Intercalating Agents with Covalent Bond Forming Capability. A Novel Type of Potential Anticancer Agents. 2.[1] Derivatives of Chrysophanol and Emodin

Masao Koyama,[†] Kiyobumi Takahashi,[†] Ting-Chao Chou,[‡] Zbigniew Darzynkiewicz,[§] Jan Kapuscinski,[§] T. Ross Kelly,[∥] and Kyoichi A. Watanabe*,[†]

Laboratories of Organic Chemistry, Pharmacology, and Experimental Cell Research, Sloan-Kettering Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021, and Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02167.
Received July 14, 1988

Fifty-one new *C*-methyl-modified derivatives of the anthraquinones chrysophanol and emodin or their various methyl ethers were prepared for structure–activity relationship studies of anticancer activity against mouse leukemia L1210 and human leukemia HL-60 cells. Representative compounds were spectrophotometrically studied for their capacity to interact with natural and denatured DNA. In general, those anthraquinones bearing an amino function interact with DNA. 1,8-Dimethoxyanthraquinones are incapable of intercalating into DNA. 1- or 8-Monohydroxymonomethoxyanthraquinones, however, interact with DNA to some extent. No straightforward correlation is apparent between the DNA-affinity data of the compounds studied spectrophotometrically and their cytotoxic effects. Cytotoxic potencies of these compounds on cell growth inhibition during a 72-h period are inversely correlated to their potencies when inhibiting [^{3}H]TdR incorporation into DNA during the initial 30 min of exposure. Surprisingly, some compounds that showed more cytotoxicity did not inhibit initial TdR incorporation (0–30 min), while some others that strongly inhibited TdR incorporation initially did not exhibit cytotoxicity in 72 h. The results suggest that the cytotoxicity produced by these compounds is time dependent and is not a direct result of initial inhibition of DNA replication.

A number of analogues of certain antitumor intercalating agents, such as ellipticine (Figure 1),[2,3] 4′-(9-acridinylamino)methanesulfon-*m*-aniside (*m*-AMSA, amsacrine),[4] and anthracycline antibiotics (e.g., doxorubicin)[5-7] have been synthesized in order to gain better therapeutic potential. However, preliminary screening data show that there is no straightforward structure–activity relationship within each group. These results seem to suggest that although intercalation may be a necessary condition, it may not be sufficient and other factors may be involved that per se potentiate the anticancer activity.

Studies on the mechanism of anticancer action of the indole antibiotic CC1065[8,9] show that it binds to the minor groove of DNA by nonintercalative means and then slowly alkylates the amino group of adenine by opening the cyclopropane ring in the antibiotic molecule. With CC1065, covalent binding of the drug with DNA, therefore, seems to be important for its potent cytotoxic activity. Mere physical interaction between the drug and DNA may not be sufficient.

Recent studies indicate that *m*-AMSA inhibits the topoisomerization and catenation reactions of DNA topoisomerase II,[10] probably by trapping the enzyme–DNA complexes.[11,12] Other substances, such as etoposide (VP-16, Figure 1), adriamycin, and ellipticine[13] also stabilize the cleavable complex between DNA topoisomerase II and DNA. In the present study, we show that the incorporation of an alkylating group into some DNA intercalating agents greatly enhances their antileukemic properties.

It is well-known that one of the metabolites of ellipticine, 9-hydroxyellipticine[14] (9-OH-E), is also a potent anticancer agent.[15] 2-*N*-Methyl-9-hydroxyellipticinium (9-OH-NME) is one of the most active drugs among the ellipticine analogues.[16] The latter is easily oxidized by peroxidases to 9-oxo-2-methylellipticinium[17,18] (9-oxo-NME), which is highly electrophilic and alkylates various nitrogen,[19,20] sulfur,[21,22] and oxygen[18,19] nucleophiles. Among biological macromolecules, proteins,[23] polyadenylate,[24] RNA,[24] and DNA[25] are easily alkylated by 9-oxo-NME. This "biooxidative alkylation" has been proposed as a possible

[†] Laboratory of Organic Chemistry, Memorial Sloan-Kettering Cancer Center.
[‡] Laboratory of Pharmacology, Memorial Sloan-Kettering Cancer Center.
[§] Laboratory of Experimental Cell Research, Memorial Sloan-Kettering Cancer Center.
[∥] Department of Chemistry, Boston College.

(1) Koyama, M.; Kelly, T. R.; Watanabe, K. A. *J. Med. Chem.* **1988**, *31*, 283, is considered Part 1 of this series.
(2) Le Pecq, J.-B.; Xuong, N.-D.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(3) Guthrie, R. W.; Brossi, A.; Mennona, F. A.; Mullin, J. G.; Kierstead, R. W.; Grunberg, E. *J. Med. Chem.* **1975**, *18*, 755.
(4) Denny, W. A.; Cain, B. F.; Atwell, G. J.; Hansch, C.; Panthananickal, A.; Leo, A. *J. Med. Chem.* **1982**, *25*, 276.
(5) Mosher, C. W.; Wu, H. Y.; Fujiwara, A. N.; Acton, E. M. *J. Med. Chem.* **1982**, *25*, 18.
(6) Seshadri, R.; Isreal, M.; Pegg, W. J. *J. Med. Chem.* **1983**, *26*, 11.
(7) Myers, C. *Cancer Chemother.* **1986**, *8*, 52.
(8) Chidester, C. G.; Krueger, W. C.; Mizsak, S. A.; Duchamp, D. J.; Martin, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 7629.
(9) Li, L. H.; Swenson, D. H.; Schpok, S. L. F.; Kuentzel, S. L.; Dayton, B. D.; Krueger, W. C. *Cancer Res.* **1982**, *42*, 999.
(10) Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665.
(11) Nelson, E. M.; Tewey, K. M.; Liu, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1361.
(12) Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560.
(13) Kuhn, K. W.; Pommier, Y.; Kerrigan, D.; Markowitz, J.; Cuvey. J. M. *Natl. Cancer Inst. Monogr.* **1987**, *4*, 61.
(14) Lesca, P.; Lecointe, P.; Paoletti, C.; Mansuy, D. *Biochem. Pharmacol.* **1977**, *26*, 2169.
(15) Le Pecq, J.-B.; Gosse, C.; Xuong, N. D.; Cros, S.; Paoletti, C. *Cancer Res.* **1976**, *36*, 3067.
(16) Bernadou, J.; Meunier, B.; Meunier, G.; Auclair, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1297.
(17) Auclair, C.; Paoletti, C. *J. Med. Chem.* **1981**, *24*, 289.
(18) Bernadou, J.; Meunier, G.; Paoletti, C.; Meunier, B. *J. Med. Chem.* **1983**, *26*, 574.
(19) Meunier, G.; Meunier, B.; Auclair, C.; Bernadou, J.; Paoletti, C. *Tetrahedron Lett.* **1983**, *26*, 574.
(20) Auclair, C.; Voisin, E.; Banoun, H.; Paoletti, C.; Bernadou, J.; Meunier, B. *J. Med. Chem.* **1984**, *27*, 1161.
(21) Monsarrat, B.; Maftouh, M.; Meunier, G.; Dugue, B.; Bernadou, J.; Armand, J. P.; Picard-Fraire, C.; Meunier, B.; Paoletti, C. *Biochem. Pharmacol.* **1983**, *32*, 3887.
(22) Bernadou, J.; Monsarrat, B.; Roche, H.; Armand, J. P.; Paoletti, C.; Meunier. B. *Cancer Chemother. Pharmacol.* **1985**, *15*, 63.
(23) Auclair, C.; Meunier, B.; Paoletti, C. *Biochem. Pharmacol.* **1984**, *32*, 3883.
(24) Dugue, B.; Paoletti, C.; Meunier, B. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 416.
(25) Auclair, C.; Dugue, B.; Meunier, B.; Paoletti, C. *Biochemistry* **1986**, *25*, 1240.

