mg), stirred at 25 °C for 30 min, and evaporated to a syrup under high vacuum. The syrup was triturated with ether (20 mL) to give a solid, which was collected and extracted with $CHCl_3$ (6 × 100 mL). The $CHCl_3$ extract was evaporated to dryness and the residue triturated with ether, collected, and dried: yield 210 mg (62%); MS, m/z 342 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 263 (13.2), in pH 7, 262 (13.2), in pH 13, 266 (7.94); ^1H NMR δ 11.29 (s, 1, H-3), 8.82 (t, 1, NH), 7.51 (s, 1, H-6), 6.15 (ψq, 1, H-1', $J_{1',2a'}$ = 6.1, $J_{1',2b'}$ = 7.9), 5.33 (d, 1, 3'-OH), 4.24 (m, 1, H-3'), 3.93 (m, 1 H-4'), 3.77 (q, 2, CH_2NNO), 3.52 (t, 2, H-5'), 2.10 (m, 2, H-2'), 1.79 (s, 3, 5-Me), 0.91 (t, 3, CH_3CH_2). Anal. ($C_{13}H_{19}N_5O_6$) C, H, N.

***N*-(4-Aminophenyl)-*N'*-methylurea (12).** A solution of methyl isocyanate (5.7 g, 0.1 mol) in anhydrous chloroform (150 mL) was added dropwise to a stirred solution of 1,4-phenylenediamine (10.8 g, 0.1 mol) in anhydrous chloroform (350 mL). The resulting precipitate was collected and washed with chloroform: yield 12.5 g (75%); mp 146–148 °C.

The analytical sample was obtained from a similar reaction by recrystallization from chloroform: mp 147–148 °C; MS, m/z 165 (EI) (M^+); ^1H NMR δ 7.87 (s, 1, NH, exchanges with D_2O), 6.98 (d, 2, aromatic H ortho to NH), 6.45 (d, 2, aromatic H ortho to NH_2), 5.73 (q, 1 CH_3NH, J = 4.7 Hz), 4.65 (s, 2, NH_2, exchanges with D_2O), 2.59 (d, 3, CH_3, J = 4.7 Hz). Anal. ($C_8H_{11}N_3O$) C, H, N.

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Preparation and Antischistosomal and Antitumor Activity of Hycanthone and Some of Its Congeners. Evidence for the Mode of Action of Hycanthone

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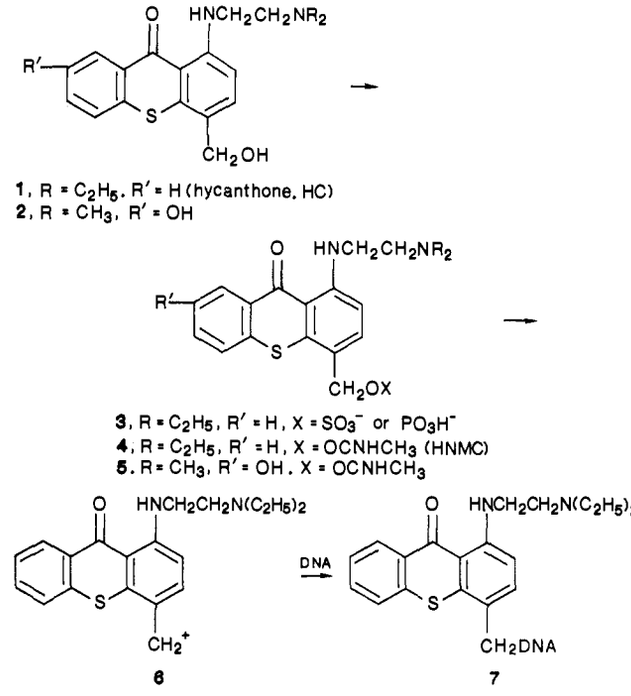
The synthesis of a series of esters of hycanthone (HC) and 7-hydroxyhycanthone, their antitumor activity, and their antischistosomal effects on HC-sensitive and HC-resistant schistosomes are reported. Binding studies using tritium-labeled HC and hycanthone *N*-methylcarbamate (HNMC) with calf thymus DNA provided evidence that HNMC but not HC alkylated the DNA. Tritiated HNMC also bound to the DNA of intact HeLa cells exposed to the drug while very little tritiated HC bound to DNA under the same conditions. The mechanism proposed previously[10] to account for the antischistosomal action of HC, namely, drug esterification followed by alkylation of DNA, applies also to the antitumor action of the drug as shown in Scheme I.

Hycanthone (HC) (1) is a schistosomicidal[1,2] and antitumor agent.[3] Its mutagenicity[4] proscribed its clinical use as a one-dose treatment for *Schistosoma mansoni* and *Schistosoma haemotobium* infections. Several years ago Hartman and Hulbert suggested a mechanism without any experimental support to account for the mutagenic properties of HC.[4] Elsewhere we proposed a slight variant of their mechanism to account for the antischistosomal action of hycanthone as shown in Scheme I.[5]

In this mechanism HC is enzymically esterified in the target cell to the ester **3**, which we propose is either a sulfate or phosphate. This ester, which now possesses a good leaving group, dissociates nonenzymically to give **6**, which alkylates DNA to give **7**. In this paper we describe the preparation and some biological properties of a group of esters derived from HC and one of its congeners and present experimental evidence that supports the view that the mechanism in Scheme I not only accounts for the schistosomicidal properties of HC but for its antitumor action as well.

Scheme I



Chemistry

The substituted carbamate esters **23–25** were prepared from HC and the appropriate substituted isocyanates. The methyl carbonate ester **26** was prepared by treatment of HC with methyl chloroformate. The 3,5-dinitrobenzoate **28** was a gift from the Sterling-Winthrop Research Institute. Since treatment of the known thioxanthenone **2** with CH_3NCO furnished the biscarbamate, the procedure shown in Scheme II was used to prepare the carbamate **5**.

