Synthesis and Structure−Activity Relationships of Potential Anticancer Agents: Alkylcarbamates of 3-(9-Acridinylamino)-5-hydroxymethylaniline

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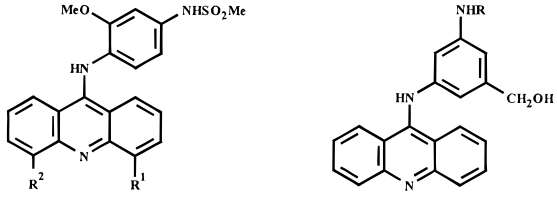
A series of potential 9-anilinoacridine antitumor agents, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA) derivatives with monosubstituent at C4′ and disubstituents at C4′ and C5′ of the acridine ring and their alkylcarbamates, were synthesized for evaluation of their antitumor activity. A structure−activity relationship (SAR) study showed that the AHMA-alkylcarbamates were more potent than their corresponding parent AHMA compounds. In addition, the cytotoxicity of the AHMA-alkylcarbamate decreased with increasing length and size of the alkyl function. Among these compounds, AHMA-ethylcarbamate (**18**) and 4′-methyl-5′-dimethylaminoethylcarboxamido-AHMA-ethylcarbamate (**34**) possess potent cytotoxicity on the inhibition of human leukemic HL-60 cell growth in culture. Further in vivo studies of these compounds displayed significant anticancer therapeutic effects in mice bearing sarcoma 180, Lewis lung carcinoma, and P388 leukemia.

Introduction

Members of the class of 9-anilinoacridine derivatives have been synthesized as potential topoisomerase II (Topo II)-mediated anticancer agents.[1−6] Structure−activity relationships (SAR) including the position of substituent(s), chemical and physical properties, lipophilicity−hydrophilicity balance, etc., have also been investigated by Atwell and Baguley et al.[1−6] The first 9-anilinoacridine derivative to achieve clinical use as an anticancer agent was amsacrine (*m*-AMSA, **1**; Chart 1) to treat leukemia and lymphoma.[7−9] However, *m*-AMSA lacks broad-spectrum clinical activity and has a low therapeutic index. In addition, this agent has been difficult to formulate because of its low aqueous solubility. Subsequently, the 4-methyl-5-methylcarboxamide-substituted *m*-AMSA (CI-921, **2**; Chart 1) was synthesized and was found to have superior antileukemic activity and a broad spectrum of antitumor activity.[10,11]

The chemical structures of *m*-AMSA and CI-921 indicate that both possess a methanesulfonyl function at the para-position of the 9-acridinylamino group and readily undergo reversible oxidation either chemically[12] or microsomally,[12,13] giving the reactive species quinonediimine (*m*-AQDI). The intermediate *m*-AQDI further forms glutathione conjugates and is excreted. The half-life of *m*-AMSA is about 30 min in the presence of fresh mouse blood at 37 °C.[14,15] Therefore, the antitumor potency of *m*-AMSA derivatives suggests a correlation with the derivatives' redox potential. However, a clear quantitative relationship between redox potential and antitumor activity could not be discerned since the isomer, *o*-AMSA, was found to be biologically inactive. To determine whether the oxidized *m*-AQDI is required for antitumor activity, we previously synthesized a series of 9-anilinoacridine analogues in which the amino was replaced by an O or S atom or compounds lacking the substituent at the para-position to the 9-amino function of acridine.[16−18] Among these compounds, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA, **3**; Chart 1) bearing an NH_2 and CH_2OH at the meta-position to the 9-amino function was found to have greater antitumor efficacy against murine leukemia and solid tumors than *m*-AMSA or VP-16 and was also less toxic to the host. We also found that AHMA possesses a longer plasma half-life (1.5 h) than *m*-AMSA.

Chart 1



1 $R^1 = R^2 = H$ *m*-AMSA
2 $R^1 = CONHMe$, $R^2 = Me$ CI-921
3 R = H AHMA
4 R = $COOMe_3$

Previously, we synthesized a series of AHMA derivatives without substituents on the acridine ring. Our SAR study was mainly focused on the modification of the substituent(s) on the amino and/or hydroxymethyl group(s).[16] However, AHMA without a substituent at 5-CH_2OH exhibited slightly better in vitro antitumor activity against human leukemic HL-60 and L1210 cell lines, while a substituent or substituents at NH_2 and/or CH_2OH, in general, did not greatly affect the cytotoxicity of AHMA. Therefore, we concluded that the antitumor activity of 9-anilinoacridine derivatives is mainly attributed to Topo II-mediated cleavage of double-stranded DNA, common to DNA-intercalating agents.[19,20] Formation of stable ternary complexes between drugs (such as AHMA), DNA, and Topo II is

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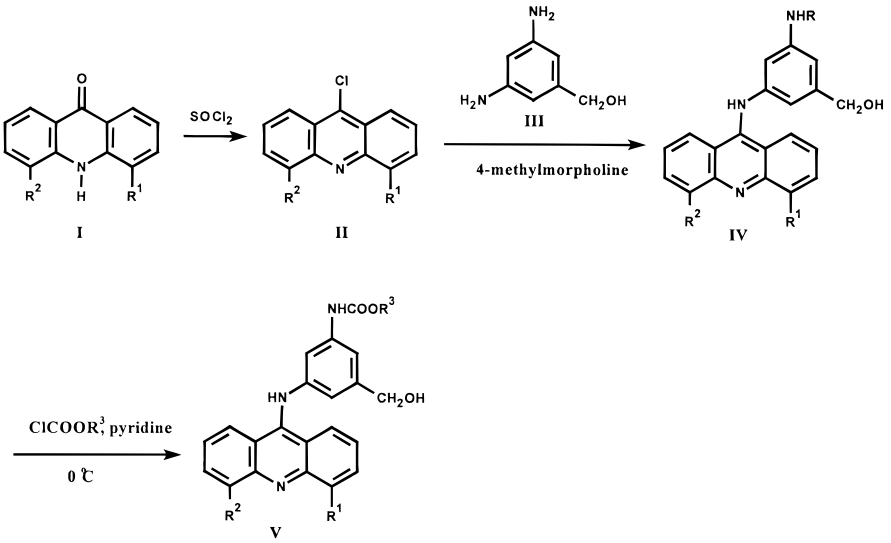
Scheme 1



essential for pharmacologically based antitumor activity.[19,20] Other factors such as water solubility, alternation of lipophilicity−hydrophilicity balance, and stereo effect for drug binding with DNA by substituent(s) at NH_2 and/or CH_2OH of AHMA may also affect AHMA's antitumor activity.

During the course of our earlier SAR studies of AHMA derivatives, we found that one of the intermediates, *tert*-butylcarbamate of AHMA (**4**; Chart 1), was more cytotoxic than the parent compound AHMA.[21] This finding prompted us to synthesize a series of alkylcarbamates of AHMA for evaluation of their antitumor activity.

A study of the DNA binding kinetics of 9-anilinoacridines suggested that, at equilibrium, amsacrine-4-caboxamides bind with the acridine chromophore in a position of (1) maximum overlap with the DNA base pairs; (2) the carboxamide side chain lying in the DNA minor groove; and (3) the bulky anilino chain lying in the major groove.[22,23] Both equilibrium and kinetic measurements show that amsacrine-4-carboxamide binds selectively to poly(dG-dC)·poly(dG-dC) compared to poly(dA-dT)·poly(dA-dT). These findings suggest that the highest affinity sites in the natural DNA sequence (calf thymus DNA) for the antitumor compound are GC-rich.[22−24] The substituent on the acridine ring may affect the drug binding in terms of their sequence-specific binding sites and binding constants. In the present study, a series of AHMA derivatives with mono- or disubstituent(s) at C4′ or/and C5′ of the acridine moiety and their alkylcarbamates were synthesized to observe the effect of these substituent(s) on the derivatives' cytotoxicity. Since our preliminary studies indicated that ethylcarbamate of AHMA was shown to be the most potent among the alkylcarbamates of AHMA in inhibiting HL-60 cell growth in cell culture, a series of ethylcarbamates of AHMA with substituent(s) on the acridine ring were also synthesized for SAR study. In this paper we describe the synthesis, SAR, and antitumor effects of the AHMA-alkylcarbamates in vitro and in vivo.

