCH_2CH_2NH$	CH_3	205	300	51.2	6.4	8
20	$Cl(CH_2)_3NH$	H	140	245	12.8	6.4	2
21	$HO(CH_2)_3NH$	H	244	306	25.6	3.2	8
22	$HO(CH_2)_3NH$	CH_3	239	239	25.6	1.6	16
23	$CH_3O_2CCH_2NH$	H	300 (2)	178	12.8	0.4	32
24	H_2NCOCH_2NH	H	217	178	6.4	0.4	16
25	$C_6H_5CH_2NH$	H	188	252	12.8	1.6	8
26	$H_2NSO_2\text{-}C_6H_4\text{-}CH_2NH$	H	150	306	25.6	6.4	4
27	$C_6H_5CH_2CH_2NH$	H	194	194	25.6	1.6	16
28		H	276 (2)	252	12.8	<0.4	>32
29		CH_3	261	242	25.6	1.6	16
30		H	289 (2)	178	25.6	0.4	64
31		H	167	194	12.8	3.2	4
32		H	167		12.8	3.2	4
33	$4\text{-}HOC_6H_4NH$	CH_3	137	168	6.4	1.6	4
34		H	211	198	3.2	<0.2	>16

[a] Determined at Bristol Laboratories, Syracuse, NY. 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 mitosane 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); TR = therapeutic ratio (opt dose/MED). The number of 30-day survivors at the optimal dose is given in parentheses beside the maximum effect. The complete testing results, including for each dose used the therapeutic effect, number of 5- and 30-day survivors, and average weight difference between the test and control group are available as supplementary material (see paragraph at the end of paper). [b] Mitomycin and analogue given once daily on days 1 and 5 only.

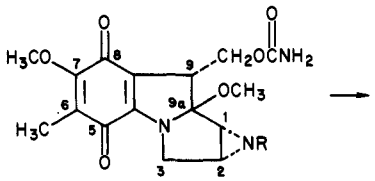
tography, because the starting materials were highly active and toxic. This precaution was especially important where mitomycin A was concerned since, as shown in Table I, it is extremely potent against P-388 leukemia. Every analogue was shown to be homogeneous by thin-layer chromatography. However, difficulty was encountered in obtaining pure samples for microanalysis, particularly since some compounds tenaciously held solvents. They could not be heated because of instability. The amounts of solvent needed to rationalize the analytical data and NMR evidence for the presence of this solvent are given Table V.

Following the observation of significant activity for analogues **5** and **6** (Table I), the corresponding homologues

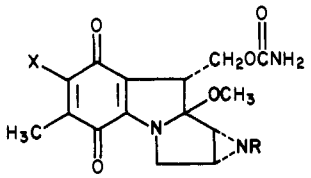
Scheme I. Mitomycin Analogues and Their Biological Activation

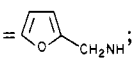
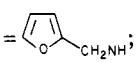
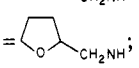
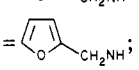
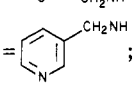
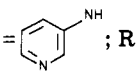


1 2



3, R = H
4, R = CH_3



5, X = ▷N; R = H
6, X = ▷N; R = CH_3
7, X = ^{CH_3}▷N; R = H
8, X = ^{CH_3}▷N; R = CH_3
9, X = ▷NH; R = CH_3
10, X = $H_2C{=}CHCH_2NH$; R = H
11, X = $H_2C{=}CHCH_2NH$; R = CH_3
12, X = $H_2C{=}C(CH_3)CH_2NH$; R = H
13, X = $H_2C{=}C(Cl)CH_2NH$; R = H
14, X = $HC{\equiv}CCH_2NH$; R = H
15, X = $HC{\equiv}CCH_2NH$; R = CH_3
16, X = $HC{\equiv}CCH_2NCH_3$; R = H
17, X = $HC{\equiv}CC(CH_3)_2NH$; R = H
18, X = $ClCH_2CH_2NH$; R = H
19, X = $ClCH_2CH_2NH$; R = CH_3
20, X = $ClCH_2CH_2CH_2NH$; R = H
21, X = $HOCH_2CH_2CH_2NH$; R = H
22, X = $HOCH_2CH_2CH_2NH$; R = CH_3
23, X = CH_3OCOCH_2NH; R = H
24, X = H_2NCOCH_2NH; R = H
25, X = $C_6H_5CH_2NH$; R = H
26, X = H_2NSO_2-C_6H_4-CH_2NH; R = H
27, X = $C_6H_5CH_2CH_2NH$; R = H
28, X =  ; R = H
29, X =  ; R = CH_3
30, X =  ; R = H
31, X =  ; R = H
32, X =  ; R = H
33, X = HO-C_6H_4-NH; R = CH_3
34, X =  ; R = H