Condensation of *p*-methoxybenzenethiol (8) with 2,6-dichlorobenzonitrile (**9**) gave the desired nitrile **10** accom-

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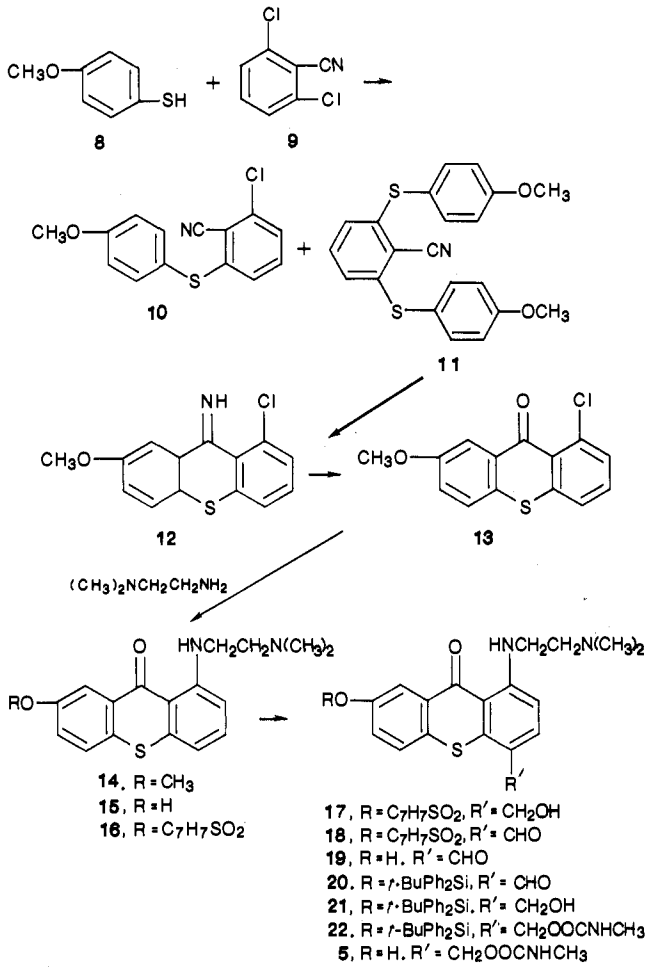
(1) Rosi, D.; Peruzzotti, G.; Dennis, E. W.; Berberian, D. A.; Freele, H.; Archer, S. *Nature (London)* **1965**, *208*, 1085.

0022-2623/88/1831-0254$01.50/0

Scheme II



panied by a small amount of the easily separable disubstituted compound **11**. Cyclization with either H_2SO_4 or polyphosphoric acid (PPA) was unsuccessful, but treatment of **10** with CF_3SO_3H afforded the imine **12**, which was easily hydrolyzed to the desired thioxanthen-9-one **13**. This procedure is superior to the one used previously as it is shorter and gives only one rather than a mixture of isomers.[3] Condensation with *N,N*-dimethylethylenediamine furnished the known base **14** in a higher state of purity than obtained earlier owing to the absence of contamination with the 3-isomer.[3] Demethylation with HI furnished the phenol **15**, which was converted to the tosyl ester **16**.

Condensation with formaldehyde in aqueous acetic acid gave **17**. Oxidation with MnO_2 gave the aldehyde **18**, which was hydrolyzed to the known phenolic aldehyde **19**. Silylation with *tert*-butylchlorodiphenylsilane[6] gave **20**, which on reduction gave **21**. Treatment with CH_3NCO afforded the carbamate **22**, which was readily purified by crystallization. Desilylation with fluoride ion gave the target compound **5**. The NMR spectrum indicated that desilylation had occurred, and the NMR and IR spectra showed that the carbamate group was still present. In contrast to **22**, the phenolic *N*-methylcarbamate **5** was unstable and was difficult to purify by the usual crystallization methods.

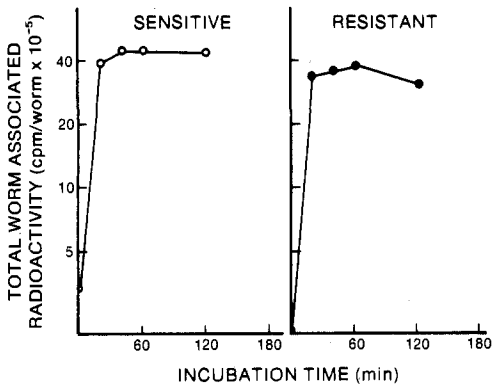


Figure 1. Total worm-associated [^{3}H]HC radioactivity as a function of time. See Experimental Section for details.

Biological Results

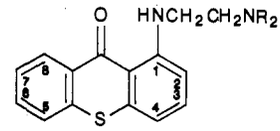
The compounds listed in Table I were tested by the National Cancer Institute against P388 lymphocytic leukemia in mice.[7] In this test system, HC showed a T/C = 166 at 60 mg/kg when administered once a day for 9 days. In the same test system, hycanthone *N*-methylcarbamate (HNMC) gave a similar T/C value but at a dose of 6.25 mg/kg. None of the other esters in this group were as active as HNMC; most were either marginally active or inactive (i.e., T/C < 125). The antitumor activity of **5** was disappointing since the corresponding alcohol **2** had a T/C > 222 at 200 mg/kg and a T/C = 194 at 100 mg/kg. It was hoped that as in the parallel case of HC and HNMC the carbamate **5** would have been as active as **2** but far more potent. In the event **5** proved to be far more potent than **2**, but it was a much less active antitumor agent.

