Antitumor Agents. 1. 1,4-Bis[(aminoalkyl)amino]-9,10-anthracenediones

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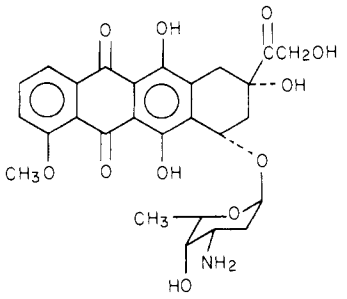
The condensation of alkylenediamines with quinizarin or with 2,3-dihydro-1,4,5,8-tetrahydroxy-9,10-anthracenedione, followed by oxidation, gave 1,4-bis[(aminoalkyl)amino]-9,10-anthracenediones. Some of these compounds and their 2,3-dihydro derivatives were markedly active against both leukemias and solid tumors in mice. Activity was maximal with 5,8-dihydroxylation and 1,4-bis[(2-aminoethyl)amino] substitution, in which the terminal nitrogen atoms were either unsubstituted (compound **50**) or carried 2-hydroxyethyl groups (compound **40**), indicating the importance of hydrophilicity. Against B-16 melanoma, **50** gave >433% increase in median life span (ILS) with 7/10 80-day survivors. Against P-388 leukemia, **40** gave >500% ILS with 4/5 60-day survivors; its efficacy and therapeutic index equaled or surpassed those of adriamycin, cyclophosphamide, daunorubicin, methotrexate, or 5-fluorouracil. Against L-1210 leukemia, B-16 melanoma, and colon tumor 26, **40** was generally as effective or more effective than adriamycin and is now undergoing preclinical toxicological evaluation.

Although adriamycin (**1**) has major clinical utility against



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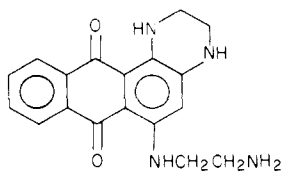
several solid tumors as well as leukemias, its usefulness is limited by toxicity and especially by an irreversible damage to the heart muscle which develops after extended therapy.[1]

Most of the related compounds reported from antitumor studies have been complexly substituted anthracyclines, usually coupled to an amino sugar.[1,2] However, we believed that useful activity might be discovered in less complex systems. Our attention focused on the anthraquinone and amino moieties of adriamycin as especially likely sites[3] for its known intercalative binding[1,4] to DNA. Various flat, tricyclic aromatic systems with basic side chains were tested. One of these, 1,4-bis[[2-(dimethylamino)ethyl]-amino]-9,10-anthracenedione (**8**), was found to give a modest but reproducible increase in life span in mice inoculated with either the L-1210 or P-388 leukemias. This report describes efforts to develop more effective compounds through structural variations from this lead.

Chemistry. The lead compound **8** was synthesized by heating quinizarin with a large excess of *N,N*-dimethylethylenediamine. This procedure (Scheme I) was satisfactory for the preparation of higher homologues (Table I, method A), except that in cases where the starting diamine was not plentiful *N,N,N',N'*-tetramethylethylenediamine was used as the solvent. Greenhalgh and Hughes[5] have shown that such condensations may be accompanied by a subsequent cyclization step to form hexahydronaphtho[2,3-*f*]quinoxaline-7,12-diones (**3**). Such



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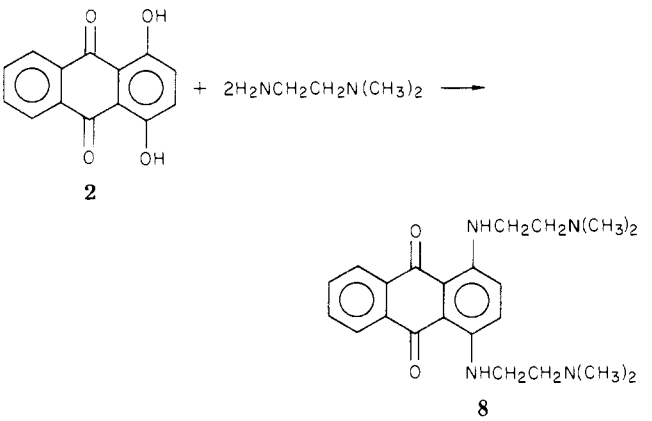
cyclizations occurred only with ethylenediamine and N-monosubstituted ethylenediamines and were favored by elevated temperatures. By using the more reactive leucoquinizarin (2,3-dihydroquinizarin), they found that condensations proceeded at a lower temperature (50 °C), thus generally avoiding cyclization; the dihydro adducts were then aromatized by oxidation with air. This con-

Scheme I



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Scheme II

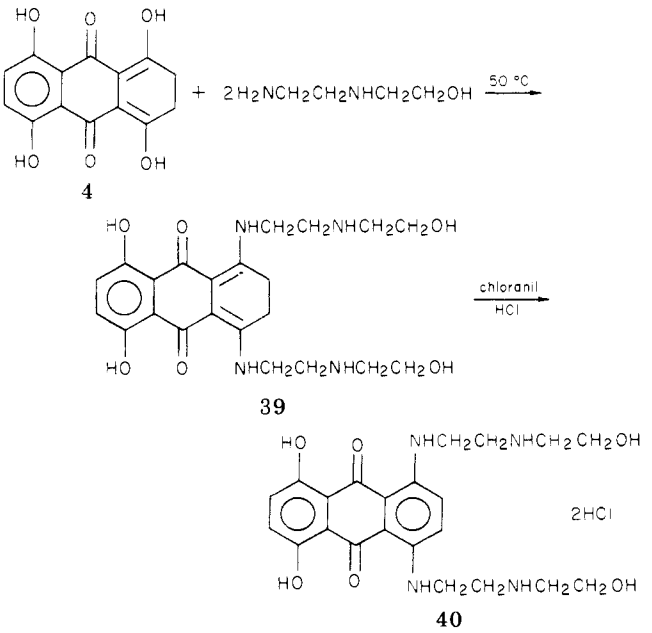

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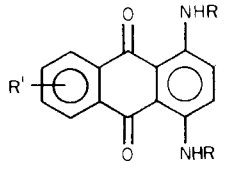
Table I. 1,4-Diamino-9,10-anthracenediones



