Potent Antitumor 9-Anilinoacridines and Acridines Bearing an Alkylating *N*-Mustard Residue on the Acridine Chromophore: Synthesis and Biological Activity

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A series of 9-anilinoacridine and acridine derivatives bearing an alkylating *N*-mustard residue at C4 of the acridine chromophore were synthesized. The *N*-mustard pharmacophore was linked to the C4 of the acridine ring with an *O*-ethyl (*O*-C_2), *O*-propyl (*O*-C_3), or *O*-butyl (*O*-C_4) spacer. It revealed that all newly synthesized compounds were very potent cytotoxic agents against human leukemia and various solid tumors in vitro. These agents did not exhibit cross-resistance against vinblastine-resistant (CCRF-CEM/VBL) or taxol-resistant (CCRF-CEM/taxol) cells. It also showed that these agents were DNA cross-linking agents rather than topoisomerase II inhibitors. Of these agents, compounds **27a** and **27c** were shown to have potent antitumor activity in nude mice bearing the human breast carcinoma MX-1 xenograft. The therapeutic efficacies of these two agents are comparable to that of taxol.

Introduction

Gene-targeted alkylating agents formed by linking *N*-mustard derivatives to DNA binding affinic molecules have been widely applied in finding drugs with high sequence-selective binding to the macromolecules and minimizing the side effects induced by highly reactive *N*-mustards.[1] The DNA binding affinic molecules, such as DNA intercalators [e.g., heterocyclic compounds,[2,3] 9-aminoacridine (e.g., **1**, Chart1),[4−6] 4-anilinoquinoline (**2**),[7,8] 9-anilinoacridine (**3** and **4**),[9] anthraquinone (**5**),[10] cyclopentanthraquinone (**6**)[11]] and DNA minor groove binders [e.g., distamycin A and related analogues,[12−20] such as tallimustine (**7**)] have been utilized for such purpose to improve the antitumor efficacy of *N*-mustard derivatives. The targeting *N*-mustards reported from these literatures were shown to be more potent than the corresponding untargeted derivatives. Despite the superior cytotoxicity of the targeting *N*-mustards, only a limited number of compounds (e.g., compound **7**) were found to have potential clinical application.[20]

Among the DNA-targeting mustards, in which 9-anilinoacridines were used as the DNA-affinic carrier, compounds **3** and **4** were synthesized by linking the alkylating aniline mustard residue either to the acridine chromophore or to the aniline ring of the clinical antileukemic amsacrine, respectively.[9] However, these agents were less cytotoxic than amsacrine in inhibiting murine leukemic P388 or Chinese hamster ovary derived tumor *AA*8 cell growth in culture and in animals, although they cross-linked DNA with high sequence-selectivity and were considerably more cytotoxic than their untargeted mustard counterparts. The 4-linked analogues (**3**) showed slightly higher in vivo antileukemic activity than their corresponding 1′-linked analogues (**4**), suggesting that the *N*-mustard residue would prefer to be linked to the acridine chromophore to have better cytotoxicity.

Our continued development of gene-targeting alkylating agents have demonstrated that alkylating *N*-mustards (aliphatic mustard) linked to the anilino ring of the 9-anilinoacridine exhibited potent antitumor effects in inhibiting various human tumor cell growth both in vitro and in vivo.[21,22] The *N*-mustard pharmacophore was linked to the C3′ or C4′ position of the anilino ring with a short spacer: *O*-ethyl (*O*-C_2), *O*-butyl (*O*-C_4), or methyl (C_1) linker. The results showed that all compounds exhibited potent in vitro cytotoxicity against human lymphoblastic leukemia cells (CCRF-CEM) in culture.[32] Studies on the structure−activity relationships of these *N*-mustards showed that their antitumor activity was slightly affected by the length of the spacer and the location of the *N*-mustard pharmacophore on the anilino ring. Among these agents, compound **8** (BO-0742) exhibited significant cytotoxicity against CCRF-CEM, with 107-fold higher potency than its parent analogue, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA, **9**).[23−25] Additionally, it also exhibited a significant cytotoxic effect against drug-resistant sublines, such as those resistant to vinblastine and taxol, CCRF-CEM/VBL and CCRF-CEM/taxol, respectively. Remarkably, compound **8** at one-tenth of the taxol's therapeutic dose resulted in complete tumor remission in nude mice bearing human breast carcinoma MX-1 xenografts. Furthermore, **8** yielded xenograft tumor suppression of 81−96% using human T-cell acute lymphoblastic leukemia CCRF-CEM, colon carcinoma HCT-116, and ovarian adenocarcinoma SK-OV-3 tumor models. Further studies suggested that the main mechanism of action for compound **8** is primarily through its DNA cross-linking activity rather than its inhibitory effect on topoisomerases.

These studies suggest that the type of *N*-mustard pharmacophore (aniline mustard or aliphatic mustard), the length of the spacer between the carrier and *N*-mustard moiety, and the location of the *N*-mustard residue on the 9-anilinoacridine (linking to the anilino ring or to the acridine chromophore) may affect their cytotoxicity and antitumor potency. In searching for more powerful gene-targeting agents, we have synthesized a series of 9-anilinoacridine and acridine analogues bearing the *N*-mustard residue, which was linked to C4 of the acridine

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Chart 1. *N*-Mustards Linked to DNA-Affinic Carriers and AHMA

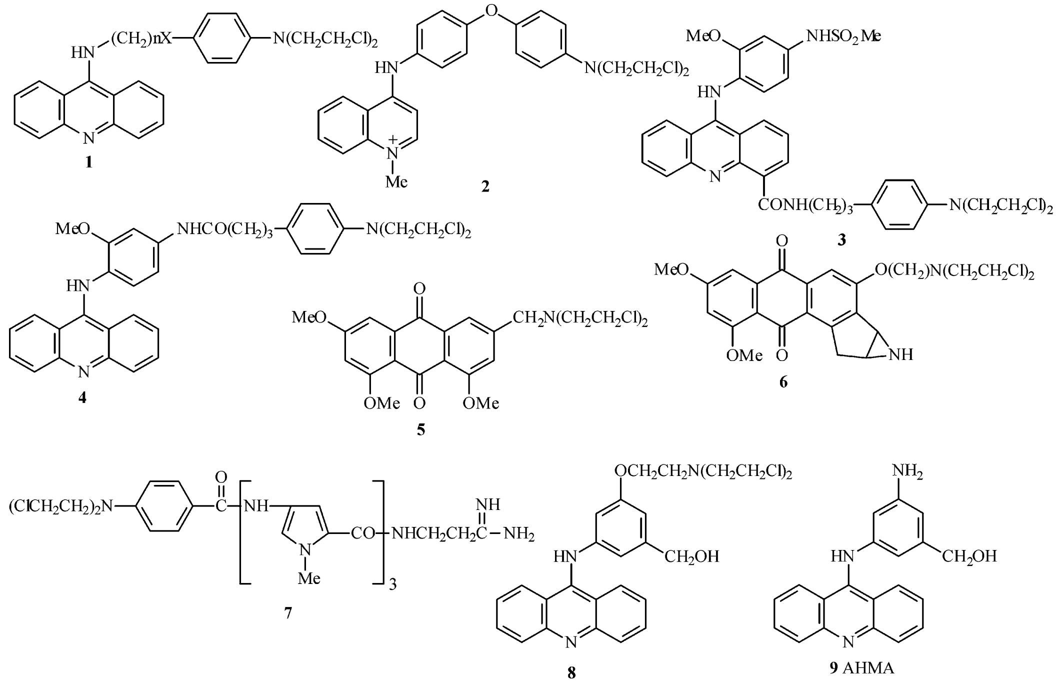
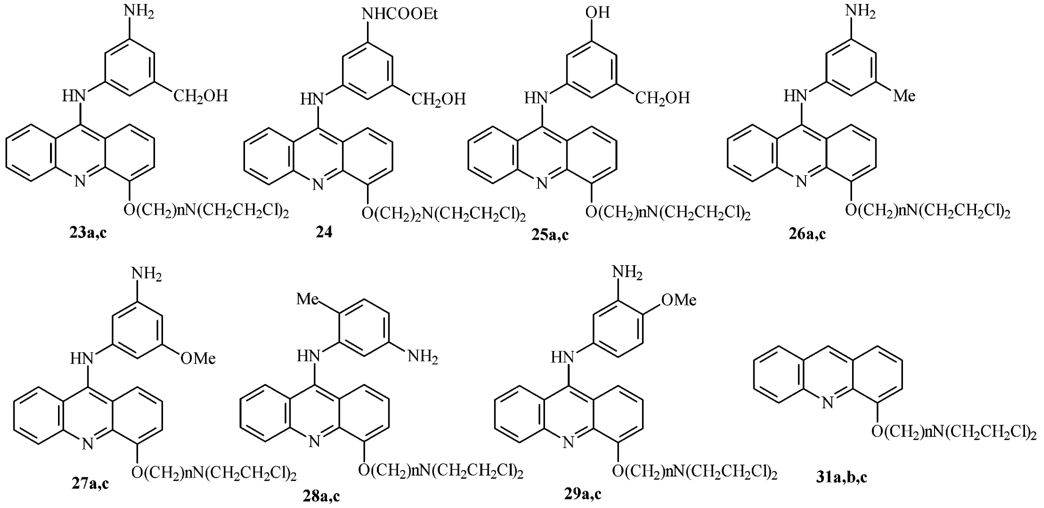


Chart 2. Newly Synthesized 9-Anilinoacridines and Acridines Bearing *N*-Mustard Residue[a]



[a] a series: $n = 2$. b series: $n = 3$. c series: $n = 4$.

