Table III—Fungicidal Activities [a]

Compound	Fungicidal G	H	I	J	K
XIX	1	1	1	30	10
XXII	1	1	3	30	10
XXVII	1	0.1	1	—[b]	10
Micronazole nitrate	30	1	30	30	10

[a] See footnotes to Table II. [b] >300.

acid. After stirring at room temperature for 3 hr, a 10% aqueous solution of sodium metabisulfite was added until starch–iodide paper gave a negative result.

Excess saturated potassium bicarbonate solution was then added, and the layers separated. The organic phase was dried (magnesium sulfate) and evaporated under reduced pressure. The residue was chromatographed, using 1% methanol–methylene chloride to elute material that crystallized from methylene chloride–hexane; 4.2 g of VIII was obtained.

Antimicrobial Assays[4]—The bioassays were done by serial broth dilutions in chemically defined media according to the procedure described by Long *et al.* (7).

[4] The bioassays were conducted under the direction of Dr. A. Braemer and Ms. S. Hitt, Institute of Agrisciences, Syntex Research.

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Potential CNS Antitumor Agents VI: Aziridinylbenzoquinones III

JOHN S. DRISCOLL [x], **LEO DUDECK** [*], **GEORGE CONGLETON** [*], and **RUTH I. GERAN** [‡]

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*Present address: Hazleton Laboratories, Vienna, VA 22180. ‡Present address: Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.

Abstract □ Thirty-one aziridinylbenzoquinones were compared against five murine tumor models *in vivo*. Two intracerebral (ependymoblastoma and L-1210 leukemia) and three intraperitoneal (P-388 and L-1210 leukemia and B16 melanoma) systems were utilized. Excellent activity was observed for many compounds. Multiple long-term survivors were produced in the ependymoblastoma, P-388, and intraperitoneal L-1210 systems. Diethyl 2,5-bis(1-aziridinyl)-3,6-dioxo-1,4-cyclohexadiene-1,4-dicarbamate demonstrated superior activity in all five test systems. This compound also was reproducibly active against two colon tumors, a mammary tumor, and the intracerebrally implanted P-388 leukemia model.

Keyphrases □ Aziridinylbenzoquinones—evaluated as potential CNS antitumor agents *in vivo*, various test systems □ CNS antitumor activity—aziridinylbenzoquinones evaluated *in vivo*, various test systems □ Antitumor agents, CNS—aziridinylbenzoquinones evaluated *in vivo*, various test systems □ Structure-activity relationships—aziridinylbenzoquinones evaluated as potential CNS antitumor agents *in vivo*, various test systems

The antitumor activity of aziridinylbenzoquinones in murine model tumor systems has been recognized for almost 25 years (1–5). Recent reports described the activity of two members of this family, trenimon (6–8) and carbazilquinone (8–11), which have had clinical trials.

As part of a program to develop agents that might be effective against neoplasms of the central nervous system (CNS), several series of aziridinylbenzoquinones were prepared and evaluated in murine brain tumor systems (12, 13). Some of these compounds produced long-term survivors in the intracerebral ependymoblastoma tumor model. To determine which aziridinylbenzoquinones might have the greatest potential for clinical trial with emphasis on CNS neoplasms, the 31 analogs available to the National Cancer Institute (NCI) were compared in two intracerebral and three intraperitoneal tumor systems.

EXPERIMENTAL

Materials—Compounds XIV–XXXI (Table I) were synthesized as described previously (12, 13), and I–XIII were obtained from other sources[1].

Tumor Test Systems—The standard NCI protocols for the intracerebral and intraperitoneal tumors are described in Table II and Ref. 14.

Treatment—Treatment was intraperitoneal in all cases. Saline (0.1%) or hydroxypropylcellulose was used as the vehicle. Treatment in all systems other than ependymoblastoma began on Day 1 and continued once daily for 9 days. In a few instances, previous data were available with

[1] Ciba-Geigy, Bayer A. G., Sankyo Co., the U.S. Department of Agriculture, and Dr. H. S. Verter of the Inter American University of Puerto Rico.

