Laboratoires, MI); virions were irradiated in Hanks solution at a density of 10^{10} particles per milliliter, as reported above for the Ehrlich cells, and in the presence of 4 μg/mL of the tested compounds. Virus titers were determined according to Adams,[33] using the same host and the same medium.

Inhibition of Epidermal DNA Synthesis in Mice. (a) By Topical Application. Furocoumarins were applied on the depilated skin of the backs of female mice by using 0.1% methanolic solutions (50 μg cm^{-2}); every compound was tested on at least six mice. The animals were then kept in a dark room for 45 min; the backs were then irradiated with UV-A light (9 J cm^{-2}, delivered in 30 min). Immediately after irradiations, [^{3}H]thymidine (10 μCi) was injected intraperitoneously; after 30 min the mice were sacrified.

The skin specimens were removed, and the epidermis was isolated according to the method of Bowden et al.,[34] with slight modifications; the whole skin was kept for 1 min in distilled water at 55 °C and chilled by immersion in ice-cold water. The skin was placed on a cooled (-20 °C) glass plate, dermis side down, and the epidermis was removed by scraping with a razor blade. The epidermis was cut with a scissor, suspended in 10 vol of ice-cold sodium saline citrate (0.15 mol of sodium chloride; 0.02 mol of sodium citrate), and then homogenized with a Vortex mixer and a Potter-Elvehjn homogenizer, with cooling in ice. The mixture was made 2% in sodium lauryl sulfate and 1 mol in sodium chloride and then left at room temperature for 2 h; the suspension was extracted three times with a chloroform–butanol (4:1) mixture (v/v), according to the method of Szybalska and Szybalski.[35]

The DNA was then precipitated with ethanol, washed with 80 °C ethanol, and dissolved in water; the solution was analyzed for DNA content and radioactivity. The percent inhibition of DNA synthesis observed in each sample was calculated assuming as a control the radioactivity incorporation obtained in the epidermal DNA of the abdomen skin of the same animal, processed in the same way. The specific activities observed in the control skins were in the range of 0.9–1.2 × 10^4 dpm mg^{-1} of DNA.

(b) By Oral Administration. The mice (NCL, 20 ± 2 g in weight) were starved for 3 h before being given, by oral intubation, a single dose of 5 mg per mouse of the furocoumarin, suspended in 0.5% methylcellulose; in these experiments also, groups of at least six animals were used. The mice were kept in a dark room for 2 h and then the skin of the back was irradiated with UV-A (9 J cm^{-2}). The subsequent steps for epidermal cell isolation, homogenization, and DNA extraction were similar to those described above. The unexposed skin of the abdomen, protected from light by black paper, was assumed, as above, as a control.

Mutagenesis Test. Furocoumarins, dissolved in absolute ethanol, were stored in the dark at room temperature prior to use. After the addition of furocoumarins (4 μg/mL), the *Escherichia coli* WP2 *trp*$^-$ suspension (10^7 cells/mL) was stored in the dark for 15 min at room temperature and then irradiated with a blacklight blue fluorescent lamp (F15T8 BLB, 15 W). Irradiated cells (0.1 mL) were added to 2 mL of molten 0.6% top agar kept at 42 °C. The contents were mixed and poured on plates containing 20 mL of SEM agar (MMA fortified with 0.1 mg/mL of Difco nutrient broth).[36] Revertant colonies were counted after 48 h of incubation at 37 °C in the dark. Survivors were counted by plating appropriate dilutions of irradiated cells on the same medium.

Acknowledgment. We are indebted to Prof. Giovanni Rodighiero for helpful discussion on this research; we thank M. Peron for her skillful technical assistance.

Registry No. **2a**, 78982-40-8; **2b**, 22975-76-4; **2c**, 5762-92-5; **3a**, 10387-49-2; **3b**, 2747-05-9; **3c**, 80813-61-2; **4a**, 6748-68-1; **4b**, 2555-29-5; **4c**, 6109-07-5; **5a**, 6835-55-8; **5b**, 16555-98-9; **5c**, 80813-62-3; **6a**, 80813-63-4; **6b**, 31479-62-6; **6c**, 5762-90-3; **7a**, 80813-64-5; **7b**, 22975-75-3; **7c**, 5762-91-4; **8a**, 80813-66-7; **8b**, 20052-10-2; **8c**, 80813-65-6.

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Synthesis and Antitumor Evaluation of Selected 5,6-Disubstituted 1(2)*H*-Indazole-4,7-diones[1]

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A series of novel aziridinyl-substituted 1(2)*H*-indazole-4,7-diones and related 1(2)*H*-indazole-4,7-diones was synthesized and tested against Ehrlich ascites carcinoma growth in male CF_1 mice. Ten of the test compounds, including two aziridinyl-substituted 1(2)*H*-indazole-4,7-diones, were found to be significantly active (inhibition of tumor growth >80%) in the Ehrlich ascites carcinoma screen. Several structure–activity relationships were indicated for antitumor activity in this screen. An aziridinyl-substituted derivative, 5-aziridinyl-6-chloro-1*H*-indazole-4,7-dione (**8a**), also exhibited significant activity against the growth of P-388 lymphocytic leukemia cells in male BDF_1 mice (% T/C = 145; % T/C > 125 is considered significant).

Many naturally occurring and synthetic compounds that contain the *p*-benzoquinone moiety have been investigated for antitumor activity.[2-16] Of these compounds, several have proven to be potent antineoplastic agents. For example, the anthracycline antibiotics are some of the most

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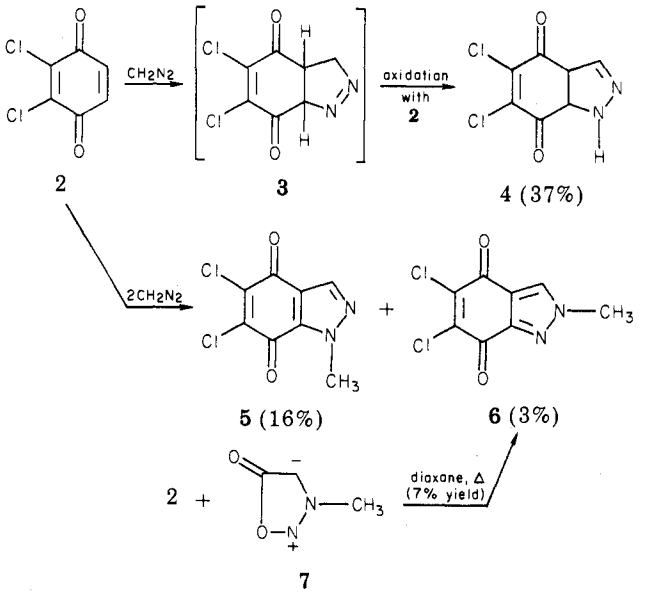
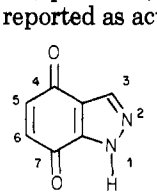
Scheme I



important agents in the treatment of human cancers,[3] and recently a series of 1,4-bis[(aminoalkyl)amino]-9,10-anthracenediones have been found to be potent antineoplastic agents.[4] Numerous nitrogen-containing heterocyclic quinones also exhibit antitumor activity.[2] Most notable of these are the antibiotics, streptonigrin[5,6] and mitomycin C.[7] The latter antibiotic is used clinically in the United States as an anticancer agent.[8] The aziridinyl-substituted *p*-benzoquinones, trenimon[2,9] and carboquone,[2,10] have undergone clinical trials in Europe and Japan, respectively. Another potent aziridinyl-substituted *p*-benzoquinone, 2,5-bis(aziridinyl)-3,6-bis(carboethoxyamino)-*p*-benzoquinone[11-13] (i.e., AZQ, NSC-182986) is now undergoing clinical trials as an anticancer drug in the Unites States.[14]