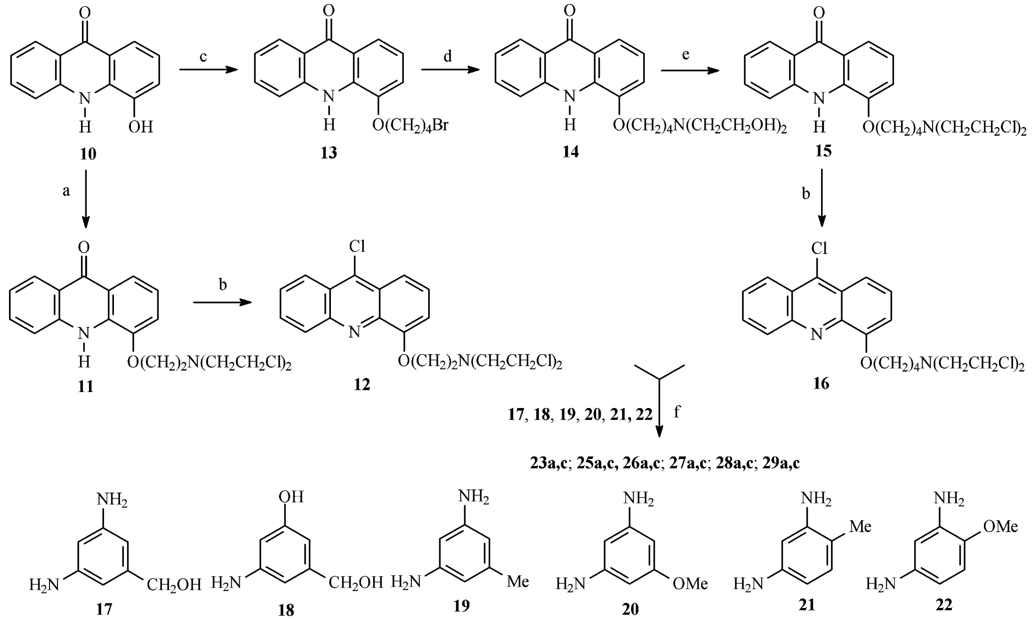
chromophore by using *O*-C_2 or *O*-C_4 as the spacer. The studies would provide more information for understanding of the effect of the carrier (9-anilinoacridine or acridine) on the cytotoxicity and the antitumor potency of the targeting *N*-mustards. The results from both in vitro and in vivo models revealed that all of these newly synthesized compounds exhibited significant antitumor activity. A thorough description on the synthesis, antitumor efficacy, and the mechanism of action of these compounds is provided herein.

Chemistry

All newly synthesized 9-anilinoacridines and acridines bearing the *N*-mustard residue are shown in Chart 2. Acridin-9-one having a *N*-mustard moiety was prepared starting from the known 4-hydroxyacridin-9-one (**10**).[26] Compound **10** was treated with tris(2-chloroethyl)amine hydrochloride in dry DMF in the presence of excess K_2CO_3 at room temperature for 20 h to give acrdin-9-one (**11**) bearing a *N*-mustard residue with *O*-C_2 as a spacer in low yield (27%) (Scheme 1). Treatment of **10** with 1,4-dibromobutane gave 4-(4-bromobutoxy)acridin-9-one (**13**), which was then reacted with diethanolamine in diglyme at 115 °C with vigorous stirring for 30 min to yield **14**. Prolongation of the reaction of **14** with methanesulfonyl chloride in CH_2Cl_2 in the presence of triethylamine afforded 4-bis(2-chloroethyl)-aminobutoxyacridin-9-one (**15**) in 70% yield. The *N*-mustard substituted acridin-9-ones (**11** and **15**) were then reacted with thionyl chloride to give the corresponding 9-chloroacridine derivatives (**12** and **16**, respectively), which were used directly for condensing with various substituted 1,3-phenylenediamines (**17**, **19**, **20**, **21**, and **22**) and 3-hydroxy-5-hydroxymethylaniline (**18**) to form the desired 9-anilinoacridines **23a**,**b**, **25a**,**b**, **26a**,**b**, **27a**,**b**, **28a**,**c**, and **29a**,**b**, which bear a *N*-mustard moiety at C4 of the acridine chromophore with *O*-C_2 or *O*-C_4 spacer (Chart 2) in moderate to good yield. Compound **23a** was converted into its ethyl carbamate derivative **24** (Chart 2) by reacting with ethyl chloroformate in dry DMF in the presence of pyridine.

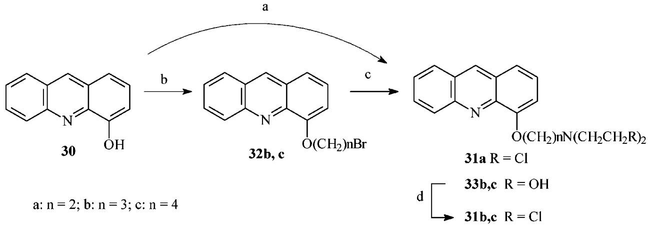
Acridine derivatives bearing a *N*-mustard pharmacophore at C4 were prepared by following the same synthetic route for **12**

Scheme 1. Synthesis Substituted 9-Anilinoacridines Having *N*-Mustard Moiety on Acridine Chromophore[a]



[a] Reagents and reaction conditions: (a) tris(2-chloroethyl)amine•HCl/KF/K_2CO_3/DMF, room temperature, 20 h; (b) $SOCl_2$/DMF, 80 °C, 40 min; (c) $Br(CH_2)_4Br/K_2CO_3$/DMF, 40 °C, 2 h; (d) $NH(CH_2CH_2OH)_2$, diglyme, 115 °C, 30 min; (e) $MeSO_2Cl/Et_3N/CHCl_3$, room temperature, 3 d; (f) 4-methylmorpholine/$CHCl_3$/EtOH, 0−25 °C, 4−8 h.

Scheme 2. Synthesis of Acridine Derivatives Bearing a *N*-Mustard Pharmacophore at C4[a]



[a] Reagents and reaction conditions: (a) tris(2-chloroethyl)amine•HCl/KF/K_2CO_3/DMF, 40−50 °C, 8 h; (b) $Br(CH_2)_3Br$ or $Br(CH_2)_4Br/K_2CO_3$/DMF, 40−50 °C, 8 h; (c) $NH(CH_2CH_2OH)_2$, diglyme, 115 °C, 4 h; (d) $MeSO_2Cl/Et_3N/CH_2Cl_2$, room temperature, 3 d.

and **16** (Scheme 2). The known 4-hydroxyacridine (**30**)[27] was converted into the desired acridines containing bis(2-chloroethyl)aminoethoxy moiety (**31a**) by treating with tris(2-chloroethyl)amine hydrochloride in dry DMF in the presence of excess K_2CO_3. Similarly, reaction of **30** with 1,3-dibromopropane or 1,4-dibromobutane gave **32b** and **32c**, respectively, which were further reacted with diethanolamine to yield **33b**,**c**. Treatment of **33b**,**c** with methanesulfonyl chloride/triethylamine afforded 4-bis(2-chloroethyl)aminopropoxy- (**31b**) or 4-bis(2-chloroethyl)aminobutoxy- (**31c**) acridines, respectively (Chart 2).

