Potential Central Nervous System Antitumor Agents. Aziridinylbenzoquinones. 2

Feng-te Chou,[1a] A. Hameed Khan,[1b] and John S. Driscoll*

Drug Design and Chemistry Section, Laboratory of Medicinal Chemistry and Biology, Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.
Received March 22, 1976

A series of 15 2,5-diaziridinyl-3,6-bis(alkylamino)-1,4-benzoquinone derivatives was synthesized and evaluated as central nervous system antitumor agents in the murine intracerebral L1210 and ependymoblastoma brain tumor systems. Intraperitoneal activity was evaluated in the leukemia L1210, P388, and B16 melanocarcinoma tumor models. The more hydrophilic hydroxyalkylamino compounds were the most effective in the intraperitoneal ascites systems (L1210, P388) with the dihydroxypropylamino (18) and hydroxyethylamino (17) analogues producing long-term survivors. The simple, more lipophilic mono- and dialkylamino derivatives were most effective in the intracerebral systems. Multiple long-term survivors were obtained with the methyl (13), ethyl (14), and dimethylamino (20) compounds in the ependymoblastoma brain tumor system. The parent amino analogue 12 was very active in several tumor models. The relationships between structure, activity, and water solubility are discussed.

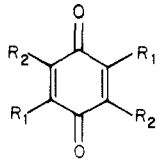
A recent review[2] of the quinones tested for antitumor activity by the National Cancer Institute (NCI) shows that a number of active materials contain this structural feature. Agents such as adriamycin,[3-5] daunorubicin,[4,5] mitomycin C,[6,7] streptonigrin,[4,8,9] and lapachol[4,10] are quinones derived from fermentation or plant products. The cancer treatment potential for another natural product, coenzyme Q_{10}, has also been discussed.[11] In vivo quinone reduction forms the basis for the concept of bioreductive alkylating agents proposed by Sartorelli and co-workers.[12]

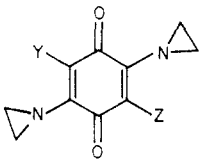
Among the many hundreds of purely synthetic compounds evaluated by the NCI, *p*-benzoquinone derivatives containing multiple aziridinyl groups were the only agents very active against murine leukemia L1210. The 2,5-diaziridinylbenzoquinone series containing various donor or electronically neutral functional groups in the 3,6 positions was shown to have substantial activity against this tumor system.[2] Members of this family are known to cross-link DNA.[13]

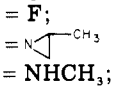
As part of a systematic search for new central nervous system (CNS) antitumor agents, it seemed reasonable to test the available aziridinylbenzoquinones[2] against intracerebrally implanted murine tumors since many of the compounds appeared to possess several of the molecular characteristics (good lipid solubility, low degree of ionization) which were claimed necessary for CNS penetration.[14] A number of these compounds were subsequently determined to be active against intracerebral L1210 leukemia.[15] Because the low water solubility of most of the available derivatives made them difficult to formulate, a synthetic program was undertaken to prepare aziridinylbenzoquinones with (a) improved solubility, (b) good CNS penetration characteristics, and (c) undiminished or improved murine intraperitoneal and intracerebral antitumor activity.

Our first approach utilized chloranil (1) as a starting material and resulted in an aziridinylquinoneurethane derivative (4) which was very active in the intraperitoneal leukemia L1210, leukemia P388, and the B16 melanoma test systems.[16] In addition, substantial intracerebral (IC) L1210 and P388 activity was observed and the compound was curative in the murine ependymoblastoma brain tumor model. While the solubility of 4 appeared to be adequate, further investigations were initiated to produce the previously unevaluated diaziridinylbenzoquinone derivatives containing amino substituents in the other two available ring positions.

Chemistry. While 2,5-diaziridinyl-1,4-benzoquinone derivatives may be prepared in a number of ways,[17] substitution reactions utilizing halo-[16,18-24] or alkoxyquinones[25-27] have been most generally employed. Tetrachloro-*p*-benzoquinone (chloranil, 1) has been used extensively as a starting material for the preparation of 2,5-diaziridinyl-3,6-disubstituted 1,4-benzoquinones.[16,18-24] Although the chlorine atoms in the 2 and 5 positions are readily replaced by nucleophiles, the electron-donating properties of the added groups usually deactivate the ring toward replacement of the other two chlorine atoms.[20] While compound **10**, for example, is easily prepared from chloranil and ethylenimine, this compound and other diaminodichloro-*p*-benzoquinone derivatives undergo no further displacement reactions with amines[16,18] This is not the case, however, with the analogous difluoro analogue, 11, which is prepared from tetrafluoro-1,4-



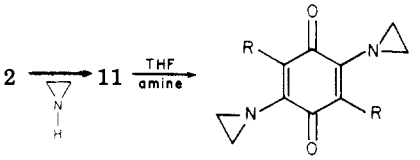


1, $R_1 = R_2 = Cl$
2, $R_1 = R_2 = F$
3a, $R_1 = F$; $R_2 =$ 
3b, $R_1 = NHCH_3$; $R_2 =$ 

4, Y = Z = $NHCOOC_2H_5$
5, Y = Z = $NHCOCH_3$
6, Y = Z = OCH_3
7, Y = H; Z = c-NC_2H_4
8, Y = CH_3; Z = $CH(OCH_3)CH_2OCONH_2$
9, Y = F; Z = c-$N(CH_2CH_2)_2O$
10, Y = Z = Cl
11, Y = Z = F

benzoquinone (fluoranil, **2**).[17,25-27] The two fluorine atoms in 11 are readily replaced by amines to form tetraaminobenzoquinone derivatives. This reaction was used as the synthetic method for the preparation of compounds **12-26** (Scheme I). These agents were evaluated for their antitumor activity in a number of murine intraperitoneal and intracerebral tumor systems.