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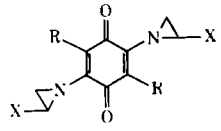
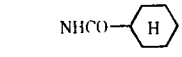
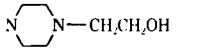
Table I—Aziridinylbenzoquinone Structures



X = H unless specified otherwise

Compound	NSC Number[a]	R
I	17262	$OCH_2CH_2OCH_3$
II	18269	$NHCOCH_3$
III	18270	OCH_2CH_3
IV	18271	$NHCOC_2H_5$
V[b]	18273	$NHCOCH_3$
VI	18274	$NHCOC_3H_7$
VII	29215	
VIII	30705	Cl
IX	31717	
X	51915	
XI	51916	$NHCOC_6H_{13}$
XII	95139	OCH_3
XIII	134679	CH_3, $CH(OCH_3)$-CH_2OCONH_2
XIV	182986	$NHCOOC_2H_5$
XV	220267	$NHCH_3$
XVI[b]	220536	$NHCH_3$
XVII	224066	NHC_2H_5
XVIII	224070	$NHCH_2CH_2OH$
XIX	224071	NHC_3H_7
XX	240026	
XXI	243037	$NHCH_2CH(OH)$-CH_2OH
XXII	246111	NH_2
XXIII	249009	$N(CH_3)CH_2CH_2OH$
XXIV	249010	NHC_4H_9
XXV	249339	
XXVI	249340	$NHCH_2CONH_2$
XXVII	249989	$N(CH_3)_2$
XXVIII	250433	
XXIX	251727	
XXX	251728	
XXXI	251729	

[a] NCI accession number. [b] X = CH_3.

Table II—Tumor Test Systems

Tumor	Tumor Site	Inoculum, number of cells	Mouse Strain	Mouse Number[a]	Termination Day[b]
Ependymoblastoma	Intracerebral	Fragment (1 mm^3)	C57BL/6	6	60
L-1210 leukemia	Intracerebral	10^4	BDF_1	6	30
L-1210 leukemia	Intraperitoneal	10^5	BDF_1	6	30
P-388 leukemia	Intraperitoneal	10^6	BDF_1	6	30
B16 melanoma	Intraperitoneal	Brei[c]	BDF_1	10	60

[a] Number of mice used per dose tested. [b] Day on which experiment ended and long-term survivors were noted. [c] Using 0.5 ml of a tumor homogenate prepared from 1.0 g of tumor blended with 10 ml of cold balanced salt solution.

treatment starting on Day 1 and continuing until death. This schedule was considered comparable to the Day 1–9 treatment schedule. The ependymoblastoma tumor was treated on the Day 1–5 schedule (14, 15).

Experiments were carried out twice when sufficient compound was available. When conflicting results were obtained, additional experiments were performed. A dose response curve was designed ranging up to toxicity and consisting of four doses, with each dose double the next lower dose. Data from experiments with other than satisfactory controls were not used unless they were the only data available. Data from doses that produced a >4-g weight loss relative to control animals on Day 5 were not used as optimum doses.

The antitumor data in Table III are the highest reproduced values observed (*i.e.*, the highest value confirmed in a separate experiment) for the L-1210, P-388, and B16 systems. The highest two values from separate experiments are reported for the ependymoblastoma system. Intracerebral L-1210 tests were scheduled only if at least one intraperitoneal L-1210 test gave a T/C value[2] $\geq$150%.

RESULTS

Intracerebral Tumor Systems—The ependymoblastoma and intracerebral L-1210 models utilize intracerebral implants of a solid fragment and ascites fluid, respectively. Antitumor activity for standard agents in the ependymoblastoma system was reviewed recently (15).

Compounds III, VI, VII, XIV, XV, XVII, XXII, and XXVII gave multiple long-term (60-day) survivors in more than one experiment. The number of long-term ependymoblastoma survivors is a more meaningful measure of activity for these compounds than the T/C value, because the T/C value is very dependent on the survival time of the control animals when long-term-treated survivors occur.