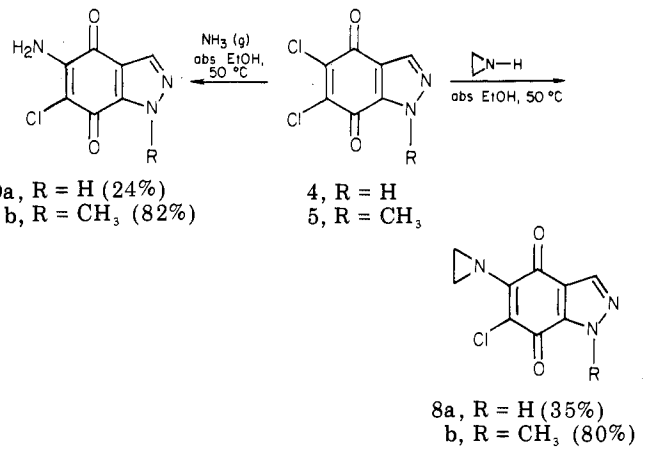
Several derivatives of an interesting group of nitrogen-containing heterocycle quinones, the 1*H*-indazole-4,7-diones (1), have been reported as active against the growth



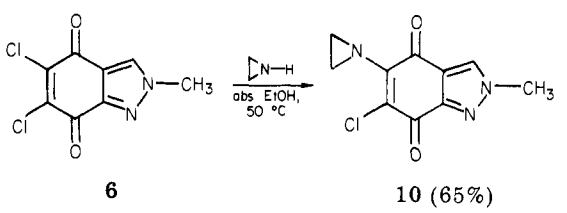
1

of certain rodent tumors.[15,16] Our goal was to incorporate the aziridinyl-*p*-benzoquinone moiety into the 1*H*-indazole-4,7-dione ring system in an attempt to synthesize

Scheme II



Scheme III



more potent antineoplastic agents.

Chemistry. The 1(2)*H*-indazole-4,7-dione ring system has been readily synthesized via 1,3-dipolar cycloaddition of diazomethane to a substituted *p*-benzoquinone,[17-21] although yields are characteristically low and compounds are difficult to purify. The method is particularly useful when a symmetrical 2,3-disubstituted *p*-benzoquinone is utilized to avoid mixtures of isomerically 5- and 6-substituted 1(2)*H*-indazole-4,7-diones.

The method of Eistert et al.[20] was employed in the synthesis of 5,6-dichloro-1*H*-indazole-4,7-dione (4) (Scheme I). The diazomethane was generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide according to the procedure of de Boer and Backer.[21] The oxidation of the intermediate cycloadduct **3** was accomplished with half of the initial 2,3-dichloro-*p*-benzoquinone **2**, yielding 2,3-dichlorohydroquinone as a byproduct[20] in the synthesis of **4**.

The *N*-methyl derivatives **5** and **6** were synthesized, albeit in very low yield, by a modification of the procedure described by Entwistle et al.[22] (Scheme II); however, Entwistel and colleagues did not describe the 2-*N*-methyl derivative **6** as a reaction product. Compounds **5** and **6** were easily separated by column chromatography. In order to establish the structure of compound **6** unequivocally, the compound was also synthesized in low yield via the cycloaddition–oxidation–decarboxylation reaction of 3-methylsydnone (7)[23] with 2,3-dichloro-*p*-benzoquinone (**2**) (Scheme I). Other similar sydnone–benzoquinone reactions have been reported.[24]

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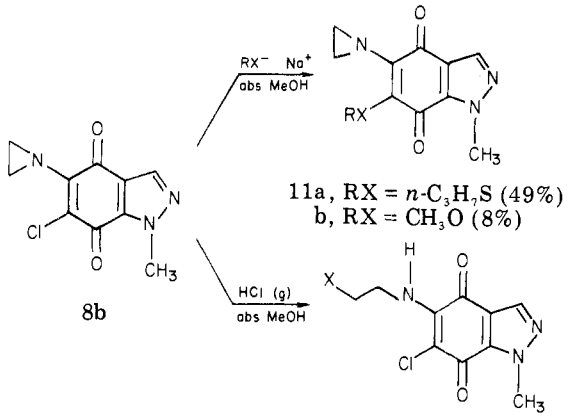
Scheme IV



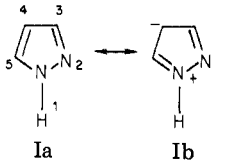
8b

11a, RX = n-C_3H_7S (49%)
b, RX = CH_3O (8%)

12a, X = Cl (35%)
b, X = CH_3O (12%)

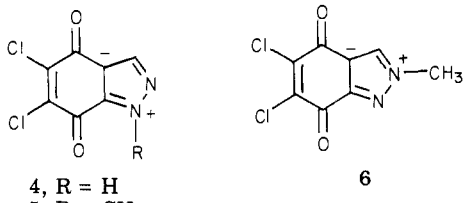
Facile substitution of a chlorine atom in the 5,6-dichloro derivatives **4–6** with ethylenimine provided aziridinyl compounds **8a**, **8b**, and **9**, respectively, in fair to good yields (Schemes II and III). X-ray crystallographic analysis of crystalline **8b** established the structure as 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione,[25] as visualized clearly in an ORTEP[26] drawing. The molecule is essentially planar in crystalline form with the exception of the 5-aziridine ring, which is tilted 51.7° out of the plane of the indazole system.[27]

The preferential replacement of the 5-chloro group in 5,6-dichloro-1-methyl-1*H*-indazole-4,7-dione (**5**) by ethylenimine may be explained in part by the electronic nature of the pyrazole ring in the 1*H*-indazole-4,7-dione system. Pyrazole (Ia) appears to have a large amount of



Ia Ib

aromatic character based upon the chemical reactions, the X-ray bond distances, and the resonance energies of the molecule.[28] One resonance structure Ib, which contributes to the electronic character of pyrazole Ia, is shown. The contribution of this resonance structure Ib to the electronic character of pyrazole agrees with electron-density calculations, which suggest that electrophilic attack occurs at position 4.[28] If the fused pyrazole ring in the 5,6-dichloro-1(2)*H*-indazole-4,7-diones **4–6** has electronic char-

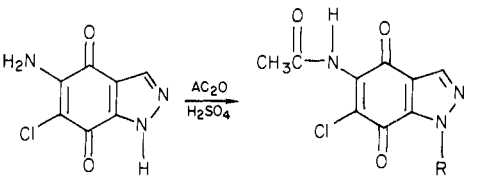


4, R = H
5, R = CH_3

6

acter similar to pyrazole Ia, then the resonance structures shown would be expected to contribute to the overall resonance hybrid of the molecules. In these resonance structures one can see that the electron density would be higher at the 4-carbonyl position than at the 7-carbonyl position. The 4-carbonyl group would likewise decrease the electropositive character of the chloro-substituted 6-carbon. This proposed difference in positive character between the 5- and 6-carbons may account for the selective replacement of the 5-chloro group by ethylenimine in the synthesis of compound **5**. Based on the resonance structures of **4–6**, along with the proven X-ray structure of **8b**, it was proposed that the 5-chloro group was replaced in the substitution reaction of compounds **4**, **5**, or **6** with an amine (e.g., ethylenimine or ammonia). All the amino-substituted 1(2)*H*-indazole-4,7-diones have been depicted accordingly.

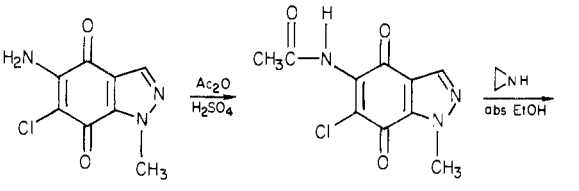
Scheme V



9a

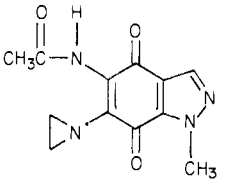
13, R = H (0.8%)
14, R = CH_3CO (10%)

Scheme VI



9b

15 (46%)



16 (22%)

The 5-aziridinyl-6-(thiopropyl) derivative **11a** and the 5-aziridinyl-6-methoxy derivative **11b** were synthesized from 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione (**8b**) by modifications of the procedure described by Khan and Driscoll[11] (Scheme IV). In both reactions the sodium salt of the thiol or alcohol was formed in absolute methanol at room temperature and then cooled to −77 °C before quinone addition. Upon warming, the substitution reaction occurred. The sodium salt of propanethiol replaced the 6-chloro group of compound **8b** more readily and at a lower temperature than sodium methoxide. The less selective methoxide ion provided only a very low yield of the desired product **11b** (8% yield).

Cleavage of the aziridinyl ring in compound **8b** with HCl gas in absolute methanol afforded the 2-chloroethylamino derivative **12a** in a 35% yield (Scheme IV). The 2-methoxyethylamino derivative **12b** was also formed in this reaction by methanolysis of the aziridinyl ring (12.4% yield).

