(C=O, carbamate), 1595 cm^{-1} (C=O at C-9).

Anal. Calcd for $C_{20}H_{23}N_3O_4S$: C, 59.83; H, 5.78; N, 10.46. Found: C, 58.08; H, 5.97; N, 9.32.

In another run, 110 mg (0.172 mmol) of **22** and 0.6 mL (0.6 mmol) of the TBAF solution furnished the crude product, which, after being washed with hexane, weighed 60 mg (87%). The NMR spectrum showed little, if any, of the *tert*-butyl signal, and the IR spectrum was satisfactory. Material of this quality was submitted for testing at the NCI. A sample was dissolved in dry benzene at room temperature and the solution was filtered. The benzene was allowed to evaporate slowly at room temperature. The crystals that separated were filtered, washed with hexane, and dried. The elemental analysis was poor (found: C, 62.89; H, 6.92; N, 9.15), indicating that decomposition occurred.

Determination of Total Worm-Associated Radioactivity after Incubation with [^{3}H]Hycanthone ([^{3}H]HC). A. As a Function of Incubation Time. One hundred HC-sensitive and 100 HC-resistant male schistosomes were incubated at 37 °C, separately in the presence of 1×10^{-4} M [^{3}H]HC. The schistosomes were suspended in 102 mL of RPMI-1640 medium buffered with 25 mM HEPES and supplemented with 10% calf serum. At various time intervals after the beginning of the incubation, five worms were harvested from each culture and processed as follows. The worms were washed three times with cold saline, resuspended in 2 mL of 1×10^{-2} M Tris·HCl, pH 7.4, and 1×10^{-2} M EDTA, and homogenized at 0 °C with about 50 strokes of a tight-fitting Dounce. Aliquots of the homogenates were taken at four time intervals up to 2 h and were counted directly to determine total worm-associated radioactivity (Figure 1).

B. As a Function of [^{3}H]HC Concentration. The same conditions as described directly above were used except that the worms were treated with different concentrations of [^{3}H]HC. At the end of 2 h of incubation at 37 °C, the worms were processed as described above. The results are summarized in Figure 2.

Binding of [^{3}H]Hycanthone ([^{3}H]HC) and [^{3}H]Hycanthone *N*-Methylcarbamate ([^{3}H]HNMC) to HeLa Cell DNA. HeLa cells were exposed for 1 h to either [^{3}H]HC or [^{3}H]HNMC at the desired concentrations, washed three times with saline, and incubated for an additional 30 min at 37 °C in a drug-free medium. The cells were washed with cold saline and disrupted by the addition of a 0.9% SDS solution. The resulting suspension was treated with ethanol, and the precipitate was collected and washes successively with methanol, CH_2Cl_2, and ether. The pellet was resuspended in a 0.3 M NaCl solution, and RNase, Pronase P, and proteinase K were added. After digestion for 2 h at 37 °C, the mixture was treated with ethanol to precipitate the crude DNA, which was purified by CsCl density gradient centrifugation. The DNA peak was dialyzed extensively, and the DNA was precipitated with ethanol. The DNA was redissolved, and the total amount of DNA was estimated by UV absorption at 260 nm. The sample was split into aliquots, one of which was treated with deoxyribonuclease. The DNA- and deoxyribonuclease-sensitive radioactivity were determined, and the results were expressed as the number of drug molecules per base pair (Table V).

Acknowledgment. A part of this work was supported by a grant from the National Cancer Institute (CA-19674). This investigation also received financial support from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases. We thank the late Dr. I. H. Lepow and A. E. Soria of the Sterling-Winthrop Research Institute for hycanthone and hycanthone 3,5-dinitrobenzoate.

Registry No. 1, 3105-97-3; **5**, 111324-50-6; **8**, 696-63-9; **9**, 1194-65-6; **10**, 101339-45-1; **11**, 111324-51-7; **12**, 111324-52-8; **13**, 86456-01-1; 14, 86456-05-5; **15**, 86456-31-7; **16**, 86456-32-8; **17**, 101339-44-0; **18**, 111324-53-9; **19**, 86456-11-3; **20**, 111324-54-0; **21**, 111324-55-1; **22**, 111324-56-2; **23**, 3612-74-6; **24**, 111324-57-3; **25**, 111324-58-4; **26**, 111324-59-5; **27**, 3612-72-4; **28**, 3612-73-5; $(CH_3)_2NCH_2CH_2NH_2$, 108-00-9; phenyl isocyanate, 103-71-9; *n*-propyl isocyanate, 110-78-1; *n*-butyl isocyanate, 111-36-4.