Scheme I



12-26

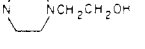
12, R = NH_2
13, R = $NHCH_3$
14, R = NHC_2H_5
15, R = NHC_3H_7
16, R = NHC_4H_9
17, R = $NHCH_2CH_2OH$
18, R = $NHCH_2CH(OH)CH_2OH$
19, R = $NHCH_2CONH_2$
20, R = $N(CH_3)_2$
21, R = $N(CH_3)CH_2CH_2OH$
22, R = c-NC_4H_8
23, R = c-NC_5H_{10}
24, R = 
25, R = c-$N(CH_2CH_2)_2O$
26, R = 

Table I. Physical and Chemical Properties of 2,5-Diaziridinyl-3,6-diamino-1,4-benzoquinones

No.	Recrystn solvent[a]	Yield, %	Mp, °C	Color	$\lambda_{max}^{CH_3OH}$ (log ε)	Approx. H_2O solubility, mg/ml	Log P[b]	Mol formula	Analyses
3b	A	84	179	Green	369 (4.13)	<0.1		$C_{14}H_{20}N_4O_2$	C, H, N
9	B	51	157	Gray	325 (3.88)	2		$C_{14}H_{16}FN_3O_3$	C, H, N, F
12	C	73	220	Green		≪0.1		$C_{10}H_{12}N_4O_2$	C, H, N
13	A	70	220	Green	374 (4.16)	<0.1		$C_{12}H_{16}N_4O_2$	C, H, N
14	A	57	157	Green	374 (4.20)	0.5		$C_{14}H_{20}N_4O_2$	C, H, N
15	A	60	140	Green	375 (4.17)	<0.1		$C_{16}H_{24}N_4O_2$	C, H, N
16	C	91	95	Green	377 (4.12)	<0.1		$C_{18}H_{28}N_4O_2$	C, H, N
17	A	48	188	Green	375 (4.08)	2	−1.48	$C_{14}H_{20}N_4O_4$	C, H, N
18	D	77	273	Green	365 (4.15)	30	−1.97	$C_{16}H_{24}N_4O_6$	C, H, N
19	D	81	200	Green	365 (3.72)	0.5		$C_{14}H_{18}N_6O_4$	C, H, N
20	E	59	112	Green	455 (3.72), 298 (3.77)	10		$C_{14}H_{20}N_4O_2$	C, H, N
21	F	74	125	Green	455 (3.67), 310 (3.71)	60	−0.38	$C_{16}H_{24}N_4O_6$	C, H, N
22	G	64	160	Brown	440 (3.80), 274 (3.65)	0.5		$C_{18}H_{24}N_4O_6$	C, H, N[c]
23	G	62	180[d]	Brown	466 (3.80), 299 (3.80)	<0.1		$C_{20}H_{28}N_4O_2$	C, H, N
24	G	54	235	Red	454 (3.69), 300 (3.72)	1		$C_{20}H_{28}N_4O_4$	C, H, N[e]
25	B	28	224	Brown	453 (3.20), 307 (3.42)	<0.1	0.52	$C_{18}H_{24}N_4O_4$	C, H, N
26	F	64	170	Green	452 (3.71), 297 (3.76)	20	−0.75	$C_{22}H_{34}N_6O_4$	C, H, N

[a] A = ethanol, B = benzene, C = water wash, D = methanol wash, E = hexane, F = toluene, G = THF + hexane. [b] Octanol-H_2O values determined by C. Hansch and M. Yamakawa, Pomona College. [c] N: calcd, 17.06; found, 16.57. [d] Lit.[26] mp 177°. [e] N: calcd, 14.42; found, 13.95.

Although several tetraamino-1,4-benzoquinone derivatives are known,[17] only the tetraaziridinyl derivative appears to have been evaluated as an antitumor agent, and its activity in the L1210 leukemia system was only moderate (ILS 39%).[2] The selection of the appropriate amino substituents for **12–26** was important since they might influence both the biological transport properties and the chemical reactivity of the compounds.[28,29] The partition coefficient, an important factor in determining the biological transport properties of a compound, was especially significant since this parameter also gives a rough guide to aqueous solubility properties which are important in this family of sparingly soluble compounds. The electronic properties of the R-group substituents (Scheme I) should influence the redox potential of the quinone system.[30–33] This parameter is known or postulated to be an important factor in determining the antitumor activity in aziridinylquinone systems,[34] mitomycin C analogues,[35] and bioreductive alkylating agents.[12,52] If the redox potential should be an important factor in the biological activity of the diaziridinylbenzoquinones, the substitution of the other two available positions with alkylamino groups should produce a maximum effect[33] in the parameter relative to other possible substituents. This would result in either a maximum or minimum in biological activity provided the activity is a linear function of the redox potential. The steric effects of the R groups should influence the degree of coplanarity of the ethylenimine and quinone rings. This should affect the reactivity of the alkylating group as well as the redox potential of the quinone. For these reasons, the compounds which were synthesized for antitumor evaluation (**12–26**) were chosen with the intention[28] of creating a spread in lipophilic (π),[36,37] steric,[38] and electronic[37] R-group effects.

Fluoranil (**2**) reacted with ethylenimine to produce the key intermediate 2,5-diaziridinyl-3,6-difluoro-1,4-benzoquinone (**11**).[17,25–27] Three isomers are possible from the disubstitution reaction and two were previously isolated and identified.[39] X-Ray crystallographic analysis verified that **11** was the structure of the material used in our reactions.[40] The colorless König's adducts sometimes found with β-hydroxyethylaminobenzoquinones[41] were not observed with the tetraamino derivatives described here. Although the piperidine derivative **23** had been prepared by several groups,[25–27] none of the other diaziridinyldiaminobenzoquinones described here have been reported previously. The physical and chemical properties of these compounds are given in Table I.