Treatment of the 5,6-dichloro derivatives **4** and **5** with anhydrous ammonia in absolute ethanol produced the 5-amino derivatives **9a** and **9b**, respectively (Scheme II). It was postulated that ammonia replaced the 5-chloro substituent of compounds **4** and **5** as did ethylenimine in the synthesis of the 5-aziridinyl-6-chloro derivative **8b** (Scheme II).

(25) X-ray crystallographic analyses were performed by R. Drake Eggleston and Dr. Derek Hodgson of the UNC-CH Chemistry Department, Chapel Hill, NC.
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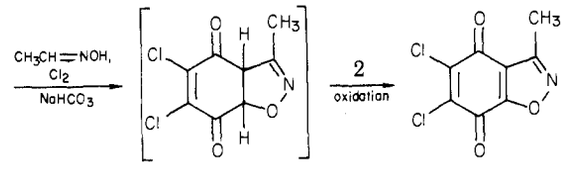
Scheme VII



17 18 (20%)

It proved essential to acetylate the 5-amino substituents of **9a** and **9b** (Schemes V and VI) if replacement of the 6-chloro group in either compound with ethylenimine was to be achieved.[29] Acetylation of compound **9a** produced two products, the monoacetylated derivative **13** (0.8% yield) and the diacetylated product **14** (10% yield) (Scheme V). Treatment of **14** with the ethylenimine at room temperature failed to give the desired 5-acetamido-6-aziridinyl-1-acetyl-1*H*-indazole-4,7-dione. Instead, the 1-acetyl group of compound **14** was hydrolyzed by ethylenimine, yielding compound **13** as a yellow precipitate. Further addition of ethylenimine or solvent (absolute ethanol) failed to produce the ethylenimine substitution product.

Acetylation of compound **9b** afforded the 5-acetamido-6-chloro derivative **15** in a 46% yield (Scheme VI). Substitution of the 6-chloro group with ethylenimine proceeded in moderate yield (22%), providing compound **16**.

A related heterocyclic quinone, 5,6-dichloro-3-methylbenzo[*d*]isoxazole-4,7-dione (**18**), was also selected as a potential lead compound for antitumor activity. Compound **18** was synthesized by the method of Entwistle et al.[22] via a 1,3-dipolar cycloaddition of acetonitrile oxide to 2,3-dichloro-*p*-benzoquinone (Scheme VII). Acetalhydroxamoyl chloride, obtained from the chlorination of acetaldoxime, generates acetonitrile oxide upon elimination of HCl in the presence of base ($NaHCO_3$). The intermediate cycloadduct **17** is oxidized by excess 2,3-dichloro-*p*-benzoquinone (**2**), providing compound **18**.

Antitumor Testing. All of the 5-aziridinyl-1*H*-indazole-4,7-diones and related derivatives were tested in the Ehrlich ascites carcinoma screen[30] at several doses (Table I). Ten out of the seventeen test compounds and the positive control compound, 6-mercaptopurine, exhibited significant inhibition of tumor growth (i.e., inhibition of tumor growth >80%) at their optimum dose. These compounds included all four of the 5,6-dichloro derivatives (**4–6** and **18**), all of the three 5-acetamido-6-chloro compounds (**13–15**), and two of the six aziridinyl-substituted derivatives (**8a** and **9**). The 5-[(2-chloroethyl)amino] derivative **12a** also demonstrated significant antitumor activity in this screen.

It should be noted here that in our experience, many compounds, especially alkylating agents, appear significantly active in the Ehrlich system as employed (>80% inhibition). However, those compounds that have shown activity in other tumor tests (P-388, Walker 256, and L-1210) have often been predicted by showing complete inhibition in the Ehrlich test (>95% inhibition). The Ehrlich system is known to be immunogenic, not highly discerning and productive of "false positives", however, and has been dropped by the National Cancer Institute for routine screening.

All of the significantly active test compounds in the Ehrlich ascites carcinoma screen possessed an alkylating functionality and a 6-chloro group. However, it has not been proven that these two features are sufficient for significant activity in this series of compounds, since compound **8b** possesses both features and yet is inactive at the doses tested. Surprisingly, aziridinyl ring opening of compound **8b** with HCl gas in absolute ethanol (Scheme V) yielded the active 5-[(2-chloroethyl)amino] derivative **12a**.

The significantly active compounds can be categorized into two groups based on their possible mechanism of alkylation: (1) 5- and/or 6-chloro-substituted 1*H*-indazole-4,7-diones, which are susceptible to nucleophilic replacement of a chlorine atom, and (2) aziridinyl-substituted derivatives and related compounds.

All four 5,6-dichloro compounds **4–6** and **18** were very active in the Ehrlich ascites carcinoma screen (98–100% inhibition of tumor growth), with 5,6-dichloro-3-methylbenzo[*d*]isoxazole-4,7-dione (**18**) exhibiting the highest activity (100% inhibition of tumor growth). The 5-acetamido-6-chloro compounds **13–15** were less active than the 5,6-dichloro compounds. Compound **13** was the least active compound of the three 5-acetamido-6-chloro derivatives (83% inhibition of tumor growth), and the 1-*N*-methyl compound **15** possessed intermediate activity (90% inhibition of tumor growth). The 1-*N*-acetyl derivative **14** was the most active 5-acetamido-6-chloro compound (96% inhibition of tumor growth). The added electron-withdrawing effect of the 1-*N*-acetyl group upon the 4-carbonyl moiety may increase the alkylating ability of compound **14** relative to the other 5-acetamido-6-chloro derivatives **13** and **15**.

The second category of active compounds, the aziridinyl-substituted 1(2)*H*-indazole-4,7-diones and related derivatives, is comprised of two 5-aziridinyl-6-chloro derivatives (**8a** and **10**) and 5-[(2-chloroethyl)amino]-6-chloro-1-methyl-1*H*-indazole-4,7-dione (**12a**). These three compounds are somewhat less active at their optimum dose (approximately 95% inhibition of tumor growth) than the 5,6-dichloro compounds (98–100% inhibition of tumor growth). Surprisingly, 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione (**8b**) was inactive. Compounds **12b**, **9a**, and **9b**, all of which lack an alkylating moiety, were not significantly active. Both the 5-aziridinyl-6-(thiopropyl) derivative **11a** and the 5-acetamido-6-aziridinyl derivative **16** were marginally active, exhibiting an inhibition of tumor growth of 78.5 and 78.0%, respectively.

The readily synthesized 5-aziridinyl-6-chloro derivatives **8a** and **8b** and their synthetic precursors **4** and **5** were selected for testing against the growth of P-388 lymphocytic leukemia cells in BDF_1 male mice.[31] These four compounds were tested at multiple doses (Table II). 5-Aziridinyl-6-chloro-1*H*-indazole-4,7-dione (**8a**) exhibited the highest activity of these four compounds with a % T/C of 145 at 1 (mg/kg)/day (% T/C > 125 was considered significant). Compounds **8b** and **5** were not active; however, 5,6-dichloro-1*H*-indazole-4,7-dione (**4**) exhibited a % T/C of 137 at 10 (mg/kg)/day. All other aziridinyl-substituted derivatives (i.e., compounds **9**, **11a,b**, and **16**) were tested in this P-388 lymphocytic leukemia screen at 1 (mg/kg)/day, the optimum dose of the active aziridinyl compound **8a**; however, no significant activity was observed for these compounds at this dose. Although 5-aziridinyl-6-chloro-1*H*-indazole-4,7-dione (**8a**) exhibited good activity in both the Ehrlich ascites carcinoma screen and