Chemistry

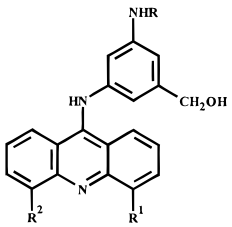
The derivatives of AHMA-alkylcarbamates were prepared in good yield by reacting AHMA[16] with various alkyl, phenyl, or benzyl chloroformates (Scheme 1) in pyridine. AHMA-alkylcarbamates with a monosubstituent at C4′ or with disubstituents at C4′ and C5′ of the acridine ring were prepared starting from the requisite known 9(10*H*)-acridones (**I**, R^1 = Me, OMe, F, Cl, Br, or NO_2, R^2 = H)[25,26] and 9-oxoacridan-4-carboxylic acids (R^1 = COOH, R^2 = H, Me, or OMe).[6,27−29] The monosubstituted 9(10*H*)-acridones were treated with thionyl chloride to yield the corresponding 9-chloroacridines (**II**). The disubstituted 9-oxoacridan-4-carboxylic acids were converted to 9-chloroacridan-4-carboxamides (**II**, where R^1 = CONHMe, R^2 = OMe or Me; R^1 = $CONH(CH_2)_2$-NMe_2, R^2 = OMe or Me) treating with thionyl chloride followed by addition of methylamine or *N*,*N*-dimethylethylenediamine.[4,6] Condensation of 9-chloroacridines (**II**) with 3,5-diaminobenzyl alcohol dihydrochloride (**III**) in a mixture of $CHCl_3$/MeOH in the presence of 4-methylmorpholine gave various AHMA derivatives (**IV**) by following our previous method.[16] In a similar manner, AHMA derivatives with substituent(s) (**III**) on the acridine ring were treated with ethyl chloroformate in pyridine to give the desired AHMA-alkylcarbamates (**V**).

Biological Results and Discussion

DNA Topo II has emerged as the chemotherapeutic target for a diverse group of antitumor agents.[30] It was suggested that the interaction of drug with DNA and DNA Topo II to produce a ternary complex is essential for the antitumor activity of drug. In a ternary drug−DNA−Topo II complex, the drug association site could reside a priori on either the enzyme or DNA exclusively, or could be at a DNA−enzyme interface in which the drug exhibits close contact with both components. However, the nature of drug−DNA−Topo II ternary complex is not fully clear. To design an effective anticancer drug, such as 9-anilinoacridine derivatives, one has to take account of the drug−DNA and drug−Topo II associations for consideration. In addition, other factors such as pK_a value and lipophilicity−hydrophilicity associated with drug distribution affect the drug's bioavailability.

To find AHMA derivatives with superior antitumor activity as compared to the parent compound, we

Table 1. In Vitro Cytotoxicity of Derivatives of AHMA and Their Ethylcarbamates on the Inhibition of Human Leukemic HL-60 Cell Growth



R^1	R^2	AHMA derivatives	inhibn of human leukemic HL-60 IC_{50} (μM)[a]	AHMA-ethylcarbamates	inhibn of human leukemic HL-60 IC_{50} (μM)
H	H	3	0.027	18	0.0044
OMe	H	5	0.011	23	0.010
Me	H	6	0.0076	24	0.0047
CONHMe	H	7	0.29	25	0.088
$CONH(CH_2)_2NMe_2$	H	8	0.13	26	0.0057
NO_2	H	9	14.0	27	3.8
F	H	10	0.15	28	0.031
Cl	H	11	0.37	29	0.067
Br	H	12	1.5	30	0.13
OMe	CONHMe	13	0.12	31	0.032
OMe	$CONH(CH_2)_2NMe_2$	14	0.038	32	0.0097
Me	CONHMe	15	0.093	33	0.011
Me	$CONH(CH_2)_2NMe_2$	16	0.0074	34	0.0023

[a] The IC_{50} values were determined by computer fit of 5−6 data points from a dose−response range.

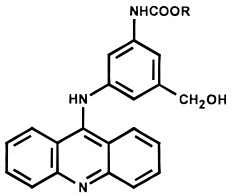
synthesized a series of AHMA derivatives with mono- and disubstituents on the acridine ring and carried out SAR studies. Table 1 shows the in vitro cytotoxicity of the parent compound AHMA (**3**) and its derivatives with mono- or disubstituent(s) on the acridine ring (**5**−**16**) against the growth of human leukemic HL-60 cell line in culture. In the series of AHMA derivatives with a monosubstituent at C4′ of the acridine ring, AHMA with a C4′-OMe or C4′-Me substituent (**5** and **6**) or with a chargeable side chain C4′-$CONH(CH_2)_2NMe_2$ (**8**) were more cytotoxic than AHMA by 2−4 times, while AHMA with C4′-CONHMe, C4′-NO_2, or C4′-halogen substituents (compounds **7**, **9**, and **10**−**12**, respectively) were less potent than the parent compound AHMA. Compound **9** bearing a strong electron-withdrawing nitro function decreased the cytotoxicity of AHMA dramatically. In the series of AHMA derivatives with disubstituents at C4′ and C5′, the compound was approximately 3 times more potent than AHMA. The cytotoxicity of compound **5** or **6** is decreased by introducing the CONHMe group to the C5′-position.

In contrast to *m*-AMSA derivatives,[6] introduction of the monosubstituent OMe or Me to the C4′-position enhanced the in vitro antitumor activity of AHMA, while introduction of disubstituents OMe or Me and CONHMe to C4′- and C5′-positions, respectively, decreased the activity. Unlike *N*-[2-(*N*,*N*-dimethylamino)-ethyl]-9-aminoacridine-4-carboxamide,[4] the in vitro antitumor activity of *N*-[2-(*N*,*N*-dimethylamino)ethyl]-AHMA-4-carboxamide (**8**) was not parallelyl increased by adding OMe or Me to the C5′-position, since compound **14** was about 3 times less cytotoxic than **5**, while compound **16** was 2 times more potent than the parent compound.

So far the most significant influence on the antitumor activity of these classes of drug was found to be the steric effects of groups placed at various positions on the 9-anilinoacridine skeleton.[31] Previous QSAR studies showed that monosubstituted derivatives of *m*-AMSA with an electron-withdrawing group (such as halogen and NO_2) having smaller pK_a values and similar or smaller DNA-binding constants were found to be less active against leukemia L1210 in cell culture.[26] Introduction of Me or OMe group to the C4′-position of *m*-AMSA provides a considerable increase in binding to poly(dA-dT) over that of the parent compound, but the carbamoyl or methylcarbamoyl group at the same position gives no such increase as they bind selectively to poly(dG-dC). The effects on the inhibition of L1210 cell growth in culture showed that 4′-Me- or 4′-OMe-*m*-AMSA was almost as potent as or a little more potent than the parent *m*-AMSA, while the in vivo antitumor activity against mice bearing leukemic P388 revealed enhanced activity. In the same studies, the carbamoyl derivative showed a large decrease in its in vitro antitumor activity but showed an increase in the case of in vivo studies. The disubstituted 5′-Me- or 5′-OMe-4′-CONHMe-*m*-AMSA showed enhancement of tightness of drug−DNA binding as shown by increase of the binding constant. The in vitro activity of these two compounds is as potent as or more potent than that of *m*-AMSA, while their in vivo activities are higher. Among these compounds, CI-921 (**2**) is the most active.