Antitumor Activity. Two aziridinylbenzoquinones are presently undergoing laboratory and clinical studies. Trenimon (**7**) is being studied mainly in Europe[42,43] while carbazilquinone (**8**) has had extensive investigation in Japan.[44–46] Clinical activity has been reported with both compounds. Compounds **5** and **6** are representative of the amide and alkoxy analogues with intraperitoneal (ip) leukemia L1210 activity.[2]

The urethane **4** was shown to have intracerebral (ic) as well as ip antitumor activity.[16] In an attempt to determine the structural factors affecting the activity of the diaminodiaziridinyl analogues, the series **12–26** was prepared and evaluated in the ip murine L1210 lymphoid leukemia tumor model. When an ip L1210 test gave increase in life-span (ILS) values >50%, the compound was also tested in the ic L1210 model. Standard NCI protocols were used.[47] Aqueous saline solution (0.9%) was used as the drug vehicle. Normally, five doses were employed per experiment with the top dose chosen to produce toxicity. The lower doses were 50% of each preceding dose.

Since this family of aminoaziridinyl-1,4-benzoquinones subsequently proved to have very good L1210 activity, the members were subjected to testing in two additional intraperitoneal systems (P388 lymphocytic leukemia and B16 melanocarcinoma)[47] as well as the murine ependymoblastoma brain tumor system.[48,49] Intraperitoneal antitumor data are given in Tables II and III. Table IV lists the antitumor activity of this series against the intracerebrally implanted solid ependymoblastoma and ascites

Table II. Intraperitoneal Lymphoid Leukemia L1210 Activity

	QD1-9[a]				Q4D (1,5,9)[b]				Day 1[c]			
No.	Expt no.	OD[d]	T − C[e]	ILS[f,g]	Expt no.	OD	T − C	ILS	Expt no.	OD	T − C	ILS
3b	7968	5.0	−2.9	69	7691	12.5	−1.8	93	8744	5.0	−0.9	42
	7967	5.0	−4.4	37	7982	12.5	−2.4	52	8743	10.0	−3.3	39
9	8769	6.0	−3.5	29	8698	6.2	−1.8	21	8757	12.5	−3.2	21
	8770	1.5	−0.2	11	8697	3.1	−2.2	7	8758	6.2	−3.3	4
12	8857	0.4	−4.5	115	8546	0.5	−1.7	161 (3)	8757	1.0	−1.7	95
	8856	0.3	−0.3	100	8769	0.02	−2.8	124 (2)	8758	0.5	−0.9	26
13	8343	0.2	−1.6	73 (1)	8343	0.8	−1.6	57	8744	0.7	−3.4	34
	8004	0.2	−2.5	61	7860	0.4	−1.0	56	8743	0.7	−2.8	19
14	8004	1.5	−3.3	112 (2)	7860	3.0	−2.3	75	8744	1.5	−1.9	68
	8005	1.5	−3.3	91 (1)	7861	1.5	−1.8	57	8743	1.5	−2.2	54
15	8005	6.2	−2.9	48	7861	6.2	−1.1	28	8743	12.0	+0.2	41
	8004	6.2	−2.4	43	7860	6.2	−0.3	25	8744	1.5	−0.1	17
16	8741	10.0	−1.5	36	8663	12.5	−1.4	44	8743	10.0	−0.8	11
	8742	5.0	−1.7	30	8727	8.2	−2.9	25	8744	10.0	−1.4	5
17	5178	0.7	−3.2	205 (2)	7861	0.7	−2.0	131	8647	1.5	−3.8	175 (5)
	8005	0.7	−3.0	134 (3)	7860	0.7	−0.3	70	8743	1.5	−3.4	119 (3)
18	8647	4.0	−2.7	206 (5)	8450	4.0	−0.3	90	8743	4.0	−3.2	173 (4)
	8648	2.0	−2.1	133 (2)	8454	8.0	−2.0	79	8744	2.0	−2.3	89
19	8861	10.0	−4.9	92	8663	8.0	−2.7	93	8743	10.0	−1.6	21
	8742	10.0	−2.7	91	8727	8.0	−2.8	32	8744	10.0	−1.8	15
20	8741	0.75	−1.6	39	8740	3.0	−0.4	120 (1)	8743	1.5	+0.4	21
	8742	0.37	0.0	16	8656	3.0	−1.0	73	8744	1.5	−2.2	15
21	8742	1.5	−2.4	114 (1)	8663	0.4	−0.9	57	8744	1.0	−0.9	26
	8826	1.5	−3.6	111 (1)	8727	0.2	+4.0	19	8743	0.2	−2.6	−5
22	8769	2.0	−2.7	24	8698	6.2	−2.3	43	8757	4.0	−1.0	25
	8770	2.0	−2.2	21	8890	6.2	−2.7	53	8758	2.0	−0.9	8
23	8883	10.0	−2.2	28	8739	35	−2.4	26	8757	20.0	−1.7	16
	8890	10.0	−3.3	41	8697	12.5	−3.0	22	8758	80.0	−2.7	8
24	8741	3.0	−1.8	59	8727	6.0	−3.8	104 (2)	8743	3.0	−0.7	21
	8742	3.0	−3.6	44	8663	4.0	−2.5	84	8744	0.7	−0.9	8
25	8769	6.0	−5.7	64	8697	6.0	−2.6	49	8757	12.5	−3.0	42
	8770	1.5	−2.7	32	8698	6.0	−2.6	44	8758	3.1	−1.6	−14
26	8539	1.0	−1.7	147	8320	4.0	−2.1	180 (3)	8743	4.0	−0.7	52
	8647	0.5	−1.7	114 (1)	8739	2.0	−1.5	165 (4)	8744	4.0	−3.4	21

[a] Day 1-9 treatment schedule (nine injections). [b] Day 1,5,9 treatment schedule (three injections). [c] Day 1 only treatment schedule (one injection). [d] Optimum dose (mg/kg per injection). [e] Average weight change of test group minus average weight change of control group in grams on day 5. [f] Percentage increase in life span of treated animals [(treated survival ÷ control survival) × 100%] − 100%. [g] Number of animals alive per six test animals on the day of termination of the experiment (L1210 and P388, day 30; B16 melanoma and ependymoblastoma, day 60) is in parentheses.

leukemia L1210 tumor systems. The criterion for minimum activity (%ILS) is defined here as follows: intraperitoneal and intracerebral leukemia L1210 and intraperitoneal leukemia P388, 25%; intraperitoneal B16 melanoma and intracerebral ependymoblastoma, 40%. Consistent with NCI antitumor test protocols,[47] intraperitoneal (ip) administration of drug was employed with all five tumor systems investigated. Tumor implantation was ip in the systems designated ip leukemia L1210 and P388 as well as B16 melanoma. Intracerebral (ic) tumor implantation was utilized in the ic L1210 and ependymoblastoma tumor systems.

