9-Substituted Acridine Derivatives with Long Half-Life and Potent Antitumor Activity: Synthesis and Structure–Activity Relationships

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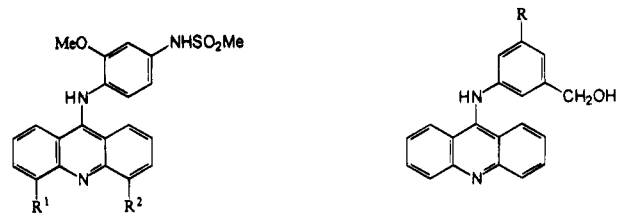
Received August 9, 1994[⊗]

A series of DNA-intercalating 9-anilinoacridines, namely 9-phenoxyacridines, 9-(phenylthio)-acridines, and 9-(3′,5′-disubstituted anilino)acridines, were synthesized as potential antitumor agents with inhibitory effects on DNA topoisomerase II. Unlike amsacrine (*m*-AMSA), these agents were designed to avoid the oxidative metabolic pathway. These acridine derivatives were, therefore, expected to have long half-life in plasma. Both 9-phenoxyacridines and 9-(phenylthio)acridines were found to have moderate cytotoxicity against mouse leukemia L1210 and human leukemic HL-60 cell growth in culture. Among 9-(3′,5′-disubstituted anilino)-acridines, 3-(9-acridinylamino)-5-(hydroxymethyl)aniline (AHMA) was found to be a potent topoisomerase II inhibitor and exhibited significant antitumor efficacy both *in vitro* and *in vivo*. Chemotherapy of solid-tumor-bearing mice with 10, 10, and 5 mg/kg (QD × 4, ip) AHMA, VP-16, and *m*-AMSA, respectively, resulted in more tumor volume reduction by AHMA than by VP-16 or *m*-AMSA for E0771 mammary adenocarcinoma and B-16 melanoma. For Lewis lung carcinoma, AHMA was as potent as VP-16 but more active than *m*-AMSA. Structure–activity relationships of AHMA derivatives are discussed.

Introduction

9-Anilinoacridine derivatives have been extensively studied as potential antitumor agents, since they are capable of binding to DNA. The synthesis and the structure–antileukemic activity relationships of a series of acridine ring-substituted 4′-(9-acridinylamino)alkanesulfonanilines were reported by Atwell and Cain *et al.*[1–4] Among these compounds, amsacrine[1] (*m*-AMSA, **1**) and its 4-methyl 5-methylcarboxamide derivatives[4] (CI-921, **2**) (Figure 1) were of particular interest; *m*-AMSA was in clinical use for the treatment of acute leukemia,[5,6] and CI-921 is currently undergoing phase II trials for treatment of leukemia and solid tumors.[7,8]

Both *m*-AMSA and CI-921 possess a methanesulfonyl and a methoxy function at C-1′ and C-3′ of the 9-anilino ring and readily undergo reversible oxidation either chemically (MnO_2)[9] or microsomally[9,10] giving the quinonediimine **3** (*m*-AQDI) (Scheme 1). Shoemaker *et al.*[9,10] and Robertson *et al.*[11,12] reported that when mouse and rat were treated with *m*-AMSA, the major biliary metabolites of this agent were the 5′- and 6′-glutathione (GSH) conjugates **4**, with no C9–GSH conjugate being found. In the case of CI-921, in addition to the 5′- and 6′-GSH conjugates, two other metabolites, namely C9–GSH and the 4-hydroxymethyl derivative of CI-921, were detected.[12] More than 50% of the dose was excreted as the glutathione conjugate in the bile when the mouse was treated with these two drugs.[12] The half life of *m*-AMSA in the presence of fresh mouse blood at 37 °C is *ca.* 30 min.[12,13] The metabolism of these agents *in vivo* by microsomes was considered to be a detoxification pathway. Wong *et al.*[14,15] also showed that *m*-AMSA was capable of cleaving pDPT275 plasmid DNA in the presence of Cu(II), whereas its inactive isomer, *o*-AMSA, was less effective. It was suggested that *m*-AMSA was oxidized to form *m*-AQDI and Cu(I) species, which were thought to form a complex that was capable of producing oxygen radical and causing single-strand DNA cleavage.



1 $R^1 = R^2 = H$ m-AMSA
2 R^1 = Me, R^2 = CONHMe CI-921
15 R = NH_2 AHMA
28 R = H

Figure 1.

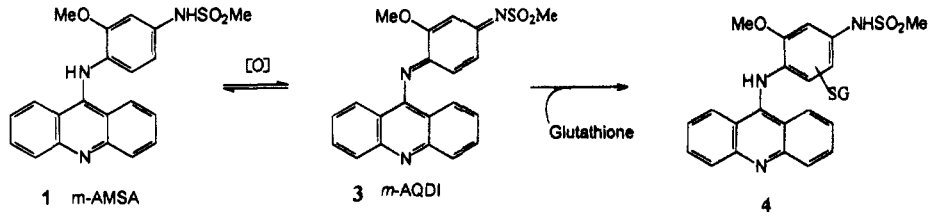
It was then suggested that the redox properties of *m*-AMSA derivatives might play an important role in their biological effect. Jurline *et al.*[16] have studied the redox potential and antitumor activity relationships of 9-anilinoacridines. The superior antitumor effect of CI-921 compared to that of *m*-AMSA may be due to lower oxidation potential required for quinonediimine formation (240 and 280 mV for CI-921 and *m*-AMSA, respectively). However, it was shown that no clear quantitative relationships between redox potential and antitumor activity could be discerned. The relevance of this redox chemistry to the mode of action of *m*-ANSA and other 9-anilinoacridines remains unclear, since the isomeric compound *o*-AMSA has little enzyme-inhibiting activity and is biologically inactive despite having similar redox potential. Later, Nelson[17] and Pommier *et al.*[18] reported that the antitumor activity of *m*-AMSA was attributed to topoisomerase II (Topo II)-mediated cleavage of double-stranded DNA, by a mechanism which appeared to be common to DNA-intercalating agents.

In order to determine whether the redox properties of this class of compounds have direct relationship to their antitumor activity, we have designed and synthesized a series of 9-anilinoacridine derivatives in which

⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1995.

0022-2623/95/1838-3226$09.00/0

Scheme 1



the 9-amino was substituted by an O or S atom or compounds lacking the substituent at the para position to the 9-amino function of acridine. These new series of 9-substituted acridine derivatives were incapable of forming diiminoquinone intermediates by oxidation and are, therefore, expected to have longer duration of drug action with higher chemotherapeutic effects.

Among these compounds, 3-(9-acridinylamino)-5-(hydroxymethyl)aniline (AHMA, **15**) possesses an intriguing chemical structure. The substituents on the anilino ring are in the meta position to each other and thus cannot form the iminoquinone structure via oxidation. AHMA was found not only to exhibit potent antitumor activity against leukemic L1210 and HL-60 and various murine tumors both *in vitro* and *in vivo*[19] but also to have long half-life in human plasma. We also found that AHMA inhibited Topo II-mediated DNA decatenation and relaxation.[20] The intriguing antitumor activity and the chemical structure of AHMA give several features for drug design and structure–activity relationship (SAR) studies by taking advantage of the amino and hydroxymethyl substituents on the phenyl ring of this agent.

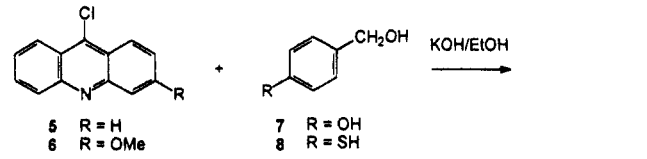
The cytotoxic effects of DNA topoisomerase inhibitors are presumably due to trapping of DNA cleavable complex. DNA and a Topo enzyme form a reversible and cleavable complex which is stabilized by the drug to form a ternary complex. Stabilization of the complex leads to interruption of DNA strand scission, passing, and resealing.[21,22] Bearing in mind this plausible mode of action of topoisomerase inhibitors, the following structural modifications are considered for studying the SAR of AHMA: (1) linking an acyl side chain of various size to the amino or hydroxymethyl function for studying the effects of the substituent size on its cytotoxicity and Topo II inhibitory activity; (2) coupling a chargeable side chain to the amino or hydroxymethyl group to enhance the stabilization of the reversible Topo II–DNA complex; and (3) attaching a leaving group to the benzylic hydroxy function so that the nucleophilic attack is expected to occur by the nucleophilic group(s) of DNA or Topo II forming a covalent bond and, hence, persistently inhibiting DNA isomerization and polymerization. In the present paper we describe the synthesis, SAR, and Topo II inhibitory effects of this new class of anilinoacridine derivatives.