Earlier QSAR studies suggested a significant correlation between antitumor activity of 9-anilinoacridines and 9-aminoacridine and DNA association constants. The drug−DNA binding constant is affected by the substituent(s) at the acridine chromorphore. However, our SAR studies indicate that the effects of the substituent(s) at the acridine ring on the in vitro cytotoxicity of AHMA are somewhat different from previous observations of the QSAR studies of *m*-AMSA[31] and 9-aminoacridine derivatives[4] as exemplified by the increase of cytotoxicity of **5**, **6**, and **8**. The difference may attribute to the different tumor cells tested and the different strength of binding with Topo II. We previously showed

Table 2. In Vitro Cytotoxicity of AHMA-alkylcarbamates on the Inhibition of Human Leukemic HL-60 Cell Growth



	AHMA (**3**)	**17**	**18**	**19**	**20**	**4**	**21**	**22**
R		Me	Et	*n*-Pro	*i*-Bu	*t*-Bu	C_6H_5	$CH_2C_6H_5$
inhibn of HL-60 IC_{50} (μM)	0.027	0.0085	0.0044	0.0080	0.0090	0.024	0.161	0.029

Table 3. Anticancer Activity of AHMA-ethylcarbamate (**18**) in $B6D2F_1$ Mice Bearing Sarcoma 180 and Lewis Lung Carcinoma[a]

		average wt change (g)			average tumor vol (treated control)		
tumor cell	dose (mg/kg)	day 7	day 10	day 14	day 7	day 10	day 14
Sarcoma 180	control	−0.5	+0.7	+1.0	1.00	1.00	1.00
	3.0	−2.0	−0.7	−0.8	1.24	1.21	0.74
	5.0	−2.6	+1.0	+1.2	0.06	0.63	0.80
	7.0	−3.4	−0.1	−0.4	0.22	<0.01	<0.01
Lewis lung carcinoma	control	−1.0	−0.1	+0.5	1.00	1.00	1.00
	3.0	−2.3	−1.3	−0.8	0.80	0.63	0.68
	6.0	−3.0	−2.6	−0.5	0.44	0.37	0.52

[a] Sarcoma 180 (3×10^6 cells) was inoculated sc on day 0. Treatment started on day 1, ip, QDx5. Control had 4 mice, and each dose had 2 mice. Lewis lung carcinoma (4×10^6 cells) was inoculated sc on day −2. Treatment started on day 1, ip, QDx5. Control had 4 mice, and each dose had 3 mice. Tumor sizes were evaluated on days 7, 10, and 14.

that AHMA exhibited superior in vitro and in vivo antitumor activity as compared to *m*-AMSA. Aside from the drug−DNA binding, one can envisage that the 3-amino-5-hydroxymethylaniline moiety of AHMA may engage in a critical hydrogen bond interaction in the drug−Topo II binding site which may effect better binding than that of *m*-AMSA. Our molecular modeling study of AHMA−DNA binding also reveals that this amino functionality, which lies outside the drug−DNA complex, may be in favor of forming a hydrogen bond with Topo II at the active binding site. It was shown[30] that DNA intercalators (9-aminoacridine phenols and amsacridine derivatives) possess good activity for Topo II-mediated DNA strand cleavage, while the activity was decreased or diminished after methoxylation at the *ortho*-position of the phenol or methanesulfonyl functions. It can be seen that *o*-AMSA is biologically inactive, suggesting the methoxy group may abolish or inhibit the drug−enzyme interaction probably due to its electron-donating properties or steric encumbrance. Therefore, the antitumor activity of 9-anilinoacridine derivatives is greatly altered by the substituent(s) on the anilino ring.

Taking the consideration of the drug−enzyme binding ability as an important account for drug activity, our previous SAR studies of AHMA derivatives demonstrated that AHMA lacking a substituent at 5-CH_2OH exhibited slightly better in vitro antitumor activity against human leukemic HL-60 and L1210 cell lines in culture, while the substituent(s) at NH_2 and/or CH_2OH, in general, had no significant effect on the cytotoxicity of AHMA. We also showed that AHMA bearing the β-acetylpropyl side chain at NH_2 only or at both NH_2 and CH_2OH revealed to be as potent as or slightly more potent than the parent compound AHMA.[16] In the present studies, we found that AHMA-alkylcarbamates in general are more potent than their corresponding parent AHMA derivatives against human leukemic HL-60 cell growth in culture.

Table 2 shows the in vitro cytotoxicity of AHMA with various alkylcarbamates that results from altering the alkylcarbamate chain length and size. This clearly demonstrates that the cytotoxicity of AHMA-alkylcarbamate decreases with increasing length and size of the alkyl group. Since AHMA-ethylcarbamate was shown to be the most potent among AHMA-alkylcarbamate derivatives, AHMA derivatives with substituent(s) at acridine ring were converted into their ethylcarbamate derivatives for SAR studies (Table 1). It is also important to note that all AHMA-ethylcarbamates (**18** and **23**−**34**) were more potent than their corresponding AHMA derivatives (**5**−**16**); among these compounds, derivative **34** was the most potent with an IC_{50} value of 0.0023 μM (10 times more potent than AHMA). Our present results suggest that introduction of an alkylformate function to the NH_2 group of AHMA forming AHMA-alkylcarbamates may alter the lipophilicity−hydrophilicity balance as well as the interaction of the compound with the active site of Topo II enzyme, thereby possibly affecting the antitumor activity of 9-anilinoacridine derivatives.

Results from the present study involving the search for a leading compound in the AHMA analogues series, such as AHMA-ethylcarbamate (**18**) and its derivative 4′-methyl-5′-*N*-(dimethylamino)carboxamido-AHMA-ethylcarbamate (**34**), formed a basis for further in vivo antitumor evaluation. The antitumor efficacies of AHMA-ethylcarbamate (**18**) in $B6D2F_1$ mice bearing sarcoma and Lewis lung carcinoma are shown in Table 3. Tumor sizes were reduced >99% and 63% by AHMA-ethylcarbamate at 7 and 6 mg/kg, respectively, by intravenous injection (iv), QDx5. The antileukemic effects of AHMA (**3**) and its derivatives, AHMA-ethylcarbamate (**18**) and AHMA-*tert*-butylcarbamate (**4**), along with *m*-AMSA (**1**) and adriamycin are compared and shown in Table 4. Percent increase in lifespan (%ILS) was measured in $B6D2F_1$ mice bearing P388 leukemia. Among these compounds, adriamycin showed better potency. How-