Notes

Heterocyclic Quinones with Potential Antitumor Activity. 2.[1] Synthesis and Antitumor Activity of Some Benzimidazole-4,7-dione Derivatives

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A series of benzimidazole-4,7-dione derivatives, bearing substituents at positions 1, 2, 5, and 6 of the benzimidazole ring, has been synthesized and tested for antitumor activity in vivo on P388 leukemia. Some of the synthesized compounds show significant antitumor activity, associated with high toxicity, however. Compunds **7**, **18**, and **27** show the highest antitumor activity in this series, whereas **17**, **19**, and **22** are scarcely active. Some hypothetical biological precursors of these quinones are devoid of antitumor activity. Some structure–activity relationships are discussed.

A great number of quinone derivatives have been extensively investigated for their biological activity.[2] The continually increasing interest in this class of compounds is related to their noticeable antitumor activity.[3-12] Although their mechanisms of action can be different for the various types of compounds, there is increasing evidence

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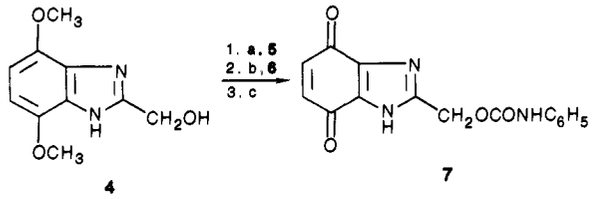
Scheme I[a]



4 7

[a] (a) HCl concentrated; (b) CrO_3; (c) C_6H_5NCO.

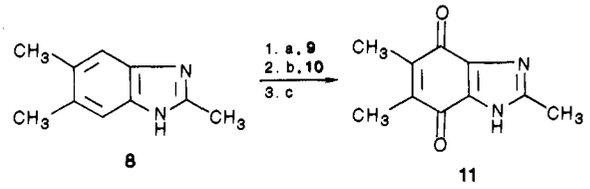
that one-electron reduction processes, involving generation of free radicals and active oxygen species (such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals), can kill cells by damaging DNA.[13-18]

Structural modifications concerning simple derivatization of the parent drugs or substituents, the sugar moiety or the chains, have been made to reduce the toxicity of these drugs.[8,19,20] Some results demonstrate that it is possible to separate the antitumor and the cardiotoxicity effects.[21-23]

If antitumor activity and cardiotoxicity are both closely related to the radical species formation and stability, structural changes in the aromatic portion of these molecules could influence various biological activities.

Significant antitumor activities have been found also in some heterocyclic quinones.[11,24,28] Some benzimidazole

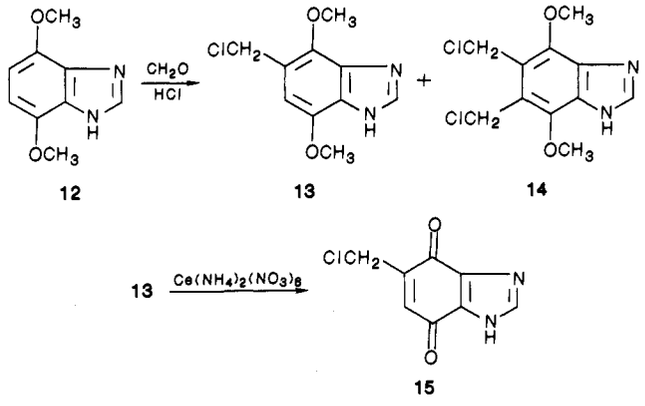
Scheme II[a]