Biological Results and Discussion

Cytotoxicity in Vitro. Table 1 shows that the new 9-anilinoacridine derivatives bearing *N*-mustard residue on the acridine chromophore (**23**−**29**, **31**) are very potent cytotoxic agents against human lymphoblastic leukemic CCRF-CEM cells. The IC_{50} values are in the nanomolar ranges. These potencies are comparable to taxol and are several 100-fold more potent than the parent compound AHMA, which lacks *N*-mustard functionality. In general, the $-(CH_2)_n-$ linker between mustard and the acridine chromophore yields higher in vitro activity when $n = 4$ than $n = 2$. We have also used the leukemic sub-cell-line resistant to taxol, CCRF-CEM/taxol, which was 457-fold resistant to taxol and 72-fold resistant to vinblastine. However, the new compounds showed only 3.1−20-fold resistance. Another drug-resistant subline CCRF-CEM/VBL that expresses the multidrug resistance (MDR1) gene product, P-glycoprotein (Pgp),[28] was also used. This cell line showed 1630-fold resistance to taxol and 517-fold resistance to vinblastine. However, the new *N*-mustard compounds showed only 2.9−26.4-fold resistance. Thus, many of these new *N*-mustard compounds have better antiproliferative activity than taxol or vinblastine against drug-resistant human tumor cells. Table 1 also shows that many of these new *N*-mustard compounds possess potent activities against the growth of human solid tumor cells such as lung carcinoma A549, colon carcinoma HCT-116, and mammary carcinoma MX-1. Overall, the human leukemic CCRF-CEM cells seem more sensitive to the new compounds than the human solid tumor cells (Table 1).

Our previous study showed that AHMA ethylcarbamate was more cytotoxic than AHMA.[24] However, in contrast to the previous studies, **24** was 3-times less cytotoxic than **23a**,**c**, indicating that compound **23a** had increased lipophilicity by converting to its ethylcarbamate derivative, which did not affect the potency of the parent compound. In this experiment we also found that the bioisosteric isomers, compounds **25a**,**c** and **23a**,**c**, were equipotent against the same tumor cell line in culture.

To appreciate the role of the carrier (9-anilinoacridines vs acridine) in the newly synthesized compounds, we linked the *N*-mustard residue to the C4 of the acridine (i.e., compounds **31a**,**b**,**c**) with *O*-ethyl, *O*-propyl, and *O*-butyl as the spacer. In the same experiment, these agents were 3−10-fold less potent than the corresponding 9-anilinoacridine derivatives. The length of the spacer, however, did not markedly affect their potency. The results suggested that the 9-anilinoacridines are a better carrier than acridine for *N*-mustard alkylating agents to achieve higher cytotoxicity.

Therapeutic Effects against Human Mammary Carcinoma MX-1 Xenograft in Nude Mice. The pharmacological and therapeutic properties of the selected new compounds in terms of dose, route, and schedule of administration, toxicity, and efficacy for **26a**, **26c**, **27a**, **27c**, and **28c** were compared in Table

Table 1. Cytotoxicity of *N*-Mustard Derivatives against Human Lymphoblastic Leukemic Cells (CCRF-CEM) and Its Drug-Resistant Sublines (CCRF-CEM/VBL and CCRF-CEM/Taxol) and Human Solid Tumor (A549, HCT-116, and MX-1 Cells) Cell Growth in Vitro[a]

	IC_{50} (μM)					
	lymphoblastic leukemia			solid tumors		
compd	CCRF-CEM	CCRF-CEM/taxol	CCRF-CEM/VBL	A549	HCT-116	MX-1
9	0.753	0.600$_{[0.8\times]}$[b]	1.60$_{[2.7\times]}$	0.047	nd	0.0035
8	0.0070	0.0340$_{[4.9\times]}$	0.0075$_{[1.1\times]}$	0.0056	0.0055	0.0035
23a	0.0067	0.0633$_{[9.6\times]}$	0.126$_{[18.8\times]}$	0.0324	0.0077	0.0066
24	0.0257	0.126$_{[4.9\times]}$	0.231$_{[9.0\times]}$	0.0940	0.0595	0.0555
23c	0.0042	0.0405$_{[9.6\times]}$	0.111$_{[26.4\times]}$	0.0053	0.0095	0.0024
25a	0.0069	0.139$_{[20.1\times]}$	0.152$_{[22.0\times]}$	0.1080	0.1250	0.0580
25c	0.0059	0.117$_{[19.7\times]}$	0.338$_{[57.3\times]}$	0.0251	0.0091	0.0072
26a	0.0041	0.0217$_{[5.3\times]}$	0.0188$_{[4.6\times]}$	0.0449	0.0232	0.0219
26c	0.0037	0.0611$_{[16.6\times]}$	0.0545$_{[14.7\times]}$	0.0089	0.0091	0.0100
27a	0.0119	0.0498$_{[4.2\times]}$	0.0469$_{[2.9\times]}$	0.0902	0.0538	0.0281
27c	0.0042	0.0439$_{[10.5\times]}$	0.0513$_{[12.3\times]}$	0.0190	0.0138	0.0056
28a	0.0080	0.0465$_{[5.9\times]}$	0.0312$_{[2.9\times]}$	0.0316	0.0090	0.0071
28c	0.0043	0.0578$_{[13.4\times]}$	0.0423$_{[9.8\times]}$	0.0154	0.0136	0.0092
29a	0.0123	0.0575$_{[4.7\times]}$	0.0601$_{[4.9\times]}$	0.1183	0.0482	0.0241
29c	0.0059	0.0899$_{[15.3\times]}$	0.0865$_{[14.7\times]}$	0.0190	0.0083	0.0056
31a	0.0376	0.117$_{[3.1\times]}$	0.163$_{[4.3\times]}$	0.1367	0.0949	0.0708
31b	0.0353	0.150$_{[4.2\times]}$	0.253$_{[7.2\times]}$	0.1663	0.1354	0.0827
31c	0.0512	0.393$_{[7.7\times]}$	0.308$_{[6.0\times]}$	0.2260	0.1175	0.0820
taxol	0.0011	0.4843$_{[457\times]}$	1.731$_{[1630\times]}$	0.0021	0.0015	0.0225
vinblastine	0.0004	0.0287$_{[71.8\times]}$	0.2066$_{[517\times]}$	0.0011	0.0007	0.0020

[a] Cell growth inhibition for leukemic cells and solid tumor cells were measured by the XTT tetrazolium assay[30] and by the sulforhodamine B method,[31] respectively, after 72 h incubation for cell growth; nd = not determined. IC_{50} values were determined from the dose−effect relationship at six or seven concentrations of each drug in duplicate by using a mass-action law based computer program.[32−34] [b] Each set of data have a linear correlation coefficient (*R* value) of 0.960−0.995 on the median effect plots indicating the mass-action low.[32,33] Numbers in the brackets are folds of resistance of the resistant cells when compared with the IC_{50}'s of the CCRF-CEM parent cells.

Table 2. Therapeutic Effects of **26a**, **26c**, **27a**, **27c**, and **28c** against Human Mammary Carcinoma MX-1 Xenografts in Nude Mice[a]

compd	dose (mg/ kg)[b]	schedule[c] (iv injection)	antitumor effect *T*/*C* (%)	maximum body weight loss (%)
26a	4.0	Q2D5	79 (D19)	15 (D17)
26c	1.5	Q3D2	6 (D14)	8 (D14)
27a	10.0	Q2D3 (on D7, 9, 11)	96 (D15)	15 (D13)
27c	2.0	Q2D3 (on D8, 10, 12)	77 (D14)	8 (D14)
28c	2.0	Q3D2	20 (D14)	21 (D12)

[a] MX-1 tissue (50 μg) was implanted subcutaneously in mice on day 0. Every other day iv treatments were given as indicated. [b] Vehicle was 20 μL of DMSO + 180 μL of saline. [c] Mice were treated every 2 days (Q2D) or every 3 days (Q3D).