no.	R'	R	method (or ref)	mp, °C	formula	anal. or lit. mp, °C	% increase in median life span (optimum dose, mg/kg) P-388 leukemia[a]	"cures"[b]	B-16 melanoma[c]	"cures"[d]
1 (adriamycin)			(1)		$C_{27}H_{29}NO_{11}$		159 (5)	1/6	275 (0.8)	18/20
5	H	H	(15)	265-268	$C_{14}H_{10}N_2O_2$	265-268	0 [100][e]	0/5	NT[f]	
6	H	CH_2CH_3	(16)	202-203	$C_{18}H_{18}N_2O_2$		0 [100]		NT	
7	H	CH_2CH_2OH	(16)	237	$C_{18}H_{18}N_2O_4$		0 [100]		NT	
8	H	$CH_2CH_2N(CH_3)_2$	A	172-173	$C_{22}H_{28}N_4O_2$	C, H, N	68 (100)	0/6	39 (50)	0/10
9	H	$CH_2CH_2N(C_2H_5)_2$	(17)	109	$C_{26}H_{36}N_4O_2$	109-110	40 (200)	0/5	NT	
10	H	$CH_2CH_2N^+(CH_3)_3I^-$	G	282	$C_{24}H_{34}N_4O_2I_2 \cdot 0.5H_2O$	C, H, N	0 [300]	0/5	NT	
11	H	$CH_2CH_2N(CH_2CH_2OH)_2 \cdot HCl$	B, C	200-203	$C_{26}H_{36}N_4O_6 \cdot 2HCl$	C, H, N, Cl	73 (300)	0/6	88 (50)	1/10
12	H	CH_2CH_2-c-$N(CH_2CH_2)_2O$	(18)	158-159[g]	$C_{26}H_{32}N_4O_4$	157	119 (400)[h]	0/5	33 (400)	0/10
13	H	CH_2CH_2-c-NC_5H_{10}[i]	A	140-141[g]	$C_{28}H_{36}N_4O_2$	C, H, N	27 (200)	0/5	NT	
14	H	CH_2CH_2-(N-pyridyl)	A	114-115[j]	$C_{28}H_{24}N_4O_2$	C, H, N	0 [250]	0/5	NT	
15	H	$CH_2CH_2NHCH_3$[i]	(5)	131-132	$C_{20}H_{24}N_4O_2$	136-137	144 (25)	2/5	114 (6.2)	0/10
16	H	$CH_2CH_2NHCH_2CH_2OH$[i]	(14, 12)	156-158	$C_{22}H_{28}N_4O_4$	156-158	154 (25)	0/5	129 (6.2)	1/10
17[k]	H	$CH_2CH_2NH_2$	(5)	162-165[l]	$C_{18}H_{22}N_4O_2$	168-169	145 (200)	0/5	103 (50)	0/10
18	H	$CH_2CH_2NH_2$[i]	(5)	170-171	$C_{18}H_{20}N_4O_2$	170-172	144 (25)	0/6	82 (12.5)	0/10
19	H	$(CH_2)_3N(CH_3)_2$	A (19)	55-56	$C_{24}H_{32}N_4O_2$	60-62	0 [200]	0/5	0 [50]	0/10
20	H	$(CH_2)_4N(CH_3)_2$	A	93-94	$C_{26}H_{36}N_4O_2$	C, H, N	0 [250]	0/5	NT	
21	H	$(CH_2)_5N(CH_3)_2$	A	58-60	$C_{28}H_{40}N_4O_2 \cdot {}^1/_3H_2O$	C, H; N[m]	0 [200]	0/5	NT	
22	6-CH_3	$CH_2CH_2NMe_2$	H	158-160	$C_{23}H_{30}N_4O_2 \cdot 0.25H_2O$	C, H, N	0 [200]	0/5	NT	
23	6-COOH	$CH_2CH_2NMe_2$	I	145-150	$C_{23}H_{28}N_4O_4 \cdot 2.5H_2O$	H, N; C[n]	0 [250]	0/5	NT	
24	6,7-$(Cl)_2$	$CH_2CH_2NMe_2$	J	147-149	$C_{22}H_{26}N_4O_2Cl_2$	H; C, N[o]	0 [200]	0/5	NT	
25	5,6-$(OH)_2$	$CH_2CH_2NMe_2$	K	133-135	$C_{22}H_{28}N_4O_4 \cdot 0.5H_2O$	C, H, N	57 (50)	0/5	60 (25)	0/10

[a] BDF or CDF_1 mice were injected ip with 10^6 ascitic leukemia cells, dosed ip on days 1, 5, and 9, and deaths were recorded or 60 days. [b] "Cures" = number of survivors/total at 60 days. [c] BDF_1 mice were implanted ip with a homogenate from 0.05 g of tumor, dosed ip on days 1-9, and deaths were recorded for 80 days. [d] "Cures" = number of survivors/total at 80 days. [e] Dosages in brackets indicate the highest dose tested for inactive compounds. [f] Not tested. [g] From EtOH. [h] Dosed on days 1-9. [i] These compounds were also independently reported to have antitumor activity by Zee-Cheng and Cheng (ref 12). [j] From CH_2Cl_2-*n*-heptane, boiling out CH_2Cl_2. [k] Tested as the 2,3-dihydro derivative. [l] Heating rate = 9 °C/min. [m] Calcd: N, 11.9. Found: N, 11.4. [n] Calcd: C, 58.8. Found: 58.3. [o] Calcd: C, 58.8; N, 12.5. Found: C, 58.0; N, 11.7.

densation method (method B) was used for the synthesis of adducts from ethylenediamine and *N*-methylethylenediamine (**15** and **17** in Table I) and for a series of 5,8-dihydroxylated analogues (Table II) derived from leuco-1,4,5,8-tetrahydroxyanthraquinone (**4**, Scheme II).

In general, we found that the aromatization step went to completion more reliably when chloranil was used as the oxidant (method C) instead of air (method D). Hot nitrobenzene was also convenient for oxidations (method E) in which cyclization was not a hazard. Diethyl azodicarboxylate was effective (method F) for the preparation of **32**, where the presence of the labile aziridine function called for a rapidly acting oxidant that required neither heat nor acid. The oxidations and other reactions are readily monitored by TLC, since spots from the dihydro compounds are generally orange-brown (yellow for **11** and **17**), while the aromatized forms are intensely blue. The overall yields and purities of the oxidized products were generally better when the dihydro intermediates were isolated. These labile dihydro compounds usually separated as solids from the condensation reaction when tetramethylethylenediamine or ethanol was used as the solvent and then were used without further purification.