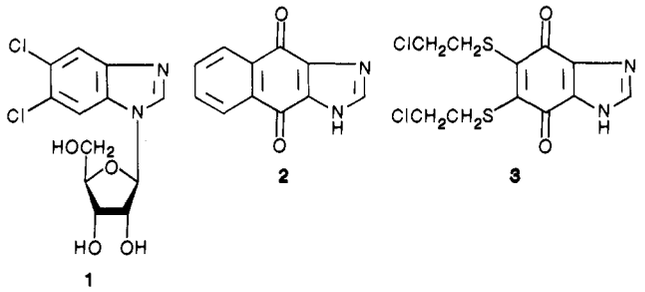
8 11

[a] (a) HNO_3 concentrated/H_2SO_4 concentrated; (b) Pd(c)/H_2; (c) $FeCl_3$.

Scheme III



derivatives have similar activities, namely, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (1) selectively inhibits HeLa cell proliferation,[29] naphtho[2,3-*d*]imidazole-4,9-dione (**2**) inhibits hypoxanthine phosphoribosyltransferase,[30] and 5,6-bis-[(2-chloroethyl)thio]benzimidazole-4,7-dione (**3**) has, in some degree, antitumor activity against Walker carcinosarcoma 256.[31] Furthermore, benzimidazole-4,7-diones possess redox properties similar to those of other quinones, and ESR studies have demonstrated that anion radicals are obtained in their reduction processes.[32]



1 2 3

On the basis of the above considerations, we have synthesized and tested for antitumor activity a series of variously substituted benzimidazole-4,7-diones. Substituents were selected with different electronic and solubility characteristics, while others were chosen for their known alkylating properties. We have also synthesized and tested some benzimidazole-4,7-diones bearing methyl groups at positions 5 and 6 of the nucleus. On the basis of the well-known reactivity of certain fully alkylated quinones with nucleophiles, through a tautomeric form,[33-35] these

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compounds could be considered as possible alkylating agents.

Chemistry

A small number of benzimidazole-4,7-dione derivatives have been previously reported in the literature. The synthesis of unsubstituted and 2-substituted benzimidazole-4,7-diones was based on the classical Day's synthesis.[36] According to this synthetic scheme, the 2-(hydroxymethyl)benzimidazole-4,7-dione (**6**) was obtained by oxidation with chromic anhydride of the corresponding 2-(hydroxymethyl)-4,7-dihydroxybenzimidazole (**5**), which, in turn, was obtained by hydrolysis of 2-(hydroxymethyl)-4,7-dimethoxybenzimidazole (**4**)[36] with concentrated hydrochloric acid in a sealed tube at 100 °C (Scheme I). Under the strongly acidic conditions of hydrolysis, the hydroxymethyl group of **4** remains unchanged, as witnessed by IR and mass spectrometry. Oxidation of **5** with ferric chloride gave unsatisfactory results. Reaction of **6** with phenyl isocyanate gave the corresponding [(phenylcarbamoyl)oxy]methyl derivative **7**.

The synthesis of 2,5,6-trimethylbenzimidazole-4,7-dione (**11**) was performed according to a previously described method[37] (Scheme II). Nitration of 2,5,6-trimethylbenzimidazole (**8**) with concentrated nitric and sulfuric acids at 10 °C gave the dinitro derivative **9** as a pure compound. Hydrogenation of **9** with palladium as the catalyst gave the diamino derivative **10**, which, in turn, by oxidation with ferric chloride in aqueous solution at room temperature gave **11**.

The synthesis of 5-(chloromethyl)benzimidazole-4,7-dione (**15**) is outlined in Scheme III. Chloromethylation of 4,7-dimethoxybenzimidazole (**12**) gave a mixture of 5-(chloromethyl)-4,7-dimethoxybenzimidazole (**13**) and 5,6-bis(chloromethyl)-4,7-dimethoxybenzimidazole (**14**), which were separated by column chromatography. The structure of these compounds was assigned on the basis of NMR and IR spectra. In particular, the IR spectra of **13** and **14** showed an NH absorption band at 3300 cm^{-1}. NMR spectra of **13** showed signals at δ 4.70, which were attributed to the CH_2Cl protons; at δ 6.87, for the aromatic proton; and at δ 8.15, corresponding to the 2-H proton. The NMR spectrum of **14** showed the disappearance of the aromatic protons and a signal at δ 5.0, corresponding to four protons of the CH_2Cl group at the 5- and 6-positions. The structure of compound **15** was derived on the basis of the NMR spectrum, which showed a disappearance of the signals corresponding to the methoxy groups. Signals at δ 4.45 and 6.75 were assigned to the CH_2Cl group and to the 6-H proton, respectively. The total yields of this process were low. Different synthetic approaches to **15** failed since direct chloromethylation of benzimidazole-4,7-dione[36] (**16**) gave a complex mixture of unidentified compounds, and hydrolysis of **13** with concentrated hydrochloric acid gave an insoluble polymeric product.