0022-2623/89/1832-1594$01.50/0

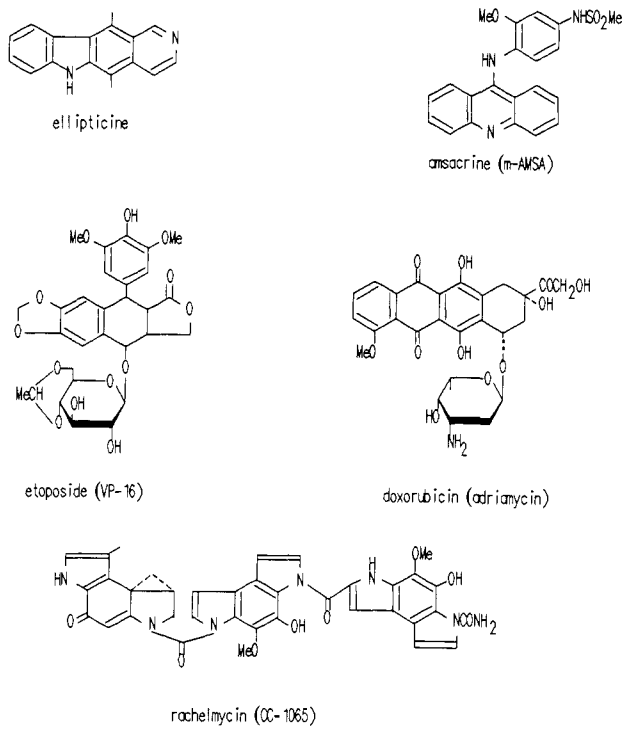


Figure 1.

mode of anticancer action.[26]

For other intercalating anticancer agents, such as amsacrine (*m*-AMSA), and the anthracycline antibiotics, extensive studies on their mechanism of anticancer action[11,12,27-31] and QSAR studies directed toward the development of more selective drugs have been conducted.[32-34] Whether these intercalators bind covalently to biomolecules has not been established.

In order to test our hypothesis that intercalating agents with covalent bond forming capability may exert potent cytocidal activity, we chose chrysophanol Ia and emodin Ib (Figure 2) as the starting materials: Ia and Ib were isolated from crude rhubarb extract.[35] Both compounds exhibit little anticancer or cytocidal activity. Their structural aromatic features indicate that these compounds and their derivatives (particularly positively charged ones) may intercalate into double helix of nucleic acids.

Chemistry

The hydroxy groups at the 1- and 8-positions of I were methylated with methyl sulfate and K_2CO_3 in acetone[36]

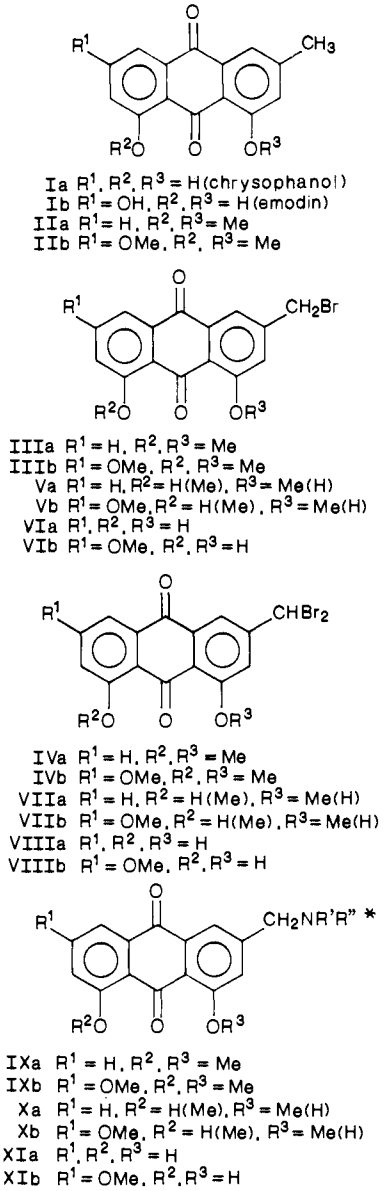
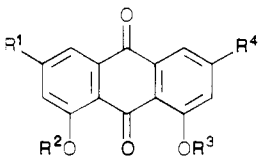


Figure 2. (*) See Table I for NR'R".

to the known 1,8-dimethoxy-9,10-anthraquinones II. The *C*-methyl group of II was then brominated with NBS[37] or 1,3-dibromo-5,5-dimethylhydantoin (BDH)[38,39] in carbon tetrachloride in the presence of benzoyl peroxide to give monobromide III as the major product along with a small amount of dibromide IV. Treatment of III with various amines including mono(2-hydroxyethyl)amine and bis(2-hydroxyethyl)amine afforded the corresponding alkylamino derivatives IX (Table I). Chlorination of the (2-hydroxyethyl)amino derivatives IX-2 gave the corresponding 3-[[(2-chloroethyl)amino]methyl]-9,10-anthraquinones IX-3. On the basis of reports by Anderson et al.[40-43] that certain carbamates are susceptible to nucleo-