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Table I. Antitumor Activity of 1(2)*H*-Indazole-4,7-dione Derivatives in CF_1 Mice Bearing Ehrlich Ascites Carcinoma[a]

compd	dose, (mg/kg)/day	survival at day 9	ascrit, % packed cell vol	ascites vol, mL/mouse	% inhibn[b]
0.05% Tween 80		23/23	41.1[c]	2.8[d]	
5% abs EtOH in 0.05% Tween 80		42/42	40.0[e]	1.8[f]	
	1*H*-Indazole-4,7-diones				
5,6-dichloro (4)	5.0[i]	5/5	4.5	0.48	98.6
	6.0[j]	5/6	24.1	0.60	85.4
5-aziridinyl-6-chloro (8a)	0.5[j]	5/6	44.0	0.38	ia[g]
	1.0[i]	6/6	5.8	0.32	95.0
	5.0[j]	6/6	49.4	0.15	92.5
5-amino-6-chloro (9a)	1.0[i]	6/6	37.6	2.1	ia[g]
	5.0[j]	5/6	32.3	2.7	ia[g]
	8.0[j]	6/6	29.1	3.0	ia[g]
5-acetamido-6-chloro (13)	1.0[j]	6/6	40.0	3.6	ia[g]
	3.0[j]	6/6	38.1	3.6	ia[g]
	5.0[j]	6/6	20.2	0.33	83.2
	8.0[j]	5/6	30.8	1.8	46.9
5-acetamido-6-chloro-1-acetyl (14)	3.0[j]	5/6	45.5	2.9	ia[g]
	5.0[j]	4/6	5.9	0.30	95.6
	8.0[j]	4/6	31.7	0.75	25.5
	1-Methyl-1*H*-indazole-4,7-diones				
5,6-dichloro (5)	5.0[i]	6/6	9.7	0.20	98.2
	10.0[i]	3/6	3.0	0.27	99.5
5-aziridinyl-6-chloro (8b)	1.0[i]	6/6	35.2	2.0	ia[g]
	5.0[i]	6/6	41.4	2.5	5.8
	10.0[j]	5/6	37.3	3.4	ia[g]
5-aziridinyl-6-thiopropyl (11a)	1.0[j]	5/6	37.2	0.26	74.1
	5.0[i]	5/5	37.8	0.88	78.5
	10.0[j]	5/6	39.4	1.5	ia[g]
5-aziridinyl-6-methoxy (11b)	1.0[j]	6/6	39.8	4.2	ia[g]
	5.0[j]	6/6	34.6	0.70	39.4
	8.0[j]	6/6	36.2	0.32	64.1
	10.0[j]	6/6	30.7	2.2	ia[g]
5-[(2-chloroethyl)amino]-6-chloro (12a)	3.0[j]	5/6	46.3	3.7	ia[g]
	5.0[i]	5/5	37.8	0.20	95.1
	8.0[j]	6/6	36.8	0.58	32.9
5-[(2-methoxyethyl)amino]-6-chloro (12b)	5.0[j]	6/6	42.9	0.62	33.8
	8.0[j]	6/6	28.7	1.1	49.8
	10.0[j]	5/6	39.4	4.8	ia[g]
5-amino-6-chloro (9b)	3.0[j]	6/6	44.0	2.0	25.3
	5.0[j]	6/6	35.4	1.0	8.4
	8.0[j]	6/6	33.9	1.9	ia[g]
5-acetamido-6-chloro (15)	3.0[j]	6/6	20.0	1.7	67.8
	5.0[i]	5/5	29.0	0.56	90.0
	8.0[j]	5/6	37.9	0.62	61.7
5-acetamido-6-aziridinyl (16)	3.0[j]	6/6	41.4	3.2	ia[g]
	5.0[i]	5/5	37.4	0.90	78.3
	8.0[j]	5/6	38.6	1.2	25.8
	2-Methyl-2*H*-indazole-4,7-diones				
5,6-dichloro (6)	3.0[j]	6/6	1.6	0.17	99.2
	5.0[i]	6/6	3.2	0.60	94.9
	8.0[j]	2/6	0.0	0.0	100
5-aziridinyl-6-chloro (10)	1.0[i]	6/6	12.7	0.80	72.9
	5.0[i]	5/5	36.2	0.70	83.7
	10.0[j]	5/6	8.4	0.44	96.3
	12.0[j]	0/6			toxic
	3-Methylbenzo[*d*]isoxazole-4,7-dione				
5,6-dichloro (18)	3.0[j]	3/6	0.0	0.0	100
	5.0[i]	5/5	0.0	0.0	100
6-mercaptopurine[h]	50.0[i]	16/18	10.5	0.20	96.3

[a] Reference 30. [b] Percent inhibition >80% is considered significantly active. [c] Ascrit plus or minus standard deviation of the mean was 41.1 ± 7. [d] Ascites volume plus or minus standard deviation of the mean was 2.8 ± 0.3. [e] Ascrit plus or minus standard deviation of the mean was 40.0 ± 2.0. [f] Ascites volume plus or minus standard deviation of the mean was 1.8 ± 0.26. [g] ia = inactive. Observed inactivity at high dose levels (large fluid volumes) may have resulted from peritoneal irritation produced by the compound. [h] Sigma Chemical Co. [i] 0.05% Tween 80 was used as vehicle for test compound and control. [j] 5% absolute EtOH in 0.05% Tween 80 was used as vehicle for test compound and control.

the P-388 lymphocytic leukemia screen, this series of 5,6-disubstituted 1(2)*H*-indazole-4,7-diones does not appear to possess the highly potent agents that would stimulate further exploration.

Experimental Section

Chemical Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Precoated TLC plates (5 × 10 cm) of silica gel 60 F-254

Table II. Antitumor Activity of 1(2)*H*-Indazole-4,7-dione Derivatives in BDF_1 Mice Bearing Lymphocytic Leukemia P-388

compd (N = 5)[a]	dose,[b] (mg/kg)/day	av days survival[c]	% T/C[d]
1*H*-Indazole-4,7-diones			
5,6-dichloro (4)	0.5	10.5/9.2	114
	1.0	11.0/9.2	120
	5.0	10.9/9.2	118
	10.0	12.6/9.2	137
	12.0	9.0/9.2	98
	15.0	9.2/9.2	104
	20.0	3.6/9.2	39
5-aziridinyl-6-chloro (8a)	0.5	10.5/9.2	114
	1.0	13.3/9.2	145
	2.0	12.6/9.2	137
	3.0	10.2/9.2	111
	5.0	11.0/9.2	120
	10.0		toxic
1-Methyl-1*H*-indazole-4,7-diones			
5,6-dichloro (5)	1.0	10.2/9.2	111
	5.0	9.4/9.2	102
	10.0	9.2/9.0	102
	20.0	8.0/9.2	87
5-aziridinyl-6-chloro (8b)	1.0	9.6/9.0	107
	5.0	8.8/9.2	96
	10.0	6.4/9.2	70
	20.0	4.8/9.2	52
	30.0	3.0/9.2	33
5-aziridinyl-6-(thiopropyl) (11a)	1.0	9.4/9.2	102
5-aziridinyl-6-methoxy (11b)	1.0	10.0/9.2	109
5-acetamido-6-aziridinyl (16)	1.0	10.0/9.2	109
2-Methyl-2*H*-indazole-4,7-diones			
5,6-dichloro (6)	1.0	10.6/9.2	115
5-aziridinyl-6-chloro (10)	1.0	10.8/9.2	117
3-Methylbenzo[*d*]isoxazole-4,7-dione			
5,6-dichloro (18)	1.0	10.2/9.2	111
5-fluorouracil[e]	25	17.6/9.5	186

[a] N = number of mice per test group. [b] 0.05% Tween 80 was used as the vehicle for test compounds and control. [c] Average days survived for treated/average days survived for control. [d] % T/C > 125% is considered significantly active. No significant weight loss vs. control was observed for animals in this experiment. [e] Sigma Chemical Co.

(layer thickness 0.25 mm) from E. M. Reagents were used for TLC analysis and the spots were detected with a Mineralight (UV, shortwave). The preparative TLC plates used for separations were Analtech Uniplates precoated with silica gel GF (1000-μm thick). Column chromatography was accomplished with silica gel 60 (70–230 mesh ASTM) from E. M. Reagents. Elemental Analyses were performed by Atlantic Microlab, Inc., Atlanta, GA and were within ±0.4% of the theoretical values. Mass spectra were obtained from the Research Triangle Institute Mass Spectrometry Laboratory, Research Triangle Park, NC, with electron impact for ionization (EIMS in the text denotes electron-impact mass spectrum). X-ray crystallographic studies were performed by Hodgson and Eggleston of the UNC-CH Department of Chemistry, Chapel Hill, NC.[25] ORTEP drawings[26] were used to depict the molecular structures. Infrared spectra were obtained from a Perkin-Elmer Model 297 infrared spectrophotometer. The UV absorption spectra were determined with a Cary 15 spectrophotometer. Routine proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a JEOL JNM-FX60 spectrometer (59.75 MHz), unless otherwise indicated. In all ^{1}H NMR spectra, tetramethylsilane was used as an internal reference for determining chemical shifts on the δ scale. Chemical shifts quoted in the case of multiplets are measured from the approximate center unless otherwise indicated, and the abbreviations used in the descriptions of the NMR spectra are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. All solvents used were reagent grade, and all starting materials were used as obtained from the suppliers unless otherwise indicated.