Other 5-substituted benzimidazole-4,7-diones were obtained by nucleophilic addition reactions on benzimidazole-4,7-dione, as previously reported in the literature.[31] Introduction of an aziridino group on benzimidazole-4,7-dione by direct nucleophilic addition failed. Because of the insolubility of this quinone in nonpolar solvents, the reaction, carried out in methanol, led to destruction of aziridine and to the formation of polymeric compounds. The synthesis of 5-aziridinyl-6-bromobenzimidazole-4,7-dione (**18**) was performed by nucleophilic substitution on 5,6-dibromobenzimidazole-4,7-dione[38] (**17**) in 1,2-dimethoxyethane. In an analogous manner, the synthesis of 5-bromo-6-(methylaziridinyl)benzimidazole-4,7-dione (**19**) was carried out.

Results and Discussion

The structures of the benzimidazole-4,7-dione derivatives and their antitumor activity against lymphocytic leukemia P388 in mice are reported in Table I.

In order to evaluate the best treatment for the most toxic compounds, two dose regimens were used for the different compounds. In some cases the same compound was tested in both regimens (injection in a single ip dose on day 1 after tumor implantation or in five doses on days 1–5). However, although the schedule of the treatment on days 1–5 generally gave better results for antitumor activity, and obviously lower toxicity, there is not a significant difference in the results obtained. For these reasons, only a few compounds were tested in both ways. Some of the tested compounds show significant antitumor activity ($T/C \geq 125$) but most are also toxic at low doses. Compounds **7**, **18**, and **27** show the highest antitumor activity at relatively low doses. Compounds **17**, **19**, and **22** were active, but only one is scarcely toxic. All other compounds were inactive against the tumor tested.

All the benzimidazole-4,7-dione derivatives reported in Table I have very low solubility in water and in organic solvents. For the biological test, they required as vehicle a mixture, DMSO–H_2O. In order to investigate if this poor solubility and the above-mentioned noticeable toxicity of these compounds could be overcome by employing some biological precursors, a comparative test was performed between the quinone **20** and its precursors, namely, water-soluble dihydroxybenzimidazole hydrochloride (**28**) and the more lipophilic 2-methyl-4,7-dimethoxybenzimidazole (**29**). The comparative data for these compounds show that the quinone and its potential biological precursors are devoid of antitumor activity. However, in comparison with **20**, compounds **28** and **29** show lower toxicity (12.5 and 800 mg/kg, respectively, in comparison with 6.25 mg/kg for **20**).

The results obtained with the different substituents do not permit a conclusive analysis of structure–activity relationships. However, it appears that the presence of a basic substituent in the quinone ring in most cases gives better antitumor properties. The most interesting results are obtained when the substituents are a (carbamoyloxy)methyl group in the 2-position or an aziridine group in the 5-position. These results confirm once more the importance of these groups for antitumor activity. The 2-methylaziridinyl group, on the other hand, gave poorer antitumor activity. This result confirmed that methyl substitution in the aziridine ring diminishes activity.[39] Alkylation of the imidazole ring did not produce good results. It can be observed that 1-alkyl-substituted derivatives generally have lower T/C values and higher toxicity in comparison with their corresponding 1-H derivatives (**21** versus **16** and **23** and **24** versus **22**).