The most active compounds (T/C > 150%) in the intracerebral L-1210 model were VI, XIII–XV, and XXII. No long-term survivors were produced.

Intraperitoneal Tumor Systems—Activity against two leukemias (intraperitoneal P-388 and L-1210) and one solid tumor (intraperitoneal B16 melanocarcinoma) was evaluated. Of these three intraperitoneally implanted models, the P-388 and B16 models were the most and least sensitive to drug treatment, respectively. All compounds were active in the P-388 system. Indeed, a majority produced T/C values >200%.

Excellent activity was also shown by many compounds in the less sensitive intraperitoneal L-1210 system. Six compounds (II, XIV, XVIII, XX, XXI, and XXIII) gave intraperitoneal L-1210 T/C values >200% and, in some instances, produced long-term (30-day) survivors. In the more refractory B16 melanoma model, the minimum criterion for statistical activity (T/C $\geq$ 140%) was met by six analogs (XII–XIV, XVI, XVIII, and XXI).

DISCUSSION

All 31 compounds met the current minimum statistical criterion for activity in the P-388 (T/C 120%) system, and all except VIII were active in the ependymoblastoma model (T/C 125%). Twenty-five qualified in the intraperitoneal L-1210 (T/C 125%) system and six in the B16 melanoma (T/C 140%) system. Seventeen of the 21 compounds tested against intracerebral L-1210 leukemia were active (T/C 125%). With almost all compounds meeting the minimum statistical criterion for activity in all tumor systems (except B16 melanoma), stricter criteria were devised (Table IV) to differentiate among the compounds.

Table IV lists the compounds that passed at least one of the following stricter criteria for activity: ependymoblastoma, multiple long-term survivors in both experiments; intracerebral L-1210, confirmed T/C 150%; intraperitoneal L-1210, confirmed T/C 200%; and B16 melanoma as previously stated, confirmed T/C 140%. This classification reduced the number of compounds for comparison to 17. When a confirmed T/C $\geq$ 200% criterion in the P-388 system was applied to these 17 compounds, all passed except XV and XXVII. Only one compound, XIV, passed the criterion in all five test systems. Five derivatives passed in three systems. Two of the five (VI and XXII) were active in the intracerebral systems plus P-388; two (XVIII and XXI) were active in the intraperitoneal systems plus P-388; one, carbazilquinone (XIII), which has been studied clinically, was active against one intracerebral (L-1210) plus one intraperitoneal (B16) model as well as the P-388 tumor.

[2] See Table III for definition.