Table V lists those compounds with outstanding activity in two or more tumor systems. The initial antitumor experiments were carried out in the ip L1210 system on the Q4D (day 1,5,9) treatment schedule. These experiments confirmed the L1210 activity in this series and established the proper dose ranges for the determination of any schedule dependency in the ip L1210 system (Table II). The chronic (QD1–9) treatment schedule was found to be superior for almost all the compounds studied. Significant activity was obtained for all the diaziridinyl derivatives except the pyrrolidino (**22**) and piperidino (**23**) compounds. Some of the data variation may be attributable to the problem of reproducibly injecting suspensions of the more insoluble compounds.

Superior activity was obtained with several derivatives among which the dihydroxypropylamino (**18**) and hydroxyethylamino (**17**) compounds were outstanding with maximum ILS values in excess of 200% being observed (the theoretical maximum ILS values in a fully acceptable experiment ranges between 173 and 275%, consistent with the control animal death limits set at 8–11 days[47] and experiment termination on day 30). Multiple cures, defined as survivors on day 30 in the L1210 system, were observed for both compounds. Excellent activity was also observed with **17** and **18** on the day 1 and Q4D treatment schedules. The day 1 schedule was generally the poorest of the three studied. Several compounds (**12**, **20**, **24**, and **26**) showed maximum activity with intermittent (Q4D) treatment.

Excellent activity was observed in the leukemia P388 system on the chronic treatment schedule (Table III). Again, compounds **17**, **18**, and **26** were very active producing multiple cures (30-day survivors). As might be expected, compounds with outstanding L1210 activity usually possessed excellent P388 activity (Table V). All the compounds studied in this series (Table III) were active against leukemia P388, including the monoaziridinyl derivative **9**.

Only three compounds in the series were reproducibly active in the B16 melanoma tumor model (Table III). Once again **17** and **18** proved active as did the 2′-methylaziridinyl analogue **3b**. The aziridinyl analogue of **3b** (**13**) approached minimal reproducible activity as did **21**, the *N*-methyl derivative of **17**, and the 3′-hydroxypiperidino analogue **24**.

Table IV shows the data obtained in the intracerebrally

Table III. Intraperitoneal Lymphocytic Leukemia P388 and B16 Melanocarcinoma Activity[a]

	Lymphocytic leukemia P388				B16 melanocarcinoma			
No.	Expt no.	OD[b]	T − C	ILS	Expt no.	OD	T − C	ILS
3b					301	5.0	−1.0	70
					300	5.0	−0.7	48
9	5282	3.0	−2.3	57	302	0.7	−0.3	18
	5281	6.0	−2.5	54	303	0.7	0.0	9
12	5281	0.2	−1.7	171 (1)	302	0.1	−0.5	31
	5439	0.2	−1.9	156	303	0.02	−0.1	19
13	5112	0.2	−3.6	95	301	0.4	−0.7	39
	5111	0.1	−3.3	69	294	0.05	−3.1	29
14	4902	1.5	−2.6	160	300	0.4	−0.3	26
	4903	0.7	−2.0	103	301	0.7	−0.4	24
15	5111	6.0	−3.0	151 (2)	288	5.0	−0.5	36
	5112	6.0	−3.3	108	287	5.0	−2.9	32
16	5281	5.0	−0.4	64	302	10.0	−1.0	36
	5282	10.0	−2.0	49	303	5.0	−0.1	11
17	4903	0.4	−2.6	175 (3)	288	0.4	−0.6	61
	4902	0.4	−2.3	160 (2)	287	0.4	−2.7	51
18	5262	4.0	−3.4	143 (6)	301	4.0	−2.5	50
	5405	4.0	−3.5	141 (4)	314	6.0	−3.0	42
19	5281	2.0	−2.3	85	302	8.0	−1.4	37
	5282	2.0	−1.1	71	303	0.5	+0.5	22
20	5282	1.0	−2.7	74	303	0.2	+0.3	16
	5281	0.5	−1.8	37	302	0.2	−0.5	12
21	5439	1.6	−2.4	162	302	0.8	−2.3	47
	5440	0.8	−2.7	145	303	0.8	−1.2	31
22	5282	1.0	−1.8	72	303	2.0	+0.8	28
	5281	0.2	−4.3	55	302	2.0	−0.5	18
23	5525	5.0	+2.3	46	317	5.0	−1.5	21
	5526	10.0	−1.5	77	316	2.5	−2.3	−3
24	5282	1.0	−2.5	95	302	1.0	−1.1	40
	5281	1.0	−0.5	78	303	1.0	−0.1	37
25	5525	0.5	−0.1	52	302	1.5	−1.8	24
	5281	0.7	−0.4	48	303	0.7	+1.0	17
26	5263	0.5	−0.8	149 (3)	301	0.5	−0.6	22
	5262	1.0	−2.4	143 (6)	300	0.2	+0.1	22

[a] QD1-9 treatment schedule. [b] See footnotes in Table II for definitions.

implanted tumor systems. Only those compounds which had given L1210 intraperitoneal ILS values >50% were tested in the intracerebral L1210 system. While nine of eleven compounds tested were reproducibly active, the parent amino compound (**12**) and mono- (**13**) and dimethylamino (**20**) analogues were the most active against ic L1210 leukemia. Compounds **17**, **18**, and **26** which produced cures in the ip L1210 model gave activity levels which may be consistent with the inhibition of systemic disease produced by migration of L1210 cells from the implant site. The marginal ic L1210 activity associated with these three compounds (which produce long-term survivors in the ip system) may be related to their hydrophilic nature (see log *P* values in Table I) and a reduced ability to cross the blood–brain barrier.