Structure–Activity Relationships. Tables I and II show that when administered by the intraperitoneal (ip) route the anthracenediones showed roughly comparable efficacies against two different ip implanted tumors: the P-388 leukemia and B-16 melanoma. Good efficacy and potency required an ethylenediamine substituent at both the 1 and 4 positions.[6] 5,8-Dihydroxylation of the aromatic nucleus substantially increased activity (Table II), although 5,6-dihydroxylation did not (**25**), and nuclear substitution with 6-methyl, 6-carboxyl or 6,7-dichloro groups gave inactive compounds (**22–24**). Increases in the chain length between N atoms reduced or abolished activity (**19–21** and **51–56**) as did increases in the number and size of aliphatic or cyclized alkylene substituents on the terminal N atoms. Branching between the N atoms was compatible with activity but not beneficial (**58** and **59**).

Pyridyl, *N*-aryl, or *N*-acyl compounds were inactive (**14**, **31**, and **60**). Thus, it appears that the terminal N atoms of the present series of "two-armed" compounds must be fully basic, in accord with our initial search for basic side chains that could bind to phosphoric acid residues of a DNA chain. "One-armed" analogues were inactive or less active,[6] suggesting that the presence of two or more binding centers enhances binding, as Canellakis[7] and Cain[8] have demonstrated with intercalating bisacridine derivatives and as is also well established with nonintercalating polyamines.[9] The high activity shown by ethylenediamines with minimal N-terminal substitution (**15**, **17**, **36**, and **50**) was surpassed by several of those with *N*-(hydroxyalkyl) substituents (**16** and **39–45**), suggesting that a suitably high hydrophilicity is more important than mere steric brevity. As compared with **50**, attachment of additional aminoalkyl groups was detrimental (**46–49**). A cyclized compound[5] showed modest activity: tetrahydronaphthoquinoxalinedione **3** gave a 47% increase in life span vs. P-388 leukemia at an optimum dosage of 200 mg/kg. It is to be noted that **3** retains only one fully basic, aliphatic amino group, in contrast with its much more active, uncyclized counterpart, **50**.

It was our hope that the aziridine groups of **32** might serve as physiological alkylating agents to give added variety and permanence in binding to DNA or other relevant receptors. Although the activities of **32** do not yet mark it as unique, we do plan to test it against other types of tumors.

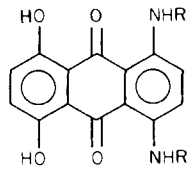
Dihydro derivatives **17** and **39** had activities comparable to those of their fully aromatic counterparts **18** and **40** but at four- to eightfold higher dosages; similar activity correlations were also observed within most other dihydro-aromatic pairs. (For most of the fully aromatic compounds of Table II, the corresponding dihydro compounds[10] were also tested, though for clarity of presentation that data is not included in Table II.) Dihydro compounds **17**, **37**, **38**, **52**, and **59** were also active. It is likely that the dihydro structures (designated by footnote *k* in Tables I and II) are at least partially oxidized in vivo, since mice given high dosages of dihydro compound **17** were later observed to have developed blue ears and tails. This "bluing" response was commonly observed on high dosages of many aromatic compounds but was not noticeable even on autopsy within the effective dosage range of such potent compounds as **40** and **50**.

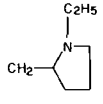
The antileukemic activity and therapeutic index of *N*-(hydroxyethyl) derivative **40** were found to compare favorably with those of a group of anticancer agents that are in wide clinical use (Table III). An extensive pharmacological study of **40**, reported separately,[11a] includes the details of our antitumor testing protocols, which follow those of the U.S. National Cancer Institute. This study established that in mice **40** is generally as effective as or more effective than adriamycin against P-388 and L-1210 leukemias, B-16 melanoma, and colon tumor 26. Neither compound was effective vs. Lewis lung carcinoma, whether the tumor was implanted subcutaneously or inoculated intravenously. 2-Hydroxypropyl derivative **42** and adriamycin caused comparable inhibitions[11b] of Ridgway osteogenic sarcoma; **40** was ineffective. Combination therapy of L-1210 leukemia with **40** and methotrexate was more effective than with either compound alone. Intraperitoneal injections of **40** were the most effective; subcutaneous and intravenous injections were also effective, but it was not active orally. In mouse L-5178Y cells in vitro it was seven times more potent than adriamycin in inhibiting RNA synthesis and four times more potent in inhibiting DNA synthesis. The replication of mouse bone marrow cells was suppressed but less so than with adriamycin or cyclophosphamide. Like adriamycin, **40** can kill cells in all phases of the cell cycle, an apparent advantage in the inhibition of slower growing types of tumors. There was extensive nuclear fragmentation and chromosome scattering in cell cultures incubated with **40**, suggesting nuclear penetration and interference with the mitotic process. In common with many useful antitumor agents, **40** was mutagenic in the Ames test. Testing of **40** for cardiotoxicity is in progress.

After our discovery of **40** and related compounds, Zee-Cheng and Cheng reported their independent synthesis and brief antitumor evaluation of the free base corresponding to **40** and of 11 related anthraquinones and a cyclized derivative which were unsubstituted in the 5 and 8 positions.[12] We subsequently prepared their lead compound[13,14] (**16**) and have included it in Table I for purposes of comparison.

Conclusion. Polyhydroxylated amines **40** and **44** each embody a total number of hydrophilic groups similar to that of adriamycin, with comparable or superior antitumor efficacies and markedly greater synthetic availability and ease of structural modification. The number of "cures" obtained with these and several related compounds against the P-388 leukemia and B-16 melanoma is especially promising (Table II, 60- and 80-day survivors, respectively).