7 and 8 were prepared by the use of 2-methylethylenimine. Attempts to prepare a 7-(*N*-azetidino) analogue were unsuccessful. A new substance was obtained by treating *N*-methylmitomycin A (4) with azetidine, but it appeared to be polymeric and it was completely inactive. Cyclopropylamine readily converted 4 into the desired analogue

Table II. Activity of Mitosanes against Murine B-16 Melanocarcinoma[a]

compd	dose, (mg/kg)/day	effect MST: % T/C	45 day survivors: cures	45 day survivors: tumored
mit C	8	164		1
	4	193		
NSC 026980	2	159		
	1	136	1	1
	0.5	122		
12	16	226	3	1
	8	186		
NSC 328209	4	160	1	
	2	146		
	1	131		
	0.5	122		
28	36	247	6	
	18	219	3	1
NSC 328208	9	186		
	4.5	170		
	2.25	148		
	1.13	125		
30[b]	24	326	4	3
	12	240	1	1
NSC 327986	6	179		
	3	173		
	1.5	146		

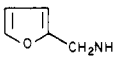
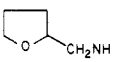
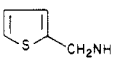
[a] Determined at Arthur D. Little, Cambridge, MA, according to the standard NCI protocol in male BDF mice. Ten mice were used at each dose of the mitosane on day 1 of the experiments and 40 control mice were injected with Tween 80 in distilled water. Effect = MST treated/MST control × 100. All mice were alive on day 5 of the experiments. [b] Doses given on days 1, 4, and 7 of the experiment.

9. Another structural type that appeared to offer useful analogy to the aziridine ring was the allylic amine. Treatment of **3** and **4** with allylamine readily furnished the 7-(allylamino) analogues **10** and **11**, respectively. The closely related 7-[(2-methylallyl)amino] analogue **12** and 7-[(2-chloroallyl)amino] analogue **13** were prepared similarly. Extending this structural analogy further, we prepared the propargylamino derivatives **14** and **15** from **3** and **4**. When it was shown that these propargylamino analogues were highly effective against P-388 murine leukemia (Table I), additional propargylamino-type derivatives were made from **3**. The *N*-methylpropargylamino derivative **16** and the 1,1-dimethylpropargylamino derivative **17** were prepared, although they required longer reaction times than most other analogues. Dipropargylamine did not react with **3**.

We were interested in mitomycins containing 7-[(2-chloroethyl)amino] substituents because this type of substituent might form a "one-armed mustard" upon bioreduction of the quinone ring. This feature could, in principle, afford a third alkylation site (as could the 7-aziridino analogues). Thus, analogues **18** and **19** were prepared from **3**, **4**, and 2-chloroethylamine. The related 3-chloropropyl analogue **20** was also prepared.

Certain types of substituents had received only brief mention in the literature, but they appeared intuitively interesting. Consequently, we prepared some new compounds related to them. For example, 2-hydroxyethylamino analogues had been reported in both the mitosane[12,14] and indoloquinone series. The mitosane analogue was not as effective as mitomycin C. However, as shown in Table I, our corresponding 3-hydroxypropyl analogue **22** was equally efficaceous. The glycine analogue of mitomycin C was briefly noted in a patent concerned with antibacterial activity.[4] We prepared the corresponding analogues **23** and **24** from mitomycin A (**3**), methyl gly-