All of the compounds in this study were reproducibly active against the ependymoblastoma (EM) murine brain tumor system. It is probably more useful to compare cures (60-day survivors) rather than ILS values in the EM model since the ILS for a highly active compound is very dependent on the life span of the control animals which normally ranges from 17 to 21 days. The best ic L1210 active derivatives (**12**, **13**, and **20**) were again among the most active materials against the EM tumor system. The ethyl (**13**) and propylamino (**15**) compounds and the 2′-methylaziridinyl analogue of **13** (compound **3b**) were also highly active. The hydrophilic dihydroxypropylamino compound **18**, which was curative in the ip L1210 and P388 leukemia systems, was active but gave a large spread in ILS values (53–186%). For this reason, **18** should be considered to have moderate to good EM activity. Again, its hydrophilic character may present transport problems in this ic tumor system. It is noteworthy that the majority of the most active compounds in the EM system are the simple monoalkylamino derivatives and their parent amine **12**.

Discussion

Several general points can be made about the diaminodiaziridinylbenzoquinone family. Its members have good antitumor activity usually in several tumor systems. They are very potent with most optimum doses in the 0.1–5.0 mg/kg range on a chronic administration schedule. Therapeutic ratios (highest active dose divided by lowest active dose) were somewhat dependent upon the tumor system. For the most active compounds, therapeutic ratios averaged 4, 8, and 2 in ip L1210, P388, and B16 melanoma, respectively. These values were approximately 2 and 8 in the ic L1210 and ependymoblastoma tumor systems. These compounds are alkylating agents giving a positive 4-(*p*-nitrobenzyl)pyridine (NBP) test.[51]

Several qualitative structure–activity relationships are apparent. The compounds that are most active in the ip ascites tumor systems are the most polar, water-soluble derivatives (**17**, **18**, **26**). The parent amino compound **12**, however, is also active and is so insoluble in any solvent that an ultraviolet spectrum was nonattainable. Among the monoalkylamines, activity appears to peak with the ethyl derivative **14** in the L1210 series. A significant reduction in activity is noted for the propyl (**15**) and butyl (**16**) analogues. Leukemia P388 is usually a more sensitive system than L1210. In this series, however, L1210 activities were often equal to or greater than P388 values with the same compounds active against both tumors. Multiple

Table IV. Intracerebral Antitumor Activity

	Ependymoblastoma[a]				Lymphoid leukemia L1210[b]			
No.	Expt no.	OD[c]	T − C	ILS	Expt no.	OD	T − C	ILS
3b	152	10.0	−2.5	294 (1)	35	5.0	−2.0	39
	153	5.0	−1.9	143 (1)	36	10.0	−1.3	25
9	169	0.8	+0.1	49				
	168	1.5	−1.3	40				
12	177	0.2	−2.7	215 (4)	43	0.3	0.0	60
	155	0.2	−2.0	177 (6)	42	0.3	−1.0	59
13	174	0.2	−1.5	219 (5)	38	0.4	−0.3	68
	175	0.4	−1.8	218 (4)	33	0.2	+0.3	60
14	175	1.5	−2.0	218 (2)	30	3.0	−2.2	44
	174	1.5	−2.3	187 (2)	31	3.0	−1.8	40
15	152	10.0	−1.9	259 (2)				
	153	5.0	−1.2	143 (1)				
16	166	6.0	−2.3	93				
	165	3.0	−0.3	46				
17	152	1.5	−3.0	83	43	0.75	+0.4	57
	174	1.5	−1.0	70	30	0.75	−1.8	48
18	155	1.0	−0.4	99 (1)	39	4.0	−1.6	43
	156	4.0	−3.3	53	40	4.0	−1.0	41
19	166	2.0	−3.2	64	42	2.5	−1.5	16
	165	1.0	−1.4	50	43	10.0	−1.8	16
20	177	1.0	−2.3	215 (5)	43	3.0	−0.4	72[d]
	176	0.5	+0.4	190 (4)	42	3.0	+0.1	56[d]
21	166	0.4	−1.6	84	43	4.0	−0.7	45
	165	0.2	−1.2	69	42	2.0	0.9	32
22	171	1.6	−1.9	101				
	170	3.1	−1.4	47				
23	171	6.2	−2.4	68				
	170	12.5	−3.1	46				
24	165	0.5	−1.2	64	43	8.0	−0.3	30[d]
	166	2.0	−4.6	48	42	6.0	−1.0	14[d]
25	168	1.5	−1.8	134				
	169	0.4	+0.8	82				
26	155	1.0	−1.2	75	39	2.0	−1.4	43
	174	1.0	−3.0	75	41	1.5	−1.4	41

[a] QD1-5 treatment schedule. [b] QD1-9 treatment schedule unless otherwise indicated. Ic L1210 experiments carried out only if ip L1210 experiments gave ILS > 50%. [c] See footnotes in Table II for definitions. [d] Q4D (1,5,9) treatment schedule.

Table V. Aziridinylbenzoquinones with Outstanding Activity in Two or More Tumor Systems[a]

	ILS, %				
No.	L1210, ip	P388	B16	L1210, ic	EM
3b			70		294 (1)
4[b]	169	138 (1)	70	84	277 (5)
12	161 (3)	171 (1)		60	177 (6)
13				68	219 (5)
14	112 (2)	160			218 (2)
15		151 (2)			259 (2)
17	205 (2)	175 (3)	61	57	
18	206 (5)	143 (6)	50		99 (1)[c]
20	120 (1)			72	215 (5)
21	114 (1)	162			
26	180 (3)	143 (3)			

[a] Highest activity on the optimum schedule from Tables II-IV. [b] Data from ref 16 included for comparison. [c] Repeat tests gave ILS values of 186 (6) and 53%.

cures were obtained in both systems.

