Synthesis of 2,3-Diaziridinyl-1,4-naphthoquinone Sulfonate Derivatives as Potential Antineoplastic Agents[1]

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A new class of 2,3-diaziridinyl-1,4-naphthoquinone sulfonates (27 compounds) has been synthesized and evaluated as potential antineoplastic agents. The most active compounds, benzenesulfonate **4**, *p*-toluenesulfonate **5**, *p*-methoxybenzenesulfonate **7**, 8-quinolinesulfonate **17**, and 2-thiophenesulfonate **20**, in the aromatic sulfonate series, at their optimum daily dosage level of 25 mg/kg × 6, produced 100%, 90%, 75%, 80%, and 100% 50-day survivors, respectively, of L1210 tumor-bearing mice. At a lower optimum daily dosage level of 20 mg/kg × 6, treatments with *p*-fluorobenzenesulfonate **11** and *p*-nitrobenzenesulfonate **15** resulted in 100% and 80% 50-day survivors. In the aliphatic sulfonate series, methanesulfonate **21** produced 80% 50-day survivors at a 10 mg/kg daily dosage level × 6. Benzenesulfonate **4**, *p*-fluorobenzenesulfonate **11**, 8-quinolinesulfonate **17**, and 2-thiophenesulfonate **20** derivatives were also tested in mice bearing the B16 melanoma; these agents gave T/C × 100 values of 180, 182, 219, and 161, respectively, in this neoplastic cell system. Structure–activity relationships of compounds of this class are discussed.

The development of successful therapeutic approaches to the cure of solid tumors requires the development of strategies to deal with the cellular heterogeneity of these neoplasms. The heterogeneity that dictates response to therapeutic agents is due both to genetic differences between cells and to physiological factors created by the immediate environment of the cancer cell. Properties such as the nutritional status of cells, the pH of the environment, and the degree of oxygenation are all physiological factors influenced by the degree of tumor vascularization.[3]

Our laboratory has been concerned with the impact of oxygen-deficient (i.e., hypoxic) tumor cells on the therapeutic outcome, since evidence exists that hypoxic tumor cells are significantly more resistant to X-irradiation than their aerobic counterparts,[4-7] and are probably more resistant to most chemotherapeutic agents. The resistance of oxygen-deficient cells to anticancer agents derives from several factors, including the fact that (a) they may be arrested or slowly moving through the cell cycle,[8-11] (b) they are distal to tumor vasculature,[12-14] making it more difficult to achieve adequate cytotoxic concentrations, and (c) they may be "spontaneously" resistant, since hypoxia has been shown to lead to gene amplification.[15]

One approach to the problem of hypoxic cells is the development of chemotherapeutic agents that, through their physical and chemical properties and mode of activation, preferentially attack this therapeutically resistant cell population. The use of bioreductive alkylating agents,[16-21] which require reductive activation to generate electrophilic species capable of covalently binding molecules necessary for cellular survival, exploits the findings that oxygen deficiency leads to an environment conducive to reductive reactions.[16,17,22,23] On the basis of these concepts, we have synthesized and evaluated for anticancer activity a variety of quinone derivatives as bioreductive alkylating agents.[16,17,24-30]

In the present report, we have designed and synthesized a new class of 2,3-diaziridinyl-1,4-naphthoquinone sulfonate derivatives; since bioreductive alkylating agents of this type should be reductively activated by both oxygenated and hypoxic tumor cells, we have evaluated them for antineoplastic activity against the L1210 leukemia and the B16 melanoma and have demonstrated that several members of this family have significant anticancer activity.

Chemistry

A new class of 2,3-diaziridinyl-1,4-naphthoquinone sulfonate derivatives has been synthesized and evaluated as potential anticancer agents. Treatment of 5-hydroxy-1,4-naphthoquinone with ethylenimine in ethanol at 0 °C gave the monosubstituted aziridinyl derivative **1**. Further treatment of compound **1** with excess net ethylenimine at 0 °C yielded the disubstituted aziridinyl derivative **2**. Reaction of compounds **1** and **2** with the appropriate

(1) This paper has been presented in part; see: Lin, T. S.; Xu, S. P.; Cosby, L. A.; Sartorelli, A. C. In *Abstracts of Papers*; 191st National Meeting of the American Chemical Society, New York City, NY, April 13–18, 1986; American Chemical Society: Washington, DC, 1986; MEDI 33.
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Table I. Effects of 2,3-Diaziridinyl-1,4-naphthoquinone Sulfonate Derivatives on the Survival of Mice Bearing the L1210 Leukemia