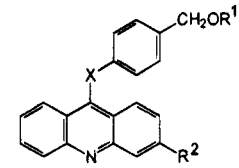
Chemistry

We first synthesized 9-phenoxy- and 9-(phenylthio)-acridine analogues **9a**, **10a**, **11a**, and **12a** (Scheme 2). These compounds were prepared by condensation of 9-chloroacridine (**5**) or 9-chloro-3-methoxyacridine (**6**) with the substituted phenol **7** or thiophenol **8** in KOH/EtOH under reflux.

In our previous paper,[23] we reported the synthesis of 9-anilinoacridine derivatives as DNA-intercalating agents

Scheme 2





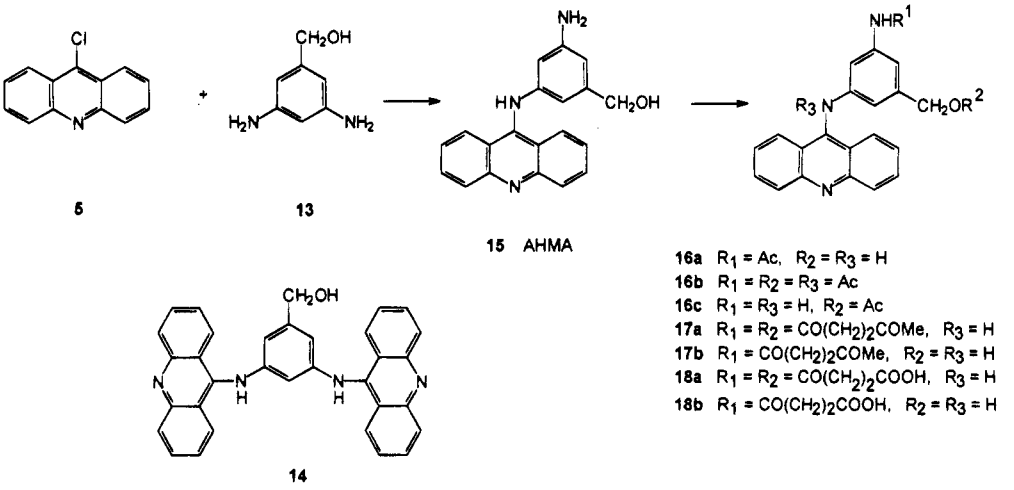
9a X = O $R^1 = R^2 = H$
9b X = O R^1 = CONHMe $R^2 = H$
9c X = O R^1 = CONHiPr $R^2 = H$
10a X = O $R^1 = H$ R^2 = OMe
10b X = O R^1 = CONHMe R^2 = OMe
10c X = O R^1 = CONHiPr R^2 = OMe
11a X = S $R^1 = R^2 = H$
11b X = S R^1 = CONHMe $R^2 = H$
11c X = S R^1 = CONHiPr $R^2 = H$
12a X = S $R^1 = H$ R^2 = OMe
12b X = S R^1 = CONHMe R^2 = OMe
12c X = S R^1 = CONHiPr R^2 = OMe

with covalent bond-forming capability. These included derivatives with a hydroxymethyl or *N*-alkylcarboxamide substituent at various positions on the anilino ring. We found that *N*-methylcarboxamide derivatives were more cytotoxic than their parent hydroxymethyl compounds, probably due to *N*-alkylcarboxamide being a leaving group and susceptible to nucleophilic attack by a negatively charged group of DNA. We, therefore, converted the hydroxymethyl-substituted acridines **9a**, **10a**, **11a**, and **12a** into their corresponding *N*-methyl- or *N*-isopropylcarboxamide derivatives **9b,c**, **10b,c**, **11b,c**, and **12b,c**, respectively, by treatment with methyl isocyanate or isopropyl isocyanate.

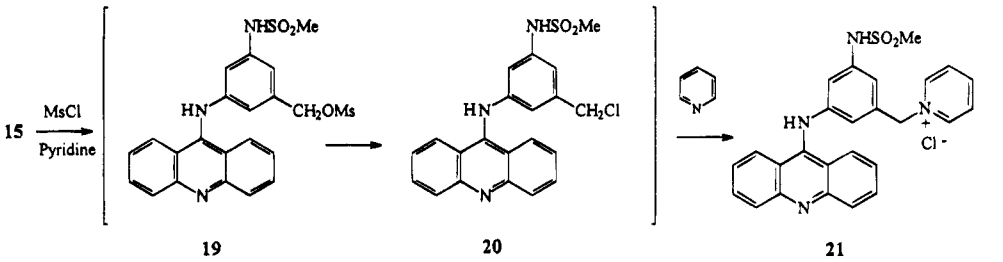
The synthesis of AHMA (**15**) was achieved in good yield by reacting 9-chloroacridine (**5**) with 3,5-diaminobenzyl alcohol (**13**) in methanol in the presence of triethylamine (Scheme 3). AHMA (65%) together with traces of its diacridinyl byproduct **14** (7%) was obtained after refluxing for 4 h. The formation of the diacridinyl derivative **14** was increased under more basic conditions such as NaOMe/MeOH or NaH/DMF. Once the side product **14** was formed, separation of the mixture became difficult due to poor solubility of these two compounds. After several attempts to optimize the reaction conditions, we found that AHMA was obtained exclusively when the reaction proceeded in a mixture of $CHCl_3$/MeOH (v/v 1:1) in the presence of 2.5 mol equiv of 4-methylmorpholine in an ice bath. This synthetic method is suitable for larger scale preparation of this agent, and the crude product can be purified by converting it into its water soluble hydrochloride salt.

The amino and/or hydroxymethyl function(s) of AHMA allowed us to derivatize by linking various substituent(s)

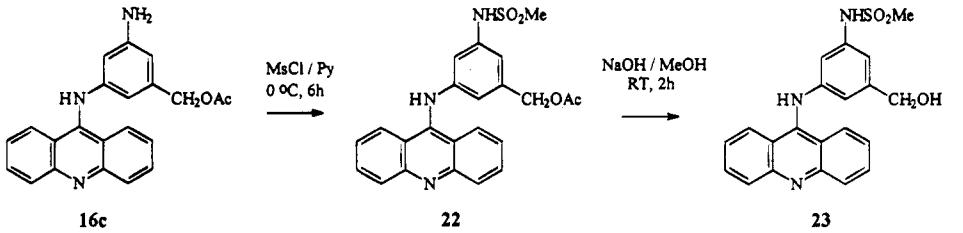
Scheme 3



Scheme 4



Scheme 5



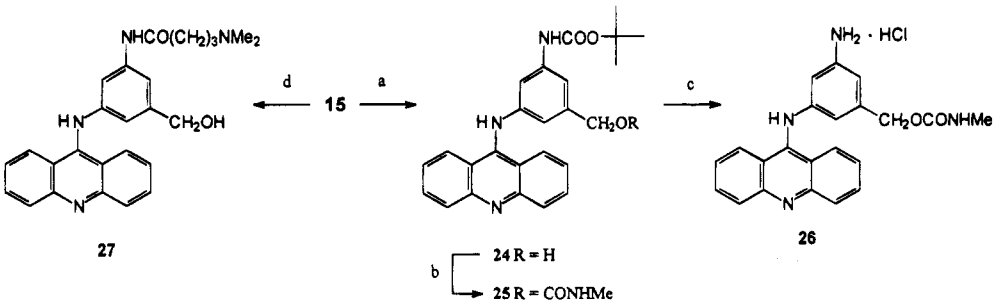
to these groups for SAR studies. AHMAs with different lengths of an acyl function attached to 1-NH_2 and/or 5-CH_2OH group(s) on the anilino ring were synthesized to study the effects of the substituent(s), the size, and the lipophilic–hydrophilic balance on its cytotoxicity. Treatment of AHMA with 1.1 equiv of acetic anhydride and pyridine in DMF in an ice bath afforded exclusively N-acetylated derivative **16a**. When excess of acetic anhydride was used without DMF, *N,N,O*-triacetyl product **16b** was obtained (Scheme 3). We also found that AHMA was selectively O-acetylated to produce **16c** in good yield by treatment with acetyl chloride in acetic acid at room temperature. Longer acyl groups were linked to 1-NH_2 or 5-CH_2OH by reaction of AHMA with levulinic anhydride or succinic anhydride in pyridine in the presence and absence of (*N,N*-dimethylamino)-pyridine (DMAP) to yield a mixture of N-mono- and N,O-diacylated products, **17a,b** and **18a,b**, respectively, which were separated by column chromatography. The N,O-diacylated products **17b** and **18b** can be converted into their corresponding N-monoacylated derivatives **17a** and **18a**, respectively, in moderate yields by treatment with methanolic sodium methoxide.