Although several derivatives possess substantial reproducible B16 melanoma activity, this tumor is the most refractory of those studied in this investigation. The relationship between outstanding ascites tumor activity and B16 activity for 17 and 18 (Table V) does not hold for several other compounds (**3b**, **26**).

In the intracerebral systems, the trend toward activity with the more lipophilic compounds is apparent. The propyl derivative **15** is still very active in the EM system and even the butyl analogue has activity. These two groups greatly reduced L1210 activity relative to their lower carbon number congeners. Exceptionally high ip L1210 activity does not guarantee good activity in the ic L1210 system (**14**, **21**, and **26**). The significant activity of the parent compound **12** as well as its methyl (**13**) and dimethyl (**20**) derivatives in the normally refractory ic L1210 system is noteworthy. The polar, water-soluble compounds were generally less active in the EM system than the nonhydroxylated derivatives.

A comparison of activity in all tumor systems (Table V) shows that those compounds which are best in the ip systems are not the most active in the IC systems. The dihydroxypropylamino analogue **18** which gives multiple long-term survivors against the leukemia L1210 and P388 tumors is probably the most active compound in the ip ascites systems. The hydroxyethylamino (**17**) and **26** are also superior in these systems. In the EM system, the unsubstituted amino compound, **12**, and several alkylamino analogues (**13**, **14**, **15**, and **20**) are most active. The recently reported[16] ethyl carbamate derivative **4** is included in Table V for comparison. While this compound does not give multiple cures in the ascites systems, it is curative in the EM system and has very substantial activity in all the other tumor systems studied. The high activity of the insoluble amino derivative **12** suggests the possibility that **12** might be the active agent and that the urethane **4** and the various active acylamino derivatives,[2] such as **5**, serve as solubility-enhancing groups which facilitate drug transport to the tumor cell and then produce **12** by hydrolysis. There is, however, no evidence for this at the present time.

Since retention of CNS antitumor activity with an improvement in water solubility by the use of nonionic hydrophilic groups was a major objective of this investigation, a comment regarding the water solubilities and partition coefficients of the diaziridinylbenzoquinones (Table I) is in order. The unsubstituted, parent amino derivative **12** is an exceptionally insoluble material, not only in water but in all solvents studied. The monoalkylamino compounds also have very limited water solubility. The addition of a second alkyl group (e.g., **20** vs. **13**), which might be expected to lower water solubility, actually increases the solubility greatly. This effect is also apparent in a comparison of **21** and **17**. In both of these cases a dramatic decrease in melting point takes place upon addition of the second alkyl (methyl) group. The increase in water solubility, therefore, may be due to a decrease in hydrogen bonding properties and a resulting decrease in the crystal forces in the solid state. This type behavior has been noted previously.[50]

The partition coefficients of a few of these compounds were determined (Table I, footnote *b*). Initial calculated approximations of the partition coefficients in this series were made using the log P value of p-benzoquinone and substituent π values in order to help determine which molecules to synthesize. The values measured after synthesis were qualitatively in the right order but contained large quantitative differences. This was expected because attempts to calculate known quinone partition coefficients values[36] from a parent quinone plus π values[37] produced very poor results.

The partition coefficient of **4** was measured and found to have a log P value of 0.05. The water solubility of **4** is ~0.5 mg/ml. A comparison of log P values and water solubilities (Table I) shows that a general qualitative correspondence between hydrophilicity and water solubility exists. However, no quantitative relationship between the two is apparent for the five derivatives with log P values.

An attempt to relate quinone structure to antitumor activity in a quantitative manner is presently under investigation.

Experimental Section

All melting points are uncorrected and recorded on a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by NIAMDD, NIH, Bethesda, Md. Fluoranil, ethylenimine, and 2-methylethylenimine were obtained commercially. When several compounds were prepared by comparable procedures, only one representative example is included in this section. Reference should be made to Table I for supplementary information for each compound. New compounds were identified by NMR and ir spectroscopy. In the tetraamino derivatives, the methylene protons of the aziridine rings usually occurred as a singlet at δ 2.0–2.2 in $CDCl_3$ or Me_2SO-d_6. This absorption appeared at δ 2.40 ($CDCl_3$) for **11**. Carbonyl absorption frequencies (Nujol mull) occurred in the range 1620–1640 cm^{-1} for the tetraamino compounds. Satisfactory elemental analyses (±0.4% of calculated values) are indicated by elemental symbols in Table I. Room temperature water solubilities were determined by the incremental addition (pipet) of water to a weighed amount of compound with shaking. The solubility values in Table I must be considered as approximate.

2,5-Diaziridinyl-3,6-difluoro-1,4-benzoquinone (11) (General Procedure for 3a). A solution of ethylenimine (33.3 g, 0.77 mol) in dry tetrahydrofuran (100 ml) was added dropwise to a stirred solution of fluoranil (25.0 g, 0.14 mol) in tetrahydrofuran (100 ml) cooled by an ice–water bath. The cold solution was stirred for 40 min; cold water (100 ml) was added. The precipitate was filtered, washed thoroughly with water, and dried. Recrystallization from toluene gave 19.4 g (62%) of red crystals, mp 220° (lit. mp 230°,[26] 212°[27]).

2,5-Diaziridinyl-3,6-diamino-1,4-benzoquinone (12). A solution of **11** (0.56 g, 2.5 mmol) in tetrahydrofuran (100 ml) was saturated with dry ammonia gas at 2°. This solution was transferred to a pressure bottle and was heated overnight at 50–55°. After cooling to room temperature, the bottle was opened carefully. The precipitate was filtered, washed with water, and dried to give 0.40 g (73%) of green crystals, mp 220°.