compd	daily dosage,[a] mg/kg	av Δ wt,[b] %	T/C × 100[c]	compd	daily dosage,[a] mg/kg	av Δ wt,[b] %	T/C × 100[c]
AZQ	3	−11.7	231 (0/5)	14	20	+14.6	127 (0/5)
1	5	+12.7	94 (0/5)		25	+11.1	146 (0/5)
	10	+5.1	90 (0/5)		30	+15.8	146 (0/5)
	15	+2.1	94 (0/5)	15	20	−11.1	402 (4/5)
2	5	+6.3	114 (0/5)		25	−12.9	293 (3/5)
	10	−3.2	96 (0/5)		30	−13.1	152 (4/5)
	15	−0.8	104 (0/5)	16	20	+2.3	111 (0/5)
3	20	+7.3	102 (0/5)		25	+4.8	105 (0/5)
	25	+5.1	103 (0/5)		30	−1.3	100 (0/5)
	30	+1.2	111 (0/5)	17	20	−6.6	332 (3/5)
4	20	−6.2	--- (5/5)		25	−6.5	418 (4/5)
	25	−8.3	--- (5/5)		30	−8.0	291 (3/5)
	30	−7.0	359 (4/5)	18	20	+14.6	107 (0/5)
5	20	−2.2	155 (7/10)		25	+9.2	104 (0/5)
	25	−3.5	490 (9/10)		30	+13.7	104 (0/5)
	30	−7.4	248 (7/10)	19	20	+2.2	110 (0/5)
6	20	+8.4	147 (0/5)		25	+3.8	115 (0/5)
	25	+13.6	151 (0/5)		30	+3.9	117 (0/5)
	30	+9.9	172 (0/5)	20	20	−2.3	165 (4/5)
7	20	−0.1	175 (1/5)		25	−4.7	--- (5/5)
	25	−2.4	222 (3/4)		30	−6.4	--- (5/5)
	30	−1.3	193 (2/5)	21	5	+3.3	130 (0/5)
8	20	+12.8	104 (0/5)		10	−7.1	185 (4/5)
	25	+11.8	98 (0/5)		15	−10.0	295 (2/5)
	30	+6.7	98 (0/5)	22	20	+0.1	131 (0/5)
9	20	−0.4	113 (0/5)		25	−10.9	124 (0/5)
	25	−2.5	113 (0/5)		30	−11.3	131 (0/5)
	30	−2.6	124 (0/5)	23	20	+13.0	96 (0/5)
10	20	+7.8	100 (0/5)		25	+9.9	91 (0/5)
	25	+9.0	102 (0/5)		30	+9.7	91 (0/5)
	30	+7.4	100 (0/5)	24	20	−7.5	123 (0/5)
11	20	+0.3	--- (5/5)		25	−12.1	145 (0/5)
	25	−5.7	200 (4/5)		30	−27.6	150 (0/5)
	30	−3.4	--- (5/5)	25	20	−3.5	132 (0/5)
12	20	+4.2	161 (0/5)		25	+1.9	130 (0/5)
	25	+1.7	200 (0/5)		30	−1.0	150 (0/5)
	30	−2.7	167 (3/5)	26	20	+2.4	127 (0/5)
13	20	+12.2	214 (0/5)		25	+3.1	153 (0/5)
	25	+11.2	165 (0/5)		30	−3.0	141 (0/5)
	30	+8.3	179 (0/5)	27	20	−9.3	136 (0/5)
					25	−3.5	148 (0/5)
					30	−9.2	168 (0/5)

[a] Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days. [b] Average change in weight from onset to termination of therapy. [c] T/C × 100 represents the ratio of the survival time of treated to control animals × 100. The average survival time of the untreated tumor-bearing control animals was 9.2 ± 0.4 days. The values in parentheses represent the number of mice that survived for >50 days relative to the number of mice treated.

sulfonyl chloride in methylene chloride in the presence of triethylamine at room temperature afforded the sulfonate derivatives **3–26** (Chart I).