Table II. 1,4-Diamino-5,8-dihydroxy-9,10-anthracenediones



no.	R	method (or ref)	mp, °C	formula	anal. or lit. mp, °C	% increase in median life span (optimum dose, mg/kg) P-388 leukemia[a]	"cures"[b]	B-16 melanoma[c]	"cures"[d]
26	CH_2CH_2OH	B, E (20)	235[p]	$C_{18}H_{18}N_2O_6$	C, H, N	0 [100][e]	0/5	NT[f]	
27	$CH_2CH_2N(CH_3)_2$	B, E	236-238	$C_{22}H_{28}N_4O_4$	C, H, N	122 (50)	0/10	70 (12.5)	0/20
28	$CH_2CH_2N(C_2H_5)_2$	B, E	202-204[q]	$C_{26}H_{36}N_4O_4$	C, H, N	88 (200)	0/11	38 (50)	0/20
29	$CH_2CH_2N[CH(CH_3)_2]_2$	B, E	233-235[r]	$C_{30}H_{44}N_4O_4$	C, H, N	0 [200]	0/5	0 [50]	0/10
30	$CH_2CH_2N(n\text{-}C_4H_9)_2$	B, E	60-62[s]	$C_{34}H_{52}N_4O_4$	C, H, N	0 [200]	0/5	0 [50]	0/10
31	$CH_2CH_2N(CH_3)C_6H_5$	B, E	200-202[t]	$C_{32}H_{32}N_4O_4$	C, H; N[u]	0 [200]	0/5	0 [50]	0/10
32	CH_2CH_2-c-NC_2H_4	B, F	168-170	$C_{22}H_{24}N_4O_4 \cdot 0.5H_2O$	H, N; C, H_2O[v]	185 (100)	0/5	58 (12.5)	0/10
33	CH_2CH_2-c-NC_4H_8	B, E	193-196[w]	$C_{26}H_{32}N_4O_4$	C, H, N	118 (100)	0/5	58 (12.5)	0/10
34	CH_2CH_2-c-NC_5H_{10}	B, E	217-219	$C_{28}H_{36}N_4O_4$	C, H, N	40 (300)	0/5	0 [50]	0/10
35	CH_2CH_2-c-$N(CH_2CH_2)_2O$	B, E	241-243	$C_{26}H_{32}N_4O_6$	C, H, N	0 [200]	0/5	NT[f]	
36	$CH_2CH_2NHCH_3 \cdot HCl$	B, C		$C_{20}H_{24}N_4O_4 \cdot 2HCl \cdot H_2O$	C, H, N; H_2O[x]	118 (6.2)	0/5	210 (3.1)	0/10
37[k]	$CH_2CH_2NHCH_2CH_2CH_3$	B	107-110	$C_{24}H_{34}N_4O_4 \cdot 0.5H_2O$	C, H, N	36 (50)	0/6	0 [50]	0/10
38[k]	$CH_2CH_2NHCH(CH_3)_2$	B	139-143	$C_{24}H_{34}N_4O_4 \cdot 0.5H_2O$	C, N; H[y]	73 (50)	0/5	88 (25)	0/10
39[k]	$CH_2CH_2NHCH_2CH_2OH$	B	130-131	$C_{22}H_{30}N_4O_6 \cdot 0.25H_2O$	C, H, N	278 (6.2)	10/20	120 (3.1)	2/10
40	$CH_2CH_2NHCH_2CH_2OH \cdot HCl$[i]	C	203-205	$C_{22}H_{28}N_4O_6 \cdot 2HCl \cdot 0.5H_2O$	C, H, N; Cl[z]	>500 (1.6)	4/5	>272 (0.8)	8/10
41	$CH_2CH_2NH(CH_2)_3OH \cdot HCl$	B, C	190-192	$C_{24}H_{32}N_4O_6 \cdot 2HCl \cdot 0.5H_2O$	C, H, N, H_2O; Cl[a,a]	191 (12.5)	2/6	357 (6.2)	5/10
42	$CH_2CH_2NHCH_2CH(CH_3)OH \cdot HCl$	B, C	177-185	$C_{24}H_{32}N_4O_6 \cdot 2HCl$	C, H, N; Cl[a,b]	255 (12.5)	1/6	240 (3.1)	1/10
43	$(CH_2)_3NHCH_2CH_2OH \cdot HCl$	B, C	>350	$C_{24}H_{32}N_4O_6 \cdot 2HCl$	C, H, N, Cl	191 (50)	1/6	100 (25)	0/10
44	$CH_2CH_2NHCH_2CH(OH)CH_2OH$	B,[a,c] D	167-168	$C_{24}H_{32}N_4O_8 \cdot 0.5H_2O$	C, H, N, H_2O	>422 (50)	4/6	79 (12.5)	0/10
45	$CH_2CH_2N(CH_2CH_2OH)_2 \cdot HCl$	B, C	210-216	$C_{26}H_{36}N_4O_8 \cdot 2HCl$	C, H, N; Cl[a,d]	306 (200)	1/6	212 (200)[a,e]	1/10
46	$(CH_2CH_2NH)_2CH_2CH_2OH \cdot 2HCl$	B,[a,f] C	199-202	$C_{26}H_{38}N_6O_6 \cdot 4HCl$	H, N; C, Cl[a,g]	73 (100)	0/6	135 (50)	0/10
47	$CH_2CH_2NHCH_2CH_2NHCH_3 \cdot 2HCl$	B, C	190-203	$C_{24}H_{34}N_6O_4 \cdot 4HCl$	C, H; N, Cl[a,h]	120 (100)	0/6	122 (50)	0/10
48	$CH_2CH_2NHCH_2CH_2$-c-$N(CH_2CH_2)_2O \cdot 2HCl$	B, C	265	$C_{30}H_{42}N_6O_6 \cdot 4HCl$	H, N; C, Cl[a,i]	136 (200)	0/6	233 (100)	3/10
49	CH_2CH_2-c-$N(CH_2CH_2)_2NH$	B, D	200-203	$C_{26}H_{34}N_6O_4 \cdot 0.75H_2O$	C, H, N	133 (100)	0/5	103 (50)	0/10
50	$CH_2CH_2NH_2 \cdot HCl$	B, C	>350	$C_{18}H_{20}N_4O_4 \cdot 2HCl \cdot 0.5H_2O$	C, H, H_2O; N, Cl[a,j]	173 (3.1)	4/6	>433 (0.78)	7/10
51	$(CH_2)_3NH_2$	(5)	130	$C_{20}H_{24}N_4O_4$	146-150	83 (25)	2/5	87 (6.2)	0/10
52[k]	$(CH_2)_4NH_2$	B	>350	$C_{22}H_{30}N_4O_4 \cdot H_2O$	C; H, N[a,k]	25 (200)	0/5	0 [100]	0/10
53	$(CH_2)_3N(CH_3)_2$	B, D	154-157	$C_{24}H_{32}N_4O_4$	C, H, N	29 (50)	0/5	25 (25)	0/10
54	$(CH_2)_4N(CH_3)_2$	B, E	151-152[a,l]	$C_{26}H_{36}N_4O_4$	C, H, N	0 [200]	0/5	0 [50]	0/10
55	$(CH_2)_5N(CH_3)_2$	B, E	122-124	$C_{28}H_{40}N_4O_4$	C, H, N	0 [200]	0/5	0 [50]	0/10
56	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	B, E	110-115	$C_{32}H_{48}N_4O_4 \cdot 1.5C_4H_6O_6$[a,m]	C, H, N	30 (100)	0/5	0 [50]	0/10
57	$(CH_2)_3$-c-$N(CH_2CH_2)_2O \cdot HCl$	B, E	277-280[a,n]	$C_{28}H_{36}N_4O_6 \cdot 2HCl$	H, N, Cl; C[a,o]	40 (200)	0/5	0 [50]	0/10
58[k]	$CH_2CH(CH_3)N(CH_3)_2$	B	>350	$C_{24}H_{34}N_4O_4 \cdot 0.5H_2O$	C, H; N[a,p]	100 (200)	0/5	68 (50)	0/10
59[k]		B	184-188	$C_{28}H_{38}N_4O_4 \cdot {}^1/_3H_2O$	C, H, N	45 (200)	0/5	30 (50)	0/10
60	$CH_2CH_2NHCOCH_3$	B, C	270-273[a,q]	$C_{22}H_{24}N_4O_6$	C, H, N	0 [200]	0/6	0 [50]	0/10