Table III—Aziridinylbenzoquinone Antitumor Activity

Compound	Intracerebral Tumors				Intraperitoneal Tumors[a]					
	Ependymoblastoma		L-1210[a]		P-388		B16		L-1210	
	O.D.[b]	T/C[c]	O.D.	T/C	O.D.	T/C	O.D.	T/C	O.D.	T/C
I	1	138	4	121	2	223 (1)	2	136	2	183
II	0.5	147 (1)[d]	1	146	0.5	270 (1)	1	131	0.5	220
	0.5	150								
III	2	>304 (4)	1	135	2	242	1	136	1	157
	2	211 (2)								
IV	1	193 (1)	1	129	2	225	2	113	1	181
V	2.5	192 (1)	5	120	5	233	1	105	5	184
	5	233								
VI	1	>398 (3)	4	152	1	213	2	118	4	167
	1	>368 (2)								
VII	0.08	>294 (3)	0.02	138	0.02	265 (3)	0.02	126	0.05	145
	0.16	>398 (3)								
VIII	10	123	1	120	5	172	10	124	0.6	128
	2.5	123								
IX	0.04	>361 (3)	—	NT[e]	0.04	268 (1)	0.04	125	0.01	139
	0.02	218								
X	100	218 (1)	400	116	400	170	25	109	175	145
	200	175								
XI	80	253	—	NT	100	245	50	135	115	132
	160	184								
XII	0.5	284 (1)	1	129	2	>252 (5)	2	169	2	136
	2	238								
XIII	1	159	0.05	154	0.25	211	0.5	179	0.25	159
	0.25	148								
XIV	1	>272 (6)	3	179	0.8	212 (1)	3	162	3	213
	2	>376 (5)								
XV	0.2	>319 (5)	0.2	160	0.1	169	0.05	129	0.2	161
	0.4	>318 (4)								
XVI	5	243 (1)	10	125		NT	5	148	2.5	116
	10	394 (1)								
XVII	1.5	287 (2)	3	140	1.5	228	0.75	124	1.5	191 (1)
	1.5	318 (2)								
XVIII	0.75	183	0.75	148	0.4	260 (2)	0.4	161	0.75	234 (3)
	0.75	170								
XIX	5	243 (1)	—	NT	6	208	5	132	6	143
	10	359 (2)								
XX	1	175	1.5	141	1	243 (6)	0.25	122	0.5	214 (1)
	1	175								
XXI	0.5	>288 (6)	4	141	4	241 (4)	6	142	2	233 (2)
	1	199 (1)								
XXII	0.25	277 (6)	0.3	159	0.2	256	0.02	119	0.3	194
	0.2	315 (4)								
XXIII	0.2	175 (1)	2	132	0.8	245	0.8	131	1.5	211 (1)
	0.2	169								
XXIV	3	175 (1)	—	NT	10	149	5	111	5	130
	3	146								
XXV	4	165 (1)	—	NT	1	178	1	137	3	144
	1	129								
XXVI	2	164 (1)	10	116	2	171	0.5	122	10	180
	1	150								
XXVII	1	>315 (5)	—	NT	0.5	137	0.25	112	0.4	116
	1	>290 (4)								
XXVIII	3	201	—	NT	1	155	2	118	2	121
	1.6	147								
XXIX	1.5	149 (1)	—	NT	0.75	147	0.75	117	1.5	120
	1.5	234								
XXX	0.8	149	—	NT	6	154	0.75	109	0.75	114
	1.5	140								
XXXI	12.5	146	—	NT	5	146	2.5	97	5	117
	6	168								

[a] Highest reproduced T/C value; see *Experimental.* [b] Optimum dose (milligrams per kilogram per day). [c] The %T/C = (treated survival/control survival) × 100%. [d] Numbers in parentheses are animals alive on the termination day; see Table II. [e] Not tested.

The relatively low intraperitoneal L-1210 activity found for XIII may be related to the schedule used in this protocol. Other murine test data indicate that XIII may be most active on the Day 1 treatment schedule. It was feasible, however, to compare all compounds only on one schedule. Since chronic treatment (QD 1–9) appeared to give optimum activity for analogs that previously had schedule dependency testing, this schedule was chosen for comparison studies. The two relatively low ependymoblastoma activity values obtained for XIII were checked a third time in light of the active intracerebral L-1210 results obtained with this compound. A comparable third ependymoblastoma value (T/C 143%) was obtained. Trenimon (VII), the other compound with prior clinical studies, possessed excellent ependymoblastoma and P-388 activity but was marginal in the two L-1210 models and inactive against B16 melanoma.

Of the four other compounds with good activity in three of the models (VI, XVIII, XXI, and XXII), the bis(dihydroxypropylamino) analog, XXI, probably comes the closest to XIV in overall superiority. With one more long-term survivor in the second ependymoblastoma experiment and a slightly higher intracerebral L-1210 value, XXI would have met the strict criteria for all five tests. The amino compound, XXII, which is the parent for many of these analogs, almost met the standard in the intraperitoneal L-1210 but was inactive in two B16 tests (T/C 131 and 119%). Compound VI was moderately active against intraperitoneal L-1210 but was inactive against B16 melanoma. Compound XVIII almost met the criterion in the intracerebral L-1210 but was only moderately active in the ependymoblastoma system.