Table 4. Therapeutic Effects of AHMA Hydrochloride, AHMA-ethylcarbamate (**18**), and AHMA-*tert*-butylcarbamate (**4**) in B6D2F$_1$ Mice Bearing P388 Leukemia[a]

drug	dose (mg/kg) & schedule, iv	average wt change (g) day 1	day 7	day 9	day 11	average survival time[b] (day ± SD)	increase in lifespan (%)	toxicity death
control	0	21.8					0	0/5
3	16 (Q2Dx5)	23.3	+0.8	−0.5	−2.1	14.9 ± 1.1	133	0/5
	22 (Q2Dx5)	23.8	−1.3	−1.5	−2.8	21.1 ± 2.2	230	0/5
	30 (Q2Dx5)	22.6	+0.2	−0.1	−0.2	21.8 ± 4.0	240	0/4
18	10 (Q2Dx5)	22.9	−0.2	−0.4	−0.1	18.4 ± 1.2	188	0/5
	15 (Q2Dx5)	23.0	−1.6	−2.0	−3.7	13.2 ± 4.2	106	4/5
4	12 (Q2Dx5)	23.1	−0.10	−0.7	+0.3	18.0 ± 1.3	181	0/5
	18 (Q2Dx5)	23.9	−1.3	−1.2	−1.6	22.1 ± 2.0	245	0/5
	24 (Q2Dx5)	22.3	−1.4	−1.4	−1.8	21.0 ± 4.0	228	2/4
m-AMSA	8 (Q2Dx5)	22.8	+0.1	−2.1	−3.8	12.2 ± 0.5	91	0/5
	12 (Q2Dx5)	22.7	−0.7	−1.4	−3.2	14.1 ± 1.6	120	1/5
	18 (Q2Dx5)	24.5	−4.3	- -	- -	7.6 ± 0.8	19	5/5
adriamycin	3 (Q2Dx5)	23.0	−0.4	−03.2	−3.6	14.2 ± 1.1	122	0/5
	4 (Q2Dx5)	23.6	−1.4	−1.6	−3.2	27.4 ± 6.7	328[c]	0/5

[a] P388/0 ascites cell (0.2 mL) (ascites:saline = 1.10) was implanted iv on day 0. Tumor grew in liver in mice; Q2D, iv treatment was given on days 1, 3, 5, 7, and 9. [b] Survival time was recorded based on following death times: 8 a.m. (0.0), 1 p.m. (0.25), 5 p.m. (0.5). [c] 1 of 5 mice died of tumor on day 40.

ever, 1 of 5 mice died of tumor on day 40 at 4 mg/kg, Q2Dx5. Of AHMA derivatives, AHMA-*tert*-butylcarbamate (**4**) is as potent as AHMA-ethylcarbamate (**18**) but has less toxicity to the host. Although compound **18** revealed better antitumor efficacy than AHMA at 10 mg/kg, Q2Dx5, **18** is more toxic than AHMA in the testing system. From this study, it demonstrats that AHMA derivatives are more potent than *m*-AMSA and yet have less toxicity to the host.

The in vivo therapeutic studies presented in this paper are preliminary results. More elaborate experiments such as the antitumor efficacy of AHMA-alkylcarbamate against human xenografts in nude mice will be carried out. Actually, some compounds are shown to have promising antitumor effects in this testing system. The pK_a values and DNA-binding measurements as well as the computational modeling calculation of AHMA and AHMA-alkylcarbamate derivatives are in progress at our laboratory. We hope these studies will lead to a clear QSAR for new anticancer drug design.

Experimental Section

Melting points were determined on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel G60 (70−230 mesh, ASTM; Merck). Thin-layer chromatography was performed on silica gel G60 F_{254} (Merck) with short-wavelength UV light for visualization. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. ^{1}H NMR spectra were recorded on a Brucker-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.

The synthesis of the requisite C4-monosubstituted 9(10*H*)-acridones (**I**, R^1 = 4-OMe, 4-Me, 4-NO_2, 4-F, 4-Cl, and 4-NO_2)[25,26] and 9-oxoacridan-4-carboxylic acids (**I** , R^1 = COOH)[27] and the C5-substituted 9-oxoacridan-4-carboxylic acids (**I**, R^1 = COOH, R^2 = OMe or Me)[27−29] were prepared by following the literature procedures. Conversion of the C4-monosubstituted acridones to their 9-chloroacridines (**II**) or the C5-substituted 9-oxoacridan-4-carboxylic acids to their methylcarbamoyl or *N*,*N*-dimethylethylenecarbamoyl derivatives was performed by the known methods.[4,6] All 9-chloroacridines were then used directly to condense with 3,5-diaminobenzyl alcohol (**III**) without further purification.

Synthesis of AHMA Derivatives (IV) in Table 1. 4′-Methoxy-AHMA Hydrochloride (5). 4-Methoxymorpholine (4.29 mL, 39 mmol) was added dropwise into a suspension of **III** (2.74 g, 13 mmol) in $CHCl_3$/EtOH (1:3 v/v) in an ice bath. After stirring for 1 h, the crude 9-chloro-4-methoxyacridine (prepared from 4-methoxy-9(10*H*)-acridone,[26] 2.83 g, 13 mmol) in $CHCl_3$ (15 mL) was added dropwise into the mixture at 0 °C and stirred vigorously for 4 h. The crude solid product was collected by filtration, washed with EtOH, and then recrystallized from MeOH to give **5**: 2.02 g (70%); mp 247−8 °C; ^{1}H NMR (DMSO-d_6) δ 4.16 (1H, s, OMe), 4.38 (2H, s, CH_2), 5.20 (1H, br, OH), 5.82 (2H, br, NH_2), 6.46, 6.51 and 6.64 (each 1H, s, 3 × ArH), 7.41 (1H, t, J = 8.7 Hz, ArH), 7.46 (1H, t, J = 8.7 Hz, ArH), 7.54 (1H, d, J = 8.0 Hz, ArH), 7.88 (1H, d, J = 8.7 Hz, ArH), 7.96 (1H, t, J = 8.0 Hz, ArH), 8.27 and 8.37 (each 1H, d, J = 8.6 Hz, 2 × ArH), 11.33 (1H, brs, NH). Anal. ($C_{21}H_{19}N_3O_2$·2HCl) C, H, N.

Using the same procedure as that for the synthesis of **5**, the following AHMA derivatives were prepared.

4′-Methyl-AHMA hydrochloride (6) was synthesized from 4-methyl-9(10*H*)-acridinone (1.20 g, 5.70 mmol)[26] and **III** (1.21 g, 5.70 mmol): yield 1.67 g (88%); mp 264−5 °C; ^{1}H NMR (DMSO-d_6) δ 2.78 (1H, s, Me), 4.37 (2H, s, CH_2), 5.18 (1H, br, OH), 5.50 (2H, br, NH_2), 6.39, 6.46 and 6.59 (each 1H, s, 3 × ArH), 7.37 (1H, t, J = 8.0 Hz, ArH), 7.43 (1H, t, J = 8.0 Hz, ArH), 7.86 (1H, d, J = 8.0 Hz, ArH), 7.96 (1H, t, J = 8.0 Hz, ArH), 8.23 and 8.26 (each 1H, d, J = 8.8 Hz, 2 × ArH), 8.41 (1H, d, J = 8.0 Hz, ArH), 11.33 (1H, brs, NH). Anal. ($C_{21}H_{19}N_3O$·HCl·H_2O) C, H, N.

4′-Methylcarboxamido-AHMA (7) was synthesized from crude 9-chloroacridan-4-methylcarboxamide (3.64 g, 13 mmol)[6] and **III** (2.744 g, 13 mmol): yield 2.86 g (59%); mp 275−6 °C; ^{1}H NMR (DMSO-d_6) δ 2.96 (3H, s, NHMe), 4.38 (2H, s, CH_2), 5.21 (1H, br, OH), 5.80 (2H, br, NH_2), 6.46, 6.52 and 6.64 (each 1H, s, 3 × ArH), 6.19 (2H, s, 2 × ArH), 7.44 (1H, m, ArH), 7.51 (1H, m, ArH), 7.96 (1H, m, ArH), 8.11 (1H, m, ArH), 8.26 (1H, m, ArH), 8.49 (2H, br, ArH), 9.34 and 11.63 (each 1H, br, 2 × NH). Anal. ($C_{22}H_{20}N_4O_2$·HCl·$^1/_2H_2O$) C, H, N.