2,5-Diaziridinyl-3,6-bis(ethylamino)-1,4-benzoquinone (14) (General Procedure for 3b, 13, 15–19, 21, 23, and 24). Anhydrous ethylamine (25 ml, 17.3 g, 380 mmol) was added to a stirred ice-cold solution of **11** (2.0 g, 9 mmol) in 600 ml of tetrahydrofuran (methylamine used in the preparation of **13** was a 40% aqueous solution). The reaction mixture was stirred at 2° for 2 h and then at 23° for 80 h. Evaporation of the solvent in vacuo gave a dark green solid which was washed with ice water and dried in vacuo over KOH pellets. Recrystallization from ethanol gave 1.40 g (57%) of dark green prisms, mp 156–157°.

2,5-Diaziridinyl-3,6-bis(dimethylamino)-1,4-benzoquinone (20). Dimethylamine hydrochloride (16.3 g, 0.2 mol) was slowly added to a stirred ice-cold methanolic sodium methoxide solution (4.6 g, 0.2 mol of Na in 100 ml of methanol). The resulting mixture was stirred at room temperature for 0.5 h and a solution of **11** (1.13 g, 0.005 mol) in tetrahydrofuran (300 ml) was added. The reaction mixture was stirred overnight at ambient temperature. The solvent was evaporated in vacuo to give a dark solid which was extracted with benzene. The solid obtained from evaporation of the benzene was recrystallized from hexane to give 1.1 g (59%) of green solid, mp 110–112°.

2,5-Diaziridinyl-3,6-dipyrrolidino-1,4-benzoquinone (22). Compound **11** (1.13 g, 5 mmol) and pyrrolidine (2.0 ml, 1.7 g, 24 mmol) were added to benzene (250 ml) and the mixture was stirred at 23° for 3 days. The dark solution was heated to 50–60° for 2 h. The resulting precipitate was filtered and discarded. The filtrate was concentrated to give a brown solid which was recrystallized from tetrahydrofuran–hexane to give 1.05 g (64%) of brown product, mp 160°.

2,5-Diaziridinyl-3,6-dimorpholino-1,4-benzoquinone (25) and 2,5-Diaziridinyl-3-fluoro-5-morpholino-1,4-benzoquinone (9). Morpholine (1.75 g, 20 mmol) was added to an ambient solution of **11** (1.13 g, 5 mmol) in tetrahydrofuran (250 ml). After stirring overnight, the solvent was removed in vacuo. The solid was washed with water and recrystallized from benzene to give 0.50 g of **25** as a brown solid (28%), mp 224°. Addition of the recrystallization filtrate to hexane gave 0.75 g (51%) of gray **9**, mp 157°.

Acknowledgment. We thank Mr. Leo Dudeck and Mr. George Congleton of Hazleton Laboratories for the determination of the antitumor test data and Drs. C. Hansch and M. Yamakawa of Pomona College for the determination of the octanol–water partition coefficient values.

References and Notes

(1) (a) NIH Visiting Postdoctoral Fellow, 1974–1975; (b) NIH Visiting Postdoctoral Fellow, 1971–1974.
(2) J. S. Driscoll, G. F. Hazard, H. B. Wood, and A. Goldin, *Cancer Chemother. Rep., Part 2*, **4** (2), 1 (1974).
(3) (a) F. Arcamone, G. Franceschi, S. Penco, and A. Selva, *Tetrahedron Lett.*, **13**, 1007 (1969); (b) Adriamycin Symposium, *Cancer Chemother. Rep., Part 3*, **6**, 83 (1975).
(4) Proceedings of the Symposium on Quinones as Antitumor Agents, *Cancer Chemother. Rep., Part 2*, **4** (4), 1 (1974).
(5) N. Bachur, *Biochem. Pharmacol., Suppl. 2*, 207 (1974).
(6) S. Kinoshita, K. Uzu, K. Nakanao, et al., *J. Med. Chem.*, **14**, 103 (1971).
(7) R. Kojima, J. Driscoll, N. Mantel, and A. Goldin, *Cancer Chemother. Rep., Part 2*, **3** (1), 121 (1972).
(8) K. V. Rao, K. Biemann, and R. B. Woodward, *J. Am. Chem. Soc.*, **85**, 2532 (1963).
(9) J. W. Pearson, T. S. Papas, W. A. Woods, H. B. Wood, and G. Spahn, *Cancer Chemother. Rep.*, **57**, 305 (1973).
(10) K. V. Rao, T. J. McBride, and J. J. Oleson, *Cancer Res.*, **28**, 1952 (1968).
(11) K. Folkers, *Cancer Chemother. Rep., Part 2*, **4** (2), 19 (1974).
(12) A. J. Lin, B. J. Lillis, and A. C. Sartorelli, *J. Med. Chem.* **18**, 917 (1975).