Attempts at introduction of a methanesulfonyl function to 1-NH_2 of AHMA without protecting the hydroxymethyl function under various conditions failed. When AHMA was treated with methanesulfonyl chloride (MsCl) or methanesulfonic anhydride in pyridine, the pyridium hydrochloride salt **21** was obtained. The formation of the pyridium salt occurred probably by initial formation of N,O-dimesylated intermediate **19**, which underwent nucleophilic attack by chloride anion to form benzyl chloride derivative **20**, which subsequently formed the water soluble pyridium salt **21** (Scheme 4). Hence, we used *O*-acetyl-AHMA (**16c**) as the starting material. Compound **16c** was then smoothly converted into the desired *N*-methylsulfonyl AHMA (**23**) by treatment with MsCl to yield **22** followed by saponification with NaOMe/MeOH (Scheme 5).

Introduction of a leaving group, *N*-methylcarboxamide, to the 5-CH_2OH group of AHMA was also achieved as shown in Scheme 6. The amino function was first selectively protected by BOC protecting group by treatment with di-*tert*-butyl carbonate in pyridine; the product **24** was then reacted with methyl isocyanate and triethylamine at room temperature to give **25**. The protecting function was removed by reaction with aluminum chloride and anisole in CH_2Cl_2/CH_3NO_2 to afford **26** in high yield.

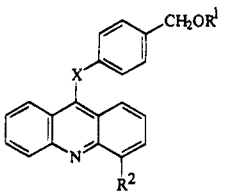
Linking a chargeable side chain (such as (*N,N*-dimethylamino)ethyl group) to the amino function via N-alkylation was rather difficult. Under various condi-

Scheme 6[a]



[a] Reaction conditions: (a) di-*tert*-butyl dicarbonate in pyridine, 0 °C, 16 h; (b) MeNCO in DMF/Et_3N, rt, 8 h; (c) $AlCl_3$, anisole in $MeNO_2$/CH_2Cl_2, rt, 5 min; (d) $Me_2NCH_2CH_2COOH$, 2,2′-dithiodipyridine, Ph_3P in DMF, rt, 0.5 h.

Table 1. *In Vitro* Cytotoxicity of 9-Phenoxy- and 9-(Phenylthio)acridines



compd	X	R^1	R^2	inhibtn of Topo II-mediated k-DNA decatenation[a]	IC_{50} for cell growth inhibtn, HL-60 (μM)
9a	O	H	H	−	12.85
9b	O	CONHMe	H	+	0.68
9c	O	CONH-*i*-Pr	H	+	1.49
10a	O	H	OMe	+	1.04
10b	O	CONHMe	OMe	±	0.95
10c	O	CONH-*i*-Pr	OMe	−	0.42
11a	S	H	H	−	3.33
11b	S	CONHMe	H	+	1.96
11c	S	CONH-*i*-Pr	H	±	2.83
12a	S	H	OMe	±	1.83
12b	S	CONHMe	OMe	−	2.71
12c	S	CONH-*i*-Pr	OMe	−	1.55

[a] Relative potency of inhibiting Topo II-mediated k-DNA decatenation was graded by the concentration required for 50% inhibition: ++++ (<1 μM), +++ (1−5 μM), ++ (5−20 μM), + (20−50 μM), ± (50−100 μM), and − (>100 μM). ND, not determined.

tions, reaction of AHMA with (*N*,*N*-dimethylamino)ethyl chloride ($Me_2NCH_2CH_2Cl$) led to the formation of a mixture of starting material and decomposition products. However, we found that N-acylation of AHMA with alkyl carboxylic acid could be easily achieved by following the known procedure.[24] Thus, treatment of AHMA with (*N*,*N*-dimethylamino)butyric acid in the presence of 2,2′-dithiopyridine and triphenylphosphine afforded exclusively the *N*-acyl product **27** in good yield (Scheme 6).

Biological Results and Discussion

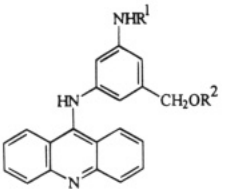
Table 1 shows the *in vitro* cytotoxicity of 9-phenoxy- and 9-(phenylthio)acridines and their corresponding *N*-methyl- and *N*-isopropylcarboxamide derivatives **9**−**12**. The preliminary experiments indicated that both 9-phenoxy- and 9-(phenylthio)acridines exhibit moderate cytotoxicity against human leukemic HL-60 cell growth and inhibition of DNA Topo II. Although many derivatives showed inhibitory effects on k-DNA decatenation mediated by Topo II, the inhibitory activities, however, were not always correlated with cytotoxicity suggesting that some compounds may have different or more than one mode of action. In the 9-phenoxyacridine series, the cytotoxicity of the parent compound **9a** was enhanced after introduction of the *N*-carboxamide to the hydroxymethyl function or a methoxy group to the C-3 position of the acridine ring. However, introduction of the same carboxamide or methoxyl group only slightly increased the cytotoxicity of 9-(phenylthio)acridine derivatives. The similar results were obtained in our previous studies.[23]

Of the series of AHMA derivatives, it was revealed that addition of a short acyl side chain, acetyl group (compounds **16a**,**b**), or a long levulinyl function (compounds **17a**,**b**) to the 1-NH_2 and/or 5-CH_2OH of the anilino ring did not greatly affect the cytotoxicity of the parent compound (AHMA) against leukemic HL-60 cell growth *in vitro* (Table 2), but these compounds were less cytotoxic than AHMA against leukemic L1210 and S180 cell growth in culture. Compound **17b** was shown to be more potent inhibitor than AHMA and *m*-AMSA in terms of their effect on the inhibition of Topo II with k-DNA (Figure 2). On the contrary, AHMAs linking with succinyl side chain (compounds **18a**,**b**) were much less cytotoxic, probably due to their poor solubility. The Topo II-mediated DNA cleavage patterns induced by AHMA and *m*-AMSA are compared in Figure 3 using PBR_{322}DNA.

The *in vitro* cytotoxicity of AHMA and its derivatives against human leukemic HL-60 cell growth was also studied as shown in Table 3. Both AHMA and its water soluble HCl salt (**15a**) have the same IC_{50} value (0.025 μM). Compound **28** (Figure 1) lacking one amino function was 10 times less active than AHMA. 1-*N*-Methylsulfonyl-AHMA (**23**), which is structurally closer to *m*-AMSA, was shown to be 2 times less active than AHMA and *m*-AMSA. On the other hand, compound **26** possessing a slow leaving group, *N*-methylcarboxamide, at 5-CH_2OH was found to be less cytotoxic than AHMA. It is also clear that compounds with substitution at 5-CH_2OH, whether the 1-NH_2 is substituted or not, were as active as or less active than their parent compounds. Water soluble compound **21** with pyridium chloride salt, however, was the least cytotoxic.

The interaction of 9-anilinoacridines with DNA has been studied by spectrophotometrical methods[25,26] as well as by computer calculation.[27] The results showed that the correlation between DNA−drug binding and drug antitumor activity could not be determined, since *m*-AMSA formed one of the weakest intercalation complexes among the compounds studied (inactive *o*-AMSA was shown to have a higher affinity for DNA than *m*-AMSA). In addition, linking a bulky side chain

Table 2. *In Vitro* Cytotoxicity of AHMA and Its Acylated Derivatives



compd	R^1	R^2	inhibtn of Topo II-mediated k-DNA decatenation[a]	IC_{50} for cell growth inhibtn (μM)		
				HL-60	L1210	S180
15 (AHMA)	H	H	+++	0.025	0.016	0.030
16a	Ac	H	ND	0.018	0.028	ND
16c	H	Ac	ND	0.028	0.034	ND
17a	$CO(CH_2)_2COMe$	$CO(CH_2)_2COMe$	++++	0.015	0.098	0.097
17b	$CO(CH_2)_2COMe$	H	++++	0.025	0.045	0.070
18a	$CO(CH_2)_2COOH$	$CO(CH_2)_2COOH$	−	4.295	0.990	1.83
18b	$CO(CH_2)_2COOH$	H	+	4.995	0.330	1.07
m-AMSA			+++	0.021	ND	ND

[a] See Table 1 footnote.