Table III. Effect of Mitosanes on Total White Blood Cell Count[a]

compd	X	Y	dose, mg/kg	% change from control day 3	day 5	day 7
3 (mit A)	CH_3O	H	1.7	-28**	-24	+5
			1.3	-12	-15	-9
			0.96	-11	-25*	+4
mit C	H_2N	H	7.5	-58**	-67	-53**
			5.63	-57**	-58**	-26*
			4.22	-55**	-53	-1
			3.16	-48**	-42	-38
5		H	3.0	-12	+3	+13
			2.55	+15	34	+18*
			1.69	+16*	+9	-11
			1.27	-16*	+27	+3
6		CH_3	5.3	-15	-20*	-13**
			3.98	-12.3	-17	+7
			2.98	+5	+25	+31**
			2.24	+1	+11	+39**
7		H	3.0	-37**	-39**	-12
			2.25	-36**	-32**	-6
			1.69	-24*	-5	+3
			1.27	-16	-5	+18*
8		CH_3	6.7	-28**	-49	-37**
			5.03	-31**	-44**	-4
			3.77	-30**	-25**	-11
			2.83	-31**	-21*	+2
10	$H_2C{=}CHCH_2NH$	H	12.8[b]	-33	-42*	6
			6.4	-35**	-37**	-0.6
12	$H_2C{=}C(CH_3)CH_2NH$	H	51.2[b]	-60*	-25	D
			25.6	-60**	-68**	-34
14	$HC{\equiv}CCH_2NH$	H	17.0	-80**	-75**	-73
			12.57	-68**	-69**	-52**
			9.56	-59**	-56**	-28**
15	$HC{\equiv}CCH_2NH$	CH_3	59.0	-80**	-84**	-77**
			44.25	-74**	-69**	-67**
			33.18	-64**	-66**	-49**
18	$ClCH_2CH_2NH$	H	68.0	-71**	-63**	-35**
			51.0	-55**	-61**	-23*
22	$HOCH_2CH_2CH_2NH$	CH_3	90.5	-78*	-72*	D
			67.9	-85*	D	D
23	$CH_3O_2CCH_2NH$	H	25.6[b]	-65**	D	D
			12.8	-55*	-68	D
			6.4	-39*	-55**	-21
28		H	50.9	-76*	D	-64**
			39.4	-84**	-74*	D
			29.6	-78**	-67*	0
			22.2	-70**	62*	-1
30			45	-76**	-72**	-25
			33.8	-58**	-62**	-13
			25.3	-44**	-54**	-19
31		H	25.6[b]	-32*	-33*	-11
			12.8	-26	-5	-2
34		H	9.8	-36**	-42**	+24
			7.4	-43**	-46**	-0.2
			5.5	-41**	-24*	-14

[a] The mitosane was given in a single dose, ip, on day 0 to BDG_1 male mice. The highest dose level was at the LD_{50}. D indicates death of one or more mice. * = significant change, $p \leq 0.05$; ** = significant change, $p \leq 0.01$. [b] The highest dose level was twice the optimal dose.

cinate, and glycinamide. Attempts to prepare an analogue from **3** and aminoacetonitrile gave only a polymeric substance. In the same patent,[4] the preparation of the 7-(benzylamino) analogue of mitomycin B was noted. (Mitomycin B resembles structure **4**, but with 9a-OH group and epimeric at 9.) We prepared the corresponding analogue **25** of mitomycin C. The related analogues **26** and **27** were prepared from **3**, mafenide [4-(aminomethyl)-benzenesulfonamide], and phenethylamine.

Although a number of heterocyclic amino derivatives had been prepared from the mitomycins, there were no examples of heterocycles bearing aminomethyl groups (heterocyclic analogues of benzylamine). It was relatively simple to prepare the furfurylamino analogues **28** and **29** and the tetrahydrofurfurylamino analogue **30**. The 2-(aminomethyl)thiophene analogue **31** and 3-(aminoethyl)pyridine analogue **32** also were made. Only the anilino derivative was reported among analogues in which the amino group was attached directly to an aromatic ring.[14] We prepared the 4-hydroxyanilino derivative **33** from **4** and the 3-aminopyridyl derivative **34** from **3**. The preparation of derivatives from 3-aminopyridine and mi-

tomycin B and porfiromycin were briefly noted in a patent.[4] Our compound **34** differed in color (purple vs. orange) from these derivatives. 2-Aminopyridine and 4-aminopyridine failed to react with **3**, presumably because of very low electron density on the amino nitrogens. A successful reaction between **3** and 4-aminopyridine was previously reported.[4]

Mitomycin C analogues in which the 7 position is substituted with hydrazino groups appeared to offer interesting possibilities for structure–activity relationships. Treatment of *N*-methylmitomycin (**4**) with hydrazine in methanol gave a vigorous reaction, resulting in extensive decomposition. *N*-Aminomorpholine and 1-amino-4-methylpiperazine gave mild reactions with **3**, but complex mixtures of products were obtained. Hydroxylamine gave with **4** a product that could not be purified.