Several of the esters of HC were tested for their effect on the survival in culture of HC-sensitive and HC-resistant *S. mansoni* and also on their effect on [^{3}H]uridine incorporation by the same worms (Table II). This latter assay is of interest since it has been shown[5] that HC inhibits uridine incorporation irreversibly in an HC-sensitive strain but only transiently in an HC-resistant strain of *S. mansoni*. Irreversible inhibition of nucleic acid synthesis appears to correlate with worm death.

According to the mechanism in Scheme I, the conversion of HC to the ester **3** is the only enzymic step in the process. It is tempting to postulate that HC-resistance is due to the absence of an esterifying enzyme in HC-resistant worms, a suggestion that is in accord with genetic studies.[8] If our proposed mechanism is correct and certain esters of HC may act as surrogates for **3**, these esters should be effective in HC-resistant as well as in HC-sensitive worms. We had reported earlier[5] that HNMC inhibits [3H]uridine incorporation in and also causes the death of HC-resistant worms. We now report that the *N*-phenylcarbamate (**23**), the methyl carbonate (**26**), and the acetate (**27**) esters of HC behave similarly but none was more active than HNMC itself. In general, the inhibition of [^{3}H]uridine incorporation is greater in HC-sensitive than in HC-resistant *S. mansoni*. The 3,5-dinitrobenzoate **28** is a striking example of this phenomenon; at a concentration of 25 μg/mL, this ester inhibited [^{3}H]uridine incorporation in

(2) Rosi, D.; Peruzzotti, G.; Dennis, E. W.; Berberian, D. A.; Freele, H.; Tullar, B. F.; Archer, S. *J. Med. Chem.* **1967**, *10*, 867.
(3) Archer, S.; Zayed, A.-H.; Rej, R.; Rugino, T. A. *J. Med. Chem.* **1983**, *26*, 1240.
(4) Hartman, P. E.; Hulbert, P. B. *J. Toxicol. Environ. Health* **1975**, *2*, 243.
(5) Cioli, D.; Pica-Mattoccia, L.; Rosenberg, S.; Archer, S. *Life Sci.* **1985**, *37*, 161.
(6) Hanessian, S.; LaVallee, P. *Can. J. Chem.* **1977**, *55*, 2975.
(7) Geran, R. L.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 1.
(8) Cioli, D.; Pica-Mattoccia, L. *Am. J. Trop. Med. Hyg.* **1984**, *33*, 80.

Table I. Antitumor Activity of Thioxanthen-9-one Esters



no.	substituents	R	empirical formula	mp, °C	in vivo antitumor act. vs P-388 lymphocytic leukemia in mice: dose, mg/kg	T/C
1	4-CH_2OH (hycanthone, HC)	C_2H_5			60	165
4	4-$CH_2OCONHCH_3$ (HNMC)	C_2H_5			12.5	toxic
					6.25	166
					3.13	132
23	4-$CH_2OCONHC_6H_5$	C_2H_5	$C_{27}H_{29}N_3O_3S$	161–162	12.5–50	toxic
					6.25	111
24	4-$CH_2OCONHC_3H_7$	C_2H_5	$C_{24}H_{31}N_3O_3S$	128–129	12.15	toxic
					6.25	134
					3.12	117
					1.50	99
25	4-$CH_2OCONHC_4H_9$	C_2H_5	$C_{25}H_{33}N_3O_3S$	141–142	25	129
					12.5	104
					6.25	135
					3.12	119
5	4-$CH_2OCONHCH_3$, 7-OH	CH_3	$C_{22}H_{23}N_3O_4S$	145–165	12.5	112
					6.25	127
					3.12	130
					1.5	134
26	4-$CH_2OCOOCH_3$	C_2H_5	$C_{22}H_{26}N_2O_4S$	120–122	20	122
					10	124
					5	124
					2.5	118
27	4-CH_2OCOCH_3	C_2H_5	$C_{22}H_{26}N_2O_3S$	120–121	50	118
					25	145 (125)[a]
					12.5	121 (129)
					6.25	109 (123)
28	4-$CH_2OCOC_6H_3(NO_2)_2$	C_2H_5	$C_{27}H_{26}N_4O_7S$	144–145		

[a] Values for a duplicate run are in parentheses.

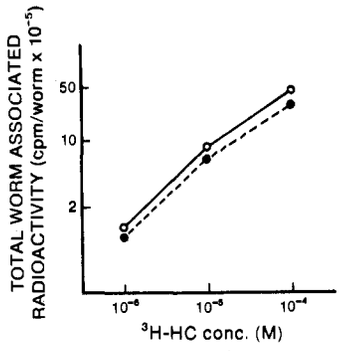


Figure 2. Total worm-associated radioactivity as a function of concentration of [^{3}H]HC. Solid line is the HC-sensitive strain, and broken line is the HC-resistant strain. See Experimental Section for details.

HC-sensitive worms but was essentially inactive in the HC-resistant strain. The reason for this is not clear.

As shown in Figures 1 and 2, the uptake of [^{3}H]HC[9] is the same in HC-sensitive and HC-resistant worms regardless of whether the total worm-associated radioactivity is measured as a function of time or drug concentration. The lack of difference in uptake between the two strains effectively rules out any mechanism of resistance based on decreased drug permeability or uptake in the HC-resistant strain.