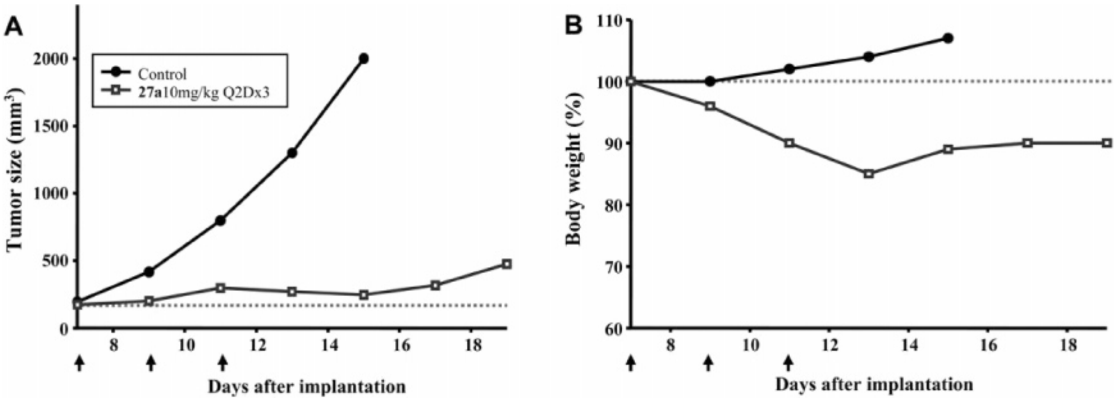


Figure 1. Therapeutic effects of **27a** (10 mg/kg, Q2D×3, iv injection) in nude mice bearing human mammary carcinoma MX-1 xenograft (A) and the body weight changes during treatments as indicated by arrows (B). Mice were treated every 2 days (Q2D).

2. Nude mice bearing human mammary MX-1 xenografts were used. Among the new compounds, **27a** and **27c** showed more impressive therapeutic results.

Under optimal therapeutic conditions, intravenous injection of **27a**, 10 mg/kg every other day (days 7, 9, 11) after tumor implantation, yielded as much as 96% tumor suppression (day 15) against MX-1 xenografts in nude mice (Figure 1A). The maximal toxicity as indicated by body weight decrease was a 15% drop from the initial pretreatment body weight (on day 13), two days after the last dose (Figure 1B). The body weight slightly recovered, but with a 10% decrease on days 17 and 19 (Figure 1B).

Intravenous injection of **27c** at a dose as low as 2 mg/kg, given every other day on days 8, 10, and 12 after tumor implantation resulted in 77% tumor suppression on day 14 (Figure 2A). The maximal body weight decrease was 8% on day 14, two days after the last dose (Figure 2B).

Although **27a** and **27c** were slightly less potent than taxol in vitro (Table 2), the optimal therapeutic doses of 10 mg/kg (Figure 1) for **27a** and 2 mg/kg (Figure 2) for **27c**, respectively,

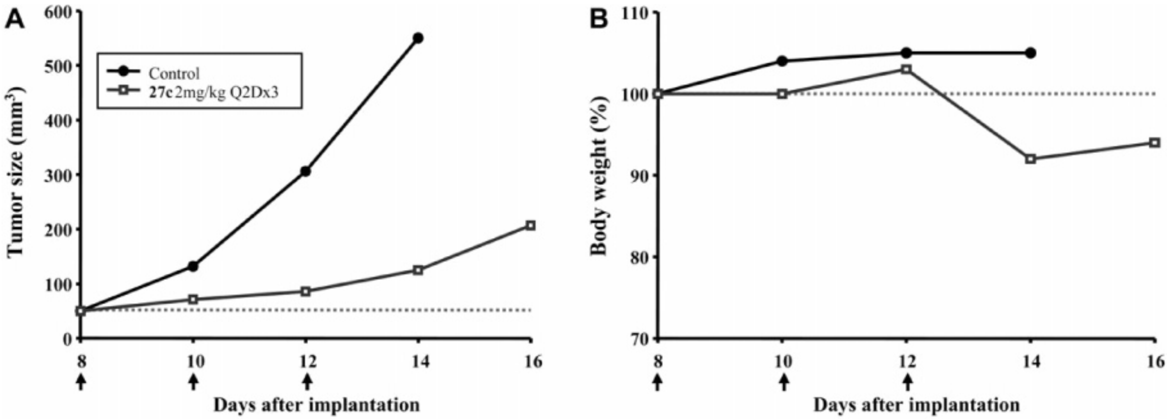


Figure 2. Therapeutic effects of **27c** (10 mg/kg, Q2D×3, iv injection) in nude mice bearing human mammary carcinoma MX-1 xenograft (A) and the body weight changes during treatments as indicated by arrows (B). Mice were treated every 2 days (Q2D).

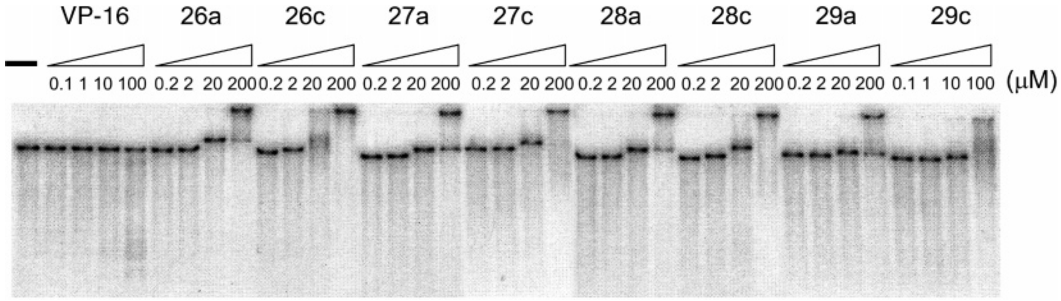


Figure 3. Topoisomerase II-mediated DNA cleavage by VP-16 and *N*-mustard derivatives **26a**,**c**, **27a**,**c**, **28a**,**c**, and **29a**,**c**. The first lane is the control without enzyme.

were considerably lower (i.e., more potent) than the optimal therapeutic dose of taxol (20 mg/kg, Q2D×6) against the same MX-1 xenograft tumor.[21,22]

Topoisomerase II-Mediated DNA Cleavage. Many antitumor acridines are known to induce topoisomerase II-mediated DNA cleavage. To test whether our newly synthesized compounds act similarly, a topoisomerase II-mediated DNA cleavage assay was performed. As shown in Figure 3, unlike VP-16, which induced topoisomerase II-mediated DNA cleavage at 100 μM, none of the newly synthesized compounds induced any detectable topoisomerase II-mediated DNA cleavage at 100−200 μM. However, treatment of linear DNA with any of our newly synthesized compounds resulted in dose-dependent reduction of gel electrophoretic mobilities (Figure 3). Furthermore, similar reduction of gel electrophoretic mobility was observed in the absence of topoisomerase II (data not shown). This result suggests that our newly synthesized agents were able to cause intramolecular cross-linking of the linear DNA resulting in reduced electrophoretic mobilities. This result is also consistent with results obtained from our previous studies which showed that 9-anilinoacridine derivatives bearing an *N*-mustard residue are DNA cross-linking agents.[21,22] Together, these results suggest that our new compounds are DNA cross-linking agents but not topoisomerase II poisons. Consequently, they may exert their cytotoxicity primarily through their DNA cross-linking activity.

Conclusions

Gene-targeting agents, such as DNA alkylators, have played an important part in anticancer drug development. A drawback of using DNA-alkylating agents includes their high reactivity resulting in loss of the drug's therapeutic activity against malignancy by reacting with other cellular components such as proteins, thiols, or genes, lacking of intrinsic DNA binding affinity of the core *N*,*N*-bis(2-chloroethyl)amine pharmacophore, and a requirement for bifunctional cross-linking of DNA to be fully cytotoxic, resulting in lower their potency and producing a high ratio of genotoxic monoadducts to cross-linkers (up 20:1).[29] It has demonstrated that the targeting of mustards to DNA by attaching to DNA-affinic carriers facilitates in finding compounds of higher cytotoxicity and potency than the corresponding untargeted *N*-mustard moiety.

Among DNA-targeting mustards using 9-anilinoacridines as a DNA-affinic carrier, as mentioned previously, compounds **3** and **4** were less cytotoxic than amsacrine, and the 4-linked analogues (**3**) showed slightly higher in vivo antileukemic activity than their corresponding 1′-linked analogues (**4**), indicating that the *N*-mustard residue would prefer to be linked to the acridone chromophore to have better cytotoxicity. In contrast, compound **8** and the newly synthesized compounds (Chart 2) were significantly more cytotoxic and potent than **9**.[21,22] In the present studies, we have synthesized a series of *N*-mustard derivatives in which the aliphatic *N*-mustard residue was linked to C4 of the acridine chromophore of 9-anilinoacridines and clearly demonstrated that these new compounds were significantly more potent than **9** and comparable to compound **8** in inhibiting various human tumor cell growth in vitro. It also revealed that the acridines bearing a *N*-mustard residue were 3−10-fold less potent than the corresponding 9-anilinoacridine derivatives. These results demonstrated that the length of the spacer between *N*-mustard moiety and carrier, the type of the *N*-mustard (aniline mustard or aliphatic mustard), the linking position of the *N*-mustard moiety to 9-anilinoacridine (linking to the anilino ring or acridine chromophore), and the sort of the carrier (9-anilinoacridine or acridine) affect the cytotoxicity of the targeting mustards. Additionally, we have found that the cytotoxicity of the newly synthesized compounds was not affected by the substituent(s) (CH_2OH, Me, or OMe) on the anilino ring. Among these agents, compounds **27a** and **27c** exhibited potent in vivo therapeutic effect in nude mice bearing human breast carcinoma MX-1 xenograft. To realize the formation of DNA cross-linking or monoadduct and their sequence-specific binding, the interaction of **27a**,**c** and other derivatives with DNA doubled strands are currently being studied in our laboratories.