2,3-Dichloro-*p*-benzoquinone (2). Method A. Clark's Oxidation.[32] As a modification of Clark's oxidation procedure, a suspension of 250 g of MnO_2 (80% native or artificial powder) and 25.0 g of 2,3-dichloro-1,4-hydroquinone[32] (0.07 mol) in 1 L of 9 N H_2SO_4 was steam distilled. Recrystallization of the yellow solid from the distillate in absolute EtOH yielded 8.2 g of 2,3-dichloro-*p*-benzoquinone (**2**; 66% yield), mp 97–100 °C (lit.[32] mp 100–101 °C).

Method B. Chromic Acid Oxidation.[33] The method used was a modification of the procedure of Vliet.[33] 2,3-Dichloro-1,4-hydroquinone[32] (40 g, 0.22 mol.) was mostly dissolved in 1.5 L of distilled H_2O by warming to 50 °C. After cooling the stirred suspension to 20 °C, 14 mL of concentrated H_2SO_4 was added, followed by the dropwise addition of 35.0 g of $Na_2Cr_2O_7$ in 20 mL of distilled H_2O, keeping the reaction temperature below 30 °C. The dark greenish-yellow suspension was stirred for 1 h at room temperature, then cooled, to 5 °C, and filtered. The yellow solid was taken up in hot benzene, and the benzene-soluble portion was decanted. The benzene layer was dried over $MgSO_4$ and filtered, and the filtrate was evaporated in vacuo to a green solid. Recrystallization in absolute EtOH yielded 28 g of crystalline yellow product (**2**; 71% yield): mp 99–102 °C (lit.[32] mp 100–101 °C); TLC R_f 0.5 ($CHCl_3$); IR (Nujol) 3060 weak (=CH), 1675 (strong, conj C=O), 1565 (strong, conj C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.0 (s, 2, 5-H and 6-H).

5,6-Dichloro-1*H*-indazole-4,7-dione (4). As a modification of the procedure used by Eistert et al.,[20] 64 mL of 0.3 M cold ethereal diazomethane[34] (0.02 mol) was added dropwise to a cold (0–5 °C), stirred suspension of 3.5 g of 2,3-dichloro-*p*-benzoquinone (**2**; 0.02 mol) in 40 mL of anhydrous ethyl ether. The resulting brown suspension was stirred at 5 °C for 20 min, followed by stirring overnight (10 h) at room temperature (behind a protective shield). The brown solid was filtered behind the shield: yield 2.9 g. Recrystallization from Me_2SO gave 1.6 g of yellow solid (37% yield): mp >290 °C (lit.[20] mp 282 °C dec); TLC R_f 0.4 ($CHCl_3$/MeOH, 10:1); IR (Nujol) 3260 (broad, N-H), 1678 (strong, conj C=O) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 8.63 (s, 1, 3-H). Anal. ($C_7H_2Cl_2N_2O_2$) C, H, Cl, N.

5,6-Dichloro-1-methyl-1*H*-indazole-4,7-dione (5) and 5,6-Dichloro-2-methyl-2*H*-indazole-4,7-dione (6). A modification of the procedure described by Entwistle et al.[22] was used to prepare the 5,6-dichloro-1-*N*-methyl derivative (**5**). The 5,6-dichloro-2-*N*-methyl derivative (**6**) was found to be a minor product not described in the work of Entwistle et al.[22] Behind a safety shield and under fume hood, 2,3-dichloro-*p*-benzoquinone (**2**; 7.7 g, 0.04 mol) was added portionwise to 230 mL of cold (0–5 °C), stirred 0.5 ethereal diazomethane solution[36] (0.1 mol). The reaction temperature was kept below 10 °C during the addition of the quinone. The yellow reaction suspension was stirred for 3 h in an ice bath and filtered behind a protective shield. The yellow product (2.5 g) was chromatographed on a column of silica gel (80 g), eluted with $CHCl_3$. The first compound eluted was the 5,6-dichloro-1-*N*-methyl derivative (**5**; 1.6 g, 16% yield), which was recrystallized from absolute EtOH: mp 178–180 °C (lit.[22] mp 176–177 °C); TLC R_f 0.56 ($CHCl_3$); IR (Nujol) 1680 (strong, conj C=O) cm^{-1}; ^{1}H NMR (acetone-d_6) δ 4.24 (s, 3, CH_3), 8.00 (s, 1, 3-H). Anal. ($C_8H_4Cl_2N_2O_2$) C, H, Cl, N. The second compound eluted from the column was the 5,6-dichloro-2-*N*-methyl derivative

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(33) Vliet, E. B. In "Organic Syntheses", 2nd ed.; Gilman, H., Ed.; Wiley: New York, 1941; Collect Vol. I, p 482.
(34) The solution of 0.3 M diazomethane in ethyl ether was generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide according to the procedure of DeBoer and Backer.[21] The ethereal diazomethane was titrated according to the procedure of Arndt.[35]
(35) Arndt, F. In "Organic Syntheses"; Blatt, A. H., Ed.; Wiley: New York, 1943; Collect. Vol. II, p 165.
(36) The diazomethane was generated according to the procedure in Note 3 of ref 35 and dried over KOH pellets for 1 h prior to use. The solution was titrated according to note 1 of ref 35 by using benzoic acid and 0.2 N NaOH.

(6; 0.26 g, 3% yield): mp 260–264 °C; TLC R_f 0.35 ($CHCl_3$); IR (Nujol) 1690, 1676 (strong, conj C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.05 (s, 3, CH_3), 8.63 (s, 1, 3-H); EIMS, m/e 230 (M^+). Anal. ($C_8H_4Cl_2N_2O_2$) C, H, N.

5,6-Dichloro-2-methyl-2*H*-indazole-4,7-dione (6) via 3-Methylsydnone Cycloaddition. The method of Brockmann and Reschke[24] was employed. A solution of 3.5 g of 2,3-dichloro-*p*-benzoquinone (2; 0.02 mol) and 2.0 g of 3-methylsydnone[37,38] (0.02 mol) in 20 mL of peroxide-free dioxane[39] was refluxed for 3 days. The reaction mixture was filtered to remove a black residue. The dioxane filtrate yielded a black liquor upon evaporation. This liquor was adsorbed onto 5.0 g of silica gel, placed on a 90-g silica gel column, and eluted with $CHCl_3$. The first major eluate was a brown solid. The solid was boiled in absolute EtOH, cooled, and filtered, and the filtrate was washed with absolute EtOH: yield 0.2 g of yellow 5,6-dichloro-2-methyl-2*H*-indazole-4,7-dione (6; 7% yield): mp 265–268 °C; TLC R_f 0.36 ($CHCl_3$/EtOAc, 85:15); IR (Nujol) 1690, 1676 (strong, conj C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.04 (s, 3, CH_3), 8.63 (s, 1, 3-H). Anal. ($C_8H_4Cl_2N_2O_2$) C, H, N.

5-Aziridinyl-6-chloro-1*H*-indazole-4,7-dione (8a). The method of Entwistle et al.[22] was modified in this procedure. 5,6-Dichloro-1*H*-indazole-4,7-dione (4; 1.0 g, 5.0 mmol) was boiled in 400 mL of absolute EtOH to dissolve most of the starting material. The stirred suspension was than slowly cooled to 40 °C, and ethylenimine[40] (2.0 mL, 39 mmol) was added dropwise. The starting material (4) dissolved, giving an orange solution from which the light orange product (8a) precipitated upon cooling to room temperature (0.35 g, 35% yield): mp >290 °C; TLC R_f 0.3 ($CHCl_3$/EtOAc, 1:4); IR (Nujol) 1682, 1670 (strong, conj C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.50 (s, 4, aziridinyl H), 8.49 (s, 1, 3-H). Anal. ($C_9H_6ClN_3O_2$) C, H, Cl, N.