Direct evidence for covalent binding of [^{3}H]HC and [^{3}H]HNMC to schistosomal DNA was reported recently. In separate experiments, the radiolabeled drugs at concentrations at 5×10^{-5} M were incubated with HC-sensitive and HC-resistant worms. The DNA from both strains was isolated and rigorously purified. In two determinations the amounts of DNA-bound HC in sensitive worms were 48 and 80 pmol/mg of DNA whereas in the resistant worms less than 0.1 pmol/mg of DNA was found. In the case of HNMC, 79 and 57 pmol/mg of DNA were observed in the sensitive strain and the corresponding values for the HC-resistant strain were 49 and 34 pmol/mg of DNA. These results support the hypothesis that resistance to HC in schistosomes is due to the absence of an esterifying enzyme which converts HC to an alkylating agent.[10]

When [^{3}H]HNMC was incubated with calf thymus DNA at 37 °C for 1.5 h and the mixture dialyzed at 4 °C against saline for 4 days, it was observed that a substantial amount of the radioactivity did not dialyze out. About one-third of the counts per minute were precipitated by the addition of the trichloroacetic acid (TCA), indicating that the [^{3}H]HNMC is very tightly bound to the DNA. When the same experiment was repeated with [^{3}H]HC, practically none of the radioactivity was associated with the TC-precipitable material (Table III). This evidence for covalent binding, although indirect, supports the mechanism shown in Scheme I.

HeLa cells were incubated with [^{3}H]uridine in the presence and absence of HC and HNMC. In one series

(9) [^{3}H]HC (sp. act. ca. 18 Ci/mmol) was prepared by reduction of HC aldehyde with $NaBT_4$. [^{3}H]HNMC was prepared by treating [^{3}H]HC with CH_3NCO. These preparations were carried out at New England Nuclear, Boston, MA, and also by Amersham, Inc., Arlington Heights, IL.

(10) Pica-Mattocia, L.; Cioli, D.; Archer, S. *Biochem. Soc. Trans.* **1987**, *15*, 76.

Table II. In Vitro [^{3}H]Uridine Incorporation and Survival in Culture of HC-Sensitive and HC-Resistant Adult *S. mansoni* Treated with Esters of Hycanthone[a,b]

		sensitive worms		resistant worms	
no.	concn, M × 10^6	[^{3}H]uridine incorp, % control	day 50% of worms were dead in culture	[^{3}H]uridine incorp, % control	day 50% of worms were dead in culture
1	0.7	20		–	>12
	1.4	30	7	98	
2	1.3	43	9	96	>12
	2.6	20	–	108	–
	5.2	23	–	91	>12
4	1.2	21	9	39	8
	6.0	3	–	11	8
23	1.0	18	8	33	12
	5.0	7		32	8
24	1.1	40	>12	100	>12
	5.5	22	10	56	>12
25	1.1	34	>12	100	>12
	5.5	31	>12	100	>12
26	1.2	32	7	80	13
	6.0	6	6	28	6
27	1.3	13	7	19	>15
	6.3	4	–	–	7
28	0.9	50	7	95	>17
	4.5	2		105	

[a] The methods used in these experiments are described in ref 5. [b] Cultures were terminated at different times in different experiments. Survival for >12 days is considered to be indefinite, i.e., no drug effect.

Table III. In Vitro Binding of [^{3}H]HC and [^{3}H]HNMC to Calf Thymus DNA. Nondialyzable Radioactivity (Counts per Minute) in Aliquots Taken from Mixtures of Calf Thymus DNA plus [^{3}H]HC or [^{3}H]HNMC[a]

time	[^{3}H]HC alone	[^{3}H]HC + DNA	[^{3}H]HNMC alone	[^{3}H]HNMC + DNA
	a. Total Radioactivity (cpm)			
0	250 000	235 000	168 000	134 000
2 h	20 500	50 000	27 500	52 000
1 day	860	3460	1750	15 000
2 days	1170	2300	1200	14 800
3 days	700	1600	1350	16 000
4 days	1900	1100	1500	15 000
	b. Alcohol and TCA Precipitable Radioactivity			
0	260	350	160	11 500
2 h	100	430	220	12 300
1 day	40	330	150	9500
2 days	40	275	170	8000
3 days	62	265	100	6900
4 days	58	200	150	5100

[a] The ^{3}H-labeled drugs (0.02 μCi) were added to solutions of 50 μg of calf thymus DNA in 20 μL of 0.1 M citrate–phosphate buffer, pH 7. The solutions (including controls) were incubated in the dark for 1.5 h at 37 °C. They were diluted to 0.1 mL of saline and dialyzed against saline at 4 °C. Duplicate 10-μL samples were taken at various times from inside the bags and counted directly. Another set of duplicate samples were counted after TCA precipitation.

of experiments the incorporation of the [^{3}H]uridine was measured in the presence of the drugs, and in another the same measurements were carried out 2 h after the drugs were removed by washing. The results are summarized in Table IV. Significant inhibition of [^{3}H]uridine incorporation was observed in the presence of either HC or HNMC. However, when the cells were washed prior to the determination of [^{3}H]uridine incorporation, it was found that in the case of HC the inhibition of incorporation diminished whereas in the case of HNMC, if anything, the inhibition of incorporation of [^{3}H]uridine was more pronounced, suggesting that irreversible binding to DNA had occurred.

A series of experiments similar to those undertaken for resistant and sensitive schistosomes were carried out in HeLa cells. The DNA was isolated and purified by CsCl gradient density centrifugation, and the purified material was dialyzed and then hydrolyzed with DNase. The results, summarized in Table V, are expressed in terms of drug molecules per base pair.

Table IV. Incorporation of [^{3}H]Uridine in HeLa Cells in the Presence and Absence of HC and HNMC[a]

		TCA precipitable radioact, % control	
drug	concn, M	drug present	2 h after drug removal
HC	10^{-5}	32	88
	2×10^{-5}	37	68
HNMC	10^{-6}	63	64
	2×10^{-6}	57	24
	10^{-5}	11	4
	2×10^{-5}	6	2

[a] Duplicate tubes containing 10^6 HeLa cells in 0.5 mL of medium were incubated for 1 h in the presence of the drug to be tested. Tritiated uridine was added, and incubation was continued for 1 h more. At the end of the incubation, the cells were washed with cold saline solution, 10% TCA was added, and the precipitates were collected on glass filter disks, washed with TCA, and counted. Another set of duplicate tubes were incubated for 1 h in the presence of the drug, washed three times with medium, and then incubated for an additional 2 h in a drug-free medium. At the end of this time, [^{3}H]uridine was added, and after 1 h, the cells were treated with 10% TCA as described above. The results are expressed as % TCA-precipitable radioactivity compared with nontreated controls.