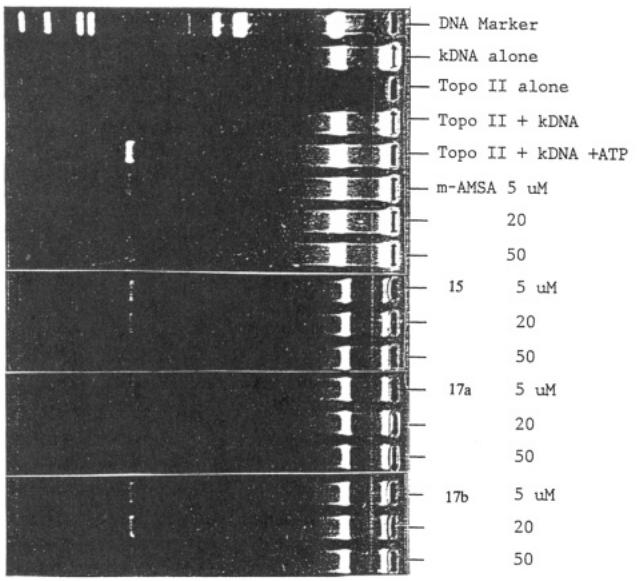


Figure 2. Inhibition of Topo II-mediated k-DNA decatenation by AHMA (**15**), **17a**,**b**, and *m*-AMSA using purified Topo II from calf thymus.[20] Electrophoresis was carried out on 1% agarose in TBE buffer as described previously.[28,29]

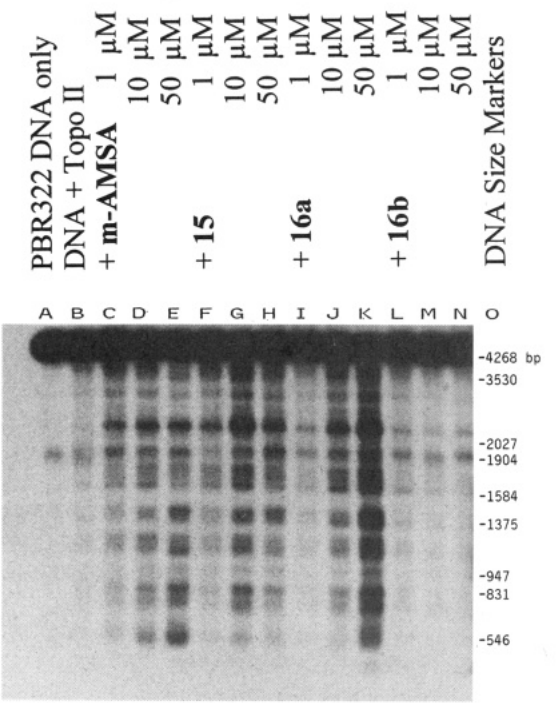


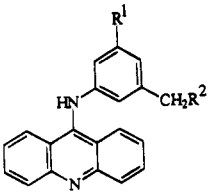
Figure 3. Comparison of Topo II-mediated cleavage of PBR_{322}-DNA induced by AHMA (**15**), **16a**,**b**, and *m*-AMSA. These acridines showed similar DNA cleavage patterns with varied potency.

to the anilino ring has little effect on the kinetic stability of the drug−DNA complexes. It is difficult to interpret the SARs of this class of compounds in terms of a striking difference in their strength or mode of binding to DNA. From our above findings, it could be concluded that change in the size of substituent(s) at NH_2 and/or CH_2OH function(s) of the anilino ring has little effect on their cytotoxicity. Our results also suggested that antitumor activity of AHMA analogues, like the acridine-sulfonanilides, may exert through the formation of ternary complexes in which the planar acridine moiety serves as the DNA-binding anchor, whereas the amino- and hydroxymethyl-substituted anilino ring may interact with macromolecular enzymes such as topoisomerases as proposed previously.[27]

In our preliminary studies on human leukemic CCRF-CEM cells and their sublines resistant to teniposide (CEM/VM-1, a cell line with mutated Topo II gene) and vinblastin (CEM/VBL, a P-glycoprotein multiple drug resistant (MDR) cell line), we found that *m*-AMSA and AHMA were 13.7- and 6.1-fold resistant to the CEM/VM-1 cell line and 3.8- and 14.4-fold resistant to the CEM/VBL cell line, respectively (Table 4). Thus, AHMA and *m*-AMSA are cross-resistant. We also studied hamster lung cells (CD-3F) and their subline resistant to actinomycin D (CD-3F/ADII, a P-glycoprotein MDR cell line). AHMA and *m*-AMSA showed similar degrees of cross-resistance.

The antitumor activity of AHMA and its water soluble hydrochloride salt against various solid tumors in mice was also studied. The half-lethal doses (LD_{50}s) in BD_2F_1 mice (QD × 5, ip) for AHMA, VP-16, and *m*-AMSA were 20.1, 15, and 8.2 mg/kg, respectively. Survivors recovered the lost body weight rapidly. Chemotherapy of solid-tumor-bearing mice with 10, 10, and 5 mg/kg (QD × 4, ip) of AHMA, VP-16, and *m*-AMSA, respectively, resulted in more tumor volume reduction by AHMA than by VP-16 or *m*-AMSA for E0771 mammary adeno-

Table 3. Structure–Activity Relationships of AHMA Derivatives



compd	R^1	R^2	IC_{50} for cell growth inhibtn, HL-60 (μM)
15a (AHMA·HCl)	NH_2	OH	0.025
16a	NHAc	OH	0.018
16c	NH_2	OAc	0.028
21	$NHSO_2Me$	NC_5H_5·HCl	2.970
22	$NHSO_2Me$	OAc	0.063
23	$NHSO_2Me$	OH	0.045
25	$NHCOOMe_3$	OCONHMe	0.020
26	NH_2	OCONHMe	0.116
27	NHCO-$(CH_2)NMe_2$	OH	0.054
28	H	OH	0.226
m-AMSA			0.021

carcinoma and B-16 melanoma (Table 5). For Lewis lung carcinoma, AHMA was as potent as VP-16 but more active than *m*-AMSA.

Conclusions

Two types of 9-anilinoacridine derivatives, namely 9-phenoxy- or 9-(phenylthio)acridines and 3-(9-acridinylamino)-5-(hydroxymethyl) aniline derivatives, were synthesized. These compounds were designed to avoid formation of the bioinactive diiminoquinone–glutathione conjugate as metabolic inactivation of *m*-AMSA. Our compounds were expected to have long half-life due to their inability to form the diiminoquinone intermediate via oxidation. The preliminary antitumor studies of these acridine derivatives showed that 9-phenoxy- and 9-(phenylthio)acridines exhibited moderate cytotoxicity against mouse leukemic L1210 and human leukemic HL-60 cell growth *in vitro*. 4-(9-Acridinyloxy)benzyl alcohol (**9a**) was much less cytotoxic than the corresponding 9-acridinylthio derivative **11a** with IC_{50} values of 12.85 and 3.33 μM, respectively. In order to design acridine derivatives with both DNA-intercalating and -alkylating potential, we have introduced a slow leaving function, *N*-methylcarbamate, to the benzylic hydroxy function. These compounds are expected to be attacked by nucleophilic function(s) of DNA or topoisomerase to form more stable unbreakable complexes. It was shown that the cytotoxicity of 9-phenoxyacridines was greatly increased by adding the *N*-methylcarbamate to the benzylic alcohol function, while in the case of 9-(phenylthio)acridines, their cytotoxicity was increased only slightly. It should be noted that both 9-phenoxy- and 9-(phenylthio)acridines were not good inhibitors of Topo II.

In contrast to the derivatives of 9-phenoxy- and 9-(phenylthio)acridines with two substituents at the para position, AHMA derivatives bearing three substituents all in the meta position to each other on the anilino ring exhibited significant antitumor activity both *in vitro* and *in vivo* and were found to be potent inhibitors of Topo II (Figures 2 and 3). The preliminary antitumor efficacy of AHMA derivatives described above indicated that (1) AHMA and its derivatives represent a novel type of antitumor agents which have a longer plasma half-life (1.5 h) than *m*-AMSA, since these compounds, unlike m-AMSA, do not bio-oxidize to form the iminoquinone intermediate, (2) AHMA has greater efficacy against murine leukemia and solid tumors than *m*-AMSA or VP-16 and yet is less toxic toward the host, (3) AHMA without substituent at 5-CH_2OH exhibited slightly better antitumor activity against leukemic HL-60 and L1210 cell lines in culture, and (4) substituent(s) at NH_2 and/or CH_2OH, in general, did not greatly affect the cytotoxicity of AHMA. Formation of stable ternary complexes between drug (AHMA derivatives), DNA, and enzyme (Topo II) may be demanded for their antitumor activity. Other factors such as water solubility and alternation of lipophilicity/hydrophilicity balance by these substituent(s) may also affect the cytotoxicity of these derivatives.