5-Aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione (8b). A modification of the method of Entwistle et al.[22] was used in this synthesis. 5,6-Dichloro-1-methylindazole-4,7-dione (5; 6.2 g, 0.027 mol) was dissolved in 660 mL of absolute EtOH by warming the suspension to 70 °C. After the solution was cooled to 50 °C and before a large amount of the starting material precipitated, 9.0 mL of ethylenimine[40] (0.17 mol) was added dropwise with stirring of the solution. After completion of the ethylenimine addition, an orange precipitate formed on cooling to room temperature. The orange suspension was stirred for 45 min in an ice bath and filtered, and the orange product was air-dried (8b; 5.1 g, 80% yield), mp 145–148 °C. Recrystallization (absolute EtOH) gave mp 147–152 °C; TLC R_f 0.4 ($CHCl_3$/EtOAc, 10:1); IR (Nujol) 1678, 1657 (strong, conj C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.57 (s, 4, aziridinyl H), 4.21 (s, 3, CH_3), 7.83 (s, 1, 3-H). Anal. ($C_{10}H_8ClN_3O_2$) C, H, Cl, N. An X-ray crystallographic analysis[25] unequivocally proved the product (8b) to be 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione, as visualized clearly in an ORTEP drawing[25] of the molecular structure.

5-Aziridinyl-6-chloro-2-methyl-2*H*-indazole-4,7-dione (10). A modification of the procedure described by Entwistle et al.[22] was employed. 5,6-Dichloro-2-methyl-2*H*-indazole-4,7-dione (6; 0.3 g, 1.3 mmol) was suspended in 150 mL of absolute EtOH and warmed to 55 °C with stirring, and the suspension was allowed to cool slowly. Ethylenimine[40] (0.7 mL or 0.58 g, 14 mmol) was added dropwise when the suspension temperature had cooled to 50 °C. Addition of the ethylenimine imparted an orange color to the suspension, and most of the solid dissolved. The desired orange product (10) precipitated upon cooling to room temperature (0.2 g, 65% yield): mp 210–212 °C; TLC R_f 0.25 ($CHCl_3$/EtOAc, 75:25); IR (Nujol); 1670 (broad, strong, conj C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.50 (s, 4, aziridinyl H), 3.98 (s, 3, CH_3), 8.49 (s, 1, 3-H). Anal. ($C_{10}H_8ClN_3O_2$) C, H, N.

5-Aziridinyl-6-(thiopropyl)-1-methyl-1*H*-indazole-4,7-dione (11a). This procedure was adapted from the method of Khan and Driscoll.[11] Sodium metal (0.06 g, 2.7 mmol) was added to a solution of thiopropane (0.28 mL, 3.0 mmol) in 40 mL of dried absolute MeOH. After dissolution of the sodium metal, the solution was cooled in a dry ice–acetone bath (−77 °C) and 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione (8b; 0.4 g, 2.0 mmol) was added. The resultant orange suspension was warmed slowly, facilitating the dissolution of the solid. While a purple solid formed, the suspension was allowed to warm to 0 °C. The purple product was collected by filtration, washed with H_2O, and air-dried. Recrystallization (absolute MeOH) yielded 0.23 g (49% yield): mp 99–101 °C; TLC R_f 0.38 ($CHCl_3$/EtOAc, 10:1); IR (Nujol) 1662 (strong, conj C=O), 1648 (medium, conj C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (t, 3, J = 6.0 Hz, $CH_3CH_2CH_2S$), 1.53 (m, 2, $CH_3CH_2CH_2S$), 2.58 (s, 4, aziridinyl H), 2.88 (t, 2, $CH_3CH_2CH_2S$), 4.21 (s, 3, CH_3N), 7.82 (s, 1, 3-H). Anal. ($C_{13}H_{15}N_3O_2S$) C, H, N.

5-Aziridinyl-6-methoxy-1-methyl-1*H*-indazole-4,7-dione (11b). A modification of the procedure of Khan and Driscoll[11] was employed. Sodium (0.19 g, 8.0 mmol) was dissolved in 100 mL of dried absolute MeOH at room temperature. The stirred methoxide solution was cooled in a dry ice–acetone bath, and 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione (8b; 0.6 g, 2.5 mmol) was added. The orange suspension was allowed to warm to room temperature and stirred overnight. The resulting red solution was evaporated in vacuo to a dark red residue, taken up in 300 mL of distilled H_2O, and extracted with $CHCl_3$ (2 × 250 mL). The $CHCl_3$ extracts were dried ($MgSO_4$) and filtered, and the filtrate was evaporated to a red solid (0.7 g). Chromatography on a silica gel column (9 g, $CHCl_3$ eluent) yielded the desired red product. Recrystallization from absolute MeOH gave 0.05 g (8% yield): mp 173–175 °C; TLC R_f 0.33 ($CHCl_3$/EtOAc, 10:1); IR (KBr) 1658 (shoulder, strong), 1648 (strong, conj C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.26 (s, 4, aziridinyl H), 3.94 (s, 3, CH_3O), 4.18 (s, 3, CH_3N), 7.80 (s, 1, 3-H). Anal. ($C_{11}H_{11}N_3O_3$) C, H, N.

5-[(2-Chloroethyl)amino]- (12a) and 5-[(2-Methoxyethyl)amino]-6-chloro-1-methyl-1*H*-indazole-4,7-dione (12b). The procedure was adapted from the method of Kahn and Driscoll.[11] A stirred suspension of 5-aziridinyl-6-chloro-1-methyl-1*H*-indazole-4,7-dione (8b; 0.5 g, 2.1 mmol) in 50 mL of absolute MeOH was treated with anhydrous HCl gas for 15 min, keeping the temperature at 22–27 °C by means of an ice bath. After stirring for 3 h at room temperature, the red solution was concentrated on a rotary evaporator to 20 mL. After the solution was cooled, the resultant red solid (0.2 g) was collected and air-dried. Additional product was obtained from the filtrate by neutralization (5% $NaHCO_3$) and extraction (ethyl ether, 3 × 150 mL) to yield a red solid (0.3 g). Both products were combined and column chromatographed (20 g of silica gel, $CHCl_3$ eluent). The first compound from the column was 5-[(2-chloroethyl)-amino]-6-chloro-1-methyl-1*H*-indazole-4,7-dione (12a; 0.23 g, 35% yield). The product was recrystallized from absolute MeOH: mp 126–129 °C; TLC R_f 0.29 ($CHCl_3$/EtOAc, 10:1); IR (Nujol) 3290 (strong (N–H), 1680 (strong, conj C=O), 1642 (medium, conj C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.75 (t, 2, J = 5.5 Hz, CH_2N), 4.10 (t, 2, J = 5.5 Hz, CH_2Cl), 4.20 (s, 3, CH_3N), 6.42 (very broad, 1, N-H), 7.85 (s, 1, 3-H). Anal. ($C_{10}H_9Cl_2N_3O_2$) C, H, N. The second compound collected was 5-[(2-methoxyethyl)amino]-6-chloro-1-methyl-1*H*-indazole-4,7-dione (12b; 0.07 g, 12.4% yield): mp 105–107 °C; TLC R_f 0.14 ($CHCl_3$/EtOAc, 10:1); IR (Nujol) 3245 (medium, N-H), 1690 (strong, conj C=O), 1637 (medium, conj C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.42, (s, 3, CH_3O), 3.60 (t, 2, J = 4.5 Hz, CH_2N), 4.03 (t, 2, J = 4.5 Hz, OCH_2), 4.21 (s, 3, CH_3N), 6.50 (very broad, 1, N-H), 7.82 (s, 1, 3-H). Anal. ($C_{11}H_{12}CLN_3O_3$) C, H, N.

5-Amino-6-chloro-1*H*-indazole-4,7-dione (9a). Ammonia gas was bubbled vigorously into a stirred suspension of 5,6-dichloro-1*H*-idazole-4,7-dione (4; 10.6 g, 0.046 mol) in absolute EtOH (1 L) at room temperature. The yellow starting material afforded a red solution, which spontaneously warmed to a maximum temperature of 40 °C. After the solution was stirred for 7 h at room temperture, a dark purple residue (M_r = 358 by MS) was collected by filtration. The desired red product (9a) was obtained from the filtrate by evaporating the solvent, slurrying in 150 mL

(37) 3-Methylsydnone was synthesized by the method of Hammick and Voaden;[38] however, the 3-methylsydnone was used after in vacuo removal of acetic anhydride without further purification. ^1H NMR and IR indicated correct product.

(38) Hammick, D. L.; Voaden, D. J. *J. Chem. Soc.* **1961**, 3033.