Biological Activity. The comparative activities of certain mitomycins and our analogues against P-388 leukemia in mice are given in Table I. Although these assays were not all done concurrently, each analogue was standardized against mitomycin C in the same experiment. The maximum increase in life span (% T/C) for mitomycin C at its optimal dose is given in column 5 beside the data for each analogue. It should be noted that there is considerable variation in the effect of mitomycin C in different experiments. Consequently, analogues should not be compared directly with each other but should be compared according to how each relates to mitomycin C in a particular experiment. For example, compound **21** would appear to be more effective than compound **34** in prolonging life span (MST 244 vs. 211), but the reverse order of activity is found when both compounds are compared with their mitomycin C controls.

From Table I it is apparent that three analogues with propargylamino (**14**), methyl glycinate (**23**), and tetrahydrofurfurylamino (**30**) substituents gave much greater increases in life span than mitomycin C. Analogues **6**, **24**, **28**, and **34** were somewhat more effective than mitomycin C and analogues **22**, **27**, and **29** were approximately equal to it. Every compound in this table was active in terms of the assay (T/C ≥125) and most of them showed significant prolongation of life. Compounds **5**, **6**, **14**, **23**, **28**, and mitomycin C produced "cures" (30-day survivors) at two or more different doses, with **14** and **23** being the most notable in this respect. Potency greater or equal to that of mitomycin C, as measured by the lowest dose at which T/C ≥125, was observed for compounds **5–7**, **14**, **34**, and mitomycin A (**3**). Among these compounds, the aziridine analogue **5** was active at 0.025 mg/kg and mitomycin A was active at 0.05 mg/kg. Thirteen analogues showed greater therapeutic ratios than mitomycin C (which has a value of 16), as determined by the ratio of the optimal dose to the minimum effective dose, with analogues **5**, **14**, **24**, and **30** having a therapeutic ratio of 64.

In order to evaluate further the antitumor potential of certain analogues, the L-1210 ascites leukemia assay was utilized. Unfortunately, it was not particularly useful in highlighting differences in relative antitumor activities of the analogues. Compounds **6**, **12**, **14**, **15**, **22**, **28**, **29**, and **30** all were active (T/C >125), but none showed a substantial improvement in activity over mitomycin C. Details of the assay are given as supplementary material (paragraph at the end of the paper).

A few of the more interesting analogues have been screened against B-16 melanocarcinoma. As shown in Table II, they are highly active in this sytem and clearly superior to mitomycin C. Thus, analogue **28** gave cures in 6 of 10 mice at its optimal dose and analogue **30** gave 4 cures and 3 tumored survivors.

Table IV. Polarographic Half-Wave Potentials of Mitosanes[a]

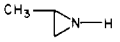
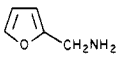
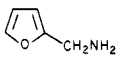
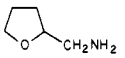
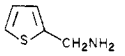
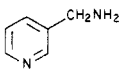
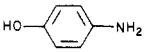
compd	X	$E_{1/2}$, V	MED,[b] mg/kg	leukopenia: % change in white cells on day 5 at the opt dose
3	CH_3O	−0.21	0.05	−2
5		−0.31	0.025	−11[d]
34		−0.31	≤0.2	−33
7		−0.32	0.1	−24[c]
28		−0.39	≤0.4	−54
14	$HC{\equiv}CCH_2NH$	−0.41	0.2	−69
30		−0.41	0.4	−54
12	$H_2C{=}C(CH_3)CH_2NH$	−0.41	1.6	−59
21	$HOCH_2CH_2CH_2NH$	−0.42	3.2	−43
mit C	NH_2	−0.45	0.2	−42[c]

[a] Determined by differential pulse polarography on a Model 174A EG8G 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. 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. [b] Values from Table I. [c] Value for day 3. [d] Value for day 7.