Table 4. Sensitivity and Cross-Resistance Studies[a]

	cell line used for IC_{50} (μM)				
compd	CCRF-CEM	CEM/VM-1	CEM/VBL	DC-3F	DC-3F/ADII
AHMA	0.263	1.60 (6.08×)	3.79 (14.4×)	0.005	0.054 (10.8×)
m-AMSA	0.139	1.91 (13.7×)	0.52 (3.76×)	0.004	0.047 (11.8×)

[a] Human leukemic CCRF-CEM cells and their sublines resistant to teniposide (CEM/VM-1, a cell line with mutated Topo II gene) and vinblastin (CEM/VBL, a P-glycoprotein MDR cell line). Hamster lung cells (DC-3F) and their subline resistant to actinomycin D (DC-3F/ADII, a P glycoprotein MDR cell line).

Table 5. Antitumor Activity of AHMA against Solid Tumors in BD_2F_1 Mice[a]

		E0771 mammary adenocarcinoma		B-16 melanoma		Lewis lung carcinoma	
compd	dose (mg/kg)	AWC (g)	avg tumor vol. (*T/C*)	AWC (g)	avg tumor vol. (*T/C*)	AWC (g)	avg tumor vol. (*T/C*)
CTRL		+0.8	1.00	+1.6	1.00	+1.8	1.00
ctrl (DMSO)		+2.4	0.93	+1.1	0.93	+2.2	0.98
AHMA (**15a**)	7.5	+0.6	0.13	−0.3	0.23	+0.1	0.55
	10	+0.6	0.14 ± 0.03	−1.9	0.18 ± 0.01	−0.4	0.23 ± 0.09
VP-16	10	+0.6	0.26 ± 0.01	+0.8	0.21 ± 0.02	+0.1	0.20 ± 0.15
m-AMSA	5.0	+0.2	0.31 ± 0.01	−2.1	0.24 ± 0.01	−1.4	0.46 ± 0.08

[a] BD_2F_1 female mice, 19–21 g, were inoculated subcutaneously with 0.2 mL of solid tumor debris, and the treatment was started on day 3, daily for 5 days intraperitoneously with 10, 10, and 5 mg/kg AHMA, VP-16, and *m*-AMSA, respectively. Average weight changes (AWC) in grams were measured on day 14, and the average tumor volumes in mm^3 on day 14 were measured by length (mm) × width (mm) t width (mm)/2. Each dose (including the untreated control and DMSO-treated control) consists of five mice, and averaged values are shown. The ranges given are the mean of two experiments with the range of actual variations.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Column chromatography was carried out on silica gel G60 (70–230 mesh, ASTM; Merck). Thin layer chromatography was performed on Analtech uniplates with short-wavelength UV light for visualization. Elemental analyses were done by M.H.W. Laboratory, Phoenix, AZ. ^{1}H NMR spectra were recorded on a JEOL-FX90Q, Brucker ACF-250, or Brucker AMX-400 spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in ppm (δ), and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and brs (broad singlet). Values reported for coupling constants are first order.

4-(9-Acridinyloxy)benzyl Alcohol (9a). 4-Hydroxybenzyl alcohol (**7**; 248 mg, 3 mmol) in EtOH (3 mL) was added dropwise to a mixture of KOH (112 mg, 2 mmol) in EtOH (3 mL). After being stirred under reflux for 4 h, 9-chloroacridine (**5**; 384 mg, 1.8 mmol) in EtOH (30 mL) was added, and the mixture was refluxed for 3 days. The solvent was removed *in vacuo* to dryness, and the residue was dissolved in 2% NaOH aqueous solution (5 mL) and extracted with $CHCl_3$ (3 × 20 mL). The combined organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated *in vacuo* to dryness. The residue was chromatographed on a silica gel column (2 × 30 cm) using hexane/EtOAc (2:1, v/v) as the eluant. Compound **9a** was obtained as yellow crystals (EtOH): 206 mg (38%); mp 156–157 °C; ^{1}H NMR ($CDCl_3$) δ 1.66 (1H, br, exchangeable, OH), 4.64 (2H, s, CH_2), 6.84 and 7.28 (each 2H, d, J = 8.75 Hz, ArH), 7.26–8.34 (8H, m, ArH). Anal. ($C_{20}H_{15}NO_2 \cdot {}^1/_2H_2O$) C, H, N.

By following the same procedure, the following 4-(9-acridinyloxy)- and 4-(acridinylthio)benzyl alcohol derivatives were synthesized.

4-[(3-Methoxy-9-acridinyl)oxy]benzyl alcohol (10a): synthesized by condensation of 4-hydroxybenzyl alcohol (248 mg, 2 mmol) with 9-chloro-3-methoxyacridine (438 mg, 1.8 mmol); yield 216 mg (36%); mp 144–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.56 (1H, br, exchangeable, OH), 3.99 (3H, s, OMe), 4.62 (2H, s, CH_2), 6.82 and 7.26 (each 2H, d, J = 8.75 Hz, ArH), 7.37–8.23 (7H, m, ArH). Anal. ($C_{21}H_{17}NO_3 \cdot {}^1/_4H_2O$) C, H, N.

4-(9-Acridinylthio)benzyl alcohol 11a: prepared by condensation of 4-mercaptobenzyl alcohol (280 mg, 2 mmole) with 9-chloroacridine (384 mg, 1.8 mmole); yield 126 mg (22%); mp 152–153 °C; ^{1}H NMR (DMSO-d_6) δ 4.35 (2H, s, CH_2), 5.50 (1H, br, exchangeable, OH), 7.00 and 7.26 (each 2H, d, J = 8.20 Hz, ArH), 7.61–8.69 (8H, m, ArH). Anal. ($C_{20}H_{15}NOS$) C, H, N, S.

4-[(3-Methoxy-9-acridinyl)thio]benzyl alcohol (12a): prepared by condensation of 4-mercaptobenzyl alcohol (280 mg, 2 mmol) with 9-chloroacridine (384 mg, 1.8 mmol); yield 216 mg (17%); mp 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 1.56 (1H, br, exchangeable, OH), 4.20 (3H, s, OMe), 4.67 (2H, s, CH_2), 6.98 and 7.12 (each 2H, d, J = 8.51 Hz, ArH), 7.36–8.32 (7H, m, ArH). Anal. ($C_{21}H_{17}NO_2S$) C, H, N, S.

4-(9-Acridinyloxy)benzyl *N*-Methylcarbamate (9b). A mixture of **9a** (100 mg, 0.33 mmol), methyl isocyanate (500 mg, 9 mmol), and triethylamine (0.5 mL) in dry CH_2Cl_2 was stirred at room temperature for 6 h. The mixture was concentrated *in vacuo* to dryness, and the residue was chromatographed on a silica gel column (2 × 20 cm) using hexane/EtOAc (v/v 3:1) as the eluant. Compound **9b**, 57 mg (48%), was obtained after recrystallization from $CHCl_3$/hexane: mp 161–162 °C; ^{1}H NMR ($CDCl_3$) δ 2.79 (3H, d, Me), 4.61 (1H, br, exchangeable, NH), 5.03 (2H, s, CH_2), 6.85 and 7.27 (each 2H, d, J = 8.51 Hz, ArH), 7.36–8.32 (7H, m, ArH). Anal. ($C_{22}H_{18}N_2O_3$) C, H, N.

In a similar manner, the following *N*-alkylcarboxamides were prepared.

[(3-Methoxy-9-acridinyl)oxy]benzyl *N*-methylcarbamate (10b) prepared from **10a** (100 mg, 0.3 mmol); yield 70 mg (60%); mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 2.79 (3H, d, J = 4.94 Hz, NH-Me), 4.00 (3H, s, OMe), 4.33 (1H, br, exchangeable, NH), 5.03 (2H, s, CH_2), 6.81 and 7.26 (each 2H, d, J = 8.78 Hz, ArH), 7.05–8.25 (7H, m, ArH). Anal. ($C_{23}H_{20}N_2O_4$) C, H, N.

4-(9-Acridinylthio)benzyl *N*-methylcarbamate (11b): synthesized from **11a** (106 mg, 0.33 mmol); yield 78 mg (60%); mp 140–141 °C; ^{1}H NMR ($CDCl_3$) δ 2.76 (3H, d, J = 4.96 Hz, NH-Me), 4.52 (1H, br, exchangeable, NH), 4.96 (2H, s, CH_2), 6.95 and 7.12 (each 2H, d, J = 8.51 Hz, ArH), 7.25–8.73 (8H, m, ArH). Anal. ($C_{22}H_{18}N_2O_2S$) C, H, N.

