Notes

6*H*-Pyrazolo[4,5,1-*de*]acridin-6-ones as a Novel Class of Antitumor Agents. Synthesis and Biological Activity

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The 7-substituted 6*H*-pyrazolo[4,5,1-*de*]acridin-6-ones with (aminoalkyl)amino and/or (hydroxyalkyl)amino groups in the side chains were synthesized by bromination using *N*-bromosuccinimide and the subsequent reaction with amines from the 7-substituted 5-bromo-2-methyl-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one. The substitution reaction of the amines with alkyl bromide (the C2 position) and aryl bromide (the C5 position) was accomplished by choosing the proper reaction conditions. These compounds show DNA intercalating ability in ethidium fluorescence assay and antiproliferative activity against Hela S_3 cells. Impressive antitumor activity in vivo against murine P388 leukemia and murine sarcoma 180 solid tumor in mice was demonstrated for the 7-hydroxy analogs. In addition, some of these showed excellent antitumor activity against adriamycin-resistant murine P388 leukemia (P388/ADM) in mice.

Introduction

A large number of antitumor agents are known to act by virtue of their ability to interact with DNA. One fundamental constraint is the requirement for efficient binding to DNA by intercalation, and the planar, polycyclic nucleus have been currently investigated as "DNA intercalating agents".[1] As common features, the compounds containing these coplanar chromophores also have one or two polymethylenediamine fragments as side chains bearing cationic charges for electrostatically binding to the phosphate moieties of DNA.[2]

One class of synthetic polycyclic aromatic compounds has been investigated by modifying the chromophore of the anthracendione nucleus (e.g., mitoxantrone), which includes anthrapyrazoles (e.g., CI-941)[3-5] and benzothiopyranoindazoles (e.g., CI-958),[6,7] and has resulted in high-level, broad-spectrum activity in preclinical models.

On the other hand, 9-amino-substituted acridine compounds have been shown to bind to DNA by intercalation and possess antitumor activities.[8] Amsacrine was the first synthetic DNA intercalator to find widespread clinical use,[9] and nitracine has been used clinically in Poland.[1,2] Their major end products of metabolism in vivo are the corresponding acridones.[1] Noting this, a variety of acridone analogs and acridine/acridone chromophore modified compounds such as imidazoacridone,[10,11] triazoloacridone,[12] and pyrazoloacridine (e.g., PD 115,934)[13,14] were prepared and have been studied for antitumor activity.

On the basis of these reports, we designed 6*H*-pyrazolo[4,5,1-*de*]acridin-6-ones, which we might abbreviate as pyrazoloacridones, a new class of DNA intercalators. In a study of anthrapyrazoles, the added pyrazole ring, forming a modified quinonimine, was assumed to increase the electron density of the π system of the chromophore and to make the chromophore more resistant to the enzymatic reduction to radical species.[15,16] This result also encouraged us to provide pyrazoloacridones as agents with diminished cardiotoxicity. Whereas pyrazoloacridones also have the pyrazole ring system, they have no quinon-

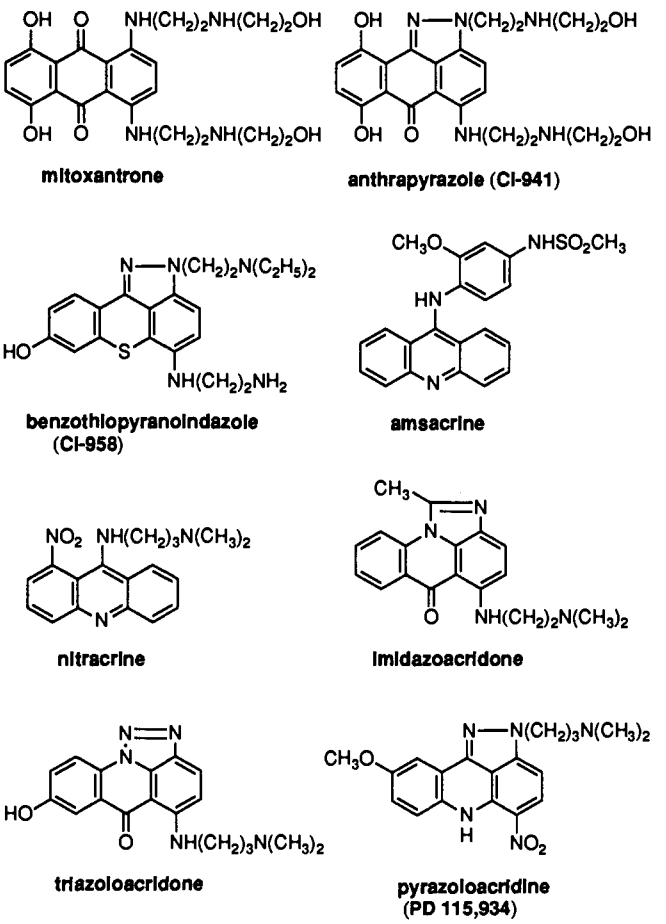

Figure 1.