Experimental Section

Melting points were determined on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out over silica gel G60 (70−230 mesh, ASTM; Merck). Thin-layer chromatography was performed on silica gel G60 F_{254} (Merck) plates with short wavelength UV light for visualization. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. ^1H NMR spectra were recorded on a Brücker DRX-600 spectrometer with Me_4Si as internal standard.

4-{2-[Bis(2-chloroethyl)amino]ethoxy}-10*H*-acridin-9-one (11). A mixture of tris(2-chloroethyl)amine hydrochloride (9.64 g, 40 mmol), KF (1.16 g, 20 mmol), and dry powdered K_2CO_3 (6.91, 50 mmol) in dry DMF (15 mL) was stirred at room temperature for 1 h. A solution of 4-hydroxy-9-oxoacridine[26] (**10**, 2.12 g, 10 mmol) in dry DMF (5 mL) was added into the above mixture, and it was stirred at room temperature for 20 h. The reaction mixture was poured onto ice water (100 mL) and extracted with EtOAc (5 × 100 mL). The organic extracts were combined, washed with ice water, dried over Na_2SO_4, and evaporated in vacuo to dryness. The residue was recrystallized from EtOH, yield 1.02 g (27%): mp 131−134 °C; ^1H NMR (DMSO-d_6) δ 3.01 (4H, t, $J = 6.8$ Hz, 2 × NCH_2), 3.20 (2H, t, $J = 5.6$ Hz, CH_2N), 3.64 (4H, t, $J = 6.7$ Hz, 2×CH_2Cl), 4.31 (2H, t, $J = 5.7$ Hz, OCH_2), 7.18 (1H, m, ArH), 7.27 (1H, m, ArH), 7.49 (1H, m, ArH), 7.72 (1H, m, ArH), 7.82 (1H, m, ArH), 7.92 (1H, m, ArH), 8.23 (1H, m, ArH), 10.78 (1H, brs, exchangeable, NH). Anal. ($C_{19}H_{20}Cl_2N_2O_2$) C, H, N.

4-(4-Bromobutoxy)-10*H*-acridin-9-one (13). A solution of 4-hydroxy-9-oxoacridine[26] (**10**, 5.01 g, 24 mmol) and K_2CO_3 (6.64 g, 48 mmol) in DMF (35 mL) was stirred for 5 min. To the mixture was added 1,4-dibromobutane (8.6 mL, 72 mmol), and the mixture was then stirred at 40 °C for 2 h. The mixture was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure to remove DMF. The residue was diluted with water (30 mL) and extracted with $CHCl_3$ (5 × 50 mL). The combined organic extracts were washed successively with 1% NaOH (50 mL) and water (30 mL), dried over Na_2SO_4, and evaporated in vacuo to dryness. The residue was chromatographed on a silica gel column (2 × 20 cm) using $CHCl_3$ as the eluant. The fractions containing the desired product were combined and evaporated, and the residue was recrystallized from EtOH to give **13**, 4.09 g (49%): mp 180−181 °C; ^1H NMR (DMSO-d_6) δ 2.15 (4H, m, CH_2CH_2), 3.60 (2H, t, $J = 6.0$ Hz, CH_2Br), 4.24 (2H, t, $J = 9.0$ Hz, OCH_2), 7.06 (1H, m, ArH), 7.15 (1H, m, ArH), 7.41 (1H, m, ArH), 7.65 (1H, m, ArH), 8.04 (1H, m, ArH), 8.48 (1H, m, ArH). Anal. ($C_{17}H_{16}BrNO_2$) C, H, N.

4-{4-[Bis(2-hydroxyethyl)amino]butoxy}-10*H*-acridin-9-one (14). A mixture of **13** (2.77 g, 8.0 mmol) and diethanolamine (5.27 g, 50 mmol) in diglyme (10 mL) was heated at 115 °C with vigorous stirring for 30 min. After cooling, the mixture was evaporated in vacuo to remove excess diethanolamine, and the residue was successively washed with hexane followed by ether. The oily residue was dissolved in $CHCl_3$ (200 mL), washed with water (6 × 80 mL), dried over Na_2SO_4, and evaporated under reduced pressure to dryness. The residue was crystallized from EtOH/hexane to give **14** as pale-yellow needles, 2.37 g, (80%): mp 124−126 °C; ^1H NMR (DMSO-d_6) δ 1.64 (2H, m, CH_2), 1.99 (2H, m, CH_2), 2.59 (2H, t, $J = 5.8$ Hz, OCH_2), 2.74 (4H, t, $J = 5.0$ Hz, 2 × CH_2OH), 3.76 (6H, m, 3 × NCH_2), 6.62 (1H, m, ArH), 6.90 (1H, m, ArH), 7.15 (1H, t, m, ArH), 7.31 (1H, m, ArH), 7.44 (1H, m, ArH), 7.92 (1H, m, ArH), 8.40 (1H, m, ArH), 9.45 (1H, brs, exchangeable, NH). Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

4-{4-[Bis(2-chloroethyl)amino]butoxy}-10*H*-acridin-9-one (15). To a solution of **14** (1.11 g, 3.0 mmol) and triethylamine (1.25 mL, 9.0 mmol) in dry $CHCl_3$ (25 mL) was added dropwise methanesulfonyl chloride (0.6 mL, 7.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 days. The solution was diluted with $CHCl_3$ (50 mL), washed successively with water (2 × 30 mL), ice cold aqueous $NaHCO_3$ (30 mL), and ice water (50 mL), dried over Na_2SO_4, and evaporated under reduced pressure to dryness. The residue was recrystallized from EtOH, yield 0.859 g (70%): mp 119−120 °C; ^1H NMR (DMSO-d_6) δ 1.64 (2H, brs, CH_2), 1.93 (2H, t, $J = 6.7$ Hz, CH_2), 2.84 (2H, brs, NCH_2), 3.34 (4H, brs, 2 × NCH_2), 3.61 (4H, brs, 2 × CH_2Cl), 4.28 (2H, t, $J = 8.5$ Hz, OCH_2), 7.18 (1H, m, ArH), 7.27 (1H, m, ArH), 7.34 (1H, m, ArH), 7.72 (1H, m, ArH), 7.80 (1H, m, ArH), 7.99 (1H, m, ArH), 8.23 (1H, m, ArH), 10.90 (1H, brs, exchangeable, NH). Anal. ($C_{19}H_{20}Cl_2N_2O_2$) C, H, N.

[3-Amino-5-(4-{2-[bis(2-chloroethyl)amino]ethoxy}acridin-9-yl-amino)phenyl]methanol Hydrochloride (23a). A mixture of **11** (1.52 g, 4.0 mmol) and $SOCl_2$ (5.0 mL) containing 2 drops of DMF was heated to 80 °C for 40 min. The reaction mixture was evaporated under reduced pressure to dryness, and the residue was coevaporated with $CHCl_3$ (3 × 20 mL). The crude yellow product **12** was dissolved in 50 mL of $CHCl_3$ and then filtered to remove undissolved byproducts. The filtrate was then added dropwise to a solution of 3,5-diaminobenzyl alcohol·2HCl (**17**, 912 mg, 4.2 mmol) and 4-*N*-methylmorpholine (2.3 mL, 21 mmol) in EtOH (60 mL) in an ice bath. After being stirred at 0 °C for 3 h, the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was acidified with excess concentrated HCl (1 mL) in EtOH (10 mL). The solvent was evaporated to dryness. The residue was chromatographed on a silica gel column (6 × 25 cm) using $CHCl_3$/MeOH (10:3 v/v) as the eluant. The fractions containing the product were combined and evaporated in vacuo to dryness. The solid residue was recrystallized from ethanol/acetone to give 1.68 g (67%): mp 105−106 °C. ^1H NMR (DMSO-d_6) δ 3.79 (4H, s, 2 × NCH_2), 3.99 (2H, s, CH_2N), 4.20 (4H, s, 2 × CH_2Cl), 4.46 (2H, s, $ArCH_2$), 4.74 (2H, s, OCH_2), 7.18 (1H, s, ArH), 7.26 (2H, s, ArH), 7.42−7.51 (2H, m, ArH), 7.60 (1H, m, ArH), 7.95 (1H, m, ArH), 8.03 (1H, m, ArH), 8.35 (1H, m, ArH), 8.99 (1H, m, ArH), 11.96 (1H, brs, exchangeable NH). Anal. ($C_{26}H_{28}Cl_2N_4O_2$·4HCl·$3H_2O$) C, H, N.