4-[(3-Methoxy-9-acridinyl)thio]benzyl *N*-methylcarbamate (12b): prepared from **12a** (69 mg, 0.2 mmol); yield 50 mg (62%); mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 2.76 (3H, d, J = 4.94 Hz, NH-Me), 4.01 (3H, s, OMe), 4.35 (1H, br, exchangeable, NH), 4.96 (2H, s, CH_2), 6.94 and 7.12 (each 2H, d, J = 8.51 Hz, ArH), 7.02–8.67 (7H, m, ArH). Anal. ($C_{23}H_{20}N_2O_3S$) C, H, N, S.

4-(9-Acridinyloxy)benzyl *N*-isopropylcarbamate (9c): prepared from **9c** (100 mg, 0.3 mmol) with isopropyl isocyanate (0.4 mL); yield 59 mg (47%); mp 193–194 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 and 1.18 (each 3H, s, Me), 3.85–3.99 (1H, m, CH), 4.51 (1H, br, exchangeable, NH), 5.20 (2H, s, CH_2), 6.81 and 7.27 (each 2H, d, J = 8.78 Hz, ArH), 7.25–8.34 (8H, m, ArH). Anal. ($C_{24}H_{22}N_2O_3$) C, H, N.

4-[(3-Methoxy-9-acridinyl)oxy]benzyl *N*-isopropylcarbamate (10c): prepared from **10b** (100 mg, 0.3 mmol); yield 68 mg (55%); mp 168–169 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 and 1.18 (each 3H, s, Me), 3.68–3.91 (1H, m, CH), 4.45 (1H, br, exchangeable, NH), 5.02 (2H, s, CH_2), 6.82 and 7.26 (each 2H, d, J = 8.78 Hz, ArH), 7.26–8.30 (7H, m, ArH). Anal. ($C_{25}H_{24}N_2O_4$) C, H, N.

4-(9-Acridinylthio)benzyl *N*-isopropylcarbamate (11c): prepared from **11b** (106 mg, 0.3 mmol); yield 90 mg (70%); mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 1.11 and 1.19 (each 3H, s, Me), 3.59–3.84 (1H, m, CH), 4.36 (1H, br, exchangeable, NH), 4.99 (2H, s, CH_2), 6.99 and 7.16 (each 2H, d, J = 8.78 Hz, ArH), 7.29–8.77 (8H, m, ArH). Anal. ($C_{24}H_{22}N_2O_2S$) C, H, N.

4-[(3-Methoxy-9-acridinyl)thio]benzyl *N*-isopropylcarbamate (12c): prepared from **12b** (69 mg, 0.2 mmol); yield 56 mg (65%); mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.14 and 1.21 (each 3H, s, Me), 3.72–3.79 (1H, m, CH), 4.50 (1H, br, exchangeable, NH), 5.01 (2H, s, CH_2), 7.01 and 7.19 (each 2H, d, J = 8.51 Hz, ArH), 7.17–8.74 (7H, m, ArH). Anal. ($C_{25}H_{24}N_2O_3S$) C, H, N, S.

3,5-Bis(9-acridinylamino)benzyl Alcohol (14) and 3-(9-Acridinylamino)-5-(hydroxymethyl)aniline (AHMA, 15). A mixture of **5** (523 mg, 2.5 mmol), 3,5-diaminobenzyl alcohol dihydrochloride (**13**; 527 mg, 2.5 mmol), and triethylamine (500 mg, 5.0 mmol) in MeOH (10 mL) was heated under reflux for 4 h (two products were formed with R_f values of 0.48 and 0.35). After cooling, the orange precipitates were collected by filtration. The solid was dissolved in MeOH and coevaporated with silica gel (3 g) *in vacuo* to dryness. The residue was put on the top of a silica gel column (3 × 40 cm), which was washed with $CHCl_3$/MeOH (10:1, v/v). Compound **14** with the higher R_f value was eluted with $CHCl_3$/MeOH (9:1, v/v) followed by the second product (AHMA, **15**) ($CHCl_3$/MeOH, 4:1, v/v).

Compound 14: 86 mg (7%); mp 226–227 °C; ^{1}H NMR (DMSO-d_6) δ 4.53 (2H, s, CH_2), 5.50 (1H, br, OH), 6.34 (1H, s, ArH), 7.39 (2H, s, ArH), 7.54 (4H, t, J = 7.71 Hz, ArH), 8.02 (4H, t, J = 7.65 Hz, ArH), 8.13 (4H, d, J = 8.55 Hz, ArH), 8.39 (4H, d, J = 8.73 Hz, ArH), 11.65 (2H, br, NH). Anal. ($C_{33}H_{24}N_4O$) C, H, N.

Compound 15 (AHMA): 511 mg (65%); mp 251–252 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.38 (2H, s, CH_2), 5.20 (1H, br, OH), 5.52 (2H, br, NH_2), 6.45, 6.50, and 6.62 (each 1H, s, ArH), 7.44, 7.98, 8.05, and 8.31 (each 2H, m, ArH), 11.39 (1H, br, NH). Anal. ($C_{20}H_{17}N_3O$) C, H, N.

Larger Scale Preparation of AHMA (15a). A mixture of **13** (12.66 g, 0.06 mol) and 4-methylmorpholine (12.2 g, 0.12 mol) in EtOH (400 mL) was stirred for 1 h in an ice bath. A solution of **5** (12.8 g, 0.06 mol) in a mixture of EtOH/$CHCl_3$ (100 mL, v/v 1:1) containing 4-methylmorpholine (7.0 g, 0.07 mol) was then added dropwise to this mixture. After being stirred for 2 h at 0 °C, the mixture was warmed to room temperature and continuously stirred for an additional 1 h.

The solid product formed was collected by filtration; the filter cake was washed successively with cold 95% EtOH, acetone, and ether and dried. Compound **15a** was obtained as orange crystals, 17.4 g (92%). A small amount of crude product was purified by recrystallization from MeOH. The TLC, IR, and ^{1}H NMR of the product were identical with those of an authentic sample.

3-(9-Acridinylamino)-5-(hydroxymethyl)aniline Hydrochloride (15a). The crude AHMA was purified by hydrochloride salt formation; 1 N HCl (32 mL) was added slowly to a suspension of AHMA (9.46 g, 30 mmol) in MeOH (300 mL). After being stirred for 1 h, the orange solid (6.81 g) was collected by filtration. The filtrate was concentrated, and the second crop of products (2.81 g) was obtained to give a total yield of 9.62 g (77%): mp >290 °C. Anal. ($C_{20}H_{17}N_3O$·2HCl·$^3/_2H_2O$) C, H, Cl, N.

***N*-Acetyl-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (16a).** To a solution of AHMA (1.58 g, 5 mmol) in DMF (20 mL) containing pyridine (0.8 mL, 10 mmol) was added dropwise 0.62 mL (6.5 mmol) of acetic anhydride at 0 °C. After being stirred for 2 h, the mixture was evaporated *in vacuo* to dryness, and the residue was coevaporated several times with EtOH. The solid residue was recrystallized from EtOH to give **16a**: 1.60 g (89%); mp 293–294 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.05 (3H, s, NH-Ac), 4.46 (2H, s, CH_2), 5.61 (1H, br, OH), 7.00, 7.65, and 7.71 (each 1H, s ArH), 7.39–7.58 (2H, m, ArH), 8.00–8.10 (4H, m, ArH), 8.26–8.28 (2H, m, ArH), 11.50 and 14.70 (each 1H, brs, NH). Anal. ($C_{22}H_{19}N_3O_2$·$^5/_2H_2O$) C, H, N.

***N*-Acetyl-3-(*N*-acetyl-9-acridinylamino)-5-(acetoxymethyl)aniline (16b).** A mixture of AHMA (3.15 g, 10 mmol) in acetic anhydride (10 mL) and pyridine (10 mL) was stirred at room temperature for 4 h. The mixture was diluted with cold EtOH in an ice bath, and the solid was collected by filtration. The filtrate was evaporated *in vacuo* to dryness, and the residue was crystallized from EtOH. The solid was combined with the product obtained before and recrystallized from EtOH to give **16b**: 3.62 g (86%); mp 161–162 °C; ^{1}H NMR (DMSO-d_6) δ 1.91, 1.99, and 2.04 (each 3H, s, Ac), 4.98 (2H, s, CH_2), 6.46, 7.00, and 7.20 (each 1H, ArH), 7.33 (2H, br, ArH), 7.51 (4H, br, ArH), 8.14 (2H, br, ArH), 9.87 (1H, brs, NH). Anal. ($C_{26}H_{23}N_3O_4$·H_2O) C, H, N.