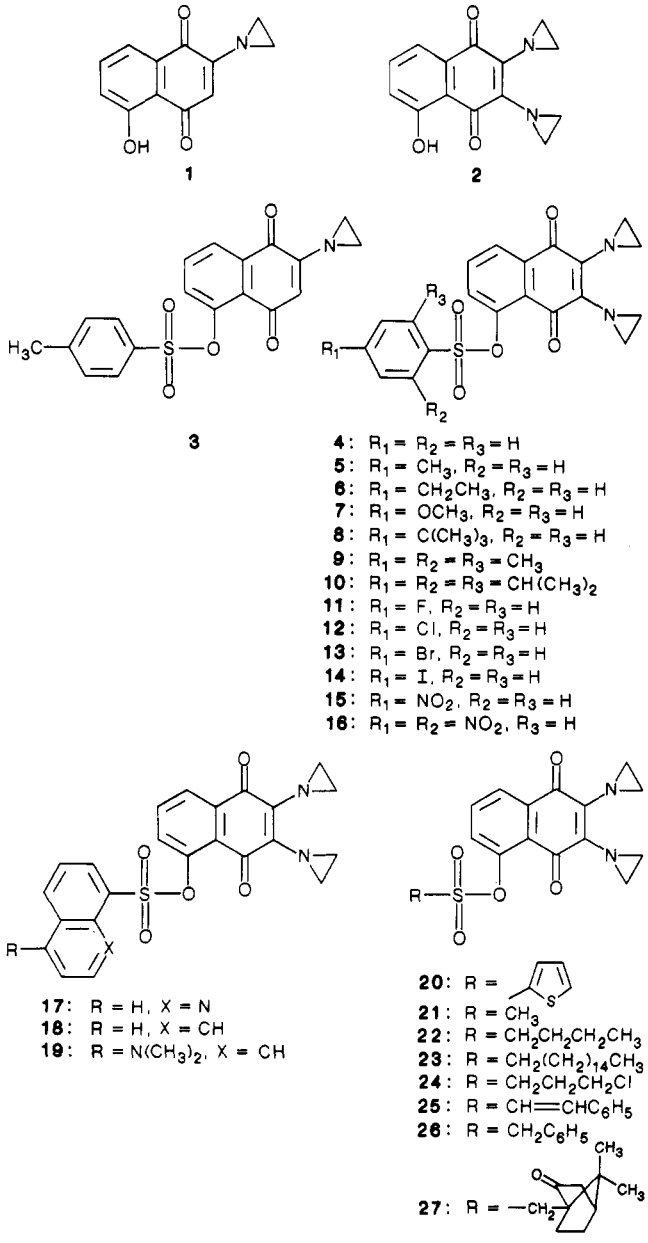
Anticancer Activity

The most active compounds in the aromatic sulfonate series were the 5-substituted benzenesulfonate **4**, *p*-toluenesulfonate **5**, *p*-methoxybenzenesulfonate **7**, *p*-fluorobenzenesulfonate **11**, *p*-nitrobenzenesulfonate **15**, 8-quinolinesulfonate **17**, and 2-thiophenesulfonate **20** of 2,3-bis(aziridinyl)-1,4-naphthoquinone. At their optimum daily dosage levels of 20–25 mg/kg × 6, these compounds produced 100%, 90%, 75%, 100%, 80%, 80%, and 100% 50-day survivors, respectively, of L1210 tumor-bearing mice. In contrast, *p*-ethylbenzenesulfonate **6**, *p*-chloro-, *p*-bromo-, and *p*-iodobenzenesulfonates **12–14** were less active. Conversely, *p-tert*-butylbenzenesulfonate **8**, 2,4,6-trimethylbenzenesulfonate **9**, 2,4,6-tris-*tert*-butylbenzenesulfonate **10**, 2,4-dinitrobenzenesulfonate **16**, naphthalene-1-sulfonate **18**, and 5-(dimethylamino)-1-naphthalenesulfonate **19** derivatives were inactive against this transplanted leukemia. In the aliphatic sulfonate series, only the methanesulfonate derivative **21** was active, producing 80% 50-day survivors at a 10 mg/kg daily dosage level × 6. At the optimum daily dosage level of 3 mg/kg × 6, 2,5-bis(carbethoxyamino)-3,6-diaziridinyl-1,4-benzoquinone (AZQ), included as a positive control, gave a T/C × 100 value of 231, and did not produce any long-term survivors. The results of these tests are summarized in Table I. Rechallenge of the mice that survived 50 days with 10^5 L1210 leukemia cells did not result in tumor take, indicating the presence of a supporting immune reaction in the anticancer response obtained with this neoplasm. For this reason, the most active compounds against the L1210 leukemia were also evaluated in mice bearing the B16 melanoma. Benzenesulfonate **4**, *p*-fluorobenzenesulfonate **11**, 8-quinolinesulfonate **17**, and 2-thiophenesulfonate **20** derivatives were all found to exhibit significant anticancer activity, producing T/C × 100 values of 180, 182, 219, and 161, respectively, at their optimum daily dosage level in this tumor system. The positive control of AZQ, at its optimum daily dosage of 3 mg/kg × 6, produced in this tumor system a T/C × 100 value of 147. These findings are shown in Table II.

Structure–Activity Relationships

Both the 5-*O*-sulfonyl moiety and the 2,3-disubstituted aziridinyl groups appear to be required for anticancer activity in this series of compounds. If either of these requirements were lacking, the synthesized compound was

Chart I. Structure Formulae of Various 2,3-Diaziridinyl-1,4-naphthoquinone Sulfonate Derivatives



inactive (compounds **2** and **3**). In the aromatic sulfonate series, replacement of the hydrogen at the para position of the benzene ring in the sulfonyl moiety of compound **4** with a methyl, methoxy, fluoro, or nitro group (compounds **5**, **7**, **11**, and **15**, respectively) resulted in the retention of anticancer activity. However, replacement of the para hydrogen in **4** with an ethyl, chloro, bromo, or iodo group (compounds **6**, **12**, **13**, and **14**, respectively) produced compounds with considerably less antineoplastic activity. Conversely, substitution of the para hydrogen in **4** with a bulky *tert*-butyl group resulted in a complete loss of tumor-inhibitory activity. Substitution of the ortho hydrogen(s) in the benzenesulfonyl moiety of compound **4** with one or more methyl, *tert*-butyl, or nitro group(s) (compounds **9**, **10**, and **16**, respectively) completely abolished the capacity of this agent to prolong survival time. Replacement of the nitrogen in the 8-quinolinesulfonyl group of compound **17** with –CH– produced the inactive compound **18**. In the aliphatic sulfonate series, only the methanesulfonate derivative **21** exhibited significant anticancer activity. Lengthening the aliphatic carbon chain in **21** markedly decreased its capacity to inhibit tumor growth. Thus, there seems to be a relationship between anticancer activity and the size of the substituent at the para position in the benzenesulfonyl moiety, with tumor-inhibitory activity decreasing with an increase in the size of the substituent. Conversely, there appears to be no clear relationship between the capacity of these agents to inhibit tumor growth and either the electron-withdrawing or the electron-donating properties of the substituent in the para position of the benzenesulfonyl group. It is conceivable that the solubility of individual compounds may have a role in the structure–activity relationships exhibited by this class of agents.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Brucker WM-500 spectrometer with Me_4Si as the internal reference. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