(26) Dugue, B.; Auclair, C.; Meunier, B. *Cancer Res.* **1986**, *46*, 3828.
(27) Riou, J. F.; Vilarem, M. J.; Larsen, C. J.; Riou, G. *Biochem. Pharmacol.* **1986**, *35*, 4409.
(28) Crooke, S. T., Reich, S. D., Eds. *Anthracyclines: Current Status and New Developments*; Academic Press: New York, 1980.
(29) Myers, C. E. In *Pharmacologic Principles of Cancer Treatment*; Chabner, B., Ed.; Saunders: Philadelphia, 1982; p 416.
(30) DiMarco, A. In *Cancer Medicine*; Holland, J. F., Frei, E., Eds.; Lea and Febiger: Philadelphia, 1982; 2nd ed., p 872.
(31) Muggia, F. M., Young, C. W., Carter, S. K. Eds.; *Anthracycline Antibiotics in Cancer Therapy*; Martinus Nijhoff: Hague/Boston/New York, 1982; pp 71-174.
(32) Ferguson, L. R.; Denny, W. A. *J. Med. Chem.* **1980**, *23*, 269.
(33) Denny, W. A.; Cain, B. F.; Atwell, G. J.; Hansch, C.; Panthananickal, A.; Leo, A. *J. Med. Chem.* **1982**, *25*, 276.
(34) Alexander, J.; Khanna, I.; Lednicer, D.; Mitscher, L. A.; Veysoglu, T.; Wielogorski, Z.; Wolgemuth, R. L. *J. Med. Chem.* **1984**, *27*, 1343.
(35) Kelly, T. R.; Chandrakumar, N. S.; Walters, N.; Blancaflor, J. *J. Org. Chem.* **1983**, *48*, 3573.
(36) Alexander, J.; Bhatia, A. V.; Mitscher, L. A.; Omoto, S.; Suzuki, T. *J. Org. Chem.* **1980**, *45*, 20.
(37) Banville, J.; Brassard, P. *J. Chem. Soc., Perkin Trans. 1*, **1976**, 613.
(38) Sargent, M. V.; Smith, D. O. N.; Elix, J. A.; Roffey, P. *J. Chem. Soc., C* **1969**, 2763.
(39) Cava, M. P.; Ahmed, Z.; Benfaremo, N.; Murphy, R. A.; O'Malley, G. J. *Tetrahedron* **1984**, *40*, 4767.
(40) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 812.
(41) Anderson, W. K.; Halat, M. J. *J. Med. Chem.* **1979**, *22*, 977.
(42) Anderson, W. K.; Chang, C.-P.; McPherson, H. W. *J. Med. Chem.* **1983**, *26*, 1333.
(43) Zwelling, L. A. *Cancer Metastasis Rev.* **1985**, *4*, 263.

Table I. Synthetic Derivatives of Chrysophanol and Emodin



compd[a]	R^1	R^2	R^3	R^4	mp, °C[b,c]	formula
IIa	H	Me	Me	Me	189–190	$C_{17}H_{14}O_4$
IIb	OMe	Me	Me	Me	228–229	$C_{18}H_{16}O_5$
IIIa	H	Me	Me	CH_2Br	176–178	$C_{17}H_{13}BrO_4$
IIIb	OMe	Me	Me	CH_2Br	250–254	$C_{18}H_{15}BrO_5$
IVa	H	Me	Me	$CHBr_2$	207–210	$C_{17}H_{12}Br_2O_4$
IVb	OMe	Me	Me	$CHBr_2$	254–257	$C_{17}H_{14}Br_2O_5$
Va	H	H or Me	Me or H	CH_2Br	213–215	$C_{16}H_{11}BrO_4$
Vb	OMe	H or Me	Me or H	CH_2Br	199–201	$C_{17}H_{13}BrO_5$
VIa	H	H	H	CH_2Br	220–222	$C_{15}H_9BrO_4$
VIb	OMe	H	H	CH_2Br	249–250	$C_{16}H_{11}BrO_5$
VIIa	H	H or Me	Me or H	$CHBr_2$	176–178	$C_{16}H_{10}Br_2O_4$
VIIb	OMe	H or Me	Me or H	$CHBr_2$	202–203	$C_{17}H_{12}Br_2O_5$
VIIIa	H	H	H	$CHBr_2$	211–213	$C_{15}H_8Br_2O_4$
VIIIb	OMe	H	H	$CHBr_2$	237–238	$C_{16}H_{10}Br_2O_5$
IXa-1	H	Me	Me	CH_2NEt_2	154–158	$C_{21}H_{23}NO_4 \cdot HCl \cdot H_2O$
IXa-2	H	Me	Me	$CH_2N(CH_2CH_2OH)_2$	202–205d	$C_{21}H_{23}NO_6 \cdot HCl$
IXa-3	H	Me	Me	$CH_2N(CH_2CH_2Cl)_2$	205–206d	$C_{21}H_{21}Cl_2NO_4 \cdot HCl$
IXa-4	H	Me	Me	$CH_2N(CH_2CH_2OCONHMe)_2$	120d	$C_{25}H_{29}N_3O_8 \cdot HCl$
IXa-5	H	Me	Me	CH_2NHEt	254–255d	$C_{19}H_{19}NO_4 \cdot HCl \cdot H_2O$
IXa-6	H	Me	Me	$CH_2NHCH_2CH_2OH$	251–252d	$C_{19}H_{19}NO_5 \cdot HCl$
IXa-7	H	Me	Me	$CH_2NHCH_2CH_2Cl$[d]	208–209d	$C_{19}H_{18}ClNO_4$
IXb-1	OMe	Me	Me	CH_2NEt_2	222–223d	$C_{22}H_{25}NO_5 \cdot HCl$
IXb-2	OMe	Me	Me	$CH_2N(CH_2CH_2OH)_2$	225–227d	$C_{22}H_{25}NO_7 \cdot HCl$
IXb-3	OMe	Me	Me	$CH_2N(CH_2CH_2Cl)_2$	200–201d	$C_{22}H_{23}Cl_2NO_5 \cdot HCl$
IXb-5	OMe	Me	Me	CH_2NHEt	267–269d	$C_{20}H_{21}NO_5 \cdot HCl$
IXb-6	OMe	Me	Me	$CH_2NHCH_2CH_2OH$	252–253d	$C_{20}H_{21}NO_6 \cdot HCl$
IXb-7	OMe	Me	Me	$CH_2NHCH_2CH_2Cl \cdot HCl \cdot DMF$	204–205d	$C_{20}H_{20}ClNO_5 \cdot HCl \cdot C_3H_7NO$
Xa-1	H	H or Me	Me or H	CH_2NEt_2	225–227d	$C_{20}H_{21}NO_4 \cdot HBr$
Xa-2	H	H or Me	Me or H	$CH_2N(CH_2CH_2OH)_2$	209–216d	$C_{20}H_{21}NO_6 \cdot HCl$
Xa-3	H	H or Me	Me or H	$CH_2N(CH_2CH_2Cl)_2$	203–205d	$C_{20}H_{19}Cl_2NO_4 \cdot HCl$
Xa-4	H	H or Me	Me or H	$CH_2N(CH_2CH_2OCONHMe)_2$	178–182d	$C_{24}H_{27}N_3O_8 \cdot HCl$
Xb-1	OMe	H or Me	Me or H	CH_2NEt_2	110–112	$C_{21}H_{23}NO_5$
Xb-2	OMe	H or Me	Me or H	$CH_2N(CH_2CH_2OH)_2$	221–223	$C_{21}H_{23}NO_7 \cdot HCl$
Xb-3	OMe	H or Me	Me or H	$CH_2N(CH_2CH_2Cl)_2$	152–154	$C_{21}H_{21}Cl_2NO_5$
XIa-1	H	H	H	CH_2NEt_2	235–238d	$C_{19}H_{19}NO_4 \cdot HCl \cdot {}^1/_2H_2O$
XIa-2	H	H	H	$CH_2N(CH_2CH_2OH)_2$	204–207d	$C_{19}H_{19}NO_6 \cdot HCl$
XIa-3	H	H	H	$CH_2N(CH_2CH_2Cl)_2$	211–214d	$C_{19}H_{17}Cl_2NO_4 \cdot HCl$
XIa-4	H	H	H	$CH_2N(CH_2CH_2OCONHMe)_2$	125–131	$C_{23}H_{25}N_3O_8 \cdot HCl$
XIa-5	H	H	H	CH_2NHEt	>275	$C_{17}H_{15}NO_4 \cdot HCl$
XIa-6	H	H	H	$CH_2NHCH_2CH_2OH$	255–261d	$C_{17}H_{15}NO_5 \cdot HCl$
XIa-7	H	H	H	$CH_2NHCH_2CH_2Cl$[d]	255–261d	$C_{17}H_{14}ClNO_4 \cdot HCl$
XIa-8	H	H	H	CH_2NH_2	240–245d	$C_{15}H_{11}NO_4 \cdot HCl \cdot H_2O$
XIa-9	H	H	H	CH_2NMe_2	282–283d	$C_{17}H_{15}NO_4 \cdot HCl$
XIa-10	H	H	H	$CH_2NH(CH_2)_3Me$	>275	$C_{19}H_{19}NO_4 \cdot HCl$
XIa-11	H	H	H	$CH_2NHCH_2CH_2CH_2OH$	251–252d	$C_{18}H_{17}NO_5 \cdot HCl$
XIa-12	H	H	H	$CH_2N(CH_2CH_2OCONH\text{-}iPr)_2$	159–160	$C_{27}H_{33}N_3O_8 \cdot HCl$
XIa-13	H	H	H	$CH_2N(CH_2)_4$	255–257d	$C_{19}H_{17}NO_4 \cdot HCl$
XIa-14	H	H	H	$CH_2N(CH_2)_5$	246–247d	$C_{20}H_{19}NO_4 \cdot HCl$
XIa-15	H	H	H	CH_2(imidazol-1-yl)	270–272d	$C_{18}H_{12}N_2O_4 \cdot HCl \cdot H_2O$
XIb-1	OMe	H	H	CH_2NEt_2	240–241d	$C_{20}H_{21}NO_5 \cdot HBr$
XIb-2	OMe	H	H	$CH_2N(CH_2CH_2OH)_2$	225–227d	$C_{20}H_{21}NO_7 \cdot HCl$
XIb-3	OMe	H	H	$CH_2N(CH_2CH_2Cl)_2$	203–206d	$C_{20}H_{19}Cl_2NO_5 \cdot HCl$
XIb-5	OMe	H	H	CH_2NHEt	>275	$C_{18}H_{17}NO_5 \cdot HBr$
XIb-6	OMe	H	H	$CH_2NHCH_2CH_2OH$	259–260d	$C_{18}H_{17}NO_6 \cdot HCl$
XIb-7	OMe	H	H	$CH_2NHCH_2CH_2Cl$	powder	$C_{18}H_{16}ClNO_5$