By following the same procedure as that for compound **23a**, the following *N*-mustard derivatives linked to the acridine chromophore of the 9-anilinoacridine were prepared:

[3-Amino-5-(4-{4-[bis(2-chloroethyl)amino]butoxy}acridin-9-yl-amino)phenyl]methanol Hydrochloride (23c). Compound **23c** was prepared from **15** (815 mg, 2.0 mmol) and **17** (317 mg, 1.5 mmol): yield 275 mg (26%); mp 247−248 °C; ^1H NMR (DMSO-d_6) δ 2.04 (4H, m, 2 × CH_2), 3.35 (2H, brs, CH_2N), 3.57 (4H, t, $J = 7.0$ Hz, 2 × NCH_2), 4.12 (4H, t, $J = 7.1$, 2 × CH_2Cl), 4.41 (2H, brs, OCH_2), 4.46 (3H, brs, CH_2OH and OH), 7.05 (1H, s, ArH), 7.08 (1H, s, ArH), 7.11 (1H, s, ArH), 7.41 (1H, m, ArH), 7.49 (1H, m, ArH), 7.57 (1H, m, ArH), 7.86 (1H, m, ArH), 8.01 (1H, m, ArH), 8.32 (1H, m, ArH), 8.77 (1H, m, ArH), 11.66 (1H, brs, exchangeable, NH). Anal. ($C_{28}H_{32}Cl_2N_4O_2$·3HCl·$1.5H_2O$) C, H, N.

3-(4-{2-[Bis(2-chloroethyl)amino]ethoxy}acridin-9-ylamino)-5-hydroxymethylphenol Hydrochloride (25a). Compound **25a** was prepared from **11** (1.90 g, 5.0 mmol) and 3-amino-5-hydroxybenzyl alcohol (**18**, 695 mg, 5.0 mmol): yield 1.73 g (73%); mp 237−238 °C; ^1H NMR (DMSO-d_6 + D_2O) δ 3.80−3.36 (6H, m, 3 × NCH_2), 4.09 (4H, s, 2 × CH_2Cl), 4.57 (2H, s, $ArCH_2$), 4.67 (2H, s, CH_2), 6.71 (1H, s, ArH), 6.88 (1H, s, ArH), 6.90 (1H, s, ArH), 7.42−7.40 (3H, m, 3 × ArH), 7.58 (1H, m, ArH), 7.85 (1H, m, ArH), 7.97 (1H, m, ArH), 8.20 (1H, m, ArH), 8.50 (1H, brs, exchangeable, NH). Anal. ($C_{26}H_{27}Cl_2N_3O_3$·2HCl·H_2O) C, H, N.

3-(4-{4-[Bis(2-chloroethyl)amino]butoxy}acridin-9-ylamino)-5-hydroxymethylphenol Hydrochloride (25c). Compound **25c** was prepared from **15** (815 mg, 2.0 mmol) and **18** (278 mg, 2 mmol): yield 244 mg (23%); mp 195−197 °C; ^{1}H NMR (DMSO-d_6) δ 2.03 (4H, m, 2 × CH_2), 3.54 (6H, m, 2 × NCH_2), 4.08 (4H, s, 2 × CH_2Cl), 4.40 (2H, s, OCH_2), 4.44 (2H, s, CH_2OH), 6.70 (1H, s, ArH), 6.79 (1H, s, ArH), 6.83 (1H, s, ArH), 7.39 (1H, m, ArH), 7.46 (1H, m, ArH), 7.55 (1H, m, ArH), 7.87 (1H, m, ArH), 7.97 (1H, m, ArH), 8.68 (1H, m, ArH), 9.90 (1H, brs, exchangeable, NH), 11.49 (2H, brs, exchangeable, 2 × OH). Anal. ($C_{28}H_{31}Cl_2N_3O_3\cdot 2.5HCl\cdot 0.5H_2O$) C, H, N.

N1-(4-{2-[Bis(2-chloroethyl)amino]ethoxy}acridin-9-yl)-5-methylbenzene-1,3-diamine Hydrochloride (26a). Compound **26a** was prepared from **11** (1.14 g, 3 mmol) and 5-methylphenylene-1,3-diamine·2HCl (**19**, 585 mg, 3 mmol): yield 756 mg (52%); mp 247−278 °C; ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s, ArMe), 3.78 (4H, s, 2 × NCH_2), 3.96 (2H, s, CH_2N), 4.19 (4H, s, 2 × CH_2Cl), 4.73 (2H, s, OCH_2), 6.99 (2H, s, ArH), 7.02 (1H, s, ArH), 7.25 (1H, s, ArH), 7.44 (1H, m, ArH), 7.49 (1H, m, ArH), 7.60 (1H, m, ArH), 7.98 (1H, m, ArH), 8.03 (1H, m, ArH), 8.38 (1H, m, ArH), 8.98 (1H, m, ArH), 11.94 (2H, s, exchangeable, NH_2). Anal. ($C_{26}H_{28}Cl_2N_4O\cdot 7HCl\cdot H_2O$) C, H, N.

N1-(4-{4-[Bis(2-chloroethyl)amino]butoxy}acridin-9-yl)-5-methylbenzene-1,3-diamine Hydrochloride (26c). Compound **26c** was prepared from **15** (1.22 g, 3 mmol) and **19** (585 mg, 3.0 mmol): yield 698 mg (46%); mp 236−237 °C; ^{1}H NMR (DMSO-d_6) δ 1.92 (4H, s, 2 × CH_2), 2.25 (3H, s, $ArCH_3$), 3.34 (2H, brs, CH_2N), 3.57 (4H, brs, 2 × NCH_2), 4.12 (4H, m, 2 × CH_2Cl), 4.41 (2H, m, OCH_2), 6.86−6.91 (3H, m, ArH), 7.42 (1H, m, ArH), 7.50 (1H, m, ArH), 7.57 (1H, m, ArH), 7.89 (1H, m, ArH), 8.01 (1H, m, ArH), 8.33 (1H, m, ArH), 8.75 (1H, m, ArH), 11.62 (2H, m, exchangeable, NH_2), 13.39 (1H, brs, exchangeable, NH). Anal. ($C_{28}H_{32}Cl_2N_4O\cdot 6HCl$) C, H, N.

N1-(4-{2-[Bis(2-chloroethyl)amino]ethoxy}acridin-9-yl)-5-methoxybenzene-1,3-diamine Hydrochloride (27a). Compound **27a** was prepared from **11** (1.14 g, 3.0 mmol) and 5-methoxyphenylene-1,3-diamine·2HCl (**20**, 633 mg, 3 mmol): yield 971 mg (65%); mp 277−178 °C; ^{1}H NMR (DMSO-d_6) δ 3.66 (3H, s, OMe), 3.78 (4H, s, 2 × NCH_2), 3.95 (2H, brs, CH_2N), 4.19 (4H, brs, 2 × CH_2Cl), 4.73 (2H, s, OCH_2), 6.67 (2H, s, ArH), 6.75 (1H, s, ArH), 7.46 (1H, m, ArH), 7.51 (1H, m, ArH), 7.60 (1H, m, ArH), 7.99 (1H, m, ArH), 8.02 (1H, m, ArH), 8.36 (1H, m, ArH), 8.97 (1H, m, ArH), 11.96 (2H, s, exchangeable, NH_2). Anal. ($C_{26}H_{28}Cl_2N_4O_2\cdot 4HCl\cdot 1.5H_2O$) C, H, N.