[a-o] See footnotes a-o in Table I. [p] From DMF-H_2O. [q] From $C_6H_5NO_2$-EtOH. [r] From C_6H_6-EtOAc. [s] From $C_6H_5NO_2$-MeOH. [t] From $CHCl_3$-$MeOC_2H_4OH$, boiling out $CHCl_3$. [u] Calcd: N, 10.4. Found: 9.8. [v] Calcd: C, 64.0; H_2O, 1.1. Found: C, 62.8; H_2O, 0.6. [w] From toluene. [x] Calcd: H_2O, 3.8. Found, 3.2. [y] Calcd: H, 7.8. Found: H, 7.0. [z] Calcd: Cl, 13.5. Found: 12.7. [a,a] Calcd: Cl, 12.8. Found: 12.1. [a,b] Calcd: Cl, 13.0. Found: 12.5. [a,c] Starting diaminodiol (mp 60-62 °C), ref 21. [a,d] Calcd: Cl, 11.7. Found: 11.2. [a,e] Dosed on days 1-4. [a,f] The starting amine is 63 (Experimental Section). [a,g] Calcd: C, 46.2; Cl, 21.0. Found: C, 46.7; Cl, 20.0. [a,h] Calcd: N, 13.6; Cl, 23.0. Found: N, 12.4; Cl, 21.6. [a,i] Calcd: C, 49.5; Cl, 19.5. Found: C, 50.4; Cl, 17.5. [a,j] Calcd: N, 12.8; Cl, 16.2. Found: N, 12.2; Cl, 15.0. [a,k] Calcd: H, 7.5; N, 13.0. Found: H, 6.5; N, 11.6. [a,l] From EtOAc at -10 °C. [a,m] Sesqui-L-tartrate, from ether-acetone. [a,n] From *i*-PrOH. [a,o] Calcd: C, 56.3. Found: 55.0. [a,p] Calcd: N, 12.4. Found: N, 11.9. [a,q] From CF_3COOH-MeOH.

Table III. Comparison of 40 with Other Anticancer Agents vs. P-388 Leukemia[a]

drug	min effective dose,[b] mg/kg: no. of inject. 1	min effective dose,[b] mg/kg: no. of inject. 3	max tolerated dose,[c] mg/kg: no. of inject. 1	max tolerated dose,[c] mg/kg: no. of inject. 3	ILS,[d] %: no. of inject. 1	ILS,[d] %: no. of inject. 3	therapeutic index, MTD/MED: no. of inject. 1	therapeutic index, MTD/MED: no. of inject. 3
40	0.15	0.023	4.2	3.4	172	300	28	148
adriamycin	0.35	0.14	13.1	2.7	176	104	37	19
cyclophosphamide	26.3	12.0	350.0	100.0	147	142	13	8
daunorubicin	0.52		5.2		98		10	
methotrexate	27.9	1.5	108.0	17.5	56	102	4	11
5-fluorouracil	88.0	11.4	280.0	48.0	47	70	3	4

[a] Drugs administered ip once as a single dose or once each 4 days for three total injections. Determinations were made from regression lines of plotted log dose–response data. [b] Dose providing an increase in life span of 40% over control in P-388 tumor-bearing mice. [c] Lethal dose for 10% of the normal mice; animals observed for deaths during 21 days. [d] Percent increase in life span at the MTD in P-388 tumor-bearing mice.

Note Added in Proof: Clinical trials of **40** (CL 232, 315; NSC 301739D) are in progress.

Experimental Section

Melting points are uncorrected. For intensely dark-colored compounds melting points were observed with a hot-stage microscope or a Maquenne block. Solids were pressed with KBr for IR spectral determinations on Perkin-Elmer Model 21 or Nicolet Model 7199-FT spectrophotometers. NMR spectra were obtained with a Varian Model HA-100 spectrophotometer; chemical shifts (δ) are reported in parts per million relative to Me_4Si. A Cary Model 14 spectrophotometer was used for UV spectral determinations. The structures of all new compounds were confirmed by IR and NMR spectra and by elemental analyses that were within 0.4% of theoretical values, except where specified otherwise. Although the lability of the 2,3-dihydro-9,10-anthracenediones and diaziridine **32** and the high hygroscopicity of most of the salts and polyhydroxy compounds often correlated with unsatisfactory analyses, it was judged better to report these compounds than to exclude them. Microanalytical data for water of hydration was obtained by the Karl Fischer method.

Method A. 1,4-Bis[[2-(dimethylamino)ethyl]amino]-9,10-anthracenedione (8). A mixture of 12.01 g (0.05 mol) of quinizarin, 40.00 g of 2-(dimethylamino)ethylamine, and 70 mL of water was stirred and heated under reflux for 2 h and then allowed to cool, and the solid was collected and washed with water. Crystallization from EtOH gave 12.18 g (51%) of blue-black needles with a reddish reflection: mp 172–173 °C; IR 6.33, 6.22, 6.11 (sh) μm; NMR ($CDCl_3$) δ 2.34 (s, 12 H, NMe_2), 2.66 (t, 4 H, CH_2NMe_2), 3.48 (q, 4 H, $ArNCH_2$), 7.20 (s, 2 H, 2,3-H_2), 7.65 (q, 2 H, 6,7-H_2), 8.32 (q, 2 H, 5,8-H_2); UV (MeOH) λ_{max} 232 nm (ϵ 24 300), 253 (31 800), 274 sh (17 000), 313 sh (4800), 555 sh (8100), 593 (14 000), 637 (15 400).