[a] All the compounds were analyzed for C, H, X (Br or Cl), and/or N. Analyses for these elements were within ±0.4% of the theoretical values required unless specifies otherwise. [b] For nitrogen-containing compounds, melting points were of the HX salt. [c] d = decomposition. [d] Unstable, and satisfactory analyses could not be obtained.

philic attack, we synthesized *N*-methylcarbamate IX-4 by treatment of IX-2 with *N*-methyl isocyanate.

The methyl protecting groups at 1 and 8 could be removed stepwise at various stages (Figure 2). Thus, treatment of IX with HBr in acetic acid at room temperature afforded a crystalline mixture of 1-*O*-methyl- and 8-*O*-methylanthraquinones X, whereas acid hydrolysis at reflux temperature for a few hours resulted in complete demethylation, giving rise to XI. Later, it was found that partially methylated chrysophanol and emodin could be directly brominated to the corresponding mixtures of the monobromides V (major products) and dibromides VII. The former were treated with amines to give X, which were further converted to the corresponding XI. Alternatively,

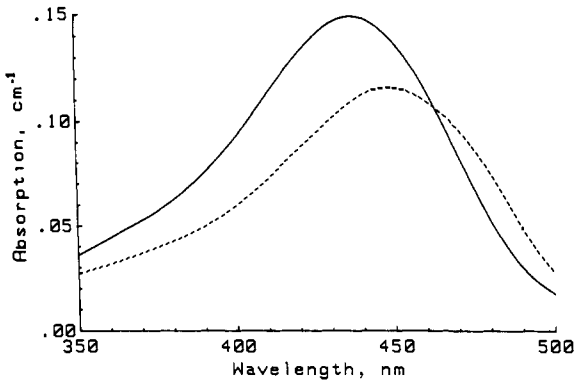


Figure 3. Visible light absorption spectrum of drivative XIb-6 (11.3 μM in the buffer containing 0.01 M NaCl) alone (solid line) and in the presence of 0.2 μM calf thymus DNA (Sigma type 1) (broken line).

the 3-[(alkylamino)methyl] derivatives XI were prepared by amination of VI. Chlorination of XIa-2 with thionyl chloride afforded XIa-3. *N*-Methyl- and *N*-isopropylcarbamates, XIa-4 and XIa-12, respectively, were prepared by treatment of XIa-2 with the corresponding *N*-alkyl isocyanates.

Spectrophotometric Studies for DNA Interactions

Some representative compounds were studied for their ability to interact with nucleic acids in solution by comparison of the electronic spectrum of drug alone with that of the drug in the presence of an excess of nucleic acid. All drugs studied have an absorption band in the visible region, separate from the absorption band of the nucleic acids, and therefore any changes in band intensity and position were indicative of drug chromophore–DNA interaction. It was observed that both chrysophanol (Ia) and emodin (Ib) and their derivatives lacking the basic center do not appear to interact with DNA to any significant extent. Since these compounds have low solubility in aqueous solutions, the spectral measurement alone may not be sufficient however to allow one to draw a definite conclusion.