2-Aziridinyl-5-hydroxy-1,4-naphthoquinone (1). Ethylenimine (5.0 g, 115.7 mmol, 6 mL) in 60 mL of ethanol was added slowly to a stirred solution of 5-hydroxy-1,4-naphthoquinone (5.0 g, 28.7 mmol) in 940 mL of ethanol at 0 °C (ice–water bath) over a period of 0.5 h. The solution was stirred for another 4 h at the same temperature and then concentrated in vacuo. The residue was chromatographed on a silica gel column ($EtOAc/C_6H_{14}/CH_2Cl_2$, 3:4:5, v/v) to afford 3.2 g (44%) of product: R_f 0.57, mp 170–172 °C; NMR ($CDCl_3$) δ 2.31 (s, 4 H, 2-aziridinyl), 6.27 (s, 1 H, 3-H), 7.23 (m, 1 H, 6-H), 7.60 (m, 2 H, 7- and 8-H), 11.92 (s, 1 H, 5-OH, D_2O exchangeable). Anal. ($C_{12}H_9NO_3$) C, H, N.

2,3-Diaziridinyl-5-hydroxy-1,4-naphthoquinone (2). 2-Aziridinyl-5-hydroxy-1,4-naphthoquinone (**1**, 1.0 g, 4.7 mmol) was added in small portions to 5.8 g (7 mL, 134.9 mmol) of ethylenimine at 0 °C with stirring. The reaction mixture was stirred at the same temperature for an additional 20 h. The excess ethylenimine was removed in vacuo, and the resulting solid residue was redissolved in 150 mL of CH_2Cl_2. The solution was washed with water (3 × 20 mL) and dried (anhydrous Na_2SO_4). The drying agent was removed by filtration. The filtrate was evaporated to dryness under reduced pressure to give 1.1 g (92%) of product: mp 181–183 °C; R_f 0.38 ($EtOAc/C_6H_{14}$, 1:1, v/v); NMR ($CDCl_3$) δ 2.42 (s, 8 H, 2- and 3-aziridinyl), 7.26 (d, 1 H, 6-H), 7.48–7.54 (m, 1 H, 7-H), 7.55 (d, 1 H, 8-H), 12.16 (s, 1 H, 5-OH, D_2O exchangeable). Anal. ($C_{14}H_{12}N_2O_3$) C, H, N.

2-Aziridinyl-5-[(*p*-tolylsulfonyl)oxy]-1,4-naphthoquinone (3). A solution of triethylamine (1.45 g, 14.4 mmol, 2 mL) and 1.20 g (6.30 mmol) of *p*-toluenesulfonyl chloride in 10 mL of CH_2Cl_2 was added to a solution of 0.70 g (3.25 mmol) of 2-aziridinyl-5-hydroxy-1,4-naphthoquinone (**1**) in 30 mL of CH_2Cl_2. The reaction mixture was stirred for 2 h at room temperature. The solution was concentrated to a small volume and chromatographed on a silica gel column ($EtOAc/C_6H_{14}/CH_2Cl_2$, 2:3:5, v/v) to give 0.5 g (42%) of the desired product: R_f 0.48; mp 152–154 °C; NMR ($CDCl_3$) δ 2.26 (s, 4 H, 2-aziridinyl), 2.47 (s, 3 H, 4′-CH_3), 6.23 (s, 1 H, 3-H), 7.36 (d, 2 H, 3′- and 5′-H), 7.41 (d, 1 H, 6-H), 7.66 (m, 1 H, 7-H), 7.87 (d, 2 H, 2′- and 6′-H), 8.07 (d, 1 H, 8-H). Anal. ($C_{19}H_{15}NO_5S$·EtOAc) C, H, N.

2,3-Diaziridinyl-5-[(*p*-tolylsulfonyl)oxy]-1,4-naphthoquinone (5). A solution of *p*-toluenesulfonyl chloride (0.50 g, 2.60 mmol) and triethylamine (0.73 g, 7.20 mmol, 1 mL) in 10 mL of CH_2Cl_2 was added to a stirred solution of 2,3-diaziridinyl-5-hydroxy-1,4-naphthoquinone (**2**) in 10 mL of CH_2Cl_2. The reaction mixture was stirred for another 20 h at room temperature. The solution was then concentrated to a small volume and chromatographed on a silica gel column ($EtOAc/C_6H_{14}$, 1:1, v/v, and followed by EtOAc) to afford 0.2 g (41%) of **5**: R_f 0.2; mp 157–159 °C; NMR ($CDCl_3$) δ 2.35 and 2.37 (2 s, 8 H, 2- and 3-aziridinyl), 2.45 (s, 3 H, 4′-CH_3), 7.33 (d, 1 H, 6-H), 7.35 (d, 2 H, 3′- and 5′-H), 7.54 (dd, 1 H, 7-H), 7.88 (d, 2 H, 2′- and 6′-H), 8.01 (d, 1 H, 8-H). Anal. ($C_{21}H_{18}N_2O_5S$·0.5EtOAc) C, H, N.