A number of the most active analogues have been tested for their effect on the total white blood cell count in mice. The results of this leukopenic assay are given in Table III. As anticipated, mitomycin C is strongly leukopenic, reducing the white blood cell count to below 50% of normal at a dose (4.22 mg/kg) that is 55.5% of the LD_{50}. Some recovery in the count occurs by day 7. Most of the other results in Table III are very surprising. For example, mitomycin A, a highly toxic compound (LD_{50} = 1.7 mg/kg in BDF_1 male mice) is not significantly leukopenic. The 7-aziridino analogues **5** and **6** also are not very leukopenic, but the closely related 7-(2-methylaziridino) analogues are moderately leukopenic. To our disappointment, the highly active propargylamino analogues **14** and **15** proved to be extremely leukopenic, as did the 7-(methyl glycinate) analogue **23** and the 7-(furfurylamino) analogue **28**. Since analogue **28** showed an appreciable improvement in total white blood cell count by day 7, its effect on bone marrow suppression was analyzed by more specific hematology. It was found by Dr. J. E. Schurig of Bristol Laboratories that reticulocytes and lymphocytes were strongly suppressed, but the neutrophils were only partially suppressed. Finally, Table III shows that the 7-[(2-chloroethyl)amino] analogue **18** was strongly leukopenic, whereas the 7-(3-aminopyridyl) analogue **34** was moderately leukopenic with complete recovery by day 7.

At this point in the development of new mitomycin analogues it is important to examine the published structure–activity relationships (SAR) and see if they can correlate the data in Tables I–III. The earliest attempt at mitomycin SAR involved the correlation of quinone half-wave reduction potentials with antibacterial and antitumor activities. A correlation appeared to exist between the ease of reducing the quinone and the potency against bacterial cultures, especially the Gram-positive species.