Experimental Section

Chemistry. Melting points were determined with a Buchi apparatus and are uncorrected. IR spectra were recorded on a

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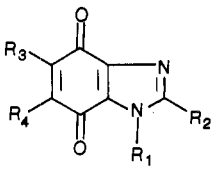
Table I. Structure and Antitumor Properties of the Benzimidazole-4,7-dione Derivatives



					antitumor activity against P338 Leukemia[a]					
					treatment on day 1 only			treatment on days 1–5		
no.	R_1	R_2	R_3	R_4	opt dose, mg/kg ip	max,[b] % T/C	toxic level, mg/kg	opt dose, mg/kg ip	max,[b] % T/C	toxic level, mg/kg
6[c]	H	CH_2OH	H	H	3.12	105	12.5	6.25	110	12.5
7	H	$CH_2OCONHC_6H_5$	H	H				6.25	146	25
11	H	CH_3	CH_3	CH_3				12.50	110	100
15	H	H	CH_2Cl	H				3.12	95	12.5
16	H	H	H	H	6.25	105	12.5	6.25	110	25
17	H	H	Br	Br				1.56	130	12.5
18	H	H	$-\overline{NCH_2CH_2}$	Br				6.25	156	12.5
19	H	H	$-\overline{NCH(CH_3)CH_2}$	Br				6.25	136	12.5
20	H	CH_3	H	H				3.12	100	6.25
21	CH_3	H	H	H	0.40	76	3.12	0.40	95	6.25
22	H	H	CH_3	CH_3				25	130	200
23	CH_3	H	CH_3	CH_3	25	95	100			
24	C_2H_5	H	CH_3	CH_3	25	100	100			
25	H	H	Cl	H	6.25	117	12.5			
26	H	H	$C_6H_5CH_2NH$	H	25	90	100	3.12	105	150
27	H	H	NH_2	Br				6.25	154	25

[a] Groups of five CD2F1 mice were inoculated ip with 10^6 P388 lymphocytic leukemic cells on day 0 and treatment was started on day 1. Two different schedules were used. In the first one, animals were injected ip with the drug on day 1; in the second one, the treatment was continued through days 1–5. [b] % T/C = (average survival time of treaed mice/control mice) × 100. Antitumor activity must be ≥125% for active compounds. [c] In (hydroxypropyl)cellulose.

Perkin-Elmer Model 257 spectrophotometer. NMR spectra were obtained with a Varian EM-390 90-MHz spectrometer with tetramethylsilane (TMS) as internal standard and are reported as δ (ppm). NMR abbreviations used are as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were obtained on a low-resolution Hewlett-Packard 5980 A mass spectrometer with an ionization potential of 79 eV. TLC was carried out on precoated TLC plates with silica gel 60 F-254 Merck. For column chromatography, silica gel 60 (Merck) was used. Microanalytical results, indicated by atomic symbols, are within ±0.4% of theoretical values and were obtained from the microanalytical laboratory of the University of Camerino with a Perkin-Elmer 240 autoanalyser. 1,4-Dimethoxybenzene and 2,5,6-trimethylbenzimidazole were purchased from Aldrich Chemical Co. (European Division) whereas 2-methylaziridine was purchased from Fluka A.G. The following compounds were synthesized according to the cited literature procedures: benzimidazole-4,7-dione (**16**), 2-methylbenzimidazole-4,7-dione (**20**), 2-methyl-4,7-dimethoxybenzimidazole (**29**), and 2-methyl-4,7-dihydroxybenzimidazole hydrochloride (**28**);[36] 1-methylbenzimidazole-4,7-dione (**21**);[32] 5,6-dimethylbenzimidazole-4,7-dione (**22**), 1,5,6-trimethylbenzimidazole-4,7-dione (**23**), and 1-ethyl-5,6-dimethylbenzimidazole-4,7-dione (**24**);[37] 5-chlorobenzimidazole-4,7-dione (**25**) and 5-(benzylamino)benzimidazole-4,7-dione (**26**);[31] 5,6-dibromobenzimidazole-4,7-dione (**17**) and 5-amino-6-bromobenzimidazole-4,7-dione (**27**).[38]