4′-Dimethylaminoethylcarboxamido-AHMA (8) was synthesized from crude 9-chloroacridan-4-dimethylaminoethylcarboxamide (2.05 g, 6.57 mmol)[4] and **III** (1.39 g, 6.57 mmol): yield 1.83 g (65%); mp 82−3 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (6H, s, NMe_2), 3.47 and 3.62 (each 2H, brs, 2 × CH_2) 4.29 (2H, s, CH_2), 5.21 (1H, br, OH), 5.52 (2H, br, NH_2), 5.90 (1H, s, ArH), 6.19 (2H, s, 2 × ArH), 7.45 (1H, t, J = 8.0 Hz, ArH), 7.51 (1H, t, J = 8.0 Hz, ArH), 7.87 (1H, d, J = 8.0 Hz, ArH), 8.14 (1H, d, J = 8.8 Hz, ArH), 8.20 (1H, d, J = 8.8 Hz, ArH), 8.37 (1H, d, J = 8.8 Hz, ArH), 8.66 (1H, d, J = 8.0 Hz, ArH), 9.23 (1H, br, NH), 12.23 (1H, brs, NH). Anal. ($C_{25}H_{27}N_5O_2$·HCl·H_2O) C, H, N.

4′-Nitro-AHMA hydrochloride (9) was synthesized from 4-nitro-9(10*H*)-acridinone[26] (4.8 g, 20.0 mmol) and **III** (4.22 g,

20.0). The TLC (SiO_2, $CHCl_3$/MeOH, 10:1 v/v) of the crude products (5.63 g, 81%) showed a mixture of two main compounds (R_f = 0.6 and 0.3), which were not separated due to their poor solubility in organic solvent. The mixture was directly used for the next reaction without further purification. A small amount of the compound with low R_f value was isolated by column chromatography (SiO_2, $CHCl_3$/MeOH, 10:1 v/v) and then treated with 1 N HCl to form the hydrochloride salt and recrystallized from EtOH: mp 219−20 °C; ^{1}H NMR (DMSO-d_6) δ 4.50 (2H, s, CH_2), 5.21 (1H, br, OH), 6.09 (2H, br, NH_2), 7.36 (1H, s, ArH), 7.39 (2H, s, 2 × ArH), 7.50 (1H, t, J = 8.0 Hz, ArH), 7.58 (1H, t, J = 8.0 Hz, ArH), 8.02 (1H, d, J = 8.0 Hz, ArH), 8.25 (1H, d, J = 8.0 Hz, ArH), 8.37 (1H, d, J = 8.8 Hz, ArH), 8.81 (1H, d, J = 8.8 Hz, ArH), 8.86 (1H, d, J = 8.0 Hz, ArH), 9.36 (1H, br, NH). Anal. ($C_{20}H_{16}N_4O_3$·HCl·H_2O) C, H, N.

In a similar manner, compounds **10**−**12** were synthesized and purified as described for **9**.

4′-Fluoro-AHMA (10) was synthesized from 4-fluoro-9(10*H*)-acridinone (2.13 g, 10.0 mmol)[26] and **III** (2.11 g, 10.0 mmol). The crude product yielded 2.40 g, 76%. A small amount of crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH, 20:1 v/v) to give pure **10** for analysis: mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 4.31 (2H, d, J = 4.2 Hz, CH_2), 5.01 (1H, t, J = 4.2 Hz, OH), 5.98, 6.14, 6.27 (each 1H, s, 3 × ArH), 7.12 (2H, m, 2 × ArH), 7.24 (2H, m, 2 × ArH), 7.37 (2H, brs, NH_2), 7.56, 7.71, 7.94 and 8.10 (each 1H, m, 4 × ArH). Anal. ($C_{20}H_{16}FN_3O$·H_2O) C, H, N.

4′-Chloro-AHMA hydrochloride (11) was synthesized from 4-chloro-9(10*H*)-acridinone (2.17 g, 10.0 mmol)[26] and **III** (2.11 g, 10.0 mmol). The crude product yielded 2.42 g 72%. **11**−HCl salt: mp 232−4 °C; ^{1}H NMR (DMSO-d_6) δ 4.49 (2H, s, CH_2), 5.21 (1H, br, OH), 6.62 (2H, br, NH_2), 7.48 (3H, s, 3 × ArH), 7.51 (1H, t, J = 8.0 Hz, ArH), 7.55 (1H, t, J = 8.0 Hz, ArH), 8.03 (1H, d, J = 8.0 Hz, ArH), 8.21 (1H, d, J = 8.0 Hz, ArH), 8.47 (1H, d, J = 8.8 Hz, ArH), 8.55 (1H, d, J = 8.8 Hz, ArH), 8.60 (1H, d, J = 8.0 Hz, ArH), 9.16 (1H, br, NH). Anal. ($C_{20}H_{16}ClN_3O$·HCl·H_2O) C, H, N.

4′-Bromo-AHMA hydrochloride (12) was synthesized from 4-bromo-9(10H)-acridinone (2.74 g, 10.0 mmol)[26] and **III** (2.11 g, 10.0 mmol). The crude product yielded 2.97 g 78%. **12**−HCl salt: mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 4.46 (2H, s, CH_2), 5.18 (1H, br, OH), 6.62 (2H, br, NH_2), 7.10 (1H, s, ArH), 7.22 (2H, s, 2 × ArH), 7.39 (1H, t, J = 8.0 Hz, ArH), 7.50 (1H, t, J = 8.0 Hz, ArH), 7.96 (1H, d, J = 8.0 Hz, ArH), 8.30 (1H, d, J = 8.0 Hz, ArH), 8.32 (1H, d, J = 8.8 Hz, ArH), 8.47−8.50 (2H, m, 2 × ArH), 8.98 (1H, br, NH). Anal. ($C_{20}H_{16}BrN_3O$·HCl·$2H_2O$) C, H, N.

4′-Methoxy-5′-methylcarboxamido-AHMA hydrochloride (13) was synthesized from crude 4-methoxy-9-chloroacridan-5-methylcarboxamide (5.55 g, 18.5 mmol)[6] and **III** (3.91 g, 18.5 mmol): yield 4.40 g (60%); mp 222−3 °C; ^{1}H NMR (DMSO-d_6) δ 2.82 (3H, s, NHMe), 4.13 (3H, s, OMe), 4.38 (2H, s, CH_2), 5.20 (1H, br, OH), 5.80 (2H, br, NH_2), 6.45, 6.49 and 6.63 (each 1H, s, 3 × ArH), 7.36 (1H, t, J = 8.0 Hz, ArH), 7.51 (2H, m, 2 × ArH), 7.80 (1H, d, J = 8.0 Hz, ArH), 8.51 (1H, d, J = 8.0 Hz, ArH), 8.56 (1H, d, J = 8.0 Hz, ArH), 9.43 (1H, br, NH). Anal. ($C_{23}H_{22}N_4O_3$·HCl·$^1/_2H_2O$) C, H, N.