Table V. Binding of [^{3}H]HC and [^{3}H]HNMC to HeLa Cell DNA

drug	concn, M	drug molecules per base pair
[^{3}H]HC	1×10^{-4}	$<4 \times 10^{-8}$
	5×10^{-5}	7×10^{-7}
	1×10^{-5}	$<4 \times 10^{-8}$
[^{3}H]HNMC	1×10^{-4}	1.05×10^{-3}
	5×10^{-5}	2.03×10^{-4}
	1×10^{-5}	8.4×10^{-5}

After 1-h incubation of [^{3}H]HC with HeLa cells, practically no incorporation of radiolabel was observed. [^{3}H]HNMC was incorporated into HeLa cell DNA in a dose-dependent manner as would be expected if the *N*-

methylcarbamate ester **4** acted as a surrogate for **3**.

Discussion

The results reported herein coupled with those reported earlier[5,8,10] strongly support the mechanism shown in Scheme I, which serves as the molecular basis for the antischistosomal, antitumor, and, as Hartmann and Hulbert[4] proposed earlier, the mutagenic activity of hycanthone. Resistant worms appear to be less susceptible to the action of hycanthone esters. This is strikingly demonstrated in the case of hycanthone 3,5-dinitrobenzoate (**28**), which is essentially inactive against the HC-resistant strain of *S. mansoni*. It is possible that other unknown factors affecting resistance may be operative.

In the case of HeLa cells, the observation that at concentrations of 1×10^{-4} M and 1×10^{-5} M only traces of [^{3}H]HC are bound to DNA lends support to the postulated mode of action, which requires that enzymic esterification precede alkylation. Apparently, 1 h of drug exposure is inadequate time to allow the required ester to accumulate in sufficient quantities to furnish more than trace amounts of ^{3}H-labeled DNA.

In the experiment in which 5×10^{-5} M [^{3}H]HC was employed, greater binding of the drug to the DNA was observed than at the other concentrations. This may be an artifact, or it is possible that a small amount of enzymic esterification did occur to permit the detection of a measurable quantity of DNA alkylation. It should be noted that even in this case the number of drug molecules bound per base pair was about 3 orders of magnitude less than in the corresponding [^{3}H]HNMC experiment.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are corrected. Infrared spectra were run on a Perkin-Elmer Model 298 infrared spectrometer. The proton NMR spectra were run on a 60-MHz Hitachi Perkin-Elmer R-600 spectrometer in $CDCl_3$, unless stated otherwise, with $(CH_3)_4Si$ as the internal standard. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, and Galbraith Microanalytical Laboratories. Unless otherwise specified, all elemental analyses were for C, H, N and were within ±0.3% of the theoretical values.

Hycanthone Carbamates. General Preparation. Hycanthone *N*-Phenylcarbamate (23). A solution of 400 mg (1.12 mmol) of hycanthone in 20 mL of dry CH_2Cl_2 was treated with 0.2 mL (307 mg, 2.225 mmol) of phenyl isocyanate. The mixture was stirred overnight. The CH_2Cl_2 was removed in vacuo to leave ca. 530 mg (100%) of the crude carbamate, which was purified by chromatography on silica gel with ethyl acetate as the eluant. The crystals obtained were recrystallized from ethyl acetate/hexane.

A similar procedure was used for the *N*-propylcarbamate **24** and *N*-butylcarbamate **25** by starting with *n*-propyl isocyanate and *n*-butyl isocyanate, respectively.

Hycanthone Acetate (27).[11] Five grams (0.014 mol) of hycanthone was dissolved in 100 mL of dry pyridine, and to the cooled solution was added 50 mL of acetic anhydride with stirring. After 2 h, the ice bath was removed and the solution left overnight. The solution was evaporated to dryness under reduced pressure, and the residue was dissolved in ethyl acetate, washed with water, dried, and evaporated to leave to solid, which was triturated with H_2O, filtered, and dried, weight 3.5 g (63%). Trituration with dry ether gave analytically pure ester: IR 3240 (NH), 1725 (acetate C=O), 1615 cm^{-1} (C=O); NMR δ 1.15 (t, 6 H, CH_2CH_3), 2.15 (s, 3 H, CH_3CO), 2.70 (q, 4 H, CH_2CH_3), 2.50–3.00 and 3.20–3.60 (2 m, 4 H, CH_2CH_2), 5.23 (s, 2 H, CH_2O), 6.50–6.80, 7.20–7.70, and 8.40–8.70 (3 m, 6 H, aromatic H).

Hycanthone Methyl Carbonate (26). A solution of 660 mg (1.85 mmol) of hycanthone in 30 mL of CH_2Cl_2 containing 210 mg (2.65 mmol) in dry pyridine was cooled to 0 °C while a solution of 200 mg (2.11 mmol) of methyl chloroformate in 10 mL of CH_2Cl_2 was added dropwise with stirring over a period of 30 min. The mixture was cooled overnight and then stirred at room temperature for 4 h. The mixture was diluted with 60 mL of CH_2Cl_2 and washed with cold water. The dried organic layer was evaporated to dryness, leaving 420 mg of a yellow solid, which was triturated with dry ether and filtered. The filtrate was concentrated to 20 mL and then treated with 10 mL of hexane, and the solution was cooled overnight to give yellow crystals (150 mg, 19.5%): IR 3230 (NH), 1740 (C=O, carbonate), 1610 cm^{-1} (C=O); NMR δ 1.15 (t, 6 H, CH_2CH_3), 2.68 (q, 4 H, CH_2CH_3), 2.50–3.00 and 3.10–3.60 (2 m, 4 H, CH_2CH_2), 3.85 (s, 3 H, OCH_3), 5.33 (s, 2 H, CH_2O), 6.50–6.80 and 7.20–7.50 (m, 6 H, aromatic H), 5.80 (br s, 1 H, NH).