Table V. Preparation and Properties of 7-Substituted Mitosanes

product	amine used	yield, %	recrystn solvents	solvent impurity[a]	mp, °C	NMR signals for the new substituent, δ
5		40	CH_2Cl_2-hexane		98-99 dec	2.27 (s, 4)
6		51	CH_2Cl_2-hexane		89-90 dec	2.27 (s, 4)
7		52	CH_2Cl_2-hexane	1.33 hexane (δ 0.9, 1.28)	58-59 dec	1.40 (d, 3), 2.25 (m, 3)
8		73	CH_2Cl_2-hexane		126-127 dec	1.40 (d, 3), 2.25 (m, 3)
9		60	CH_2Cl_2-hexane		166-167 dec	0.70-0.90 (br s, 5), 6.30 (br s, 1)
10	$H_2C{=}CHCH_2NH_2$	30	CH_2Cl_2-hexane		81-82 dec	3.30-3.43 (m, 2), 5.10 (d, 2), 5.50-6.10 (m, 1), 6.50 (t, 1)
11	$H_2C{=}CHCH_2NH_2$	40	CH_2Cl_2-hexane		70-71 dec	3.30-3.43 (m, 2), 5.10 (d, 2), 5.50-6.20 (m, 1), 6.50 (t, 1)
12	$H_2C{=}C(CH_3)CH_2NH_2$	55	$CHCl_3$-hexane		>250 dec	1.97 (s, 3), 4.00 (d, 2), 4.85 (d, 2), 6.53 (t, 1)
13	$H_2C{=}C(Cl)CH_2NH_2$	38	$CHCl_3$-hexane	0.1 hexane (δ 0.9, 1.28)	71-72 dec	4.03-4.55 (s, 2), 5.30-5.40 (br s, 2), 6.53 (t, 1)
14	$HC{\equiv}CCH_2NH_2$	60	$CHCl_3$-hexane		95-96	2.40 (s, 1), 4.33 (s, 2), 6.37 (t, 1)
15	$HC{\equiv}CCH_2NH_2$	77	$CHCl_3$-hexane		87-88	2.40 (s, 1), 4.33 (s, 2), 6.38 (t, 1)
16	$HC{\equiv}CCH_2NH(CH_3)$	49	CH_2Cl_2-hexane	0.66 hexane	86-87 dec	2.30 (s, 1), 3.20 (s, 3), 4.20 (s, 2)
17	$HC{\equiv}CC(CH_3)_2NH_2$	30	$CHCl_3$-hexane	1 hexane 0.66 $CHCl_3$ (δ 0.9, 1.28, 7.23)	99-100 dec	1.68 (s, 6), 2.47 (s, 1), 6.23-6.60 (br s, 1)
18	$ClCH_2CH_2NH_2$	30	$CHCl_3$-hexane		58-59 dec	3.30-4.00 (m, 4), 6.40 (t, 1)
19	$ClCH_2CH_2NH_2$	46	$CHCl_3$-hexane	0.33 hexane (δ 0.9, 1.28)	73-74 dec	3.30-4.00 (m, 4), 6.57 (t, 1)
20	$ClCH_2CH_2CH_2NH_2$	61	$CHCl_3$-hexane	2 hexane, 1 water	63-64 dec	1.87-2.30 (m, 2), 3.35-3.95 (m, 4), 6.28 (t, 1)
21	$HOCH_2CH_2CH_2NH_2$	91	acetone-pentane	1.3 acetone (δ 2.03)	>250 dec	1.52-1.92 (m, 2), 3.43-4.00 (m, 4), 6.73 (t, 1)
22	$HOCH_2CH_2CH_2NH_2$	47	$CHCl_3$-hexane	0.56 hexane (δ 0.9, 1.28)	139-140	1.80-2.13 (m, 2), 3.43-4.00 (m, 4), 6.34 (t, 1)
23	$H_3COCOCH_2NH_2$	13	benzene	0.33 water	95-97	3.50 (s, 2), 3.73 (s, 3), 5.33-5.66 (br s, 1)
24	$H_2NCOCH_2NH_2$	29	benzene		124-126	3.95 (br s, 1), 5.36 (br s, 2), 6.35 (m, 1)
25	$C_6H_5CH_2NH_2$	96	$CHCl_3$-hexane		124-125 dec	3.63 (five, 2), 6.67 (t, 1), 7.30 (s, 5)
26	H_2NSO_2-C_6H_4-CH_2NH_2	47	benzene	0.44 benzene [δ 7.24 (m)]	93-96	3.66-4.00 (br s, 2), 6.47 (s, 1), 7.27-8.00 (2 d, 6)
27	$C_6H_5CH_2CH_2NH_2$	59	EtOAc-ether		94-96	3.40-3.90 (m, 4), 6.33 (t, 1), 7.27 (s, 5)
28		50	$CHCl_3$-hexane	0.60 hexane (δ 0.9, 1.28)	60-63 dec	4.67 (s, 2), 6.33-6.53 (m, 2), 6.57 (t, 1), 7.33 (s, 1)
29		53	$CHCl_3$-hexane	0.8 hexane (δ 0.9, 1.28)	140-141 dec	4.67 (s, 2), 6.33-6.53 (m, 2), 6.57 (t, 1), 7.33 (s, 1)
30		44	$CHCl_3$-pentane		236-237 dec	1.26 (s, 2), 1.66-2.37 (m, 4), 3.50-4.10 (m, 3), 6.55 (t, 1)
31		65	$CHCl_3$-hexane		92-94	3.72 (s, 2), 6.47 (t, 1), 6.77-7.33 (m, 3)
32		76	$CHCl_3$-hexane		116-118 dec	3.88 (s, 2), 6.58 (t, 1), 7.00-7.40 (d, 1), 7.43-7.90 (d, 1), 8.53 (s, 2)
33		72	$CHCl_3$-hexane		>250 dec	7.02 (m, 4)
34		75	$CHCl_3$	0.60 $CHCl_3$ (δ 7.23)	78-80	7.27 (br s, 2), 7.67 (s, 1), 8.35 (br s, 2)

[a] Molar ratios which must be added to 1 mol of the mitomycin analogue to obtain a satisfactory elemental analysis. NMR evidence for the solvent impurity is given in parentheses. It represents major peaks due to the impurity, expressed in parts per million from tetramethylsilane, with $CDCl_3$ as solvent.

The inverse correlation was apparent for activity against solid sarcoma 180.[14] In a subsequent attempt at SAR it was pointed out that activity against L-1210 leukemia was best for the most hydrophilic of the natural mitomycins.[13] More recently, a semiquantitative relationship was established between the structures of 15 mitomycins with variants in their quinone ring, aziridine ring, and 9a (OH or OCH_3) substituents and their activity against solid sarcoma 180. In general, increasing the size and electron-withdrawing power of a substituent decreased its activity.[16]