4′-Methoxy-5′-dimethylaminoethylcarboxamido-AHMA (14) was synthesized from crude 4-methoxy-9-chloroacridan-5-dimethylaminoethylcarboxamide (1.21 g, 3.71 mmol)[4] and **III** (0.783 g, 3.71 mmol): yield 0.904 g (52%); mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 2.87 (6H, s, NMe_2), 3.41 and 3.82 (each 2H, brs, 2 × CH_2), 4.15 (3H, s, OMe), 4.46 (2H, s, CH_2), 5.20 (1H, br, OH), 7.13 (1H, s, ArH), 7.19 (2H, s, 2 × ArH), 7.43 (1H, t, J = 8.0 Hz, ArH), 7.60 (2H, m, 2 × ArH), 7.83 (1H, d, J = 8.0 Hz, ArH), 8.60 (1H, d, J = 8.0 Hz, ArH), 8.94 (1H, d, J = 8.0 Hz, ArH), 10.00 (1H, br, NH), 10.08 (1H, brs, NH), 12.09 (1H, br, NH). Anal. ($C_{26}H_{29}N_5O_3$·2HCl·$10^1/_2H_2O$) C, H, N.

4′-Methyl-5′-methylcarboxamido-AHMA hydrochloride (15) was synthesized from crude 4-methyl-9-chloroacridan-5-methylcarboxamide (3.58 g, 13 mmol)[6] and **III** (2.74 g, 13 mmol), 4.80 g (95%), mp 275−7 °C; ^{1}H NMR (DMSO-d_6): δ 2.83 (3H, s, Me), 3.11 (3H, s, NHMe), 4.54 (2H, s, CH_2), 5.50 (1H, br, OH), 6.65 (2H, br, NH_2), 6.64, 6.67 and 6.82 (each 1H, s, 3 × ArH), 7.52 (1H, t, J = 8.0 Hz, ArH), 7.67 (1H, t, J = 8.0 Hz, ArH), 8.02, 8.26 and 8.67 (each 1H, d, J = 8.0 Hz, ArH), 9.78 and 11.92 (each 1H, br, NH). Anal. ($C_{23}H_{22}N_4O_2$·HCl·$2^3/_4H_2O$) C, H, N.

5′-Methyl-4′-dimethylaminoethylcarboxamido-AHMA (16) was synthesized from crude 5′-methyl-9-chloroacridan-5-dimethylaminoethylcarboxamide (1.80 g, 6.0 mmol)[4] and **III** (1.26 g, 6.0 mmol): yield 0.847 g (55%); mp 210−3 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.78 (3H, s, Me), 2.87 (6H, s, NMe_2), 3.42 and 3.84 (each 2H, brs, 2 × CH_2), 4.46 (2H, s, CH_2), 5.20 (1H, br, OH), 7.35, 7.37 and 7.40 (each 1H, s, 3 × ArH), 7.62 (1H, t, J = 8.0 Hz, ArH), 7.98 (1H, d, J = 8.0 Hz, ArH), 8.19 (1H, d, J = 8.0 Hz, ArH), 8.65 (1H, d, J = 8.0 Hz, ArH), 8.95 (1H, d, J = 8.0 Hz, ArH), 10.10 (1H, br, NH), 10.08 (1H, brs, NH), 10.80 (1H, br, NH). Anal. ($C_{26}H_{29}N_4O_2$·HCl) C, H, N.

Synthesis of AHMA-alkylcarbamate Derivatives (V) in Tables 1 and 2. AHMA-methylcarbamate (17). Methyl chloroformate (0.58 g, 5.4 mmol) was added dropwise to a solution of **3** (1.87 g, 4.50 mmol) in dry DMF (50 mL) containing pyridine (1.42 mL, 17.6 mmol) at − 20 °C. The mixture was then stirred at room temperature for 15 min and evaporated in vacuo to dryness. The residue was coevaporated several times with EtOH and was chromatographed on a silica gel column (5 × 50 cm). The impurities were eluted with $CHCl_3$, while the product was eluted by $CHCl_3$/MeOH (10:1 v/v). The fractions containing the product were collected, concentrated, and recrystallized twice from DMF/EtOH to give **17**: 1.08 g (64%); mp 273−4 °C; ^{1}H NMR (DMSO-d_6) δ 3.64 (3H, s, Me), 4.45 (2H, s, CH_2), 5.32 (1H, br, OH), 7.44 (2H, s, 2 × ArH), 7.45 (2H, d, J = 7.3 Hz, Ar), 7.49 (2H, d, J = 5.6 Hz, 2 × ArH), 8.00 (2H, t, J = 7.8 Hz, 2 × ArH), 8.09 (2H, d, J = 8.4 Hz, 2 × ArH), 8.26 (2H, d, J = 8.8 Hz, 2 × ArH), 9.91 and 11.49 (each 1H, brs, 2 × NH). Anal. ($C_{22}H_{19}N_3O_3$·HCl·H_2O) C, H, N.

Following the same procedure as that for the synthesis of **17**, the following AHMA-alkylcarbamates were prepared.

AHMA-ethylcarbamate (18) was synthesized from **3** (6.30 g, 20 mmol) with ethyl chlorofomate (3.24 g, 30.0 mmol): yield 6.32 g (82%); mp 226−8 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.06 (3H, t, J = 7.0 Hz, Me), 4.10 (2H, q, J = 7.0 Hz, CH_2), 4.45 (2H, s, CH_2), 5.35 (1H, br, OH), 6.97 (1H, s, ArH), 7.46 (2H, s, 2 × ArH), 7.48−7.51 (2H, m, 2 × ArH), 8.01, 8.03, 8.05 and 8.07 (each 1H, d, J = 8.0 Hz, 4 × ArH), 8.26 (1H, d, J = 8.8 Hz, ArH), 9.91 (1H, s, NH), 11.47 (1H, brs, NH). Anal. ($C_{23}H_{21}N_3O_3$·HCl·$1^1/_2H_2O$) C, H, N.

AHMA-propylcarbamate (19) was synthesized from **3** (0.630 g, 2.0 mmol) with propyl chlorofomate (0.30 g, 2.4 mmol): yield 0.62 g (78%); mp 208−9 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, t, J = 7.1 Hz, Me), 1.59−1.62 (2H, m, CH_2), 4.02 (2H, t, J = 6.4 Hz, CH_2), 4.46 (2H, s, CH_2), 5.25 (1H, br, OH), 6.93 (1H, s, ArH), 7.46−7.52 (4H, m, 4 × ArH), 7.99−8.03 (2H, m, 2 × ArH), 8.11−8.13(2H, m, 2 × ArH), 8.28 (1H, d, J = 8.8 Hz, ArH), 9.89 (1H, s, NH), 11.82 (1H, brs, NH). Anal. ($C_{24}H_{23}N_3O_3$·HCl·H_2O) C, H, N.

AHMA-isobutylcarbamate hydrochloride (20) was synthesized from **3** (0.63 g, 2.0 mmol) with isobutyl chlorofomate (0.41 g, 3.0 mmol): yield 0.729 g (88%); mp 255−6 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (6H, d, J = 7.0 Hz, 2 × Me), 1.84−1.91 (2H, m, CH), 3.84 (2H, d, J = 6.4 Hz, CH_2), 4.45 (2H, s, CH_2), 5.35 (1H, br, OH), 6.98 (1H, s, ArH), 7.44−7.50 (4H, m, 4 × ArH), 8.00 (2H, t, J = 8.8 Hz, 2 × ArH), 8.14 (2H, d, J = 8.8 Hz, 2 × ArH), 8.28 (2H, d, J = 8.8 Hz, 2 × ArH), 9.89 (1H, s, NH), 11.82 (1H, brs, NH). Anal. ($C_{25}H_{25}N_3O_3$·HCl·$1^1/_2H_2O$) C, H, N.