2-Chloro-6-[(*p*-methoxyphenyl)thio]benzonitrile (10) and 2,6-Bis[(*p*-methoxyphenyl)thio]benzonitrile (11). Twenty grams (0.178 mol) of potassium *t*-butoxide was suspended in 250 mL of dry DMSO, and 25.0 g (0.178 mol) of *p*-methoxybenzenethiol (**8**) was added. Twenty-nine grams (0.168 mol) of 2,6-dichlorobenzonitrile (**9**) was added portionwise at room temperature. When the addition was complete, the mixture was stirred at room temperature for 2 h and then heated on the steam bath for 3 h. The mixture was then poured onto 2.5 L of ice/water. The suspension was made strongly alkaline with NaOH solution and allowed to stand overnight. The solid was collected, filtered, washed, and dried, weight 44 g. The crude product was boiled with ethanol and the insoluble material collected and dried, weight 6.0 g (9.4%). After crystallization from ethyl acetate, it melted at 182–183 °C. This is 2,6-bis[(*p*-methoxyphenyl)thio]benzonitrile (**11**): IR 2218 cm^{-1} (CN); NMR δ 3.88 (s, 6 H, CH_3O), 6.50–7.20 (m, 11 H, aromatic). Anal. ($C_{21}H_{17}NO_2S_2$), C, H, N.

The ethanol filtrate was concentrated and cooled to give 30.0 g of a crystalline solid; mp 107–108 °C. Concentration and cooling of the mother liquor gave an additional crop of material of about the same purity; total yield 35 g (76%). After one more crystallization from ethanol, **10** melted at 109–110 °C: IR 2215 cm^{-1} (CN); NMR δ 3.85 (s, 3 H, CH_3O), 6.50–7.60 (m, 7 H, aromatic H). Anal. ($C_{14}H_{10}ClNOS$) C, H, N.

1-Chloro-7-methoxy-9*H*-thioxanthen-9-imine (12). A suspension of 1.0 g (3.63 mmol) of 2-chloro-6-[(*p*-methoxyphenyl)thio]benzonitrile in 6.5 g (44 mmol) of CF_3SO_3H was stirred at 70 °C until all the nitrile had dissolved (about 2 min). Ice/water (50 mL) was added to the cooled dark viscous solution, and after vigorous shaking, the aqueous portion was decanted from the yellow gum, which was dissolved in 50 mL of MeOH. The combined aqueous and methanolic extracts were neutralized with saturated $NaHCO_3$ and extracted three times with $CHCl_3$. The $CHCl_3$ was evaporated to leave a gum (1.0 g), which, after chromatography on silica gel with $CHCl_3$ as the eluant, gave **12**, which melted at 134–136 °C after crystallization from MeOH: IR 3290 (NH, imine), 1593 cm^{-1} (C-9, C=N); NMR δ 3.90 (s, 3 H, OCH_3), 6.80–7.60 (m, 6 H, 5 aromatic H, NH), 8.10 (m, 1 H, aromatic H at C-8). Anal. ($C_{14}H_{10}ClNOS$) C, H, N.

1-Chloro-7-methoxy-9*H*-thioxanthen-9-one (13). One gram of crude **12**, prepared as described above, was dissolved in 10 mL of pyridine and 50 mL of ethanol. Ten milliliters of 5 N KOH was added and the mixture refluxed for 24 h. The solvents were removed under reduced pressure, and the residue was dissolved in $CHCl_3$, washed with H_2O, and evaporated to leave 1.0 g of a gum, which, after chromatography on silica gel with $CHCl_3$ as the eluant, gave a crystalline solid, which melted at 132–133 °C after recrystallization from MeOH: IR 1635 cm^{-1} (C=O); NMR δ 3.95 (s, 3 H, OCH_3), 6.80–7.60 (m, 5 H, aromatic H), 8.42 (d, 1 H, aromatic H at C-8). Anal. ($C_{14}H_9ClO_2S$) C, H.

The combined cyclization–hydrolysis was carried out on a larger scale to give material that is not analytically pure but satisfactory for use in the next step. Twenty grams (0.73 mol) of **12** was added to 60 mL of CF_3SO_3H at 0 °C, and the solution was stirred at room temperature for 10 h. It was poured into H_2O, and the suspension was heated for 4–5 h on the steam bath with stirring. The solid was collected, washed with H_2O, and dried. After chromatography on silica gel with $CHCl_3$ as the eluting solvent, there was obtained 10.0 g (53%) of 1-chloro-7-methoxy-9*H*-thioxanthen-9-one, mp 128–130 °C.

(11) Miller, J. L.; Hulbert, P. B. *J. Pharm. Pharmacol.* **1976**, *28* (Suppl. 1), 18. These authors mention this ester without giving details of its preparation or physical properties.

1-[[2-(Dimethylamino)ethyl]amino]-7-methoxy-9*H*-thioxanthen-9-one (14). A solution of 1.0 g (3.8 mmol) of **13**, 0.4 g (4.5 mmol) of (dimethylamino)ethylamine, and 2 mL of pyridine was refluxed for 36 h. The solvent was removed in vacuo, and the residue was dissolved in 10 mL of HOAc and diluted with 40 mL of H_2O. The solution was clarified by filtration over a bed of Celite, and the filtrate was made alkaline to give the desired product, suitable for use in the next step, weight 1.0 g (78%). After crystallization from ethyl acetate, it melted at 130–132 °C (lit.[3] mp 113–114 °C).