Generally, those anthraquinones bearing an amino function (e.g., Xa-1, Xb-1, XIa-1, XIa-2, XIb-2, and XIb-6) interact with both native and thermally denatured DNA but more strongly with native DNA (Figure 3). As expected, 1,8-di-*O*-methylchrysophanol and 1,6,8-tri-*O*-methylemodin analogues IXa and IXb do not interact with DNA. Anthraquinones wherein one *peri*-hydroxyl group is methylated (e.g., Xa-2 and Xb-2) interact with DNA to lesser extent than the corresponding unmethylated (XIa-2 and XIb-2). It is interesting to note that while DNA induced changes in absorption spectra of some derivatives (e.g., XIa-7 and XIb-7), these do not appear to be connected with their alkylating capabilities, in view of the fact that they could be reversed by addition of Me_2SO (to 1:1 v/v). The dissociation of nonbonded ligand–DNA complexes in the presence of organic solvents is a phenomenon well documented.[44]

Changes in the absorption spectra of the drugs (Figure 3) are not inconsistent with the possibility of the intercalative mode of binding. Other types of nonbonding interactions, however, (e.g., binding to the minor groove of the double helix[45,46]) cannot be excluded. It is well-known that intercalative binding, which most often has an ionic component, is affected by a rise in concentration of salts,[47] and compounds such as Xa-2, Xb-2, XIb-3, and XIb-7 lost the ability to interact with DNA when Na^+ concentration was increased from 0.01 to 0.1 M. On the basis of this fact one can conclude that the affinity for DNA of these compounds is lower than those that interact with DNA at both ionic strengths.

No straightforward correlation is apparent between the DNA-affinity data of the drugs studied and their biological activity.

Table II. Biological Activities of Derivatives of Chrysophanol and Emodin

compd	ID_{50}, M (L1210 cell growth)	ID_{50}, M [HL-60 cell growth (72 h)] (*A*)	ID_{50}, M (HL-60 TdR into DNA) (*B*)	*B*/*A*
IIa	2.8×10^{-5}	1.0×10^{-5}	1.9×10^{-5}	1.9
IIb	1.0×10^{-4}	4.9×10^{-5}	2.1×10^{-5}	0.43
IIIa	9.2×10^{-6}	4.4×10^{-6}	1.1×10^{-5}	2.5
IIIb	6.8×10^{-7}	8.4×10^{-5}	1.2×10^{-5}	0.14
IVa	8.9×10^{-7}	2.1×10^{-6}	9.0×10^{-6}	4.3
IVb	4.1×10^{-5}	6.0×10^{-6}	5.8×10^{-5}	9.7
Va	8.8×10^{-6}	7.1×10^{-6}	1.2×10^{-5}	1.7
Vb	6.8×10^{-8}	1.7×10^{-6}	7.0×10^{-5}	41
VIa	8.6×10^{-6}	7.1×10^{-6}	1.6×10^{-5}	2.3
VIb	5.9×10^{-5}	2.5×10^{-5}	3.4×10^{-4}	13.6
VIIa	4.2×10^{-11}	6.0×10^{-8}	1.3×10^{-5}	217
VIIb	1.0×10^{-9}	9.7×10^{-8}	2.3×10^{-5}	237
VIIIa	4.4×10^{-7}	2.5×10^{-6}	3.9×10^{-5}	15.6
VIIIb	1.0×10^{-9}	1.9×10^{-7}	1.1×10^{-4}	579
IXa-1	1.3×10^{-4}	3.0×10^{-5}	1.9×10^{-5}	6.3
IXa-2	$>5.0 \times 10^{-5}$	4.9×10^{-4}	1.4×10^{-5}	0.03
IXa-3	1.3×10^{-5}	1.8×10^{-6}	1.4×10^{-5}	7.8
IXa-4	7.2×10^{-5}	9.5×10^{-6}	9.4×10^{-6}	0.99
IXa-5	1.2×10^{-6}	4.8×10^{-5}	2.9×10^{-5}	0.60
IXa-6	1.4×10^{-4}	5.5×10^{-5}	4.6×10^{-5}	0.84
IXa-7	2.6×10^{-5}	8.6×10^{-5}	1.9×10^{-5}	0.22
IXb-1	6.9×10^{-6}	7.9×10^{-6}	4.2×10^{-6}	0.53
IXb-2	$>2.7 \times 10^{-5}$	1.0×10^{-4}	1.7×10^{-5}	0.17
IXb-3	2.7×10^{-6}	2.0×10^{-6}	8.9×10^{-6}	4.5
IXb-5	9.8×10^{-6}	1.7×10^{-5}	1.3×10^{-5}	0.76
IXb-6	1.7×10^{-5}	1.1×10^{-4}	2.6×10^{-5}	0.24
IXb-7	3.2×10^{-5}	5.8×10^{-6}	1.8×10^{-5}	3.1
Xa-1	1.2×10^{-5}	5.2×10^{-6}	1.4×10^{-5}	2.7
Xa-2	$>2.4 \times 10^{-5}$	2.1×10^{-5}	8.9×10^{-5}	4.2
Xa-3	1.4×10^{-6}	3.9×10^{-7}	1.5×10^{-5}	38.5
Xa-4	8.9×10^{-5}	1.2×10^{-5}	1.2×10^{-5}	1
Xb-1	6.9×10^{-6}	6.7×10^{-6}	1.8×10^{-4}	26.9
Xb-2	8.8×10^{-6}	$>5.0 \times 10^{-4}$	9.6×10^{-6}	0.02
Xb-3	3.3×10^{-6}	5.2×10^{-7}	5.4×10^{-6}	10.4
XIa-1	2.8×10^{-6}	1.8×10^{-6}	1.4×10^{-5}	7.8
XIa-2	5.9×10^{-6}	3.3×10^{-6}	1.4×10^{-5}	4.2
XIa-3	1.3×10^{-7}	1.8×10^{-7}	6.9×10^{-5}	383
XIa-4	$>1.8 \times 10^{-5}$	2.1×10^{-4}	2.8×10^{-5}	0.13
XIa-5	7.7×10^{-7}	8.7×10^{-6}	1.8×10^{-5}	2.1
XIa-6	1.6×10^{-7}	8.6×10^{-7}	1.5×10^{-5}	17.4
XIa-7	7.1×10^{-6}	7.5×10^{-6}	3.9×10^{-5}	5.2
XIa-8	8.5×10^{-6}	1.1×10^{-5}	2.5×10^{-5}	2.3
XIa-9	2.2×10^{-6}	2.7×10^{-6}	9.3×10^{-6}	3.3
XIa-10	4.6×10^{-6}	4.1×10^{-6}	2.5×10^{-5}	6.1
XIa-11	6.7×10^{-7}	3.2×10^{-6}	1.6×10^{-5}	5.0
XIa-12	2.7×10^{-5}	2.7×10^{-5}	1.7×10^{-5}	0.63
XIa-13	1.6×10^{-6}	2.1×10^{-6}	1.0×10^{-5}	4.8
XIa-14	4.0×10^{-6}	2.8×10^{-6}	6.3×10^{-5}	22.5
XIa-15	7.4×10^{-6}	4.8×10^{-6}	4.3×10^{-5}	9.0
XIb-1	1.2×10^{-6}	3.4×10^{-6}	3.5×10^{-5}	10.3
XIb-2	1.4×10^{-5}	6.6×10^{-6}	4.5×10^{-5}	5.2
XIb-3	2.3×10^{-8}	6.1×10^{-7}	1.2×10^{-5}	19.7
XIb-5	7.2×10^{-7}	2.1×10^{-6}	1.3×10^{-5}	6.2
XIb-6	5.0×10^{-7}	1.6×10^{-6}	1.2×10^{-5}	7.5
XIb-7	1.8×10^{-5}	9.2×10^{-6}	2.2×10^{-4}	23.9