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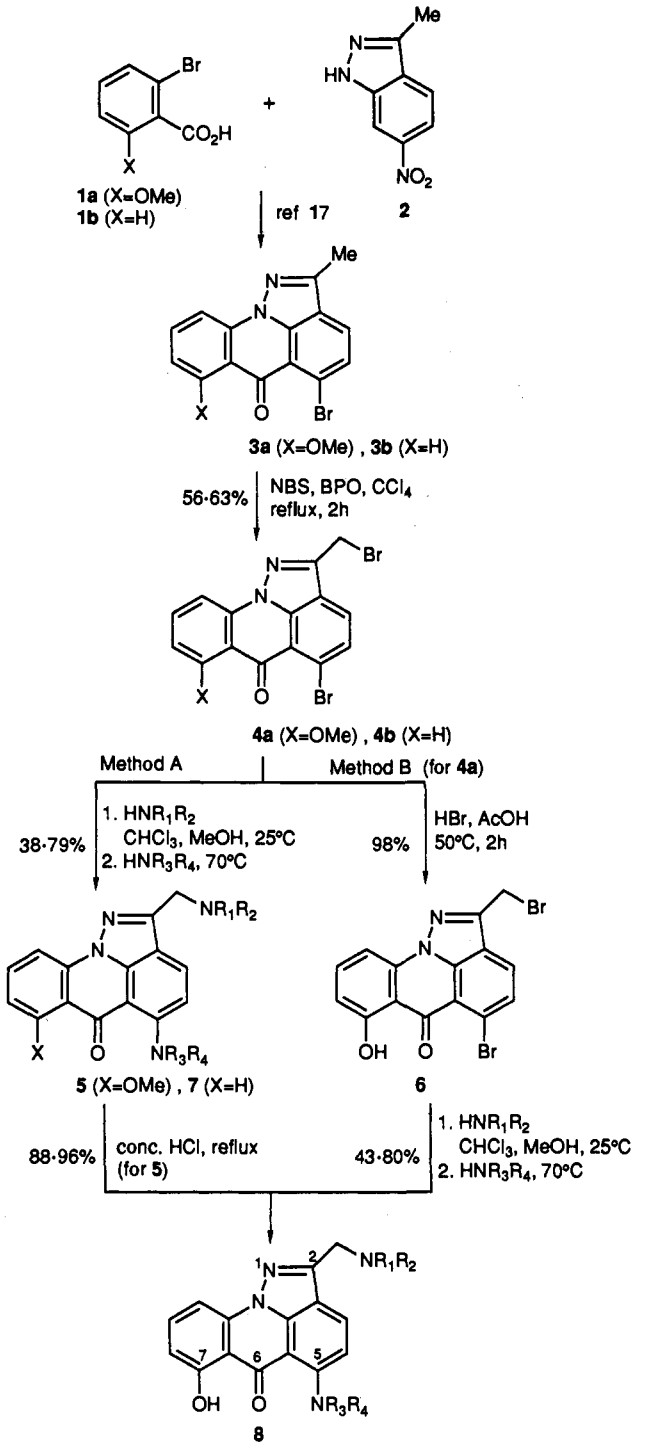
Scheme 1



imine-like structures (see Scheme 1). Thus, the chromophore of pyrazoloacridone seems more resistant to enzymatic reduction to radical species than the anthrapyrazole chromophore.

We synthesized and tested for antitumor activity of the pyrazoloacridones with alkylamino, (aminoalkyl)amino, and/or (hydroxyalkyl)amino side chains at the C2 and C5 positions.

Chemistry

We have already found a facile preparation of 5-bromo-7-methoxy-2-methyl-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one (**3a**) from 2-bromo-6-methoxybenzoic acid (**1a**) and 3-methyl-6-nitroindazole (**2**) using Ullmann coupling reaction followed by Friedel–Crafts cyclization.[17] The C7-unsubstituted compound **3b** was prepared from 2-bromobenzoic acid (**1b**) and the indazole **2** in the same way.