In syntheses of related compounds where the starting amine was not plentiful, only a 50% excess was used, replacing the rest of the excess with $Me_2NCH_2CH_2NMe_2$. The period of reflux was extended to 16 h, when TLC (typically on SiO_2 vs. $CHCl_3$–MeOH, 9:1) showed a strongly blue product spot and almost complete disappearance of a purple intermediate. With some higher homologues, the reaction mixture was evaporated to dryness on neutral alumina and the residue subjected to dry-column chromatography[22] on additional neutral alumina (480 g for 0.03 mol of starting quinizarin). The column was developed with $CHCl_3$–MeOH (6:1), and the major, blue-black band was cut out and then eluted with $CHCl_3$–MeOH–Et_3N, (6:2:1).

Method B. 2,3-Dihydro-5,8-dihydroxy-1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (39). A partial solution of 12.50 g (0.12 mol) of 2-[(2-aminoethyl)amino]ethanol in 80 mL of $Me_2NCH_2CH_2NMe_2$ was deaerated by stirring and chilling for 15 min as a stream of N_2 was bubbled through it. After the gradual addition with stirring of 10.97 g (0.04 mol) of 2,3-dihydro-1,4,5,8-tetrahydroxyanthraquinone, magnetic stirring of the tarlike mixture was continued for 5 h under N_2 while heating with an oil bath at 50 °C. The next morning the mother liquor was decanted, and the gummy solid was triturated with EtOH, collected, and then washed with EtOH: yield 15.06 g (84%) of a green-gray solid; mp 130–131 °C; IR 6.31 μm; TLC [on SiO_2 vs. THF–H_2O–HOAc (8:2:1, v/v)] showed an orange-brown spot which oxidized to blue during 2 h.

In the condensation of leucoquinizarin with $H_2NCH_2CH_2NHMe$ in an excess of that amine as solvent, followed by air oxidation (method D), very little 14 separated from solution and its purification was tedious. However, with $Me_2NCH_2CH_2NMe_2$ as solvent the dihydro adduct separated directly from solution and was washed with toluene: yield 63%; mp 105–109 °C. Anal. ($C_{20}H_{26}N_4O_2$) H, N; C: calcd, 67.77; found, 66.94. Solid dihydro precursors for most of the compounds of Table II separated from $Me_2NCH_2CH_2Me_2$ in comparable quality. No attempt was made to purify these labile compounds.

The above reaction with 2-[(2-aminoethyl)amino]ethanol could be stirred much more smoothly and was complete in 1.5 h when the solvent was 125 mL of EtOH instead of $Me_2NCH_2CH_2NMe_2$. All of the starting amine was soluble and the immiscible material was more fluid. It solidified during several days under N_2. The dihydro product **39** (yield 90%; mp 118–121 °C) gave **40** of comparable purity to that from above when oxidized with chloranil (method C).

In the synthesis of the dihydro precursors for hexol 44 and hexamine 48, the reactions did not go to completion (percent N was very low) when the solvent chosen was insufficiently polar to dissolve enough of the reactants and intermediates. Subsequent dihydro syntheses were apparently complete when MeOH was used for **44** and **50**, EtOH–$Me_2NCH_2CH_2NMe_2$ (5:4) for **47** and **49**, and EtOH for **48**, **58**, and **60**. For **43**, **48**, and **46**, the reaction solutions were filtered and the three dihydro intermediates precipitated as syrups by adding 3 volumes of ether to each filtrate; trituration with 100 mL of THF caused the first syrup to solidify, whereupon the dihydro precursor of **43** was collected and washed with EtOH. The second syrup was directly oxidized with chloranil to give **48**. The third syrup was purified by three reprecipitations from MeOH–ether before chloranil oxidation to **46**.

Method C. 5,8-Dihydroxy-1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione Dihydrochloride (40). A suspension of 17.86 g (0.04 mol) of **39** in 280 mL of 2-methoxyethanol containing 10.01 g (0.0408 mol) of chloranil was stirred and chilled with an ice bath during the gradual addition of 20.0 mL of 8 N hydrogen chloride in EtOH. The resulting, very thick mixture was stirred at ca. 24 °C for 15 h and diluted and thinned with 840 mL of ether, and the solid was collected and washed with THF, affording 21.34 g of hygroscopic, blue-black solid, mp 203–205 °C. It can be recrystallized from H_2O–EtOH: IR 6.09 (w), 6.21 (m), 6.39 (s) μm; NMR (D_2O with Me_4Si–CCl_4 as external standard) δ 3.38 (m, 8 H, CH_2NCH_2), 3.65 (m, 4 H, $ArNCH_2$), 3.94 (m, 4 H, OCH_2), 6.73 (s, 2 H, 6,7-H_2), 6.88 (s, 2 H, 2,3-H_2); UV (H_2O) λ_{max} 241 nm (ϵ 41 000), 273 (12 000), 608 (19 200), 658 (20 900).

Method D. 5,8-Dihydroxy-1,4-bis[[3-(dimethylamino)propyl]amino]-9,10-anthracenedione (53). A partial solution of 6.00 g of the corresponding dihydro compound [via method B, 79% yield, mp 116–117 °C. Anal. ($C_{24}H_{34}N_4O_4$) C, H, N] in 60 mL of $Me_2NCH_2CH_2Me_2$ was heated on a steam bath under reflux as air was bubbled in for 12 h. The product which separated on cooling was collected, washed with heptane, and then recrystallized from heptane. Washing with petroleum ether gave 3.72 g (62%) of blue-black needles, mp 154–157 °C.

The dihydro intermediate for **44** (prepared from *dl*-3-amino-1,2-propanediol[21]) separated from the reaction solvent (MeOH) as a thick syrup, which was extracted by stirring magnetically for 1.5 h with each of four, 150-mL portions of MeOH. The extracts and the mother liquor were allowed to stand at ca. 24 °C, oxidizing and partly evaporating in an open flask for 6 days. The resulting crystals of **44**, collected and washed with MeOH, amounted to 3.80 g (19%), mp 167–168 °C. The MeOH-insoluble fraction of the syrup was oxidized by chloranil (method C) to give a hydrochloride of lower purity: yield 15.25 g (64%); mp 191–193 °C. Anal. Calcd for $C_{24}H_{32}N_4O_8 \cdot 2HCl$: C, 49.9; H, 5.9; N, 9.7; Cl, 12.3. Found: C, 50.8; H, 6.0, N, 9.2; Cl, 11.2.