AHMA-phenylcarbamate hydrochloride (21) was synthesized from **3** (0.63 g, 2.0 mmol) with phenyl chlorofomate (0.468 g, 3.0 mmol): yield 0.787 g (90%); mp 228−9 °C; ^{1}H NMR (DMSO-d_6) δ 4.48 (2H, s, CH_2), 5.40 (1H, br, OH), 7.02 (1H, s, ArH), 7.22 (2H, s, 2 × ArH), 7.42−7.52 (6H, m, 6 × ArH), 8.00 (2H, m, 2 × ArH), 8.07 (2H, m, 2 × ArH), 8.29 (2H, m, 2 × ArH), 10.49 (1H, brs, NH), 11.55 (1H, br, NH). Anal. ($C_{27}H_{21}N_3O_3$·HCl·$1^1/_2H_2O$) C, H, N.

AHMA-benzylcarbamate hydrochloride (22) was synthesized from **3** (0.65 g, 2.0 mmol) with benzyl chlorofomate (0.51 g, 3.0 mmol): yield 0.75 g (78%); mp 214−5 °C; ^{1}H NMR (DMSO-d_6) δ 4.50 (2H, s, CH_2), 5.51 (2H, s, CH_2), 5.31 (1H, br, OH), 7.02 (1H, s, ArH), 7.37−7.77 (4H, m, 4 × ArH), 8.03 (2H, t, J = 8.0 Hz, 2 × ArH), 8.21 (2H, d, J = 8.0 Hz, 2 × ArH), 8.33 (2H, d, J = 8.8 Hz, ArH), 10.11 (1H, s, NH), 11.64 (1H, brs, NH). Anal. ($C_{28}H_{23}N_3O_3$·HCl·1$^1/_2$$H_2O$) C, H, N.

4′-Methoxy-AHMA-ethylcarbamate (23) was synthesized from **5** (0.345 g, 1.0 mmol) and ethyl chloroformate (0.130 g, 1.2 mmol): yield 195 mg (47%); mp 210−2 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 6.7 Hz, Me), 4.10 (2H, q, J = 6.7 Hz, CH_2), 4.16 (1H, s, OMe), 4.44 (2H, s, CH_2), 5.28 (1H, br, OH), 6.93 (1H, s, ArH), 7.39−7.53 (4H, m, 4 × ArH), 7.54 (1H, d, J = 8.0 Hz, ArH), 7.82 (1H, d, J = 8.7 Hz, ArH), 7.92 (1H, t, J = 8.0 Hz, ArH), 8.25 and 8.36 (each 1H, d, J = 8.6 Hz, 2 × ArH), 9.87 and 12.10 (each 1H, brs, NH). Anal. ($C_{24}H_{23}N_3O_4$·HCl·H_2O) C, H, N.

4′-Methyl-AHMA-ethylcarbamate (24) was synthesized from **6** (0.584 g, 1.5 mmol) and ethyl chloroformate (0.195 g, 1.8 mmol): yield 0.506 g (73%); mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 6.8 Hz, Me), 2.81 (3H, s, Me), 4.09 (2H, q, J = 6.8 Hz, CH_2), 4.43 4.44 (2H, s, CH_2), 6.92 (1H, s, ArH), 7.38−7.49 (4H, m, 4 × ArH), 7.87 (1H, d, J = 6.8 Hz, ArH), 8.01 (1H, t, J = 6.8 Hz, ArH), 8.19−8.23 (2H, m, 2 × ArH), 8.46 (1H, d, J = 8.4 Hz, ArH), 9.88 (1H, brs, NH), 11.48 (1H, br, NH). Anal. ($C_{24}H_{23}N_3O_3$·HCl·1$^1/_2$$H_2O$) C, H, N.

4′-Methylcarboxamido-AHMA-ethylcarbamate (25) was synthesized from **7** (0.63 g, 2.0 mmol) and ethyl chloroformate (0.324 g, 3.0 mmol): yield 0.506 g (73%); mp 232−3 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 6.4 Hz, Me), 2.60 (3H, s, NHMe), 4.16 (2H, q, J = 6.7 Hz, CH_2), 4.53 (2H, s, CH_2), 5.28 (1H, br, OH), 7.01 (1H, s, ArH), 7.51−7.60 (4H, m, 4 × ArH), 8.08 (1H, t, J = 8.0 Hz, ArH), 8.15 (1H, d, J = 8.0 Hz, ArH), 8.24 (1H, d, J = 8.0 Hz, ArH), 8.48 (2H, d, J = 8.0 Hz, 2 × ArH), 8.93 and 9.93 (each 1H, brs, NH). Anal. ($C_{25}H_{24}N_4O_4$·HCl·3$^1/_4$$H_2O$) C, H, N.

4′-Dimethylaminoethylcarboxamido-AHMA-ethylcarbamate (26) was synthesized from **8** (0.215 mg, 0.5 mmol) and ethyl chloroformate (0.081 g, 0.75 mmol): yield 0.174 g (69%); mp 115−7 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.0 Hz, Me), 2.88 (6H, s, NMe_2), 3.34 and 3.81 (each 2H, brs, 2 × CH_2), 4.10 (2H, q, J = 7.0 Hz, CH_2), 4.44 (2H, s, CH_2), 5.38 (1H, br, OH), 6.96 (1H, s, ArH), 7.48−7,55 (4H, m, 4 × ArH), 8.00 (1H, d, J = 8.0 Hz, ArH), 8.19 (1H, d, J = 8.8 Hz, ArH), 8.26 (1H, d, J = 8.8 Hz, ArH), 8.52 (1H, d, J = 8.8 Hz, ArH), 8.69 (1H, d, J = 8.0 Hz, ArH), 9.91 (1H, br, NH), 10.65 (1H, brs, NH). Anal. ($C_{28}H_{31}N_5O_4$·HCl·H_2O) C, H, N.

4′-Nitro-AHMA-ethylcarbamate (27) was synthesized from crude 4′-nitro-AHMA (**9**; 3.60 g, 10 mmol) and ethyl chloroformate (1.30 g, 12.0 mmol): yield 3.24 g (75%); mp 180−2 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 7.2 Hz, Me), 4.08 (2H, q, J = 7.2 Hz, CH_2), 4.41 (2H, s, CH_2), 5.48 (1H, br, OH), 6.81 (1H, s, ArH), 7.30−7.38 (4H, m, 4 × ArH), 7.50 (1H, m, ArH), 8.07 (1H, m, ArH), 8.19 (1H, m, ArH), 8.65−8.17 (2H, m, 2 × ArH), 9.52 and 9.60 (each 1H, brs, NH), 10.85 (1H, br, NH). Anal. ($C_{23}H_{20}N_4O_5$·HCl·H_2O) C, H, N.

4′-Fluoro-AHMA-ethylcarbamate (28) was synthesized from crude 4′-fluoro-AHMA (**10**; 2.19 g, 6.00 mmol) and ethyl chloroformate (0.78 g, 7.19 mmol): yield 2.19 g (90%); mp 238−9 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.2 Hz, Me), 4.11 (2H, q, J = 7.2 Hz, CH_2), 4.46 (2H, s, CH_2), 5.23 (1H, br, OH), 6.99 (1H, s, ArH), 7.36−7.52 (4H, m, 4 × ArH), 7.89−8.01 (2H, m, 2 × ArH), 8.12 (1H, d, J = 8.8 Hz, ArH), 8.27−8.29 (2H, m, 2 × ArH), 9.91 (1H, br, NH), 12.00 (1H, brs, NH). Anal. ($C_{23}H_{20}N_3O_3$·HCl·1$^1/_4$$H_2O$) C, H, N.