The whole synthetic pathway from **3** is demonstrated in Scheme 1. The bromination of the pyrazoloacridone **3** with *N*-bromosuccinimide catalyzed by benzoyl peroxide gave the brominated compound **4**. Although the yield was rather low because of the dibromination, the monobrominated compounds, **4a** and **4b**, were purified by chromatography on silica gel followed by recrystallization from chloroform–carbon tetrachloride in 63% and 56% yields, respectively.

Selective substitutions with amines at the C2 alkyl bromide and C5 aryl bromide were controlled by choosing the proper reaction conditions. Thus, at room temperature, a primary or secondary amine reacted predominantly with the C2 alkyl bromide in the solution of chloroform and methanol. When the (aminoalkyl)amine was used, a high dilution was necessary for avoiding bis substitution. The C5 aryl bromide was then substituted with another amine at 70 °C without solvents. The same amine was simultaneously introduced to both C2 and C5 using an excess amount of amine in chloroform under reflux. The 7-unsubstituted compounds with the same side chains were prepared from **4b** using this method.

For preparing the 7-hydroxy compounds, the methyl ethers **5** were cleaved in boiling concentrated hydrochloric acid after introducing the amino groups at C2 and C5 (method A). By this method, the severe condition during the demethylation gave several byproducts. Thus, we intended to proceed with the demethylation before introducing amino side chains (method B); it would be helpful to prepare 7-hydroxypyrazoloacridones bearing a variety of side chains from the same key intermediate.

The treatment of **4a** with ethyl mercaptan and anhydrous aluminum chloride in boiling chloform gave **6** in 80% yield, but the workup process was very complicated. The demethylation of **4a** with 25% hydrogen bromide in acetic acid at 50 °C gave **6** almost quantitatively. Two amino side chains were introduced separately to C2 and C5 using the method previously described. All the resulting free bases were treated with the diluted hydrochloric acid or the 2-propanol solution of dry hydrogen chloride to afford hydrochloride salts.

Results and Discussion

The relative binding affinity of the pyrazoloacridones to DNA was determined by the reduction in fluorescence of an ethidium–DNA complex in the presence of drugs as previously described.[18] The antiproliferative activity of the drugs was evaluated using Hela S_3 cells. The evaluation of antitumor activity in vivo was performed on murine P388 leukemia, murine sarcoma 180 solid tumor, and adriamycin-resistant murine P388 leukemia (P388/ADM).

In an ethidium-binding assay, the pyrazoloacridones are generally potent DNA binders as expected. (Aminoalkyl)-amino and/or (hydroxyalkyl)amino side chains at the C2 and C5 positions are apparently crucial for the activity[19] like other synthetic antineoplastic compounds designated as "DNA intercalating agents".[3-8,10-14]

The hydroxy or methoxy substituents at C7 show only a slight influence on the binding affinity to DNA. However, the 7-hydroxypyrazoloacridones apparently show higher antiproliferative activity in vitro against Hela S_3 cells than the unsubstituted and the 7-methoxy