Method E. 5,8-Dihydroxy-1,4-bis[[(2-(dimethylamino)ethyl]amino]-9,10-anthracenedione (27). A solution of 12.00 g of 2,3-dihydro-5,8-dihydroxy-1,4-bis[(2,2-dimethylethyl)amino]-9,10-anthracenedione (89% yield, via method B) was oxidized by heating in 100 mL of nitrobenzene for 15 min as the solvent refluxed on the wall of an open Erlenmeyer flask; by-product water boiled away, thus avoiding otherwise violent spattering. The solution was filtered and allowed to cool, and the resulting crystals were collected and washed with EtOH to give 8.44 g (70%) of blue-black rods: mp 236–238 °C; homogeneous by TLC [SiO_2 vs. $CHCl_3$–MeOH–Et_3N (9:1:1)]. (By TLC this oxidation was also complete in ≤6 min using activated MnO_2 in $CHCl_3$ at 24 °C.)

Method F. 1,4-Bis[[2-(1-aziridino)ethyl]amino]-5,8-dihydroxy-9,10-anthracenedione (32). To a suspension of 4.10 g of the corresponding dihydro compound (very crude, via method B) in 40 mL of $CHCl_3$ was added a solution of 1.74 g of diethyl azodicarboxylate in 25 mL of $CHCl_3$. The mixture was stirred for 20 min, the resulting dark blue solution was filtered, and the filtrate was evaporated at ≤30 °C. A solution of the residue in 40 mL of $CHCl_3$ was stirred for 5 min with 2 g of decolorizing carbon and then filtered and washed through with another 25 mL of $CHCl_3$. Addition of 100 mL of ether to the filtrates precipitated a gum, which was eliminated by decantation–filtration. The filtrates deposited crystals, which were washed sparingly with acetone. The chloroform–ether mother liquor, chilled at −60 °C, deposited a second crop of crystals, which was washed with ether and with MeOH. A solution of both crops of crystals in 20 mL of $CHCl_3$ was stirred with decolorizing carbon, filtered, evaporated at ≤25 °C to a volume of 5 mL, diluted with 20 mL of ether, and then chilled at −60 °C. The resulting blue-black crystals, washed with ether, amounted to 0.64 g: mp 168–170 °C; TLC [SiO_2 vs. $CHCl_3$–Et_3N–MeOH (27:3:1, v/v)]; NMR ($CDCl_3$) δ 1.20 (t, 4 H, aziridine), 1.30 (t, 4 H, aziridine), 2.54 (t, 4 H, $NHCH_2CH_2$), 3.50 (q, 4 H, $NHCH_2CH_2$), 7.08 (s, 4 H, 2,3,6,7-H_4). Nonequivalence of aziridine ring protons was also observed in the precursor, 1-(2-aminoethyl)aziridine [NMR ($CDCl_3$) δ 1.18 (t, 2 H), 1.76 (t, 2 H)], in accord with that observed in other N-substituted aziridines and ascribed to slowness of pyramidal inversion of N.[23]

Method G. 2,2′-[(9,10-Dioxo-1,4-anthracenediyl)diimino]bis[*N,N,N*-trimethylethanaminium Iodide] (10). A mixture of 2.0 g of **8**, 15 mL of MeI, and 30 mL of ether was stirred for 3 h, and the solid was collected and recrystallized from $MeOCH_2CH_2OH$, affording 1.4 g of black solid, mp 285 °C. Partial evaporation and cooling of the mother liquor gave another 0.8 g of product, mp 278 °C.

Method H. 1,4-Bis[[2-(dimethylamino)ethyl]amino]-6-methyl-9,10-anthracenedione (22). 6-Methylquinizarin (**61**; 4.0 g) in 17 mL of *N,N*-dimethylethylenediamine was stirred and heated under reflux for 6 h and then evaporated. To a solution of the residual thick oil in 30 mL of toluene was added 100 mL of petroleum ether. The resulting solid was removed by filtration, the filtrate was evaporated, and the residue was crystallized from EtOAc, affording 0.9 g of dark-blue solid, mp 158–160 °C.

Method I. 5,8-Bis[[2-(dimethylamino)ethyl]amino]-9,10-dihydro-9,10-dioxo-2-anthroic Acid (23). A melted mixture of 300 g of $AlCl_3$ and 60 g of NaCl was stirred at 160 °C under a reflux condenser during the portionwise addition of a finely ground, intimate mixture of 43.2 g (0.225 mol) of 1,2,4-benzenetricarboxylic anhydride and 17.7 g (0.161 mol) of hydroquinone. Stirring and heating at 165–175 °C were continued for 1 h. The molten mass was poured slowly into 2 L of ice–water, 50 mL of 12 N HCl was added, and the solid was collected and washed with water. Crystallization of this solid (34.1 g) from DMF, finally at −10 °C, and washing with ether returned 19.5 g of dark-red 5,8-dihydroxy-9,10-dioxo-2-anthroic acid, mp 345–350 °C. We found the reported[24] synthesis of this acid (lit.[24] mp >300 °C) from 1,2,4-benzenetricarboxylic acid to be less satisfactory.

A mixture of 4.75 g (16.7 mmol) of the anthroic acid, 2.5 g (14.4 mmol) of sodium hydrosulfite, 16 mL (165 mmol) of *N,N*-dimethylethylenediamine, and 32 mL of H_2O was stirred under N_2 and heated with an oil bath at 105 °C for 7 h. The next day 50 mL of H_2O and 3 drops of pyridine were added, and the mixture was stirred for 2 h as O_2 was bubbled in. Evaporation to dryness and recrystallization of the residue from EtOH gave 4.9 g of a blue solid, which was subjected to dry-column chromatography on silica gel, developing with MeOH. Very dark blue material on the top 5% of the column was rejected. A blue band below it was cut out and eluted with MeOH, the eluate was evaporated, and the residue (1.9 g) was chromatographed again on silica gel, developing with MeOH–Et_3N (100:1, v/v). The resulting blue band was cut out in sections, which were eluted with MeOH–Et_3N (100:1), and the eluates were evaporated. The residues (total 0.9 g; all homogeneous by TLC on SiO_2 vs. MeOH–Et_3N) were dissolved in H_2O–$MeOCH_2CH_2OH$, the solution was filtered, and the filtrate was evaporated, leaving 0.45 g of a hygroscopic, dark blue solid. Additionally, a center part from the 0.9 g of residues had been recrystallized from EtOH to give 0.11 g of an analytical sample, mp 145–150 °C.