(44) Wakelin, L. P. G.; Adams, A.; Hunter, C.; Waring, M. J. *Biochemistry* **1981**, *20*, 5779.
(45) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I. In *Physical Chemistry of Nucleic Acids*; Harper and Row: New York, 1974; pp 432–434.
(46) Jorgenson, K. F.; Varsheny, U.; van de Sande, J. H. *J. Biomol. Struct. Dyn.* **1988**, *6*, 1005.
(47) Kapuscinski, J.; Darzynkiewicz, Z. *J. Biomol. Struct. Dyn.* **1987**, *5*, 127.

Table III. Inverse Relationship between Cell Growth Inhibition and Inhibition of Initial Thymidine Incorporation into DNA in HL-60 Cells by Chrysophanol Derivatives[a]

no. of compds examined	value of IC_{50} (cell growth), μM		ratio of IC_{50} (dThd incorpn)/IC_{50} (cell growth), mean ± SE
	range	mean ± SE	
8	<1	0.36 ± 0.10	167.3 ± 72.4
17	1–5	2.73 ± 0.24	9.55 ± 2.30
15	5–10	7.42 ± 0.53	6.32 ± 2.10
7	10–50	28.43 ± 5.51	3.03 ± 1.83
8	>50	204.4 ± 65.4	0.22 ± 0.09

[a] Cell growth inhibition was measured at the end of 72-h exposure to each compound as described under Experimental Secton. Inhibition of [^{3}H]dThd incorporation into DNA was measured during the first 30 min of exposure to each corresponding compound as described under Experimental Section.

Biological Activities

Preliminary biological data for inhibiting cell growth of murine L1210 leukemic cells and human acute promyelocytic leukemia cells (HL-60) during 72 h of exposure to the compounds are given in Table II. The potencies for inhibiting [^{3}H]TdR incorporation into DNA in HL-60 cells during the initial 30-min period are also given in Table II. It is interesting to note that 1,8-di-*O*-methyl derivatives are uniformly devoid of activity against L1210 leukemic cells. These results are consistent with published data that compare the ratios of the potencies (ID_{50}'s) for cell growth inhibition (*A*) and inhibition of [^{3}H]TdR incorporation into DNA in HL-60 cells (*B*). The *B*/*A* ratios allow an indirect estimation of whether or not cytotoxicities exerted by these analogues are primarily due to initial inhibition of DNA synthesis. The *B*/*A* ratios for Vb, VIIa, VIIb, VIIIb, Xa-3, and XIa-3 are 41, 217, 237, 579, 39, and 383, respectively, suggesting that these compounds exert their initial effects mainly on processes other than DNA synthesis per se (Table III). These results suggest that these compounds exert their cytotoxic effects in a time-dependent manner and their initial action is targeted at the sites other than DNA elongation. Whether these analogues, like *m*-AMSA, ellipticine, or anthracyclines, act by inhibiting DNA topoisomerase II remains to be explored. It is of interest to note that the above-mentioned anthraquinones are among the most potent antileukemic analogues listed in Table II, with ID_{50} values ranging from 1.4×10^{-6} to 4.2×10^{-11} for L1210[48] cells and 1.7×10^{-6} to 6.0×10^{-8} M for HL-60 cells. Our preliminary experiments indicate that the compounds arrest cells in the S and/or G_2 phases of the cell cycle (unpublished results).

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and all new compounds with the exception of IXa-7 and XIa-7, which were unstable, analyzed correctly. ^{1}H NMR spectra were recorded on a JEOL FX90Q spectrometer with Me_4Si as the internal standard. Chrysophanol (Ia) and emodin (Ib) were isolated from rhubarb extract by the procedure of Kelly et al.[35] except CH_2Cl_2 was used instead of Et_2O throughout the isolation process.

1,8-Dimethoxy-3-methyl-9,10-anthraquinone (1,8-Di-*O*-methylchrysophanol, IIa). A mixture of chrysophanol (Ia, 7.0 g, 0.029 mol), K_2CO_3 (10 g, 0.071 mol), and Me_2SO_4 (10 mL, 0.1 mol) in Me_2CO (300 mL) was stirred under reflux for 16 h and then concentrated in vacuo. The residue was triturated well with water (300 mL), and the crystalline IIa (7.5 g, 96%) was collected by filtration and air-dried: mp 191–193 °C (lit.[49] mp 195 °C); ^{1}H NMR ($CDCl_3$) δ 2.46 (3 H, s, 3-Me), 3.98 (3 H, s, OMe), 3.99 (3 H, s, OMe), 7.08–7.86 (5 H, m, H-2,4,5,6,7). Anal. ($C_{17}H_{14}O_4$) C, H.

1,3,8-Trimethoxy-6-methyl-9,10-anthraquinone (1,3,8-Tri-*O*-methylemodin, IIb). In a similar manner, emodin (Ib, 1.0 g, 3.9 mmol) was converted into IIb (1.04 g, 90%): mp 225 °C (lit.[50] mp 225 °C); ^{1}H NMR (Me_2SO-d_6) δ 2.43 (3 H, s, 6-Me), 3.88 (3 H, s, OMe), 3.92 (6 H, s, 2 × OMe), 6.94 (1 H, d, H-7, $J_{5,7}$ = 2.2 Hz), 7.13 (1 H, d, H-5, $J_{5,7}$ = 2.2 Hz), 7.33 (1 H, s, H-2), 7.46 (1 H, s, H-4). Anal. ($C_{18}H_{16}O_5$) C, H.