The following compounds have been synthesized by the same

Table II. Effects of 2,3-Diaziridinyl-1,4-naphthoquinone Sulfonate Derivatives on the Survival of Mice Bearing the B16 Melanoma

compd	daily dosage,[a] mg/kg	av Δ wt,[b] %	T/C × 100[c]	compd	daily dosage,[a] mg/kg	av Δ wt,[b] %	T/C × 100[c]
AZQ	3	−13.8	147 (0/5)	**13**	50	+1.1	143 (0/5)
4	50	−1.3	156 (0/5)		100	+2.5	153 (0/5)
	100	−12.3	180 (0/5)		150	+0.7	151 (0/5)
	150	−13.5	148 (0/5)	**14**	50	+4.1	132 (0/5)
5	50	+4.5	144 (0/5)		100	+0.6	138 (0/5)
	100	−1.9	153 (0/5)		150	−4.1	141 (0/5)
	150	−9.8	141 (0/5)	**17**	50	−1.7	195 (0/5)
7	50	−4.7	132 (0/5)		100	−8.2	219 (0/5)
	100	−12.4	154 (0/5)		150	−14.3	239 (0/5)
	150	−15.2	165 (0/5)	**20**	50	−10.8	156 (0/5)
11	50	−5.2	159 (0/5)		100	−18.2	161 (0/5)
	100	−14.3	182 (0/5)		150	−22.7	77 (0/5)
	150	−20.3	177 (0/5)	**21**	10	−3.8	125 (0/5)
12	50	+1.1	143 (0/5)		15	−6.4	137 (0/5)
	100	−2.5	153 (0/5)		20	−8.1	153 (0/5)
	150	−3.2	178 (0/5)				

[a] Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days. [b] Average change in weight from onset to termination of therapy. [c] T/C × 100 represents the ratio of the survival time of treated to control animals × 100. The average survival time of the untreated tumor-bearing control animals was 13.2 ± 1.2 days.

methodology as described for compound **5**.