N1-(4-{4-[Bis(2-chloroethyl)amino]butoxy}acridin-9-yl)-5-methoxybenzene-1,3-diamine Hydrochloride (27c). Compound **27c** was prepared from **15** (1.22 g, 3 mmol) and **20** (665 mg, 3.15 mmol): yield 536 mg (34%); mp 260−261 °C; ^{1}H NMR (DMSO-d_6) δ 2.04 (4H, s, 2 × CH_2), 3.35 (2H, brs, CH_2N), 3.58 (4H, brs, 2 × NCH_2), 3.67 (3H, s, OMe), 4.11 (4H, m, 2 × CH_2Cl), 4.42 (2H, m, OCH_2), 6.45 (1H, brs, ArH), 6.47 (1H, brs, ArH), 6.51 (1H, brs, ArH), 7.44 (1H, m, ArH), 7.51 (1H, m, ArH), 7.58 (1H, m, ArH), 7.92 (1H, m, ArH), 8.01 (1H, m, ArH), 8.34 (1H, m, ArH), 8.70 (1H, m, ArH), 11.30−11.50 (2H, m, exchangeable, NH_2), 13.35 (1H, brs, exchangeable, NH). Anal. ($C_{28}H_{32}Cl_2N_4O_2\cdot 4HCl\cdot 0.5H_2O$) C, H, N.

N3-(4-{2-[Bis(2-chloroethyl)amino]ethoxy}acridin-9-yl)-4-methylbenzene-1,3-diamine Hydrochloride (28a). Compound **28a** was prepared from **11** (759 mg, 2 mmol) and 2,4-diaminotoluene (**21**, 244 mg, 2 mmol): yield 508 mg (53%); mp 225−226 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (3H, s, Me), 3.78 (4H, m, 2 × NCH_2), 3.95 (2H, m, CH_2N), 4.19 (4H, brs, 2 × CH_2Cl), 4.72 (2H, brs, OCH_2), 7.05 (1H, m, ArH), 7.28 (1H, m, ArH), 7.38 (1H, m, ArH), 7.44 (1H, m, ArH), 7.48 (1H, m, ArH), 7.59 (1H, m, ArH), 7.95 (1H, m, ArH), 8.01 (1H, m, ArH), 8.32 (1H, m, ArH), 8.96 (1H, m, ArH), 11.80 (2H, brs, exchangeable, NH_2), 13.68 (1H, brs, exchangeable, NH). Anal. ($C_{26}H_{28}Cl_2N_4O\cdot 4HCl\cdot 2.2H_2O$) C, H, N.

N3-(4-{4-[Bis(2-chloroethyl)amino]butoxy}acridin-9-yl)-4-methylbenzene-1,3-diamine (28c). Compound **28c** was prepared from **15** (1.22 g, 3.0 mmol) and **21** (366 mg, 3 mmol): yield 750 mg (49%); mp 216−218 °C. ^{1}H NMR (DMSO-d_6) δ 2.04 (4H, m, CH_2CH_2), 2.37 (3H, s, CH_3), 3.36 (2H, t, J = 8.7 Hz, CH_2N), 3.58 (4H, t, J = 8.7 Hz, 2 × NCH_2), 4.13 (4H, t, J = 8.7 Hz, 2 × CH_2Cl), 4.39 (2H, t, J = 8.7 Hz, OCH_2), 7.07 (1H, m, ArH), 7.29 (1H, m, ArH), 7.40 (2H, m, ArH), 7.47 (1H, m, ArH), 7.55 (1H, m, ArH), 7.87 (1H, m, ArH), 7.99 (1H, m, ArH), 8.33 (1H, m, ArH), 8.80 (1H, m, ArH), 11.74 (2H, brs, exchangeable, NH_2), 13.44 (1H, brs, exchangeable, NH). Anal. ($C_{28}H_{32}Cl_2N_4O\cdot 4HCl\cdot 4.8H_2O$) C, H, N.

N1-(4-{2-[Bis(2-chloroethyl)amino]ethoxy}acridin-9-yl)-4-methoxybenzene-1,3-diamine Hydrochloride (29a). Compound **29a** was prepared from **11** (759 mg, 2 mmol) and 4-methoxyphenylene-1,3-diamine dihydrochloride (**22**, 422 mg, 2 mmol): yield 616 mg (62%); mp 195−196 °C; ^{1}H NMR (DMSO-d_6) δ 3.79 (4H, t, J = 8.7 Hz, 2 × NCH_2), 3.91 (3H, s, OMe), 3.97 (2H, brs, CH_2N), 4.20 (4H, t, J = 8.2 Hz, 2 × CH_2Cl), 4.72 (2H, t, J = 8.2 Hz, OCH_2), 7.08−7.18 (2H, m, ArH), 7.35 (1H, m, ArH), 7.39−7.46 (2H, m, ArH), 7.57 (1H, m, ArH), 7.98 (2H, m, ArH), 8.32 (1H, m, ArH), 8.94 (1H, m, ArH), 11.87 (1H, brs, exchangeable, NH), 13.55 (1H, brs, exchangeable, NH). Anal. ($C_{26}H_{28}Cl_2N_4O_2\cdot 4HCl\cdot 1.5H_2O$) C, H, N.

N1-(4-{4-[Bis(2-chloroethyl)amino]butoxy}acridin-9-yl)-4-methoxybenzene-1,3-diamine Hydrochloride (29c). Compound **29c** was prepared from **15** (1.22 g, 3 mmol) and **22** (633 mg, 3.0 mmol): yield 499.5 mg (31.6%); mp 195−197 °C. ^{1}H NMR (DMSO-d_6) δ 2.04 (4H, m, CH_2CH_2), 3.53 (2H, t, J = 8.7 Hz, CH_2N), 3.58 (4H, t, J = 8.7 Hz, 2 × NCH_2), 3.89 (3H, s, OMe), 4.13 (4H, t, J = 8.2 Hz, 2 × CH_2Cl), 4.38 (2H, t, J = 8.2 Hz, OCH_2), 7.04 (1H, m, ArH), 7.11 (1H, m, ArH), 7.24 (1H, m, ArH), 7.37 (1H, m, ArH), 7.44 (1H, m, ArH), 7.53 (1H, m, ArH), 7.88 (1H, m, ArH), 7.96 (1H, m, ArH), 8.31 (1H, m, ArH), 8.78 (1H, m, ArH), 11.72 (2H, brs, exchangeable, NH_2), 13.27 (1H, brs, exchangeable, NH). Anal. ($C_{28}H_{32}Cl_2N_4O_2\cdot 6.7HCl\cdot 1.6H_2O$) C, H, N.

[3-(4-{2-[Bis(2-chloroethyl)amino]ethoxy}acridin-9-ylamino)-5-hydroxymethylphenyl]carbamic Acid Ethyl Ester (24). To a mixture of **23a** (500 mg, 1.0 mmol) in DMF (15 mL) and pyridine (0.096 mL, 1.2 mmol) was added dropwise ethyl chloroformate (0.12 mL, 1.2 mmol) at 0 °C. After being stirred for 50 min in ice bath, the reaction mixture was evaporated in vacuo to dryness, and the product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH, 5:1 v/v). The product was recrystallized from ethanol/hexane/acetone to give 497 mg (72%): mp 151−152 °C. ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.20 (3H, s, CH_3), 3.02 (4H, brs, 2 × NCH_2), 3.17 (2H, brs, CH_2N), 3.68 (4H, brs, 2 × CH_2Cl), 4.06 (2H, brs, OCH_2), 4.24 (2H, s, CH_2), 4.38 (2H, s, $ArCH_2$), 6.37 (1H, s, ArH), 6.75 (1H, s, ArH), 6.9−7.2 (3H, m, ArH), 7.2−7.9 (4H, m, ArH), 8.15 (1H, brs, ArH), 9.49 (1H, s, NH). Anal. ($C_{29}H_{32}Cl_2N_4O_4$) C, H, N.

[2-(Acridin-4-yloxy)ethyl]-bis(2-chloroethyl)amine Hydrochloride (31a). By following the same procedure as that for **11**, compound **31a** was prepared from 4-hydroxyacridine[27] (**30**, 1.17 g, 6.0 mmol), tris(2-chloroethyl)amine hydrochloride (1.74 g, 7.2 mmol), KF (348 mg, 6 mmol), and K_2CO_3 (4.14 g, 30 mmol): yield 0.154 g (7.1%); mp 156−157 °C; ^{1}H NMR (DMSO-d_6) δ 3.84 (4H, t, J = 8.1 Hz, 2 × NCH_2), 3.99 (2H, t, J = 6.1 Hz, NCH_2), 4.29 (4H, t, J = 8.2 Hz, 2×CH_2Cl), 4.80 (2H, t, J = 6.1 Hz, OCH_2), 7.75 (1H, m, ArH), 7.85 (1H, m, ArH), 7.93 (1H, m, ArH), 8.09 (1H, m, ArH), 8.27 (1H, m, ArH), 8.50 (1H, m, ArH), 9.03 (1H, m, ArH). Anal. ($C_{19}H_{20}Cl_2N_2O\cdot HCl\cdot 1.1H_2O$) C, H, N.