1-[[2-(Dimethylamino)ethyl]amino]-7-(toluenesulfonyloxy)-9*H*-thioxanthen-9-one (16). A solution of 1-[[2-(dimethylamino)ethyl]amino]-7-methoxy-9*H*-thioxanthen-9-one (**14**) (4.0 g) in 40 mL of 57% HI was heated under reflux for 3 h, cooled, and filtered. The HI salt was washed with ether and dried. (A small sample melted at 225–227 °C after crystallization from CH_3OH/ether.) The salt was suspended in H_2O, treated with 40% NaOH, and filtered. The filtrate was carefully neutralized to give 2.57 g of the hydroxy compound **15**. (A sample after crystallization from ethanol melted at 175–177 °C.) In another run, 11.0 g of **14** (3.35 mmol) and 110 mL of 57% HI furnished 7.0 g (67%) of the hydroxy-9*H*-thioxanthen-9-one **15**.

Compound **15** (2.3 g) was dissolved in 20 mL of dry pyridine, and the solution was cooled to 0 °C. To the cold, stirred solution was added 1.6 g (8.5 mmol) of *p*-toluenesulfonyl chloride portionwise. The reaction mixture was stirred at room temperature for 30 min, poured into ice/water, and treated with Na_2CO_3. After standing, the supernatant liquor was decanted and the residue was triturated with H_2O. The solid was filtered, washed with H_2O, and dried, weight 2.98 g (87%). After crystallization from ethanol, the tosylate **16** melted at 118–119 °C: NMR δ 2.37 (s, 6 H, $N(CH_3)_2$), 2.43 (s, 3 H, CH_3), 2.45–2.80 (t, 2 H, CH_2), 3.10–3.60 (m, 2 H, CH_2), 6.60 (d, 1 H, aromatic H), 7.10–8.10 (m, 9 H, aromatic H). Anal. ($C_{24}H_{24}N_2O_4S_2$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-4-(hydroxymethyl)-7-(*p*-toluenesulfonyloxy)-9*H*-thioxanthen-9-one (17). A solution of 2.50 g (5.4 mmol) of the toluenesulfonyloxy ester **16**, 20 mL of 20% acetic acid, and 400 mL of 37% formaldehyde was kept at 80 °C for 72 h. The mixture was cooled and made basic with 10% Na_2CO_3. It was extracted with 3 × 150 mL portions of $CHCl_3$. The dried extracts were evaporated, and the residue was chromatographed on a silica gel column, 50 × 2.0 cm (weight of silica gel = 40 g). The material was eluted with MeOH/$CHCl_3$ by gradient elution starting with 1.0% MeOH, with the MeOH concentration gradually increasing to 6%. The desired product eluted with 3–6% MeOH/$CHCl_3$ to give 1.72 g (66%) of **17**, which melted at 175–176 °C after crystallization from MeOH: NMR δ 2.33 (s, 6 H, $N(CH_3)_2$), 2.43 (s, 3 H, CH_3), 2.40–2.75 (t, 2 H, CH_2), 3.00–3.40 (m, 2 H, CH_2), 4.63 (s, 2 H, CH_2OH), 6.43 (d, 1 H, aromatic H), 7.10–8.10 (m, 8 H, aromatic). Anal. ($C_{25}H_{26}N_2O_5S_2$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-9-oxo-7-(toluenesulfonyloxy)-9*H*-thioxanthene-4-carboxaldehyde (18). A suspension of 2.3 g of MnO_2 in a solution of 2.3 g (4.6 mmol) of the 4-(hydroxymethyl)-9*H*-thioxanthen-9-one **17** in 100 mL of CH_2Cl_2 was stirred under reflux for 2 h. An additional quantity of MnO_2 (4.0 g) was added, and stirring was continued at room temperature for 16 h. The suspension was filtered and the filtrate taken to dryness, leaving 1.99 g (83%) of the aldehyde, which was satisfactory for use in the next step. After one crystallization from C_2H_5OH, the aldehyde melted at 142–143 °C: NMR δ 2.40 (s, 6 H, $N(CH_3)_2$), 2.46 (s, 3 H, CH_3), 2.50–2.70 (t, 2 H, CH_2), 3.10–3.60 (m, 2 H, CH_2), 6.60 (d, 1 H, aromatic H), 7.10–8.20 (m, 8 H, aromatic H), 9.95 (s, 1 H, CHO). Anal. ($C_{25}H_{24}N_2O_5S_2$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-9-oxo-7-hydroxy-9*H*-thioxanthene-4-carboxaldehyde (19). A suspension of 1.70 g (3.42 mmol) of toluenesulfonate **18** in 50 mL of ethanol containing 10 mL of aqueous 20% KOH was refluxed for 1 h. The ethanol was distilled off, and the residue was diluted with H_2O and neutralized. The crystals that separated were collected, washed with H_2O, and dried. The yield of crude material, mp 213–215 °C, suitable for use in the next step, was nearly quantitative. After crystallization from MeOH, the pure material melted at 222–223 °C: NMR δ 2.40 (s, 6 H, $N(CH_3)_2$), 2.60–3.00 (t, 2 H, CH_2), 3.00–3.60 (m, 2 H, CH_2), 6.60 (d, 1 H, aromatic H), 6.60–8.00 (m, 4 H, aromatic H), 9.80 (s, 1 H, CHO). Anal. ($C_{18}H_{18}N_2O_3S$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-7-[(*tert*-butyldiphenylsilyl)oxy]-9-oxo-9*H*-thioxanthene-4-carboxaldehyde (20). To a cold stirred solution of 3.2 g (9.35 mmol) of the phenolic aldehyde **19** in 60 mL of dry DMF were added 1.7 g (25 mmol) of imidazole and 3.9 g (14 mmol) of *tert*-butyldiphenylchlorosilane. The solution was stirred at room temperature, protected from moisture, for 36 h and then treated with 300 mL of ether. The mixture was washed with H_2O, dried, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel. The column was eluted with hexane followed by ethyl acetate to remove starting materials and impurities. Elution with $CHCl_3$/MeOH (3:1) gave the desired product as an oil, 5 g (93%), which was used in the next step. The material solidified and, after crystallization from ether, melted at 163–164 °C: IR 3200 (NH at C-1), 1668 (C=O at C-4), 1605 cm^{-1} (C=O at C-9); NMR δ 1.19 (s, 9 H, *tert*-butyl), 2.40 (s, 6 H, $N(CH_3)_2$), 2.60–2.90 and 3.20–3.70 (2 m, 4 H, CH_2CH_2), 6.50–7.10, 7.20–7.90 and 8.20–8.50 (3 m, 15 H, aromatic), 9.85 (s, 1 H, CHO). Anal. ($C_{34}H_{36}N_2O_3SSi$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-7-[(*tert*-butyldiphenylsilyl)oxy]-4-(hydroxymethyl)-9*H*-thioxanthen-9-one (21). Powdered $NaBH_4$ (1.60 g, 42.3 mmol) was added portionwise to a stirred suspension of 4.8 g (8.3 mmol) of the crude aldehyde **20** in ca. 100 mL of MeOH/ether (1:1) at 5 °C. The mixture was stirred at room temperature for 2 h before being poured into 200 mL of 5% NH_4Cl solution. After thorough extraction with $CHCl_3$, the combined extracts were washed with H_2O and evaporated to leave a gum, weight 4.0 g. After chromatography on silica gel, there was obtained 1.6 g of thick oil satisfactory for use in the next step. The oil crystallized, and after crystallization first from ethyl acetate/hexane and then from ethyl acetate alone, it melted at 148–150 °C: IR 3440 (OH), 3270 (NH at C-1), 1595 cm^{-1} (C=O at C-9); NMR δ 1.16 (s, 9 H, *tert*-butyl), 2.35 (s, 6 H, $N(CH_3)_2$), 2.50–2.80 and 3.10–3.55 (2 m, 4 H, CH_2CH_2), 4.72 (s, 2 H, CH_2OH), 6.40–7.00, 7.20–7.90 and 8.15–8.45 (3 m, 15 H, aromatic). Anal. ($C_{34}H_{38}N_2O_3SSi$) C, H, N).