3-(Bromomethyl)-1,8-dimethoxy-9,10-anthraquinone (IIIa). To a hot solution of IIa (4.5 g, 0.016 mol) and BDH (2.75 g, 0.019 mol) in CCl_4 (500 mL) was added Bz_2O_2 (0.7 g), and the mixture was heated under reflux for 5 h. The mixture was allowed to cool to room temperature. Insoluble hydantoin was removed by filtration, the filtrate was concentrated in vacuo, and the residue was crystallized twice from EtOAc to give IIIa (3.3 g, 57%): mp 176–178 °C (lit.[51] mp 174–175 °C); ^{1}H NMR ($CDCl_3$) δ 4.01 (3 H, s, OMe), 4.03 (3 H, s, OMe), 4.52 (2 H, s, CH_2Br), 7.26–7.84 (5 H, m, H-2,4,5,6,7). Anal. ($C_{17}H_{13}BrO_4$) C, H, Br.

3-(Dibromomethyl)-1,8-dimethoxy-9,10-anthraquinone (IVa). The mother liquors of recrystallization were concentrated, and the residue was chromatographed on a silica gel column using a mixture of C_6H_6 and EtOAc (3:1) to give IVa (0.48 g): mp 207–210 °C; ^{1}H NMR ($CDCl_3$) δ 4.01 (3 H, s, OMe), 4.07 (3 H, s, OMe), 6.67 (1 H, s, $CHBr_2$), 7.26 (5 H, m, H-2,4,5,6,7). Anal. ($C_{17}H_{12}Br_2O_4$) C, H, Br.

A further amount of IIIa (0.33 g) was eluted from the column, making the total yield of 62.7 %.

6-(Dibromomethyl)-1,3,8-trimethoxy-9,10-anthraquinone (IVb). In a similar manner, IIb (3.12 g, 0.01 mol) was brominated to give IIIb (3.06 g, 74.4%) [mp 250–254 °C (lit.[36] mp 233.5–234 °C); ^{1}H NMR (CDCl3) was identical with that reported[36]] and IVb (297 mg) [mp 254–257 °C; ^{1}H NMR ($CDCl_3$) δ 3.95 (6 H, s, 2 × OMe), 3.97 (3 H, s, OMe), 6.67 (1 H, s, $CHBr_2$), 6.78 (1 H, d, H-7, $J_{5,7}$ = 2.47 Hz), 7.32 (1 H, d, H-5), 7.54 (1 H, d, H-2, $J_{2,4}$ = 1.92 Hz), 7.90 (1 H, d, H-4)]. Anal. ($C_{17}H_{14}Br_2O_5$) C, H, Br.

3-(Bromomethyl)-1(and 8)-hydroxy-8(and 1)-methoxy-9,10-anthraquinone (Va). A mixture of IIIa (70 mg, 0.19 mmol) in HOAc (10 mL) and 30% HBr/HOAc (1 mL) was stirred overnight at room temperature and then concentrated in vacuo. The residue was chromatographed on a silica gel column using $CHCl_3$ as the eluent to give 51 mg (76%) of Va as yellow crystals: mp 213–215 °C; ^{1}H NMR ($CDCl_3$) δ 4.08, 4.10 (2 × 3 H, 2 s, 1- and 8-OMe), 4.47, 4.54 (2 × 2 H, 2 s, CH_2Br), 7.24–8.04 (10 H, m, H-2,4,5,6,7). Anal. ($C_{16}H_{11}BrO_4$) C, H, Br.

3-(Dibromomethyl)-1(and 8)-hydroxy-8(and 1)-methoxy-9,10-anthraquinone (VIIa) and 6-(Dibromomethyl)-1(and 8)-hydroxy-3,8(and 1,3)-dimethoxy-9,10-anthraquinone (VIIb). In a similar manner, from IVa (200 mg, 0.453 mmol) and IVb (100 mg, 0.213 mmol), VIIa (82 mg, 42.5%) and VIIb (82 mg, 84.4%), respectively, were prepared (see Table I).

3-(Bromomethyl)-1,8-dihydroxy-9,10-anthraquinone (VIa). A mixture of IIIa (1.05 g, 2.9 mmol), HOAc (50 mL), and 30% HBr/HOAc (5 mL) was heated at 100 °C for 5 h. After the mixture was cooled, VIa was collected by filtration, washed with HOAc and H_2O, and then air-dried to give 879 mg (91%) of the product: mp 220–222 °C; ^{1}H NMR ($CDCl_3$) δ 4.47 (2 H, s, CH_2Br), 7.22–7.90 (5 H, m, H-2,4,5,6,7), 12.01, 12.04 (2 × H, 2 s, 1-OH, 8-OH). Anal. ($C_{15}H_9BrO_4$) C, H, Br.

3-(Dibromomethyl)-1,8-dihydroxy-9,10-anthraquinone (VIIIa) and 6-(Dibromomethyl)-3-methoxy-1,8-dihydroxy-9,10-anthraquinone (VIIIb). In a similar manner, IVa (237 mg, 0.54 mmol) and IVb (472 mg, 1 mmol) were converted into VIIIa (166 mg, 75%) and VIIIb (408 mg, 95%), respectively (Table I).

3-[(Diethylamino)methyl]-1,8-dimethoxy-9,10-anthraquinone (IXa-1). To a solution of IIIa (200 mg, 0.55 mmol) in DMF (10.0 mL) was added Et_2NH (5.0 mL), and the mixture was stirred at room temperature for 3 days. The mixture was partitioned between EtOAc (20 mL) and H_2O (20 mL). The aqueous

(48) Burchenal, J. H.; Chou, T.-C.; Lokys, L.; Smith, R. S.; Watanabe, K. A.; Su, T.-L.; Fox, J. J. *Cancer Res.* **1982**, *42*, 2598.
(49) *Beilstein*, *8*, 473.
(50) *Beilstein*, *8*, 523.
(51) Blankespoor, R. L.; Schutt, D. L.; Tubergen, M. B.; De Jong, R. L. *J. Org. Chem.* **1987**, *52*, 2059.

layer was washed with EtOAc (20 mL). The combined EtOAc solutions were washed with H_2O (2 × 20 mL) and saturated NaCl (2 × 20 mL), dried (Na_2SO_4), and concentrated, and the residue was chromatographed on a silica gel column first with $CHCl_3$, which eluted the 3-(hydroxymethyl) derivative (33 mg), followed by $CHCl_3$ containing 3% MeOH. The IXa (140 mg) that eluted from the column was converted to the crystalline HCl salt (142 mg, 63%), mp 154–158 °C.