2-(Hydroxymethyl)-4,7-dihydroxybenzimidazole (5). A mixture of 1 g (4.8 mmol) of 2-(hydroxymethyl)-4,7-dimethoxybenzimidazole[36] (**4**) and 20 mL of concentrated HCl was heated at 100 °C for 20 h in a sealed tube and then left at room temperature overnight. The resulting precipitate was filtered and recrystallized from absolute ethanol–ethyl ether: yield 0.7 g (67%); mp 270–271 °C; ^{1}H NMR (DMSO) δ 4.94 (s, 2 H, CH_2OH), 6.82 (s, 2 H, 5-H and 6-H), 10.1 (br s, 2 H, OH, vanishes by addition of D_2O), 14.5 (br s, 1 H, NH, vanishes by addition of D_2O); MS, *m/z* 180 (60), 162 (100). Anal. ($C_8H_9ClN_2O_3$) C, H, N.

2-(Hydroxymethyl)benzimidazole-4,7-dione (6). Chromic anhydride (400 mg) in 10 mL of water was added at room temperature to a stirred solution of 325 mg (1.5 mmol) of **5** in 20 mL of water. The reaction mixture was stirred at room temperature for 3 h, and the resulting precipitate was filtered, washed with water, and recrystallized from ethanol: yield 215 mg (66%); mp 180–182 °C dec; IR (cm^{-1}) 3450 (OH), 3150 (NH), 1660 (C=O); ^{1}H NMR (DMSO) δ 4.60 (s, 2 H, CH_2OH), 5.60 (br s, 1 H, OH, vanishes by addition of D_2O), 6.7 (s, 2 H, 5-H and 6-H), 13.7 (br s, 1 H, NH, vanishes by addition of D_2O). Anal. ($C_8H_6N_2O_3$) C, H, N.

2-[[(*N*-Phenylcarbamoyl)oxy]methyl]benzimidazole-4,7-dione (7). Phenyl isocyanate (4 g, 2.34 mmol) was added in three portions within 15 h to a stirred suspension of 0.2 g (1.12 mmol) of **6**, and the reaction mixture was refluxed for 2 days. The resulting precipitate was filtered, washed twice with cyclohexane, and recrystallized from ethanol: yield 300 mg (90%); mp 223–225 °C; IR (cm^{-1}) 3320, 3150 (NH), 1720, 1660 (C=O); ^{1}H NMR (DMSO) δ 5.25 (s, 2 H, CH_2O), 6.60–7.70 (br s, 7 H, 5-H, 6-H, C_6H_5), 9.80 (s, 1 H, NH, vanishes by addition of D_2O), 12–14 (br s, 1 H, NH, vanishes by addition of D_2O). Anal. ($C_{15}H_{11}N_3O_4$) C, H, N.

2,5,6-Trimethyl-4,7-dinitrobenzimidazole (9). A solution of 5 g (31 mmol) of 2,5,6-trimethylbenzimidazole (**8**) in 40 mL of concentrated H_2SO_4 was added dropwise with stirring to a cold mixture of 5 mL of 98% HNO_3 and 16 mL of concentrated H_2SO_4 at 5–10 °C. It was then allowed to stand at room temperature for 3 h. The reaction mixture was poured into cracked ice and neutralized with concentrated NH_4OH. The crude precipitate was filtered, washed with water, and recrystallized from ethanol: yield 5.6 g (66%); mp 203–205 °C; ^{1}H NMR (DMSO) δ 2.42 (s, 6 H, 5-CH_3 and 6-CH_3), 2.60 (s, 3 H, 2-CH_3), 13.2 (br s, 1 H, NH, vanishes by addition of D_2O). Anal. ($C_{10}H_{10}N_4O_4$) C, H, N.

2,5,6-Trimethyl-4,7-diaminobenzimidazole Hydrochloride (10). A solution of 4 g of **9** (0.016 mol) in 500 mL of ethanol was hydrogenated in a Parr apparatus with 10 mg of 5% Pd/C at 50 psi for 2 days at room temperature. The ethanolic solution, rapidly filtered from the catalyst, was acidified with concentrated HCl. The resulting green-gray precipitate was filtered and recrystallized from absolute ethanol: yield 3.12 g (86%); mp 312–315 °C dec; IR (cm^{-1}) 3420, 3320, 3215 (NH), 3000–2800 (NH_3^+). Anal. ($C_{10}H_{15}N_4Cl$) C, H, N.