A careful examination of the data in Table I reveals that it cannot be correlated by any of the relationships described above. Perhaps this is not surprising, considering the highly complex mode of action of mitomycins and the need to produce and maintain a drastic depression in the number of tumor cells in order to gain a substantial prolongation of life. Nevertheless, we desire at least a rough guide to SAR in order to plan future analogues more rationally. From Table I it appears that the most potent analogues (lowest dose for T/C ≥125) bear 7-substituents that are least electron releasing (e.g., OCH_3, c-NC_2H_4) and thus might render the quinone system most easily reducible. Although they do not give the greatest prolongation of life, they are the least leukopenic compounds (Table III) and they appear to give substantial therapeutic ratios. Because of these properties, an examination of the quinone half-wave potentials of various analogues was made. The results are given in Table IV. Only compounds with an unsubstituted aziridine nitrogen are included in this table. In general, this type of analogue is more potent and efficaceous than the corresponding *N*-methyl analogues, although exceptions to this generalization can be found in Table I (e.g., **10** and **11**; **21** and **22**). A second $E_{1/2}$ value is given (in parentheses) for compound **14** because its polarograph showed a small peak preceding the main one. We do not know the significance of this small peak. The data in Table IV show that a rough correlation can be made among the half-wave potentials, minimum effective dosages, and degree of leukopenia produced by the mitomycin analogues at their optimal doses. the greatest deviations from expected MED values are given by mitomycin C and the aziridine analogue **5** in the direction of greater potency. Because the most easily reducible analogues show the least tendency to produce leukopenia, we intend to investigate more compounds of this type in future analogue studies.

In summary, we have partially met our goal of developing analogues that are as potent and efficaceous as mitomycin C but less leukopenic. Compounds that gave greater prolongation of life than mitomycin C were prepared, but they showed serious leukopenia. On the other hand, compounds active at very low doses and causing little leukopenia were prepared, but they did not give prolongation of life equal to mitomycin C. Among the latter type of compound were mitomycin A and the previously reported[12] aziridine analogue **5**. Compound **34**, the 3-pyridylamino analogue, appeared to be superior to mitomycin C in all three categories of activity, but the differences were small. Perhaps the most encouraging aspect of this research is the finding that a number of compounds showed better therapeutic ratios than mitomycin C in the P-388 assay. The high activities of three compounds against B-16 melanoma also is significant and this assay must receive further attention in the future. A variety of newer analogues are being prepared and tested. We will report on them at a later date.

(16) Moriguchi, I.; Komatsu, K. *Chem. Pharm. Bull.* **1977**, *25*, 2800.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Infrared spectra were determined on a Beckman IR-33 spectrophotometer as KBr pellets. Nuclear magnetic resonance spectra were recorded in $CDCl_3$ on a Varian EM-360 spectrometer using tetramethylsilane as the standard. Elemental analyses were performed by Chemalytics, Inc., Tempe, AZ. Analytical results were within ±0.4% of theoretical values unless otherwise specified.

Preparation of 7-Aminomitosanes. A solution of mitomycin A (**3**) or *N*-methylmitomycin A (**4**) in anhydrous methanol (2 mL/0.1 mmol) was treated at room temperature with 5 equiv of the appropriate amine. The reaction was monitored by thin-layer chromatography on silica gel with a 4:1 acetone–benzene system. The time required for complete disappearance of starting material varied from 1 to 4 h. Usually there was only one major (purple) spot produced. The solution was concentrated under reduced pressure and the residue was purified by chromatography on silica gel with ethyl acetate as the solvent. Concentration of eluate from the main fraction followed by crystallization from appropriate solvents (Table IV) gave the product. Some of these products contained solvents which adhered tenaciously. They were subjected to vacuum drying prior to analysis, but they could not be heated because of their instability. Thus, some of the analytical results must be corrected for the presence of solvent. In these cases, the actual presence of the solvent was apparent from the NMR spectrum, but it could not be measured precisely. The NMR evidence is given in Table V.

The amines used, products formed, yields, crystallization solvents, and melting points are given in Table IV. Infrared spectra and NMR spectra of the products were consistent with the assigned spectra. Each product had lost the 7-methoxy group methyl signals at δ 4.02 ppm and gained signals appropriate for the new 7-substituent. These signals are given in Table IV.

Acknowledgment. This investigation was supported by Grant CA 21430, awarded by the National Cancer Institute, DHEW, and by a grant from Bristol Laboratories. We thank Dr. John D. Douros of the NCI for help in obtaining L1210 leukemia and B16 melanoma testing.

Supplementary Material Available: Full screening data for compounds submitted to the P-388 (Table 1) and L-1210 (Table 2) leukemia assays (11 pages). Ordering information is given on any current masthead page.