4′-Chloro-AHMA-ethylcarbamate (29) was synthesized from crude 4′-chloro-AHMA (**11**; 1.72 g, 4.5 mmol) and ethyl chloroformate (0.586 g, 5.4 mmol): yield 1.06 g (56%); mp 174−5 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, J = 7.0 Hz, Me), 4.11 (2H, q, J = 7.0 Hz, CH_2), 4.44 (2H, s, CH_2), 5.22 (1H, br, OH), 6.96 (1H, s, ArH), 7.41−7.49 (4H, m, 4 × ArH), 8.00 (1H, d, J = 8.8 Hz, ArH), 8.19 (1H, d, J = 8.8 Hz, ArH), 8.25 (1H, d, J = 8.8 Hz, ArH), 8.35 (1H, d, J = 8.8 Hz, ArH), 8.87 (1H, d, J = 8.8 Hz, ArH), 9.91 (1H, br, NH), 12.00 (1H, brs, NH). Anal. ($C_{23}H_{20}ClN_3O_3$·HCl·7H_2O) C, H, N.

4′-Bromo-AHMA-ethylcarbamate (30) was synthesized from crude 4′-bromo-AHMA (**12**; 1.92 g, 4.5 mmol) and ethyl chloroformate (0.585 g, 5.4 mmol): yield 1.42 g (68%); mp 200−203 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.0 Hz, Me), 4.11 (2H, q, J = 7.0 Hz, CH_2), 4.44 (2H, s, CH_2), 5.20 (1H, br, OH), 6.95 (1H, s, ArH), 7.34−7.48 (4H, m, 4 × ArH), 8.02 (1H, d, J = 8.6 Hz, ArH), 8.24 (1H, d, J = 8.6 Hz, ArH), 8.35 (1H, d, J = 8.8 Hz, ArH), 8.38 (1H, d, J = 8.8 Hz, ArH), 8.53 (1H, d, J = 8.8 Hz, ArH), 9.90 (1H, br, NH), 12.05 (1H, brs, NH). Anal. ($C_{23}H_{20}BrN_3O_3$·HCl·1$^1/_2$$H_2O$) C, H, N.

4′-Methoxy-5′-methylcarboxamido-AHMA-ethylcarbamate hydrochloride (31) was synthesized from **13** (325 mg, 0.805 mmol) and ethyl chloroformate (110 mg, 0.96 mmol): yield 178 mg (46%); mp 214−5 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.4 Hz, Me), 2.94 (3H, s, NHMe), 4.1 (2H, q, J = 7.2 Hz, CH_2), 4.15 (3H, s, OMe), 4.44 (2H, s, CH_2), 5.52 (1H, br, OH), 6.96 (1H, s, ArH), 7.42 (1H, t, J = 8.0 Hz, ArH), 7.46 (2H, s, 2 × ArH), 7.52−7.58 (2H, m, 4 × ArH), 7.73 (1H, d, J = 8.8 Hz, ArH), 8.46 (1H, d, J = 8.8 Hz, ArH), 8.55 (1H, d, J = 7.2 Hz, ArH), 9.37 and 9.9210.11 and 11.71 (each 1H, br, NH). Anal. ($C_{26}H_{26}N_4O_5$·HCl) C, H, N.

4′-Methoxy-5′-dimethylaminoethylcarboxamido-AHMA-ethylcarbamate (32) was synthesized from **14** (200 mg, 0.43 mmol) and ethyl chloroformate (56 mg, 0.54 mmol): yield 103 mg (45%); mp 220−1 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.1 Hz, Me), 2.88 (6H, s, NMe_2), 3.40 and 3.81 (each 2H, brs, 2 × CH_2), 4.11 (2H, q, J = 7.0 Hz, CH_2), 4.15 (3H, s, OMe), 4.44 (2H, s, CH_2), 5.20 (1H, br, OH), 6.95 (1H, s, ArH), 7.41−7.46 (3H, m, 3 × ArH), 7.58−7.60 (2H, m, 2 × ArH), 7.79 (1H, d, J = 8.0 Hz, ArH), 8.54 (1H, d, J = 8.0 Hz, ArH), 8.86 (1H, d, J = 8.0 Hz, ArH), 9.88, 9.97 and 10.62 (each 1H, br, NH). Anal. Calcd for $C_{29}H_{33}N_5O_5$·HCl·1$^1/_2$$H_2O$: C, 58.53; H, 6.27; N, 11.78. Found: C, H, N.

4′-Methyl-5′-methylcarboxamido-AHMA-ethylcarbamate hydrochloride (33) was synthesized from **15** (732 mg, 2.0 mmol) and ethyl chloroformate (239 mg, 2.2 mmol): yield 596 g (65%); mp 252−3 °C; ^{1}H NMR (DMSO-d_6) δ 1.43 (3H, t, J = 7.2 Hz, Me), 2.93 (3H, s, Me), 3.17 (3H, s, NHMe), 4.31 (2H, q, J = 7.2 Hz, CH_2), 4.64 (2H, s, CH_2), 5.52 (1H, br, OH), 7.14 (1H, s, ArH), 7.55−7.78 (4H, m, 4 × ArH), 8.10, 8.33, 8.68 and 8.86 (each 1H, d, J = 8.0 Hz, ArH), 9.80, 10.11 and 11.57 (each 1H, br, NH). Anal. ($C_{26}H_{26}N_4O_5$·HCl·1$^3/_4$$H_2O$) C, H, N.

4′-Methyl-5′-dimethylaminoethylcarboxamido-AHMA-ethylcarbamate (34) was synthesized from **16** (310 mg, 0.75 mmol) and ethyl chloroformate (106 mg, 0.98 mmol): yield 105 mg (30%); mp 240−3 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.0 Hz, Me), 2.74 (3H, s, Me), 2.86 (6H, s, NMe_2), 3.46 and 3.82 (each 2H, brs, 2 × CH_2), 4.09 (2H, q, J = 7.0 Hz, CH_2), 4.44 (2H, s, CH_2), 5.20 (1H, br, OH), 6.97 (1H, s, ArH), 7.38 (1H, t, J = 8.0 Hz, ArH), 7.42 and 7.48 (each 1H, s, 2 × ArH), 7.58 (1H, t, J = 8.0 Hz, ArH), 7.94, 8.24, 8.59 and 8.93 (each 1H, d, J = 8.0 Hz, ArH), 9.93, 10.02 and 10.82 (each 1H, brs, 3 × NH). Anal. ($C_{29}H_{33}N_5O_4$·HCl·5H_2O) C, H, N.

Cytoxicity Assays. The HL-60 human promyelocytic leukemic cells were cultured at an initial density of 5 × 10^4 cells/mL. The cells were maintained in a 5% CO_2-humidified atmosphere at 37 °C in RPMI medium 1640 (GIBCO/BRL) containing penicillin (100 units/mL), streptomycin (100 μg/mL) (GIBCO/BRL), and 10% heat-inactivated fetal bovine serum.

For HL-cells in suspension, cytotoxicity was measured by XTT-tetrazolium microculture assay[32] in duplicate in 96-well microtiter plates following 72-h incubation. The absorbance of each well was measured with a microplate reader (EK-340, Bio-Tek, Burlington, VT) as previously discribed.[33] Each run included six to seven concentrations of the tested drugs. Dose−effect relations data were analyzed for IC_{50} (concentration for 50% inhibition) with the media-effect plot[34] by using a previously described computer program.[35]

Animals. B6D2F$_1$ mice were obtained from Toconic Farms. Eight-week-old male mice weighing 20−25 g were used. For iv injection, the drug was administrated via the tail vein. Typically, P388 leukemia-bearing mice following 10^6 cell

implantation by ip injection survived 6−7 days in the control group. The percentage increases in lifespan (%ILS) in the treatment group were measured and averaged. All animal studies were conducted in accordance with the guidelines of the National Institutes of Health: *A Guide for the Care and Use of Animals*.

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