3-(9-Acridinylamino)-5-(acetoxymethyl)aniline Hydrochloride (16c). A mixture of AHMA (3.15 g, 10 mmol), acetic acid (350 mL), and acetyl chloride (2.36 g, 30 mmol) was stirred at room temperature for 20 h. The mixture was evaporated *in vacuo* to dryness; the residue was coevaporated several times with EtOH and recrystallized from EtOH to give **16c**: 3.54 g (89%); mp 198–201 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.01 (3H, s, OAc), 4.93 (2H, s, CH_2), 5.61 (2H, br, NH_2), 6.45, 6.50, and 6.59 (each 1H, s, ArH), 7.41 and 7.97 (each 2H, t, ArH), 8.16 and 8.74 (each 2H, d, ArH), 11.50 and 14.70 (each 1H, br, NH). Anal. ($C_{22}H_{19}N_3O_2$·HCl·$^1/_4H_2O$) C, H, Cl, N.

***N*,*O*-Bis(β-acetylpropionyl)-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (17a).** A mixture of AHMA (250 mg, 0.79 mmol), freshly prepared levulinic acid anhydride (prepared from levulinic acid (290 mg, 2.5 mmol) and DCC (260 mg, 1.25 mmol)), and 4-(dimethylamino)pyridine (150 mg, 1.19 mmol) in pyridine (5 mL) was stirred at room temperature for 48 h. The mixture was evaporated to dryness *in vacuo*. The residue was diluted with H_2O (25 mL) and extracted with $CHCl_3$ (10 mL × 4). The organic extracts were combined, dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on a silica gel column (2 × 20 cm) using $CHCl_3$/MeOH (95:5, v/v) as the eluant. The main fraction was collected and evaporated to give pure **17a** as a foam: 181 mg (45%); ^{1}H NMR (DMSO-d_6) δ 2.07 and 2.10 (each 3H, s, COMe), 2.66 (8H, m, CH_2), 4.96 (2H, s, Ph-CH_2), 6.43, 6.95, and 7.20 (each 1H, s, ArH), 6.85–7.04 (2H, m, ArH), 7.51–7.86 (4H, m, ArH), 8.35 (2H, br, ArH), 9.86 and 11.23 (each 1H, br, NH). Anal. ($C_{30}H_{27}N_3O_5$) C, H, N.

***N*,*O*-Bis[β-(hydroxycarbonyl)propionyl]-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (18a) and *N*-[β-(hydroxycarbonyl)propionyl]-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (18b).** A mixture of AHMA (250 mg, 0.88 mmol), succinic acid anhydride (237 mg, 2.37 mmol), and DMAP (145 mg, 1.19 mmol) in dry pyridine (15 mL) was heated at 60 °C for 3 h (two products were formed). The mixture was concentrated, and the residue was coevaporated several times with EtOH to dryness *in vacuo*. The residue was chromatographed on a silica gel column (2 × 20 cm) using $CHCl_3$/MeOH/Et_3N (3:1:0.5) as the eluant. The diacylated product with the higher R_f value was collected first to give **18a**, 234 mg (52%), followed by the monoacylated product **18b**, 80 mg (18%).

Compound 18a: mp 193–195 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.48 (8H, m, CH_2), 4.98 (2H, s, CH_2), 6.42, 6.89, and 7.15 (each 1H, s, ArH), 7.06 (2H, brs, ArH), 7.47 (4H, brs, ArH), 7.83 (2H, brs, ArH), 9.89 and 11.05 (each 1H, brs, NH). Anal. ($C_{28}H_{25}N_3O_7$·$^3/_2H_2O$) C, H, N.

Compound 18b: mp 184–185 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.53 (4H, m, 2 × CH_2), 4.38 (2H, m, CH_2), 5.12 (1H, brs, OH), 6.42, 6.89, and 7.18 (each 1H, s, ArH), 7.10 (2H, brs, ArH), 7.51 (4H, brs, ArH), 7.95 (2H, brs, ArH), 9.89 and 10.89 (each 1H, brs, NH). Anal. ($C_{24}H_{21}N_3O_4$·2.75H_2O) C, H, N.

***N*-(β-Acetylpropionyl)-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (17b).** A mixture of compound **17a** (423 mg, 0.83 mmol) in NaOMe/MeOH (prepared from Na, 15 mg, in 5 mL of MeOH) was stirred at room temperature for 48 h and then neutralized with Dowex 50 in pyridine and filtered. The ion exchange resin was washed with pyridine/H_2O (1:1, v/v). The combined filtrate and washings were evaporated to dryness *in vacuo*. The solid residue was recrystallized from EtOH to yield **17b**: 217 mg (64%); mp 197–199 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.10 (3H, s, COMe), 2.68 (4H, m, CH_2), 4.49 (2H, s, CH_2), 5.17 (1H, brs, OH), 6.42, 7.08, and 7.15 (each 1H, s, ArH), 7.02 (2H, brs, ArH), 7.52 (4H, brs, ArH), 7.90 (2H, brs, ArH), 9.98 and 11.05 (each 1H, brs, NH). Anal. ($C_{25}H_{23}N_3O_3$) C, H, N.

In a similar manner, compound **18b** was prepared by following the same procedure as described for the synthesis of **17b** by treatment of compound **18a** (103 mg, 0.2 mmol) with NaOMe, 45 mg (54%). The spectrometric analyses (IR and NMR spectra) of the product were identical with those of **18b** previously obtained.

[3-(9-Acridinylamino)-5-[(methylsulfonyl)amino]benzyl]pyridinium Hydrochloride (21). To a suspension of AHMA (**15**; 473 mg, 1.5 mmol) in dry pyridine (20 mL) was added MsCl (229 mg, 2.0 mmol) in an ice bath. After being stirred for 1.5 h, additional MsCl (229 mg, 2.0 mmol) was added, and stirring was continued for 2 h at 0 °C. MeOH (5 mL) was added, and the mixture was evaporated *in vacuo* to dryness. The residue was dissolved in H_2O (5 mL) and filtered through a pad of Celite. The filtrate was concentrated, and the residue was recrystallized twice from 95% EtOH to give **21**: 357 mg (48%); mp 227–228 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.15 (3H, s, SO_2Me), 5.78 (2H, s, CH_2), 7.05 and 7.30 (each 1H, s, ArH), 7.40 (3H, brs, ArH), 8.01 and 8.11 (each 2H, m, ArH), 8.18 (2H, t, J = 7.67 Hz, ArH), 8.25 (2H, d, J = 8.52 Hz, ArH), 8.60 (1H, t, J = 7.67 Hz, ArH), 9.05 (2H, d, J = 5.60 Hz, ArH), 10.37, 11.74, and 15.45 (each 1H, brs, NH and HCl). Anal. ($C_{26}H_{23}ClN_4O_2S$·HCl·2H_2O) C, H, Cl, N, S.

***N*-[3-(9-Acridinylamino)-5-(acetoxymethyl)phenyl]methanesulfonamide (22).** To a suspension of **16c** (3.5 g, 8.7 mmol) in pyridine (70 mL) cooled in an ice bath was slowly added methanesulfonyl chloride (1.16 g, 15 mmol). After being stirred for 3 h, the clear solution obtained was evaporated *in vacuo* to dryness. The residue was triturated with ice–water (50 mL) and neutralized with 10% $NaHCO_3$ aqueous solution, and the solid precipitates were collected by filtration. The solid was recrystallized from $CHCl_3$/EtOH to give **22**: 2.45 g (54%); mp 250–252 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.04 (3H, s, Ac), 2.95 (3H, s, SO_2Me), 5.01 (2H, s, CH_2), 6.57, 6.59, and 6.82 (each 1H, s, ArH), 7.05, 7.54, 7.57, and 7.89 (each 2H, m, ArH), 9.72 and 11.01 (each 1H, brs, NH). Anal. ($C_{23}H_{21}N_3O_4S$) C, H, N, S.

***N*-[3-(9-Acridinylamino)-5-(hydroxymethyl)phenyl]methanesulfonamide (23).** A solution of **22** (870 mg, 2 mmol) in MeOH (50 mL) containing 1 N NaOH (3 mL) was stirred at room temperature for 3 h. The solvent was removed *in vacuo*, and the residue was triturated with ice–water and acidified to pH *ca.* 5 with 1 N HCl. The precipitates were

collected by filtration and recrystallized from 95% EtOH to yield **23**: 720 mg (92%); mp 185–187 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.01 (3H, s, SO_2Me), 4.48 (2H, s, CH_2), 5.45 (1H, brs, OH), 7.08, 7.15, and 7.19 (each 1H, s, ArH), 7.49 (2H, t, J = 7.53 Hz), 8.02 (2H, t, J = 7.10 Hz, ArH), 8.10 (2H, m, ArH), 8.29 (2H, d, J = 8.29 Hz, ArH), 10.05, 11.54, and 14.90 (each 1H, brs, NH). Anal. ($C_{21}H_{19}N_3O_3S$·HCl) C, H, Cl, N, S.