2,3-Diaziridinyl-5-[(phenylsulfonyl)oxy]-1,4-naphthoquinone (4): mp 154–156 °C; R_f 0.42 (EtOAc/CH_2Cl_2, 1:1, v/v); NMR ($CDCl_3$) δ 2.36 (s, 8 H, 2- and 3-aziridinyl), 7.32 (d, 1 H, 6-H), 7.54–7.58 (m, 3 H, 3′-, 5′-, and 7-H), 7.69 (m, 1 H, 5′-H), 8.01 (m, 3 H, 2′-, 6′-, and 8-H). Anal. ($C_{20}H_{16}N_2O_5S·0.25CH_2Cl_2$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-ethylphenyl)sulfonyl]oxy]-1,4-naphthoquinone (6): mp 120–122 °C; R_f 0.3 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 1.28 (t, 3 H, CH_3), 2.35 and 2.37 (2 s, 8 H, 2- and 3-aziridinyl), 2.74 (q, 2 H, CH_2), 7.33 (d, 1 H, 6-H), 7.38 (d, 2 H, 3′- and 5′-H), 7.57 (t, 1 H, 7-H), 7.92 (d, 2 H, 2′- and 6′-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{22}H_{20}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-methoxyphenyl)sulfonyl]oxy]-1,4-naphthoquinone (7): mp 143–145 °C; R_f 0.47 (EtOAc/CH_2Cl_2, 1:1, v/v); NMR ($CDCl_3$) δ 2.38 (s, 8 H, 2- and 3-aziridinyl), 3.90 (s, 3 H, 4′-CH_3O), 7.01 (d, 2 H, 3′- and 5′-H), 7.35 (d, 1 H, 6-H), 7.57 (m, 1 H, 7-H), 7.95 (d, 2 H, 2′- and 6′-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{21}H_{18}N_2O_6S$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-*tert*-butylphenyl)sulfonyl]oxy]-1,4-naphthoquinone (8): mp 139–141 °C; R_f 0.39 (EtOAc/C_6H_{14}/CH_2Cl_2, 3:4:5, v/v); NMR ($CDCl_3$) δ 1.36 (s, 9 H, 4′-*tert*-butyl), 2.36 (s, 8 H, 2- and 3-aziridinyl), 7.36 (d, 1 H, 6-H), 7.58 (m, 3 H, 3′-, 5′-, and 7-H), 7.93 (d, 2 H, 2′- and 6′-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{24}H_{24}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(2′,4′,6′-trimethylphenyl)sulfonyl]oxy]-1,4-naphthoquinone (9): mp 125–127 °C; R_f 0.39 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.36 (s, 3 H, 4′-CH_3), 2.39 (s, 8 H, 2- and 3-aziridinyl), 2.61 (s, 6 H, 2′- and 6′-CH_3), 7.02 (m, 3 H, 3′-, 5′-, and 6-H), 7.50 (t, 1 H, 7-H), 8.00 (d, 1 H, 8-H). Anal. ($C_{23}H_{22}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(2′,4′,6′-triisopropylphenyl)sulfonyl]oxy]-1,4-naphthoquinone (10): mp 74–76 °C; R_f 0.37 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 1.22 (s, 12 H, 2′- and 6′-methyl of isopropyl), 1.30 (d, 6 H, 4′-methyl of isopropyl), 2.38 (2 s, 8 H, 2- and 3-aziridinyl), 2.95 (m, 1 H, 4′-CH of isopropyl), 4.08 (m, 2 H, 2′- and 6′-CH of isopropyl), 6.96 (d, 1 H, 6-H), 7.24 (s, 2 H, 3′- and 5′-H), 7.50 (t, 1 H, 7-H), 8.00 (d, 1 H, 8-H). Anal. ($C_{29}H_{34}N_2O_5S$·EtOAc) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-fluorophenyl)sulfonyl]oxy]-1,4-naphthoquinone (11): mp 161–163 °C; R_f 0.39 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.36 and 2.37 (2 s, 8 H, 2- and 3-aziridinyl), 7.25 (m, 2 H, 3′- and 5′-H), 7.39 (d, 1 H, 6-H), 7.58 (m, 1 H, 7-H), 8.06 (d, 1 H, 8-H), 8.08 (m, 2 H, 2′- and 6′-H). Anal. ($C_{20}H_{15}FN_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-chlorophenyl)sulfonyl]oxy]-1,4-naphthoquinone (12): mp 159–161 °C; R_f 0.57 (EtOAc/CH_2Cl_2, 1:1, v/v); NMR ($CDCl_3$) δ 2.36 and 2.38 (s, 8 H, 2- and 3-aziridinyl), 7.38 (d, 1 H, 6-H), 7.56 (d, 2 H, 3′- and 5′-H), 7.61 (m, 1 H, 7-H), 7.98 (d, 2 H, 2′- and 6′-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{20}H_{15}ClN_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-bromophenyl)sulfonyl]oxy]-1,4-naphthoquinone (13): mp 170 °C dec; R_f 0.25 (EtOAc/C_6H_{14}, 2:3, v/v); NMR ($CDCl_3$) δ 2.30 and 2.33 (2 s, 8 H, 2- and 3-aziridinyl), 7.39 (d, 1 H, 6-H), 7.60 (m, 1 H, 7-H), 7.70 (d, 2 H, 3′- and 5′-H), 7.90 (d, 2 H, 2′- and 6′-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{20}H_{15}BrN_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-iodophenyl)sulfonyl]oxy]-1,4-naphthoquinone (14): mp 160 °C dec; R_f 0.31 (EtOAc/C_6H_{14}, 2:3, v/v); NMR ($CDCl_3$) δ 2.35 and 2.37 (2 s, 8 H, 2- and 3-aziridinyl), 7.37 (d, 1 H, 6-H), 7.58 (m, 1 H, 7-H), 7.73 (d, 2 H, 3′- and 5′-H), 7.93 (d, 2 H, 2′- and 6′-H), 8.03 (d, 1 H, 8-H). Anal. ($C_{20}H_{15}IN_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(*p*-nitrophenyl)sulfonyl]oxy]-1,4-naphthoquinone (15): mp 150–151 °C dec; R_f 0.34 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.38 (s, 8 H, 2- and 3-aziridinyl), 7.45 (d, 1 H, 6-H), 7.64 (t, 1 H, 7-H), 8.08 (d, 1 H, 8-H), 8.31 (d, 2 H, 3′- and 5′-H), 8.44 (d, 2 H, 2′- and 6′-H). Anal. ($C_{20}H_{15}N_3O_7S$·0.5EtOAc) C, H, N.

2,3-Diaziridinyl-5-[[(2,4-dinitrophenyl)sulfonyl]oxy]-1,4-naphthoquinone (16): mp 164–166 °C dec; R_f 0.26 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.30 (s, 4 H, 2-aziridinyl), 2.39 (s, 4 H, 3-aziridinyl), 7.53 (d, 1 H, 6-H), 7.69 (t, 1 H, 7-H), 8.10 (d, 1 H, 8-H), 8.59 (d, 1 H, 5′-H), 8.63 (d, 1 H, 6′-H), 8.72 (s, 1 H, 3′-H). Anal. ($C_{20}H_{14}N_4O_9S$·0.5EtOAc) C, H, N.

2,3-Diaziridinyl-5-[(8-quinolylsulfonyl)oxy]-1,4-naphthoquinone (17): mp 161–163 °C; R_f 0.30 (EtOAc); NMR ($CDCl_3$) δ 2.22 (s, 4 H, 2-aziridinyl), 2.36 (s, 4 H, 3-aziridinyl), 7.29 (d, 1 H, 6-H), 7.51 (m, 1 H, 3′-H), 7.57 (m, 1 H, 6′-H), 7.70 (m, 1 H, 7-H), 8.01 (d, 1 H, 8-H), 8.19 (d, 1 H, 4′-H), 8.31 (d, 1 H, 5′-H), 8.55 (d, 1 H, 2′-H), 9.05 (d, 1 H, 7′-H). Anal. ($C_{23}H_{17}N_3O_5S$) C, H, N: calcd, 9.39; found, 8.96.