(39) Peroxides were removed from the dioxane by elution through a column of activated neutral alumina.

(40) Allen, C. F. H.; Spangler, F. W.; Webster, F. R. In "Organic Syntheses", Rabjohn, N., Ed.; Wiley: New York, 1963; Collect. Vol. IV, p 433.

of H_2O, filtering, and then drying the collected solid in a vacuum oven (55 °C, 10 mmHg) over NaOH pellets: yield 2.2 g (24%) mp >290 °C; TLC R_f 0.2 ($CHCl_3$/MeOH, 10:1); IR (Nujol) 3465 (medium, N-H asymmetric), 3362 (medium, N-H symmetric), 3250 (broad, medium, H-bounded enol O-H and N-H), 1688 (medium, conj C=O), 1590 (broad, strong, H-bonded, conj C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.34 and 7.55 (2 br s, 2, N-H_2 of the two enol forms), 8.36 and 8.55 (2 s, 1, 3-H of the two enol forms), 14.22 (broad peak, 1, enol O-H). Anal. ($C_7H_4ClN_3O_2$) C, H, N.

5-Acetamido-6-chloro-1*H*-indazole-4,7-dione (13) and 5-Acetamido-6-chloro-1-acetyl-1*H*-indazole-4,7-dione (14). Five drops of concentrated H_2SO_4 was added to a cold (0–5 °C), stirred suspension of 5-amino-6-chloro-1*H*-indazole-4,7-dione (**9a**; 2.0 g, 10.0 mmol) in acetic anhydride (100 mL). While the solution was stirred for 3 h at 0 °C, the solid starting material slowly dissolved. After the solution was left standing at 0 °C overnight, a yellow solid (0.05 g) was collected, and the filtrate was poured into ice–water (900 mL). The aqueous mixture was extracted with $CHCl_3$ (2 × 600 mL), followed by extraction of the combined $CHCl_3$ layers with 5% aqueous $NaHCO_3$ (300 mL). The $CHCl_3$ extract was dried over $MgSO_4$ and filtered, and the filtrate was evaporated to a yellow oily solid. Column chromatography of the yellow solid on silica gel (65 g) was accomplished by elution with $CHCl_3$, followed by $CHCl_3$/EtOAc (20:1), when the first yellow band had been eluted one-fourth of the column length (column length, 16 in.; diameter, 0.75 in.). The first solid eluted was recrystallized from absolute EtOH, giving the yellow product, 5-acetamido-6-chloro-1-acetyl-1*H*-indazole-4,7-dione (**14**; 0.28 g, 10% yield): mp 212–216 °C; TLC R_f 0.26 ($CHCl_3$/MeOH, 10:1); IR (Nujol) 3255 (broad, medium, H-bonded N-H), 1746 (strong, 1-acetyl C=O), 1690 (strong, 5-acetamido C=O), 1673 (strong, conj C=O cm^{-1}; ^1H NMR (CD_3OD) δ 6.33 (s, 6, both CH_3C=O), 8.40 (s, 1, 3-H). Anal. ($C_{11}H_8ClN_3O_4$) C, H, N. The second yellow compound that was eluted was taken up in $CHCl_3$, boiled, and filtered hot. The undissolved solid collected was recrystallized (absolute MeOH), yielding yellow crystals (0.02 g, 0.8% yield), mp 250–253 °C. This product is 5-acetamido-6-chloro-1*H*-indazole-4,7-dione (**13**) and is identical with the precipitate filtered from the reaction mixture originally: TLC R_f 0.11 ($CHCl_3$/MeOH, 10:1); IR (Nujol) 3255 (very broad, medium, H-bonded N-H and enol O-H), 1672 (strong, conj C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.11 (s, 3, CH_3), 8.66 (s, 1, 3-H), 10.02 (s, 1, enol O-H). Anal. ($C_9H_6ClN_3O_3$) C, H, N.

5-Amino-6-chloro-1-methyl-1*H*-indazole-4,7-dione (9b). This method was adapted from the procedure of Entwistle et al.[22] A stirred suspension of 5,6-dichloro-1-methyl-1*H*-indazole-4,7-dione (**5**; 2.0 g, 8.6 mmol) in 225 mL of absolute EtOH was saturated with anhydrous ammonia gas at room temperature. The yellow suspension spontaneously warmed to a maximum temperature of 42 °C, and the starting material was dissolved, giving a red solution. A red solid precipitated and the saturation with ammonia gas was terminated. After cooling to room temperature, the product (**9b**) was collected by filtration, washed with absolute EtOH, and air-dried (1.5 g, 82% yield). Recrystallization from absolute EtOH gave pure product, mp 277–281 °C (lit.[22] mp 285–286 °C); TLC R_f 0.29 ($CHCl_3$/EtOAc, 85:15); IR (Nujol) 3410 (sharp, medium, N-H), 3295 (broad, medium, H-bonded N-H), 1691 (medium, conj C=O), 1625 (strong, H-bonded conj C=O), 1612 (strong, conj C=C) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.12 (s, 3, CH_3-N), 7.70 (very broad, 2, NH_2), 7.98 (s, 1, 3-H). Anal. ($C_8H_6ClN_3O_2$) C, H, N.

5-Acetamido-6-chloro-1-methyl-1*H*-indazole-4,7-dione (15). A modification of the procedure of Fieser and Martin[29] was employed. Three drops of concentrated H_2SO_4 was added slowly to a stirred suspension of 5-amino-6-chloro-1-methyl-1*H*-indazole-4,7-dione (**9b**; 1.5 g, 7.0 mmol) in 25 mL of acetic anhydride. The maximum reaction temperature was 30 °C. After stirring for 30 min, the yellow suspension was poured into ice–water (600 mL) and extracted with $CHCl_3$ (4 × 300 mL). The $CHCl_3$ extract was dried over $MgSO_4$ and filtered, and the filtrate was rotary evaporated to a volume of 20 mL. After standing for 2 days, the yellow solid product (**15**) was collected by filtration, giving 1.3 g. Recrystallization from absolute EtOH yielded yellow plate-like crystals (0.83 g, 46% yield): mp 236–239 °C; TLC R_f 0.17 ($CHCl_3$/EtOAc, 85:15); IR (Nujol) 3240 (broad, medium, N-H), 1688 (strong, 5-acetamido C=O), 1670 (strong, conj C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (s, 3, CH_3C=O), 4.25 (s, 3, CH_3-N), 7.91 (s, 1, 3-H). Anal. ($C_{10}H_8ClN_3O_3$) C, H, N.

5-Acetamido-6-aziridinyl-1-methyl-1*H*-indazole-4,7-dione (16). A stirred suspension of 5-acetamido-6-chloro-1-methyl-1*H*-indazole-4,7-dione (**15**; 0.40 g, 1.6 mmol) in absolute EtOH (125 mL) was treated dropwise with 1.0 mL (19 mmol) of ethylenimine.[40] The resulting suspension was stirred overnight. The red reaction mixture was filtered to remove a small amount of starting material, and then the filtrate was vacuum distilled with no heat by using an aspirator. Column chromatography of the red residue on silica gel (40 g, $CHCl_3$ eluent) yielded a red product after a small amount of starting material had been eluted. Recrystallization from absolute MeOH afforded 5-acetamido-6-aziridinyl-1-methyl-1*H*-indazole-4,7-dione (**16**; 0.09 g, 22% yield): mp 184–186 °C; TLC R_f 0.06 ($CHCl_3$/EtOAc, 70:30); IR (Nujol) 3360 (weak, N-H), 3220 (broad, medium, H-bonded N-H), 1690 (strong, 5-acetamido C=O), 1665, 1650 (strong, conj C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (s, 3, CH_3C=O), 2.28 (s, 4, aziridinyl H), 4.19 (s, 3, CH_3-N), 7.53 (br s, 1, N-H), 7.78 (s, 1, 3-H). Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

5,6-Dichloro-3-methylbenzo[*d*]isoxazole-4,7-dione (18). The procedure of Entwistle et al. was employed.[22] Chlorine gas (5.4 g, 0.076 mol) was bubbled into a solution of acetaldoxime (4.5 g, 0.077 mol) and $NaHCO_3$ (12.6 g, 0.15 mol) in 200 mL of distilled H_2O at 0 °C over a period of 30 min. Carbon dioxide gas evolved, and a green color developed. To this solution was added, in small portions, finely powdered 2,3-dichloro-*p*-benzoquinone (**2**; 6.7 g, 0.38 mol) over a period of 15 min at 0 °C. During the quinone addition, the light green color disappeared from the solution as a green solid adhered to the walls of the reaction flask. After the green mixture was stirred for 2 h at 0–5 °C, the reaction mixture was extracted with $CHCl_3$ (3 × 100 mL), and the green $CHCl_3$ extracts were dried over $MgSO_4$ and filtered, and the filtrate was evaporated to a yellow solid. Recrystallization (95% EtOH) yielded the starting material, 2,3-dichloro-*p*-benzoquinone (**2**; 2.2 g), mp 95–99 °C. A second crop from the recrystallization filtrate yielded a yellow solid (0.60 g), mp 185–200 °C. Three recrystallizations in 95% EtOH gave pure product (**18**; 273 mg, 2.2% yield): mp 210–213 °C; TLC R_f 0.6 (hexane/ethyl ether, 70:30); IR (KBr) 1704 (shoulder), 1685, 1675 (strong, conj C=O), 1610 (medium, conj C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.64 (s, 3, CH_3). Anal. ($C_8H_3Cl_2NO_3$) C, H, N.