Method J. 1,4-Bis[2-(dimethylamino)ethyl]-6,7-dichloro-9,10-anthracenedione (24).[25] To a stirred suspension of 1.5 g of 6,7-dichloroquinizarin[26] in 200 mL of HOAc was added 2.04 g of zinc dust in one portion. The mixture was stirred and heated at 80 °C for 45 min and filtered hot, and the filtrate was diluted with H_2O and kept at 5 °C. The solid which separated was washed well with water: yield 1.5 g of orange-brown 2,3-dihydro-1,4-dihydroxy-6,7-dichloro-9,10-anthracenedione; mp 290–293 °C. After 0.7 g of this solid in 20 mL of *N,N*-dimethylethylenediamine had been heated at 110 °C for 75 min, the excess amine was removed in vacuo and the residue purified by thick-layer chromatography on silica gel, developing with $CHCl_3$–Et_3N (9:1) to give 0.25 g of very hygroscopic, dark-blue crystals: mp 147–149 °C; IR spectrum very similar to compound **8** plus an extra modest peak at 13.20 μm; NMR (1% Me_2SO in $CDCl_3$, v/v) δ 2.36 (s, 12 H, NMe_2), 2.66 (t, 4 H, CH_2NMe_2), 3.46 (q, 4 H, $ArNCH_2$), 7.11 (s, 2 H, 2,3-H_2), 8.22 (s, 2 H, 5,8-H_2).

Method K. 1,4-Bis[[2-(dimethylamino)ethyl]amino]-5,6-dihydroxy-9,10-anthracenedione (25). A solution of 7.9 g (0.09 mol) of 2-(dimethylamino)ethylamine in 75 mL of $Me_2NCH_2CH_2NMe_2$ was heated to 80–100 °C and deaerated with N_2. After portionwise addition of 8.22 g (0.03 mol) of **62**, the mixture was stirred under N_2, heated at 90–100 °C for 6 h, and then filtered. The filtrate was cooled, diluted with 2 volumes of ether, chilled, decanted from a dark-blue gum, and then diluted with more ether. On standing, the product separated as 1.5 g of blue solid, mp 133–135 °C.

6-Methylquinizarin (61). A melted mixture of 410.6 g (3.08 mol) of $AlCl_3$ and 82.1 g of sodium chloride was stirred with a paddle stirrer having a stainless-steel shaft and heated with an oil bath at 160–165 °C during the portionwise addition of an intimate mixture of 50 g (0.308 mol) of 4-methylphthalic anhydride and 24.3 g (0.221 mol) of hydroquinone. Stirring at 170 °C was continued for 45 min, the mixture was poured into ice–water, and 67 mL of 12 N HCl was added. The solid was collected and washed with water. Recrystallization from HOAc returned 22.3 g (41%) of orange solid, mp 175–177 °C. Anal. ($C_{15}H_{10}O_4$) C, H.

1,4,5,6-Tetrahydroxy-2,3-dihydro-9,10-anthracenedione (62). After the portionwise addition of 30 g of zinc to 27.2 g of quinalizarin in a boiling solution of 40 mL of H_2O in 1.5 L of HOAc, the solution was boiled for 30 min and filtered. The filtrate deposited 19.7 g (72%) of orange-brown crystals, mp 255–257 °C (lit.[27] mp 245 °C). Anal. ($C_{14}H_{10}O_6$) C, H.

Purification of 2-[[2-[(2-Aminoethyl)amino]ethyl]amino]ethanol (63). "Technical" material from either the Union Carbide or Aldrich Chemical Co. is sold with exactly the same stated physical constants. It is apparently[28] the alkylation product from diethylenetriamine and ethylene oxide. In addition to the desired terminally N-monoalkylated product, we found that it

contains ca. 22% (by VPC) of a presumed centrally N-monoalkylated isomer (with a slightly lower boiling point) plus dialkylated material, $C_8H_{21}N_3O_2$. Fractional distillation of 200 g of Aldrich material in a spinning brush column rated at 50 theoretical plates was continued until distillation ceased (at 4.5 mm pressure, with an oil bath at 228 °C). The last cut (cut 6) amounted to 36.6 g (18%) of the desired product: bp 160.5 °C (4.5 mm), lit.[28] 153–155 °C (3.5–3.8 mm); n^{25}_D 1.4960, lit. 1.4974; VPC in a 15.4-cm column packed with 3% OV 225 (a cyanopropylsilicone) supported on 80/100 Supelcoport[29] run at 150 °C with N_2 flow at 45 mL/min showed two peaks, with retention times of 5.4 and 6.8 min and peak areas of 2 and 98%, respectively. Anal. ($C_6H_{17}N_3O$) C, H, N. Cut 2 was mostly isomeric: yield 6.4 g; bp 156 °C (4.0 mm); $n^{24.7}$ 1.4962; VPC showed the 5.4- and 6.8-min peaks in a 68:32 ratio; NMR ($CDCl_3$), in contrast to cut 6, showed the complexity expected from a mixture. Anal. Calcd for $C_6H_{17}N_3O$: C, 49.0; H, 11.6; N, 28.6. Found: C, 47.9; H, 12.1; N, 27.9. Cut 8 amounted to 18.4 g: bp 184 °C (0.1 mm); n^{25} 1.5047. Anal. ($C_8H_{21}N_3O_2$) C, H, N.

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Synthesis and Biological Evaluation of Tetramisole Analogues as Inhibitors of Alkaline Phosphatase of the 6-Thiopurine-Resistant Tumor Sarcoma 180/TG[1]

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Tetramisole and its analogues are potent inhibitors of alkaline phosphatase, including isoenzymes of Sarcoma 180/TG which appear to be involved in the mechanism of resistance of this neoplastic cell line to the 6-thiopurines. To determine the requirement for the thiazole ring system of tetramisole for inhibitory potency, 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]oxazole, 2,3-dihydro-6-phenylimidazo[2,1-*b*]oxazole, and 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*a*]imidazole were synthesized and tested for inhibitory activity against alkaline phosphatase isolated from Sarcoma 180/TG. The results indicate that 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]oxazole caused 50% inhibition at 0.21 mM, while the other synthesized compounds were inactive at a concentration of 1 mM; in contrast, tetramisole required only 0.045 mM for 50% inhibition of alkaline phosphatase activity. The findings support the concept that the thiazole ring system of the tetramisole structure is required for maximum inhibitory potency of this series against alkaline phosphatase.

Investigations by this laboratory[2-4] and by others[5] of the mechanisms by which neoplastic cells acquire resistance to the growth-inhibitory action of the 6-thiopurines (i.e., 6-mercaptopurine and 6-thioguanine) have shown that the acquired insensitivity to these drugs by the murine neoplasm Sarcoma 180/TG and by acute leukemia cells of

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