(13) M. H. Akhtar, A. Begleiter, D. Johnson, J. W. Lown, L. McLaughlin, and S. K. Sim, *Can. J. Chem.*, **53**, 2891 (1975).
(14) D. P. Rall and C. G. Zubrod, *Annu. Rev. Pharmacol.*, **2**, 109 (1962).
(15) J. S. Driscoll and R. Geran, unpublished results.
(16) A. H. Khan and J. S. Driscoll, *J. Med. Chem.*, **19**, 313 (1976).
(17) K. T. Finley in "Chemistry of Quinonoid Compounds, Part 2", S. Patai, Ed., Wiley, New York, N.Y., 1974, p 1101.
(18) Farbenfabriken Bayer A.-G., British Patent 762 723 (Dec 5, 1956).
(19) Farbenfabriken Bayer A.-G, British Patent 793 796 (April 23, 1958).
(20) A. Marxer, *Helv. Chim. Acta*, **40**, 502 (1957).
(21) Farbenfabriken Bayer A.-G., German Patent 943 166 (August 16, 1956).
(22) A. Marxer, U.S. Patent 2 802 001 (August 6, 1957).
(23) S. Petersen, W. Gauss, and G. Domagk, U.S. Patent 2 913 453 (Nov 17, 1959).
(24) A. Marxer, U.S. Patent 3 040 030 (June 19, 1962).
(25) K. Wallenfals and W. Draber, *Angew. Chem.*, **70**, 313 (1958).
(26) K. Wallenfals and W. Draber, *Justus Liebigs Ann.* Chem., **667**, 55 (1963).
(27) A. N. Makarova, V. S. Martynov, and A. Ya. Berlin, *Zh. Obshch. Khim.*, **33**, 1643 (1963).
(28) C. Hansch, *Cancer Chemother. Rep.*, **56**, 433 (1972).
(29) B. F. Cain, *Cancer Chemother. Rep.*, **59**, 679 (1975).
(30) H. Wagner and H. Berg, *J. Electroanal. Chem.*, **2**, 452 (1961).
(31) P. Zuman, *Collect. Czech. Chem. Commun.*, **27**, 2035 (1962).
(32) P. Zuman, "Substituent Effects in Organic Polarography", Plenum Press, New York, N.Y., 1967.
(33) See J. Q. Chambers in ref 17, p 737.
(34) W. C. J. Ross, "Biological Alkylating Agents", Butterworths, London, 1962.
(35) S. Kinoshita, K. Uzu, K. Nakano, and T. Takahashi, *J. Med. Chem.*, **14**, 109 (1971).
(36) C. Hansch and A. Leo, Pomona College Medicinal Chemistry Project.
(37) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
(38) R. W. Taft in "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, N.Y., 1956.
(39) V. S. Martynov, A. N. Makarova, and A. Ya. Berlin, *Zh. Obshch. Khim.*, **34**, 2807 (1964).
(40) J. A. Beisler (NCI) and J. V. Silverton (NHLI), unpublished data.
(41) K. D. McMurtrey and G. D. Davis, *J. Org. Chem.*, **35**, 4252 (1970).
(42) J. Mattern, K. Wayss, and M. Volm, *Arch.* Gynaekol., **216**, 273 (1974).
(43) J. Hartleib, *Z. Krebsforsch.*, 81, 1 (1974).
(44) H. Nakao and M. Arakawa, *Chem. Pharm. Bull.*, **20**, 1962 (1972).
(45) H. Nakao, M. Arakawa, T. Nakamura, and M. Fukushima, *Chem. Pharm. Bull.*, **20**, 1968 (1972).
(46) T. Saito, S. Ohira, A. Wakui, M. Yokoyama, et al., *Cancer Chemother. Rep.*, **57**, 447 (1973).
(47) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (2), 1 (1972).
(48) R. I. Geran, G. F. Congleton, L. E. Dudeck, B. J. Abbott, and J. L. Gargus, *Cancer Chemother. Rep., Part 2*, **4** (4), 53 (1974).
(49) P. C. Merker, I. Wodinsky, and R. I. Geran, *Cancer Chemother. Rep.*, **59**, 729 (1975).
(50) A. J. Repta, B. J. Rawson, R. D. Shaffer, K. B. Sloan, N. Bodor, and T. Higuchi, *J. Pharm. Sci.*, **64**, 392 (1975).
(51) J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955).
(52) A. J. Lin and A. C. Sortorelli, *Biochem. Pharmacol.*, **25**, 206 (1976).

Stereochemical Studies on Medicinal Agents. 21.[1] Investigation of the Role of Conformational Factors in the Action of Diphenylpropylamines. Synthesis and Analgetic Potency of 5-Methylmethadone Diastereomers

James G. Henkel, Eric P. Berg, and Philip S. Portoghese*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received April 1, 1976

The synthesis of racemic *threo*- and *erythro*-5-methylmethadone (**3a** and **3b**, respectively) was carried out and the solution conformation of each isomer was investigated through pK_a and NMR studies. The data indicate that **3a**·HCl exists exclusively in an internally hydrogen-bonded conformation while the erythro isomer **3b**·HCl is present as a mixture of conformations. The erythro racemate **3b** was found to possess 5.4 times the analgetic potency of (±)-methadone in contrast to the threo racemate **3a** which was inactive and devoid of antagonist activity. The fact that the inactive racemate **3a** contains the 5*S*,6*R* stereoisomer, which combines the configurations found in the more active enantiomers of methadone and isomethadone, suggests that the chiral centers do not behave as independent units and that conformational factors are playing an important role in governing stereoselectivity. These results, when analyzed together with earlier reports, suggest that one of the pharmacophoric conformations of the diphenylpropylamine analgetics possesses an antiperiplanar-like disposition of the Ph_2CCOEt and $^+NHMe_2$ groups.

One of the most widely studied classes of synthetic analgetics has been the diphenylpropylamines,[2] of which methadone (1) and isomethadone (2) have played a central role. Many compounds in this group have chiral centers in common with either 1 or 2, and numerous investigations pertaining to the steric requirements for analgetic activity have been reported.[3-5]

$CH_3CH_2COCPh_2CHRCHR'NMe_2$

1, R = H; R' = CH_3
2, R = CH_3; R' = H

One aspect of the structure–activity relationship which has been the subject of recent inquiry is the fact that some methadone-related compounds show inversion of antipodal stereoselectivity at analgetic receptors.[6] As no such inversions occur with analgetics related to 2, it was suggested that this could be due in part to the greater conformational flexibility of 1 relative to 2.[7] This was later given additional support by the finding that a solution of 1 consists of comparable fractions of three gauche conformers while 2 exists predominantly as the chain-extended rotamer (either as the bases or the salts).[8] However, because of the conformational heterogeneity of methadone, no correlation between conformation and activity of 1 and 2 was made.

In an effort to provide further insight into the stereostructure–activity relationship of the diphenylpropylamines and analgetic potency, we have synthesized