2,3-Diaziridinyl-5-[(naphth-1-ylsulfonyl)oxy]-1,4-naphthoquinone (18): mp 118–120 °C; R_f 0.25 (EtOAc/C_6H_{14}/CH_2Cl_2, 3:4:5, v/v); NMR ($CDCl_3$) δ 2.35 (s, 4 H, 2-aziridinyl), 2.38 (s, 4 H, 3-aziridinyl), 6.74 (d, 1 H, 5′-H), 7.34 (m, 1 H, 6′-H), 7.54 (m, 1 H, 7′-H), 7.67 (m, 1 H, 7-H), 7.78 (m, 1 H, 3′-H), 7.95 (d, 1 H, 6-H), 8.01 (d, 1 H, 8-H), 8.13 (d, 1 H, 4′-H), 8.18 (d, 1 H, 8′-H), 8.90 (d, 1 H, 2′-H). Anal. ($C_{24}H_{18}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[[5′-(dimethylamino)naphth-1-yl]sulfonyl]oxy]-1,4-naphthoquinone (19): mp 79–81 °C dec; R_f 0.29 (EtOAc/C_6H_{14}, 7:3, v/v); NMR ($CDCl_3$) δ 2.38 (2 s, 8 H, 2- and 3-aziridinyl), 2.92 [s, 6 H, 5′-$N(CH_3)_2$], 6.71 (d, 1 H, 6′-H), 7.26 (d, 1 H, 6-H), 7.35 (dd, 1 H, 7′-H), 7.51 (dd, 1 H, 7-H), 7.66 (dd, 1 H, 3′-H), 7.95 (d, 1 H, 8′-H), 8.10 (d, 1 H, 8-H), 8.54 (d, 1 H, 4′-H), 8.65 (d, 1 H, 2′-H). Anal. ($C_{26}H_{23}N_3O_5S$·0.5EtOAc) C, H, N.

2,3-Diaziridinyl-5-[(2-thiophenesulfonyl)oxy]-1,4-naphthoquinone (20): mp 140 °C; R_f 0.65 (EtOH/EtOAc/C_6H_{14}, 1:4:5, v/v); NMR ($CDCl_3$) δ 2.38 (s, 8 H, 2- and 3-aziridinyl), 7.15 (m, 1 H, 4′-H), 7.32 (d, 1 H, 6-H), 7.58 (m, 1 H, 7-H), 7.75 (d, 1 H, 5′-H), 7.79 (d, 1 H, 3′-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{18}H_{14}N_2O_5S_2$) C, H, N.

2,3-Diaziridinyl-5-[(methylsulfonyl)oxy]-1,4-naphthoquinone (21): mp 171–173 °C; R_f 0.53 (EtOAc/CH_2Cl_2, 1:1, v/v);

NMR ($CDCl_3$) δ 2.42 (d, 8 H, 2- and 3-aziridinyl), 3.45 (s, 3 H, SO_3CH_3), 7.59 (d, 1 H, 6-H), 7.66 (m, 1 H, 7-H), 8.06 (d, 1 H, 8-H). Anal. ($C_{15}H_{14}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[(butylsulfonyl)oxy]-1,4-naphthoquinone (22): isolated as a glass; NMR ($CDCl_3$) δ 1.02 (t, 3 H, 4′-CH_3), 1.59 (m, 2 H, 3′-CH_3), 2.05 (m, 2 H, 2′-CH_2), 2.38–2.39 (2 s, 8 H, 2- and 3-aziridinyl), 3.59 (m, 2 H, 1′-CH_2), 7.57 (d, 1 H, 6-H), 7.62 (m, 1 H, 7-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{18}H_{20}N_2O_5S$·0.5EtOAc) C, H, N.

2,3-Diaziridinyl-5-[(hexadecylsulfonyl)oxy]-1,4-naphthoquinone (23): mp 71–73 °C; R_f 0.49 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 0.89 (t, 3 H, 16′-CH_3), 1.27–1.30 (m, 22 H, 5′- to 15′-CH_2), 1.40 (m, 2 H, 4′-CH_2), 1.54 (m, 2 H, 3′-CH_2), 2.07 (m, 2 H, 2′-CH_2), 2.40 (s, 4 H, 2-aziridinyl), 2.41 (s, 4 H, 3-aziridinyl), 3.60 (t, 2 H, 1′-CH_2), 7.60 (d, 1 H, 6-H), 7.64 (t, 1 H, 7-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{30}H_{44}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(3-chloropropyl)sulfonyl]oxy]-1,4-naphthoquinone (24): mp 115–117 °C; R_f 0.22 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.41 (s, 8 H, 2- and 3-aziridinyl), 2.61 (m, 2 H, 2′-CH_2), 3.81 (m, 4 H, 3′- and 1′-CH_2), 7.60 (d, 1 H, 6-H), 7.65 (t, 1 H, 7-H), 8.06 (d, 1 H, 8-H). Anal. ($C_{17}H_{17}ClN_2O_5S$·0.5EtOAc) C, H, N.