In a similar manner but by using the corresponding amines, IXa-2, -5, and -6, were prepared (Table I). Also by use of the same procedure starting from IIIb and the corresponding amines, IXb-1, -2, -5, and -7 were synthesized (Table I).

3-[(Diethylamino)methyl]-1(or 8)-hydroxy-8(or 1)-methoxy-9,10-anthraquinone (Xa-1). The HCl salt monohydrate of IXa (100 mg, 0.25 mmol) was dissolved in a mixture of HOAc (5 mL) and 30% HBr/HOAc (0.4 mL), and the solution was stirred at room temperature for 24 h. After concentration in vacuo, the residue was partitioned between saturated $NaHCO_3$ (10 mL) and $CHCl_3$ (10 mL). The $CHCl_3$ layer was dried (Na_2SO_4) and concentrated and the residue chromatographed on a silica gel column using $CHCl_3$–MeOH (30:1 v/v) to give Xa-1 as a glass, which was dissolved in 1 N HBr (1 mL). Upon dilution of the solution with EtOH (5 mL), the monohydrobromide of Xa-1 (82 mg) precipitated as yellow microcrystals, mp 225–227 °C dec.

In a similar manner, Xa-2–4 and Xb-1–4 were prepared (Table I).

3-[[*N*,*N*-Bis(2-hydroxyethyl)amino]methyl]-1,8-dihydroxy-9,10-anthraquinone (XIa-2). A mixture of VIa (456 mg, 0.73 mmol) and bis(2-hydroxyethyl)amine (600 mg, 5.50 mmol) in DMF (20 mL) was stirred for 2 h and then partitioned between $CHCl_3$ (100 mL) and H_2O (100 mL). The organic layer was separated, washed (H_2O, 3 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (15:1 v/v) as the eluent. The major fraction was concentrated and the residue dissolved in 1 N HCl. After concentration of the solution in vacuo, the residue was triturated well with MeOH (5 mL), and the crystalline HCl salt of XIa-2 (496 mg, 91%) was collected by filtration, mp 204–207 °C. Anal. ($C_{19}H_{19}NO_6$·HCl) C, H, N.

In a similar manner but by use of the corresponding amines, XIa-1, -2, -5, -6, -8–11, and -13–15 were prepared. Also, from VIb, by the same procedure, XIb-1, -2, -5, and -6 were obtained (Table I).

3-[[*N*,*N*-Bis(2-chloroethyl)amino]methyl]-1,8-dihydroxy-9,10-anthraquinone (XIa-3). To a solution of XIa-2·HCl (1.05 g, 2.57 mmol) in dry DMF (40 mL) was added $SOCl_2$ (1.0 mL), and the solution was stirred at room temperature for 1.5 h. The solution was concentrated in vacuo (bath temperature <40 °C), and the residue was cooled in an ice bath. Cold MeOH (10 mL) was added to destroy DMF–HCl complex, and the mixture was concentrated in vacuo. Upon trituration of the residue with cold MeOH (10 mL), XIa-3 (977 mg, 85.1 %) was obtained, mp 211–214 °C. Anal. ($C_{19}H_{17}Cl_2NO_4$·HCl) C, H, N.

In a similar manner but by use of the corresponding amino alcohols, IXa-3 and -7, IXb-3 and -7, Xa-3, Xb-3, XIa-7, and XIb-3 and -7 were synthesized (Table I).

Spectral Studies. Absorption spectra were measured with an IBM 9410 UV-visible spectrometer interfaced to an HP 9826 computer. Small volumes of the stock drug solutions (2 mg/mL in Me_2SO) were added to 2 mL of buffer (0.01 or 0.1 M NaCl, 5 mM Hepes, pH 7) to obtain a final drug concentration of 5–15 μM, or to the solution of native or thermally denatured DNA (0.2 and 0.1 mM, respectively) in the buffer. After incubation at room temperature for 10 min, the spectra were recorded in the 300–600-nm range (increment 1 nm) and corrected by subtracting the spectrum of the blank which was measured before addition of the drug.

Biological Assays. Method A. For cell growth inhibition studies, HL-60 cells (2.0×10^5/mL) were grown in RPMI 1640 media at 37 °C in humidified 5% CO_2 for 72 h. Viable cells were counted with the trypan blue exclusion method. The fractional inhibitions at four or five concentrations of compounds (in 0.2% DMSO) were analyzed with a median-effect plot[52] by using a computer program.[53] The median-effect concentration (ID_{50}) was automatically determined for the x intercept of the median-effect plot. Cell growth in the absence of a compound and in the presence of DMSO was used as a control. DMSO (0.2%) alone inhibited cell growth 3.8 ± 1.2% during the 72-h incubation period.

Method B. For precursor incorporation studies, each compound at four to six concentrations (in 0.2% DMSO) was preincubated with HL-60 cells (2.5×10^6/mL) for 15 min prior to the addition of [^{3}H-*methyl*]TdR (1 μCi, 0.15 nmol/mL) and was incubated for 30 min. The incubation conditions and the procedures for isolating the DNA fractions were described previously.[54] The incorporation of radioactivity into DNA in the absence of an analogue in the presence of DMSO was used as a control. The control value for incorporation into DNA was 8500 ± 300 cpm/10^6 cells.

Acknowledgment. This investigation was supported in part by grants from the National Cancer Institute, NIH, U.S. DHHS [CA-08748, CA-18856 (K.A.W., T.-C.C.), CA-27569] and the Elsa U. Pardee Foundation (T.-C.C.) and by CA-28704 (Z.D., J.K.) and CA-37054 (T.R.K.). We are indebted to Miss Linda M. DeBlasio for determining the ID_{50} values of the agents on L1210 cells and Ms. L.-I. Chen and Ms. Q.-H. Tan for their assistance in carrying out the [^{3}H]TdR incorporation experiments and cytotoxicity determinations against HL-60 cells.

(52) Chou, T.-C.; Talalay, P. *Adv. Enzyme Regul.* **1984**, *22*, 27.
(53) Chou, J.; Chou, T.-C. *Dose-Effect Analyses with Microcomputer: Quantitation of ID_{50}, LD_{50}, Synergism, Antagonism, Low-dose Risk, Receptor-Binding and Enzyme Kinetics*; IBM-PC Series, Elsevier-Biosoft, Elsevier Scientific: Cambridge, U.K., 1986.
(54) Chou, T.-C.; Schmid, F. A.; Philips, F. S.; Han, J. *Cancer Res.* **1983**, *43*, 3074.