2,5,6-Trimethylbenzimidazole-4,7-dione (11). A solution of 1 g of **10** (4 mmol) in 50 mL of water was treated with an excess

of ferric chloride (2 g) and stirred overnight at room temperature. The resulting yellow-green precipitate was recovered by filtration and washed with water. The mother aqueous solution, neutralized with NH_4OH and extracted with EtOAc, gave an additional amount of the reaction product, which was recrystallized from ethanol: yield 0.86 g (80%); mp 261–265 °C dec; ^1H NMR ($C-D_3OD$) δ 2.00 (s, 6 H, 5-CH_3 and 6-CH_3), 2.45 (s, 3 H, 2-CH_3), 13.3 (br s, 1 H, NH, vanishes by addition of D_2O). Anal. ($C_{10}H_{10}N_4O_4$) C, H, N.

5(6)-(Chloromethyl)-4,7-dimethoxybenzimidazole (13) and 5,6-Bis(chloromethyl)-4,7-dimethoxybenzimidazole (14). A 40% aqueous solution of formaldehyde (4 mL) was added to a suspension of 0.4 g of 4,7-dimethoxybenzimidazole[36] (2.24 mmol) in 10 mL of concentrated HCl. Then, gaseous HCl was bubbled into the suspension at room temperature, with stirring, for 1 h. The reaction mixture was then heated on a water bath for 2 h and, after cooling at room temperature, neutralized with concentrated NH_4OH. The resulting precipitate of inorganic salts was filtered, and the aqueous solution was extracted several times with EtOAc. The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on a silica gel column eluted with EtOAc–MeOH (95:5). The first eluted fractions were evaporated to give **14**, which was recrystallized from ethanol: yield 15 mg (2.8%); mp 105–106 °C; ^1H NMR (DMSO) δ 4.0 (6 H, s, OCH_3), 5.0 (4 H, s, 5- and 6-CH_2Cl), 8.15 (1 H, s, 2-H), 12.8 (1 H, very br signal, NH, vanishes upon addition of D_2O). Anal. ($C_{11}H_{12}N_2O_2Cl_2$) C, H, N.

By further elution of the column were obtained fractions that were evaporated to give **13** as a residue, which was recrystallized from EtOAc: yield 100 mg (21%); mp 158–160 °C; ^1H NMR (DMSO) δ 3.94 (3 H, s, OCH_3), 4.06 (3 H, s, OCH_3), 4.70 (2 H, s, CH_2Cl), 6.87 (1 H, s, 6-H), 8.15 (1 H, s, 2-H), 12.9 (1 H, very br signal, vanishes upon addition of D_2O). Anal. ($C_{10}H_{11}N_2O_2Cl$) C, H, N.

5(6)-(Chloromethyl)benzimidazole-4,7-dione (15). A solution of 1 g of $Ce(NH_4)_2(NO_3)_6$ in 10 mL of water was added dropwise at room temperature, during a 10-min interval, to a stirred solution of 0.2 g of **13** (0.88 mmol) in 50 mL of CH_3CN. The resulting mixture was stirred at room temperature for 4 h and then, after addition of 10 mL of water, was extracted several times with EtOAc. The combined organic extracts were washed with water, dried (Na_2SO_4), evaporated in vacuo, and recrystallizated with EtOH: yield 140 mg (80%); mp undefined (slow decomposition at 210–220 °C); ^1H NMR (DMSO) δ 4.44 (2 H, d, J = 2 Hz, 5-CH_2Cl), 6.65 (1 H, d, J = 2 Hz, 6-H), 8.6 (1 H, s, 2-H), 9.5 (1 H, br signal, NH, vanishes upon addition of D_2O). Anal. ($C_8H_5N_2O_2Cl$) C, H, N.