2,3-Diaziridinyl-5-[(β-styrylsulfonyl)oxy]-1,4-naphthoquinone (25): mp 171–173 °C dec; R_f 0.24 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.37 (2 s, 8 H, 2- and 3-aziridinyl), 7.21 (d, 1 H, styrene-H_α), 7.43–7.47 (m, 3 H, 6-, 3′-, and 5′-H), 7.25–7.56 (m, 3 H, 2′-, 6′-, and styrene-H_β), 7.62–7.65 (m, 3 H, 4′- and 7-H), 8.03 (q, 1 H, 8-H). Anal. ($C_{22}H_{18}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[(α-tolylsulfonyl)oxy]-1,4-naphthoquinone (26): isolated as a glass; R_f 0.24 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.42 (s, 4 H, 2-aziridinyl), 2.45 (s, 4 H, 3-aziridinyl), 4.91 (s, 2 H, α-CH_2), 7.43 (m, 3 H, 3′-, 4′-, and 5′-H), 7.51 (d, 1 H, 6-H), 7.60 (m, 2 H, 2′- and 6′-H), 7.61 (t, 1 H, 7-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{21}H_{18}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[(*d*-10-camphorylsulfonyl)oxy]-1,4-naphthoquinone (27): mp 121–123 °C; R_f 0.33 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 1.00 (s, 3 H, 9′-CH_3), 1.21 (s, 3 H, 8′-CH_3), 1.47 (m, 1 H, 4′-H, a or e), 1.77 (m, 1 H, 4′-H, a or e), 1.97 (m, 1 H, 5′-H, a or e), 2.11 (m, 1 H, 2′-H, a or e), 2.16 (m, 1 H, 3′-H), 2.41 (s, 8 H, 2- and 3-aziridinyl), 2.47 (m, 1 H, 5′-H, a or e), 2.55 (m, 1 H, 2′-H, a or e), 3.78 (d, 1 H, 10′-H_A or H_B), 4.05 (d, 1 H, 10′-H_A or H_B), 7.60 (d, 1 H, 6-H), 7.65 (m, 1 H, 7-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{24}H_{24}N_2O_6S$) C, H, N.

Biological Test Procedures. Transplantation of L1210 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 female mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600 g), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter and the cell population was adjusted to 10^6 cells/mL. One-tenth milliliter of the resulting cell suspension (containing approximately 10^5 cells) was injected intraperitoneally into each animal. The drug was administered by intraperitoneal injection beginning 24 h after tumor implantation, once daily for 6 consecutive days. Test compounds were injected intraperitoneally as fine suspensions in isotonic saline in a volume of 0.25 mL. For any one experiment, animals were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Laboratory Chow pellets and water ad libitum. Controls given injections of a comparable volume of vehicle (saline) were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity.

The same methodology was used for the testing of compounds against B16 melanoma bearing mice.

Acknowledgment. This research was supported in part by U.S. Public Health Service Grant CA-43659 from the National Cancer Institute. We also acknowledge the support of the Northeast NMR Facility at Yale University for the high-resolution NMR spectra, made possible by a grant from the Chemical Division of the National Science Foundation (Grant No. CHE-7916210).

Nucleosides of Azathioprine and Thiamiprine as Antiarthritics

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Azathioprine [Imuran; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine] is a widely used immunosuppressive and antiarthritic drug. For the sake of comparison, the riboside, the 2′-deoxyriboside, and the arabinoside of azathioprine and its 2-amino congener, thiamiprine, were prepared by an enzymatic method. In vitro, the cytotoxicities of these aglycons and their nucleosides were similar (ED_{50} = 0.8–2 μM), except for the arabinosides, which were nontoxic (ED_{50} >100 μM). In vivo, their activities were compared in the rat adjuvant arthritis model. The ribosides and 2′-deoxyribosides were less potent than their corresponding aglycons. The safety indexes of these nucleosides were comparable to those of the corresponding aglycons except for the 2′-deoxyriboside of azathioprine, which had an appreciably lower safety index than did azathioprine. Both arabinosides were inactive and nontoxic. All of the aglycons tested (6-mercaptopurine, azathioprine, 6-thioguanine, and thiamiprine) were of similar potency. However, azathioprine had a more favorable therapeutic index than did 6-mercaptopurine. Similarly, thiamiprine was safer than was 6-thioguanine. In this model, the *S*-(1-methyl-4-nitro-1*H*-imidazol-5-yl) moiety imparted greater safety to these thiopurines by decreasing toxicity but not affecting potency.

6-Mercaptopurine (1) and 6-thioguanine (6) are antileukemic agents of long-standing clinical usefulness. These agents are also immunosuppressive. Not completely understood is the superiority of their *S*-(1-methyl-4-nitro-1*H*-imidazol-5-yl) derivatives as immunomodulators.[1] Early studies showed that azathioprine [2; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine; Imuran] was particularly effective in preventing rejection of kidney transplants in dogs.[2] Consequently, it was developed in the clinic as a chemotherapeutic adjunct to kidney transplant protocols. In time, the clinical use of this agent was extended to autoimmune disorders that are associated with chronic inflammation. Currently, the widest use of azathioprine is in the treatment of rheumatoid arthritis. The 2-amino congener of azathioprine, thiamiprine [7; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purin-2-amine; Guaneran], was evaluated in man as an antitumor agent[3] but has not been clinically assessed as either an

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0022-2623/89/1832-1471$01.50/0