***N*-(*tert*-Butoxycarbonyl)-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (24).** A mixture of AHMA (**15**; 630 mg, 2 mmol) and di-*tert*-butyl carbonate (436 mg, 4 mmol) in pyridine (8 mL) was stirred in an ice bath for 4 h. Additional di-*tert*-butyl carbonate (436 mg, 4 mmol) was added to the mixture and the mixture continuously stirred overnight. The mixture was then evaporated *in vacuo* to dryness, and the residue was crystallized from Et_2O/EtOH to give **24**: 651 mg (78%); mp 220–221 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.46 (3H, s, *t*-Bu), 4.43 (2H, s, CH_2), 4.99 (1H, br, OH), 6.93, 7.44, and 7.48 (each 1H, ArH), 7.48 (2H, t, J = 7.45 Hz, ArH), 8.00 (2H, t, J = 7.50 Hz, ArH), 8.12 (2H, d, J = 8.50 Hz, ArH), 8.28 (2H, d, J = 8.76 Hz, ArH), 9.62, 11.45, and 14.85 (each 1H, NH). Anal. ($C_{25}H_{25}N_3O_3$) C, H, N.

3-(9-Acridinylamino)-5-[(*tert*-butoxycarbonyl)amino]benzyl *N*-Methylcarbamate (25). A mixture of **24** (415 mg, 1 mmol), MeNCO (0.1 mL, 2 mmol), and Et_3N (202 mg, 2 mmol) in DMF (5 mL) was stirred at room temperature for 8 h. Additional MeNCO (0.1 mL) was added and the mixture continuously stirred overnight. The mixture was evaporated *in vacuo* to dryness, and the residue was chromatographed on a silica gel column (2 × 30 cm) using $CHCl_3$/MeOH (v/v 100:1) as the eluant. The main yellow fraction was collected and concentrated *in vacuo* to dryness. The residue was triturated with EtOAc, and the solid product was collected by filtration, dried, and recrystallized from EtOH to yield **25**: 328 mg (69%); mp 183–185 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.45 (9H, s, *t*-Bu), 2.51 (3H, s, NH-Me), 4.91 (2H, s, CH_2), 6.49, 6.93, and 7.22 (each 1H, s, ArH), 7.07 (2H, brs, ArH), 7.63 (4H, brs, ArH), 7.97 (2H, brs, ArH), 9.37 and 11.46 (each 1H, NH). Anal. ($C_{27}H_{28}N_4O_4$·$^3/_2H_2O$) C, H, N.

3-(9-Acridinylamino)-5-aminobenzyl *N*-Methylcarbamate (26). To a mixture of **25** (142 mg, 0.3 mmol) and anisole (195 mg, 1.8 mmol) in CH_2Cl_2/CH_3NO_2 (2:1, 10 mL) was added 240 mg (1.8 mmol) of $AlCl_3$ at room temperature. The mixture was stirred for 5 min and then concentrated *in vacuo* to dryness. The residue was diluted with ice (10 g) and treated with 5 drops of concentrated HCl, and the resulting precipitates were collected by filtration. The filter cake was washed successively with ice–water and acetone and recrystallized from EtOH to give **26**: 105 mg (28%); mp 147–148 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, d, J = 3.63 Hz, NH-Me), 4.91 (2H, s, CH_2), 6.78 (1H, s, ArH), 6.82 (3H, brs, ArH and NH_2), 7.48 (2H, t, J = 7.60 Hz, ArH), 8.03 (2H, t, J = 7.65 Hz, ArH), 8.10 and 8.30 (each 2H, d, J = 8.50 Hz, ArH), 11.46 and 14.77 (each 1H, br, NH). Anal. ($C_{22}H_{20}N_4O_2$) C, H, N.

***N*-[γ-(*N*,*N*-dimethylamino)propionyl]-3-(9-acridinylamino)-5-(hydroxymethyl)aniline (27).** A mixture of 2,2′-dithiopyridine (242 mg, 1.1 mmol) and triphenylphosphine (288 mg, 1.1 mmol) in dry DMF (6 mL) was stirred at room temperature to a clear solution (1 min). To this solution was then added AHMA (**15**; 315 mg, 1 mmol), and the mixture was stirred at ambient temperature for 20 h. The yellowish precipitates were collected by filtration; the filter cake was washed successively with acetone, $CHCl_3$, and ether and recrystallized from 95% EtOH to yield **27**: 421 mg (81%); mp 263–264 °C; ^{1}H NMR (DMSO-d_6) δ 1.95 (2H, m, $COCH_2$), 2.46 (2H, t, J = 7.20 Hz, N-CH_2), 2.73 and 2.74 (each 3H, s, N-Me), 3.02–3.08 (2H, m, CH_2), 4.47 (2H, s, CH_2), 5.35 (1H, br, OH), 7.02, 7.59, and 7.69 (each 1H, s, ArH), 7.47 and 8.01 (each 2h, t, J = 7.53 Hz, ArH), 8.09 (2H, d, J = 8.08 Hz, ArH), 8.28 (2H, d, J = 8.67 Hz, ArH), 10.32, 10.39, and 11.52 (each 1H, br, NH and HCl). Anal. ($C_{26}H_{28}N_4O_2$·$^3/_2$HCl·$2H_2O$) C, H, N.

Biological Assay. Evaluation of Antitumor Activity in Cultured Cells. The effects of the compounds on cell growth were determined in HL-60 cells and in some cases also in S180 and L1210 cells in a 72 h incubation, by XTT-tetrazolium microculture assay, as described by Scudiero *et al.*[28] After incubation with phenazine methosulfate-XTT solution at 37 °C for 6 h, absorbance at 450 and 630 nm was detected with a microplate reader (EL 340; Bio-Tek Instruments Inc., Winooski, VT). Six to seven concentrations of each compound were used. The IC_{50} and dose–effect relationships of the compounds for antitumor activity were calculated by a median-effect plot,[29,30] using a computer program on an IBM-PC workstation.[31]

Inhibition of k-DNA Decatenation. k-DNA was isolated from *Crithidia fasciculata* by a published method.[32] The standard reaction mixture for k-DNA decatenation assay, containing 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 50 mM KCl, 0.5 mM DTT, 30 μg/mL BSA, 5 mM ATP, 1.5 μg k-DNA, and 5 μg of protein of nuclear extracts, in 50 μL was incubated at 37 °C for 30 min.[33] Reaction was terminated by the addition of 5 μL of 10% SDS and 1 mg/mL proteinase K, and the reaction mixture was further incubated at 37 °C for 30 min followed by electrophoresis on 1% agarose with TBE buffer. After staining with ethidium bromide (5 μg/mL), gels were photographed under UV illumination, using Polaroid type 55 film.

Determination of Topo II-Mediated DNA Cleavage. ^{32}P-3′-end-labeled PBR_{322}DNA was prepared according to the procedure described by Chen *et al.*[34] The pattern of Topo II-mediated cleavage of the labeled DNA was determined by incubating at 37 °C for 30 min in a mixture (20 μL) containing [^{32}P]DNA (0.2 μg), purified Topo II from calf thymus (0.2 μg), and reaction buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg/mL bovine serum albumin) in the presence and absence of the test compound. The reaction was quenched by addition of 2 μL of 10% SDS and 3 μL of 1.7 mg/mL of proteinase K. Following further incubation at 37 °C for 1 h, the sample alone with the loading buffer was applied onto 1% agarose gel in TBE buffer, pH 8.0, and 10% SDS and electrophoresized at 30 V for 18 h. The gel was dried, and the autoradiography was carried out at −70 °C as described previously.[34]

Antitumor Activity in Tumor-Bearing Mice. BDF_1 mice bearing E0771 mammary adenocarcinoma, Lewis lung carcinoma, or B-16 melanoma were treated intraperitoneously with 7.5–10 mg/kg AHMA daily for 5 days beginning day 3 after tumor inoculation (subcutaneous implant with 0.2 mL of brei). Body weight changes, mortality, and average tumor volume were recorded on days 7, 10, and 14. Day 14 results were reported. Drugs were dissolved in dimethyl sulfoxide (DMSO). The injections with DMSO alone served as untreated controls. Two Topo II inhibitors, etoposide (VP-16) and amsacrine (*m*-AMSA), were also dissolved in DMSO and served as treated controls for comparing the therapeutic efficacy.

Acknowledgment. This investigation was supported in parts by funds from the National Cancer Institute, National Institutes of Health (Grant Nos. PO1-CA 18856 and RO1-AI 32350 and Elsa U. Pardee foundation).

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JM940518B