5-Bromo-6-aziridinylbenzimidazole-4,7-dione (18). To a stirred suspension of 800 mg of 5,6-dibromobenzimidazole-4,7-dione (2.61 mmol) in 200 mL of 1,2-dimethoxyethane was added 0.43 g of aziridine[40] (10 mmol). The mixture was stirred at room temperature for 3 h and during this period was decanted several times in order to remove impurities sticking to the glass. Evaporation of the solvent in vacuo at room temperature gave an oily residue, which was repeatedly washed with ether and MeOH, and finally recrystallized from MeOH–ethyl ether: yield 470 mg (67%); mp 300–302 °C dec; ^1H NMR (DMSO) δ 2.53 (4 H, s, aziridinyl-H), 8.14 (1 H, s, 2-H), 11.5–14.5 (1 H, very br signal, NH, vanishes upon addition of D_2O). Anal. ($C_9H_6BrN_3O_2$) C, H, N.

5-Bromo-6-(methylaziridinyl)benzimidazole-4,7-dione (19). To a stirred suspension of 200 mg of 5,6-dibromobenzimidazole-4,7-dione (0.66 mmol) in 50 mL of 1,2-dimethoxyethane was added 230 mg (2 mmol) of methylaziridine, and the resulting mixture was stirred at room temperature. Immediately after addition of methylaziridine, a complete solubilization was observed, and the solution became red-orange. The course of the reaction was followed by TLC (benzene–CH_3CN, 50:50). After several hours, complete disappearance of the starting quinone was observed. Evaporation of the solvent in vacuo gave an oily residue, which was washed several times with ether, redissolved in MeOH, and precipitated with ether. The obtained compound was pure on TLC (benzene–CH_3CN, 50:50): yield 70 mg (38%); mp undefined (decomposition); ^1H NMR (DMSO) δ 1.43 (3 H, d, J = 5 Hz, CH_3), 2.53 (3 H, m, H-2′ and H-3′), 3.30 (1 H, br s, NH, vanishes upon addition of D_2O), 8.12 (1 H, s, 2-H). Anal. ($C_{10}H_8BrN_3O_2$), C, H, N.

Tests against P388 Leukemia. The quinone derivatives reported in Table I were screened against P388 lymphocytic leukemia in mice. Tests were performed in accordance with the protocols of the National Cancer Institute.[41] $C_6D_2F_1$ mice were inoculated intraperitoneally on day 0 with 10^6 P388 lymphocytic leukemia cells. Treatment of groups of six of these mice was begun the following day (day 1).

For compounds **23**, **24**, and **25**, treatment was made on day 1 only; for compounds **7**, **11**, **15**, **17–20**, and **27** treatment was made on days 1–5. Compounds **6**, **16**, **21**, and **26** were tested in both regimens.

Acknowledgment. This work was supported by a grant from Italian Ministero della Pubblica Istruzione (fondi 60%). We are grateful to Dr. S. Filippeschi of Istituto Ricerche Farmacologiche "Mario Negri"-Milan for performing antitumor tests, to F. Lupidi for NMR spectra, and to M. Brandi for expert technical assistance.

Registry No. 4, 103151-22-0; **5**, 111210-09-4; **6**, 111210-04-9; **7**, 111210-05-0; **8**, 3363-56-2; **9**, 111210-10-7; **10**, 111210-11-8; **11**, 111210-06-1; **12**, 7711-50-4; **13**, 111210-12-9; **14**, 111210-13-0; **15**, 111210-07-2; **16**, 7711-39-9; **17**, 26573-35-3; **18**, 111237-56-0; **19**, 111210-08-3; **20**, 98436-82-9; **21**, 7711-63-9; **22**, 61587-95-9; **23**, 85311-42-8; **24**, 85311-43-9; **25**, 26558-10-1; **26**, 26557-29-9; **27**, 26557-83-5; **28**, 111210-14-1; **29**, 99922-32-4; aziridine, 151-56-4; 2-methylaziridine, 75-55-8.

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Cholecystokinin Antagonists. Synthesis and Biological Evaluation of 3-Substituted 1,4-Benzodiazepin-2-amines

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Cholecystokinin (CCK) is a neuropeptide[1] that is found in different molecular sizes, a major one being the octapeptide, CCK-8. CCK occurs both in mammalian peripheral tissues and in the central nervous system.[2,3] The relationship between the activation of CCK receptors in the periphery and stimulation of pancreatic and biliary

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0022-2623/88/1831-0264$01.50/0