1-[[2-(Dimethylamino)ethyl]amino]-7-[(*tert*-butyldiphenylsilyl)oxy]-4-(hydroxymethyl)-9*H*-thioxanthen-9-one *N*-Methylcarbamate (22). To a solution of 70 mg (0.12 mmol) of **21** in 6 mL of dry CH_2Cl_2 was added 190 mg (0.2 mL, 3.4 mmol) of methyl isocyanate. The flask was stoppered, and the solution was stirred at room temperature for 22 h. An additional portion (0.1 mL) of methyl isocyanate was added, and stirring was continued for 6 h. The solvents were evaporated to leave 70 mg (91%) of almost-pure product, which, after crystallization from ethyl acetate/hexane, melted at 97–98 °C: IR 3220 (NH at C-1), 1710 (C=O, carbamate), 1600 cm^{-1} (C=O at C-9); NMR δ 1.18 (s, 9 H, *tert*-butyl), 2.36 (s, 6 H, $N(CH_3)_2$), 2.85 (d, 3 H, $NHCH_3$), 2.50–3.00 and 3.20–3.60 (2 m, 4 H, CH_2CH_2), 5.18 (s, 2 H, CH_2O), 6.40–7.00, 7.20–7.90 and 8.10–8.40 (3 m, 15 H, aromatic H). Anal. ($C_{36}H_{41}N_3O_4SSi$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-7-hydroxy-4-(hydroxymethyl)-9*H*-thioxanthen-9-one *N*-Methylcarbamate (5). A solution of 1 M tetrabutylammonium fluoride (TBAF) (0.6 mL = 0.6 mmol) in 1.0 mL of THF was added dropwise with stirring to a solution of 110 mg (0.172 mmol) of the *tert*-butyldiphenylsilyl ether **22** in 20 mL of dry THF at 0 °C in an atmosphere of dry N_2. The solution was stirred at 0 °C for 5 min and then allowed to warm to room temperature over a period of 25 min. Powdered dry ice was added carefully, and the solvent was removed at room temperature in vacuo. The residue was taken up in 30 mL of acid-free $CHCl_3$ and washed with H_2O (4 × 15 mL portions). The dried solution was concentrated to dryness in vacuo at room temperature, and the residue was triturated with hexane several times to remove most of the *tert*-butyldiphenylsilyl fluoride. The residue, weight 60 mg (87%), showed a peak at δ 1.13 for $(CH_3)_3C$.

A sample was recrystallized as follows. A sample (18.1 mg) of the above quality was dissolved in 1.0 mL of dry, acid-free $CHCl_3$, filtered, and concentrated at room temperature to about 0.5 mL. On standing at −20 °C, crystals deposited, which were filtered, washed with hexane, and dried at 80 °C, 0.02 Torr: NMR δ 2.48 (s, 6 H, $N(CH_3)_2$), 2.85 (d, 3 H, $NHCH_3$), 3.00–3.70 (2 m, 4 H, CH_2CH_2), 5.18 (s, 2 H, benzylic CH_2), 6.40–7.00, 7.20–7.90, 8.10–8.40 (3 m, 15 H, aromatic H); IR (KBr) 3320 (OH), 1685

(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).

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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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