Based on the data shown in Table III, XIV is the superior compound and was tested further against an NCI panel of tumors (16). Compound

Table IV—Aziridinylbenzoquinone Activity Based on Selected Criteria [a]

Compound	Ependymoblastoma	Intracerebral L-1210	P-388	Intraperitoneal L-1210	B16
II			X	X	
III	X		X		
IV	X		X		
VI	X	X	X		
VII	X		X		
XII			X		X
XIII		X	X		X
XIV	X	X	X	X	X
XV	X	X			
XVI			NT[b]		X
XVII	X		X		
XVIII			X	X	X
XX			X	X	
XXI			X	X	X
XXII	X	X	X		
XXIII			X	X	
XXVII	X				

[a] An X indicates a compound that meets the following criterion: ependymoblastoma, multiple long-term survivors in both experiments; intracerebral L-1210, confirmed T/C >150%; P-388, confirmed T/C >200%; intraperitoneal L-1210, confirmed T/C >200%; and B16, confirmed T/C >140%. [b] Not tested.

XIV was inactive against subcutaneous and intracerebral B16 melanocarcinoma as well as subcutaneous and intravenous Lewis lung carcinoma. It had confirmed activity against nine murine models: the five models in Table IV and the four shown in Table V.

Table V—Additional Antitumor Data for XIV [a]

Tumor	O.D.	Treatment Schedule	T/C	Activity Criterion (T/C)
CD mammary (mouse)[b]	12.5	Q7D × 5	23[c]	≤42
Intracerebral P-388[d]	1.5	QD 1–9	151	≥125
C38 colon[b]	25	Q7D × 3	32[c]	≤42
C26 colon[e]	3.1	Q4D × 3	240 (4)	≥130

[a] Highest reproduced T/C; see notes of Table III for definitions. [b] Subcutaneous tumor implantation. [c] Based on mean tumor weights estimated from tumor diameter. [d] Intracerebral tumor implantation. [e] Intraperitoneal tumor implantation.

Pharmacological studies are currently underway with XIV. After development of an acceptable formulation, preclinical toxicological studies will be initiated.

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Solubility of Doxycycline in Aqueous Solution

JOSEPH B. BOGARDUS ˣ and ROBERT K. BLACKWOOD, Jr.

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Abstract □ The solubility of doxycycline monohydrate and doxycycline hydrochloride dihydrate was investigated in aqueous solution. The hydrochloride dihydrate salt was isolated and identified from solutions initially containing doxycycline hyclate in water. The pKa′ = 3.09 (μ = 0.1 and 25°) for protonation of doxycycline was determined spectrophotometrically. The pH–solubility profiles were determined for doxycycline monohydrate in water and in 1.0 *M* $NaNO_3$–HNO_3 and NaCl–HCl. The pH–solubility profile at 25° for doxycycline in aqueous hydrochloric acid without added salt reached a sharp maximum of 50 mg/ml at pH 2.16. Added chloride ion strongly suppressed the solubility of the hydrochloride dihydrate salt. The apparent solubility product was not constant but decreased as the concentration of added salt increased. A theoretical model was developed involving dimerization of doxycycline and applied to the experimental data. The dimerization constant, K_d = 24 M^{-1}, and true solubility product, $K^0_{sp} = 1.8 \times 10^{-3} M^2$, were calculated. The effect of concentration on NMR and visible spectra indicated that dimerization resulted from intermolecular hydrogen bonding of the phenolic β-diketone portion of the molecule.

Keyphrases □ Doxycycline—monohydrate and hydrochloride dihydrate salts, solubility in aqueous solution, effect of pH □ Solubility—doxycycline monohydrate and hydrochloride dihydrate in aqueous solution, effect of pH □ Antibacterials—doxycycline monohydrate and hydrochloride dihydrate, solubility in aqueous solution, effect of pH

Knowledge of quantitative solubility relationships in purely aqueous solutions is highly important to pharmaceutical research and product development. In water, solute activity is the summation of numerous associative interactions of the drug with other drug molecules and the solvent. These interactions often cause solutions of phar-

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