4-(3-Bromopropoxy)acridine (32b). By following the same procedure as that for **13**, compound **32b** was prepared from **30** (2.92 g, 15 mmol), 1,3-dibromopropane (15.3 mL, 150 mmol), and K_2CO_3 (4.14 g, 30 mmol) in DMF: yield 3.45 g (73%); mp 117−119 °C; ^{1}H NMR ($CDCl_3$) δ 2.64 (2H, m, CH_2), 3.79 (2H, t, J = 6.8 Hz, CH_2Br), 4.47 (2H, t, J = 6.8 Hz, OCH_2), 7.10 (1H, m, ArH), 7.44 (1H, m, ArH), 7.54 (1H, m, ArH), 7.60 (1H, m, ArH), 7.76 (1H, m, ArH), 7.98 (1H, m, ArH), 8.35 (1H, m, ArH), 8.73 (1H, m, ArH). Anal. ($C_{16}H_{14}BrNO\cdot 0.8H_2O$) C, H, N.

4-(4-Bromobutoxy)acridine (32c). By following the same procedure as that for **13**, compound **32c** was prepared from **30** (1.17 g, 6.0 mmol), 1,3-dibromobutane (2.3 mL, 19 mmol), and K_2CO_3 (1.17 g, 6 mmol) in DMF: yield 0.95 g (48%); mp 82−83 °C; ^{1}H NMR ($CDCl_3$) δ 2.23 (4H, m, 2 × CH_2), 3.36 (2H, t, J = 5.5 Hz,

OCH_2), 4.36 (2H, t, $J = 5.2$ Hz, CH_2Br), 7.05 (1H, m, ArH), 7.43 (1H, m, ArH), 7.54 (1H, m, ArH), 7.58 (1H, m, ArH), 7.76 (1H, m, ArH), 7.98 (1H, m, ArH), 8.35 (1H, m, ArH), 8.72 (1H, m, ArH). Anal. ($C_{17}H_{16}BrNO$) C, H, N.

2-[[3-(Acridin-4-yloxy)propyl]-(2-hydroxyethyl)amino]ethanol (33b). By following the same procedure as that for **14**, compound **33b** was prepared from **32b** (3.16 g, 10 mmol) and diethanolamine (3.15 g, 30 mmol), yield 2.64 g (79%) as syrup: ^{1}H NMR (DMSO-d_6) δ 2.04 (2H, m, CH_2), 2.59 (4H, brs, 2 × NCH_2), 2.77 (2H, brs, NCH_2), 3.48 (4H, brs, 2 × CH_2OH), 4.29 (2H, brs, OCH_2), 4.38 (2H, brs, exchangeable, 2 × OH), 7.21 (1H, m, ArH), 7.51 (1H, m, ArH), 7.63 (1H, m, ArH), 7.67 (1H, m, ArH), 7.84 (1H, m, ArH), 8.15 (1H, m, ArH), 8.21 (1H, m, ArH), 8.32 (1H, m, ArH). Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

2-[[4-(Acridin-4-yloxy)butyl]-(2-hydroxyethyl)amino]ethanol (33c). By following the same procedure as that for **14**, compound **33c** was prepared from **32c** (1.32 g, 4.0 mmol) and diethanolamine (1.26 g, 13 mmol): yield 0.98 g (69%) as syrup. ^{1}H NMR (DMSO-d_6) δ 1.66 (2H, m, CH_2), 1.94 (2H, brs, CH_2), 2.59 (4H, brs, 2 × NCH_2), 2.61 (2H, brs, NCH_2), 3.48 (4H, brs, 2 × CH_2OH), 4.24 (2H, brs, OCH_2), 4.38 (2H, brs, exchangeable, 2 × OH), 7.21 (1H, m, ArH), 7.51 (1H, m, ArH), 7.63 (1H, m, ArH), 7.67 (1H, m, ArH), 7.84 (1H, m, ArH), 8.15 (1H, m, ArH), 8.21 (1H, m, ArH), 8.32 (1H, m, ArH). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N.

[3-(Acridin-4-yloxy)propyl]-bis(2-chloroethyl)amine Hydrochloride (31b). By following the same procedure as that for **15**, compound **31b** was prepared from **33b** (840 mg, 2.24 mmol), methanesulfonyl chloride (0.4 mL, 5.2 mmol), and triethylamine (0.86 mL, 6.2 mmol): yield 368 mg (40%); mp 148−149 °C; ^{1}H NMR (DMSO-d_6) δ 2.48 (2H, m, CH_2), 3.68 (6H, m, 3 × NCH_2), 4.17 (4H, t, $J = 8.8$ Hz, 2 × CH_2Cl), 4.51 (2H, t, $J = 7.3$ Hz, OCH_2), 7.67 (1H, m, ArH), 7.79 (1H, m, ArH), 7.89 (1H, m, ArH), 8.01 (1H, m, ArH), 8.22 (1H, m, ArH), 8.45 (1H, m, ArH), 9.09 (1H, m, ArH). Anal. ($C_{20}H_{22}Cl_2N_2O \cdot 1.8HCl \cdot 1.5H_2O$) C, H, N.

[4-(Acridin-4-yloxy)butyl]-bis(2-chloroethyl)amine Hydrochloride (31c). By following the same procedure as that for **15**, compound **31c** was prepared from **33c** (3.19 g, 27 mmol), methanesulfonyl chloride (1.74 mL, 22.5 mmol), and triethylamine (3.76 mL, 27 mmol): yield 1.76 g (50%); mp 136−137 °C. ^{1}H NMR (DMSO-d_6) δ 2.08 (4H, m, 2×CH_2), 3.40 (2H, m, NCH_2), 3.59 (4H, t, $J = 8.4$ Hz, 2 × NCH_2), 4.12 (4H, t, $J = 8.4$ Hz, 2 × CH_2Cl), 4.45 (2H, t, $J = 7.3$ Hz, OCH_2), 7.68 (1H, m, ArH), 7.79 (1H, m, ArH), 7.89 (1H, m, ArH), 8.00 (1H, m, ArH), 8.21 (1H, m, ArH), 8.45 (1H, m, ArH), 9.04 (1H, m, ArH). Anal. ($C_{21}H_{24}Cl_2N_2O \cdot 2HCl \cdot 0.4H_2O$) C, H, N.

Biological Experiments. Cytotoxicity Assays. The effects of the compounds on cell growth were determined in T-cell acute lymphocytic leukemia CCRF-CEM) and human solid tumor cells (i.e., lung adenocarcinoma A549, colon carcinoma HCT-116, and breast carcinoma MX-1), in a 72 h incubation, by XTT-tetrazolium assay[30] and by sulforhodamine B method,[31] respectively. After the addition of phenazine methosulfate-XTT solution at 37 °C for 6 h, absorbance at 450 and 630 nm was detected on 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,[32,33] using a computer program on an IBM-PC workstation.[34]

In Vivo Studies. Athymic nude mice bearing the nu/nu gene were used for human breast tumor MX-1, human T cell acute lymphoblastic leukemia CCRF-CEM, human colon carcinoma HCT-116, and human ovarian adenocarcinoma SK-OV-3 xenografts. Outbred Swiss-background mice were obtained from Charies River Breeding Laboratories. Male mice 8 weeks old or older weighing 22 g or more were used for most experiments. Drug was administrated via the tail vein by iv injection. Tumor volumes were assessed by measuring length × width × height (or width) by using caliper. Vehicle used was 20 μL of DMSO in 180 μL of saline. All animal studies were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Animals and the protocol approved by the Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee.

Topoisomerase II-Mediated DNA Cleavage Assay. Topo II-mediated DNA cleavages were determined by following the procedure described previously.[35] The reaction mixture (20 μL each) containing 40 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 1 mM ATP, 30 μg/mL bovine serum albumin, 20 ng of 3′-end ^{32}P-labeled YEpG DNA, 10 ng of purified hTopo IIα, and a test compound was incubated at 37 °C for 30 min. The reactions were terminated by addition of 5 μL of a solution containing 5% SDS and 1 mg/mL proteinase K, followed by incubation for an additional 60 min at 37 °C. DNA samples were electrophoresed in 1% agarose gel containing 0.5× TPE buffer. Gels were dried onto Whatman 3MM chromatographic paper and autoradiographed at −80 °C using Kodak XAR-5 films.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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