Methods of Antitumor Testing. Test compounds were homogenized in 0.05% poly(oxyethylene)sorbitan monooleate (i.e., Tween 80) or 5% absolute EtOH, USP in a 0.05% Tween 80 solution (1 mL of absolute ethanol in 19 mL of 0.05% Tween 80). The 5% absolute EtOH in 0.05% Tween 80 was used as vehicle to improve compound solubility. Compound solutions were administered intraperitoneally (ip).

Ehrlich Ascites Carcinoma Screen.[30] Male CF_1 mice (22–25 g) were injected ip with 2 × 10^6 Ehrlich carcinoma cells on day 0. Injectible solutions of the test compounds were administered ip on days 1 through 8. Mice were sacrificed on day 9. In each test group, the ascites fluid volume per mouse and the ascrit (packed cell volume) were determined.[30] Results were expressed as percent inhibition of tumor cell growth as calculated according to the following equation:

$$\% \text{ inhibn of tumor growth} = 100 - \left[\frac{(\text{vol of treated})(\text{ascrit of treated})}{(\text{vol of control})(\text{ascrit of control})}\right]$$

6-Mercaptopurine was used as the positive control compound. An inhibition of tumor growth greater than 80% was considered significant.

P-388 Lymphocytic Leukemia Screen. On day 0, male BDF_1 mice (20–23 g) were injected ip with 1 × 10^6 P-388 lymphocytic leukemia cells as described in the NIH protocol 1.2.[31] Injectible solutions of the test compounds were administered ip once daily on days 1 through 14 (instead of days 1–9 as described in the NIH protocol).[31] 5-Fluorouracil was used as the positive control compound. Five mice per test group were used and % T/C values greater than 125 were considered significantly active.

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Registry No. 2, 5145-42-6; 4, 56054-64-9; 5, 34670-79-6; 6, 85004-79-1; 7, 6939-12-4; 8a, 85004-80-4; 8b, 85004-81-5; 9a, 85004-82-6; 9b, 34670-89-8; 10, 85004-83-7; 11a, 85004-84-8; 11b, 85004-85-9; 12a, 85004-86-0; 12b, 85004-87-1; 13, 85004-88-2; 14, 85004-89-3; 15, 85004-90-6; 16, 85004-91-7; 18, 34670-86-5; diazomethane, 334-88-3; ethylenimine, 151-56-4; acetaldoxime, 107-29-9.

Bleomycin Analogues. Phenylthiazole Models of the Bithiazole Moiety of Bleomycin A_2

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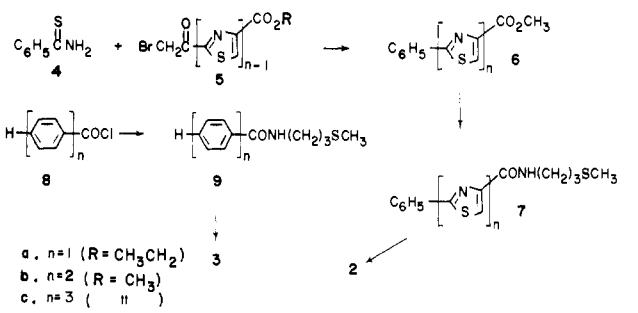
Amides of 2-phenylthiazole-4-carboxylic acid, 2′-phenyl-2,4′-bithiazole-4-carboxylic acid and 4,4′-biphenylcarboxylic acid containing the (3-aminopropyl)dimethylsulfonium chloride side chain of bleomycin A_2 have been prepared and their binding to poly(dA-dT) has been studied by proton nuclear magnetic resonance spectroscopy. Both the phenylthiazole and phenylbithiazole derivatives exhibit high-field shifts for their hydrogens in the presence of the polynucleotide which are considerably larger than those observed in the analogous completely heterocyclic systems studied previously (Sakai, T. T.; Riordan, J. M.; Glickson, J. D. *Biochemistry* **1982**, *21*, 805). The intercalative nature of the binding of these analogues was further indicated by viscometric measurements using calf thymus DNA. The data show that a phenyl ring allows the aromatic systems to interact with the base pairs of the polynucleotide to a greater extent than a thiazole ring in the same position. Possible models for the interaction of these derivatives with DNA are considered. The hydrogens of the biphenyl derivative show an interaction that is substantially less than those observed in the heterocycle-containing systems, suggesting that the ring system is oriented improperly or that the ring system is nonplanar. The analogous phenyl (benzoyl) compound does not bind, showing the requirement for an extended aromatic system for intercalation. The utility of these observations for the design of possible synthetic analogues of the bleomycins is discussed. None of the derivatives exhibited toxicity when tested against L1210 leukemia cells in culture.

Previous studies from this laboratory[1] have shown that the DNA binding region of the antitumor antibiotic bleomycin A_2 resides in the cationic terminal dipeptide of the molecule (Figure 1). This association of the drug with DNA is believed to be a requisite for subsequent (or simultaneous) reactions that degrade the nucleic acid, a process that appears to be responsible for the biological activity of the drug.[2] It has also been demonstrated that the interaction of analogues of this cationic terminus with poly(dA-dT) can be modified by the choice of substituents on the aromatic bithiazole systems.[3] In those studies, amides of 2,4′-bithiazole-4-carboxylic acid (**1b**) and of 2,4′:2′,4″-terthiazole-4-carboxylic acid (**1c**) (Figure 2) containing the (3-aminopropyl)dimethylsulfonium chloride side chain of bleomycin A_2 were found to intercalate in the nucleic acid to a greater extent than 2′-alkyl-substituted 2,4′-bithiazole derivatives structurally more like the parent compound. Introduction of the third aromatic ring markedly enhanced the interaction with poly(dA-dT). As a part of ongoing studies on synthetic fragments and analogues of bleomycin A_2, we have sought to prepare derivatives of the DNA binding region that retained the enhanced intercalative properties of derivatives such as **1b** and **1c** but which might be more amenable to chemical modification than the bithiazole system. To this end, several phenyl derivatives of the thiazole systems have been prepared, and the interaction of these analogues with poly(dA-dT) has been studied by proton nuclear magnetic resonance spectroscopy and viscometry.

The desired derivatives were prepared by using precursors described previously for the synthesis of the thiazole derivatives.[1a,3b] Thiobenzamide (4) was condensed with ethyl bromopyruvate (**5a**), methyl 2-(2-bromoacetyl)thiazole-4-carboxylate (**5b**), or methyl 2′-(2-bromoacetyl)-2,4′-bithiazole-4-carboxylate (**5c**) in anhydrous *N,N*-dimethylformamide (Scheme I) to form the phenylthiazole (**6a**), phenylbithiazole (**6b**), or phenylterthiazole (**6c**) ester, respectively, in good yield. Aminolysis of the esters with neat 3-(methylthio)propylamine gave the amides in high yield.

Scheme I



For the phenyl and biphenyl derivatives, benzoyl chloride (**8a**) or 4-phenylbenzoyl chloride (**8b**) (prepared from 4,4′-biphenylcarboxylic acid and thionyl chloride) was treated with 3-(methylthio)propylamine in anhydrous dioxane containing triethylamine to give the amides (**9a,b**) in good yield.

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