Table 1. Physical Properties and Cytotoxic Activity of Pyrazoloacridones

compd[a]	NR_1R_2	NR_3R_4	X	method[b]	formula[c]	yield[d] (%)	mp[e] (°C)	ethidium displacement IC_{50} (μM)	in vitro Hela S_3 IC_{50}[f] (μM)
5a	$NH(CH_2)_2NH_2$	$NH(CH_2)_2NH_2$	OCH_3		$C_{20}H_{24}N_6O_2 \cdot 3HCl \cdot 2.4H_2O$	79[g]	254–256	0.18	24
5b	$NH(CH_2)_2N(CH_3)_2$	$NH(CH_2)_2N(CH_3)_2$	OCH_3		$C_{24}H_{32}N_6O_2 \cdot 3HCl \cdot 3H_2O$	38[g]	229–230	0.16	28
7a	$NH(CH_2)_2NH_2$	$NH(CH_2)_2NH_2$	H		$C_{19}H_{22}N_6O \cdot 3.3HCl \cdot H_2O$	40[h]	>300	0.13	0.83
7b	$NH(CH_2)_2N(CH_3)_2$	$NH(CH_2)_2N(CH_3)_2$	H		$C_{23}H_{30}N_6O \cdot 3.3HCl \cdot 4.2H_2O$	57[h]	250–251	0.26	2.0
8a	$NH(CH_2)_2NH_2$	$NH(CH_2)_2NH_2$	OH	A	$C_{19}H_{22}N_6O_2 \cdot 3HCl \cdot 2.1H_2O$	75[g]	240–242	0.17	0.021
8b	$NH(CH_2)_2N(CH_3)_2$	$NH(CH_2)_2N(CH_3)_2$	OH	A	$C_{23}H_{30}N_6O_2 \cdot 3.3HCl \cdot 3.6H_2O$	35[g]	206–209	0.16	0.6
8c	$NHCH_2CH_3$	$NH(CH_2)_2N(CH_3)_2$	OH	B	$C_{21}H_{25}N_5O_2 \cdot 2.2HCl \cdot 3.2H_2O$	55[i]	284–286	0.11	0.0076
8d	$NH(CH_2)_2OH$	$NH(CH_2)_2NH_2$	OH	B	$C_{19}H_{21}N_5O_3 \cdot 2HCl \cdot 0.7H_2O$	59[i]	287–289	0.14	0.0034
8e	$NH(CH_2)_2OH$	$NH(CH_2)_3NH_2$	OH	B	$C_{20}H_{23}N_5O_3 \cdot 2HCl \cdot 0.9H_2O$	57[i]	278–280	0.13	0.052
8f	$NH(CH_2)_2OH$	$NH(CH_2)_4NH_2$	OH	B	$C_{21}H_{25}N_5O_3 \cdot 2.1HCl \cdot 2.7H_2O$	36[i]	267–269	0.19	0.11
8g	$NH(CH_2)_2OH$	$NH(CH_2)_2N(CH_3)_2$	OH	B	$C_{21}H_{25}N_5O_3 \cdot 2HCl \cdot 2.2H_2O$	55[i]	227–230	0.18	0.0059
8h	$NH(CH_2)_2OH$	$NH(CH_2)_2NH(CH_2)_2OH$	OH	B	$C_{21}H_{25}N_5O_4 \cdot 2HCl \cdot 2.1H_2O$	65[i]	158–161	0.14	0.0079
8i	$NH(CH_2)_2OH$	$NH(CH_2)_3OH$	OH	B	$C_{20}H_{22}N_4O_4 \cdot HCl \cdot 0.7H_2O$	43[i]	253–255	1.05	0.096
8j	$NH(CH_2)_3OH$	$NH(CH_2)_2NH_2$	OH	B	$C_{20}H_{23}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$[j]	49[i]	270–272	0.14	0.0077
8k	$NH(CH_2)_4OH$	$NH(CH_2)_2NH_2$	OH	B	$C_{21}H_{25}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$	51[i]	>300	0.17	0.014
8l	$N[(CH_2)_2OH]_2$	$NH(CH_2)_2N(CH_3)_2$	OH	A	$C_{23}H_{29}N_5O_4 \cdot 2HCl \cdot 1.8H_2O$	66[g]	243–245	0.42	0.079
8m	1-morpholino	$NH(CH_2)_3NH_2$	OH	B	$C_{22}H_{25}N_5O_3 \cdot 2.2HCl \cdot 2.6H_2O$[k]	80[i]	262–264	0.89	0.036

[a] The compounds listed are hydrochlorides of the compounds listed in Scheme 1. [b] See Scheme 1. [c] Microanalyses are within ±0.4% of theoretical values for C, H, N. [d] Yields reported refer to the hydrochlorides. [e] All hydrochlorides were recrystallized. See the Experimental Section. [f] Adriamycin was used as positive control. IC_{50}: 0.028 μM. [g] Yield from 4a. [h] Yield from 4b. [i] Yield from 6. [j] Water content: calcd 1.56%, found 1.66%. [k] Water content: calcd 8.76%, found 8.57%.

Table 2. Antitumor Activity of Pyrazoloacridones against Murine P388 Leukemia, Murine P388 Leukemia Resistant to ADM (P388/ADM), and Murine Sarcoma 180 Solid Tumor

	P388 leukemia		P388/ADM leukemia		sarcoma 180	
compd	opt dose, mg/kg per inj	T/C (%)	opt dose, mg/kg per inj	T/C (%)	opt dose, mg/kg per inj	T/C (%)
5a	25	139		NT[b]		NT[b]
7a	38	150		NT[b]		NT[b]
8a	13	>254(4)[a]	13	138	25	12
8c	3.1	196	3.1	126		NT[b]
8d	1.6	>222(2)[a]	1.6	152	6.3	6
8e	13	>230(3)[a]	6.3	156	6.0	21
8f	25	161	50	112		NT[b]
8g	6.3	>261(3)[a]	3.1	144	3.1	9
8h	3.1	>208(2)[a]	6.3	145	12	5
8i	13	152	50	123		NT[b]
8j	3.1	>181(1)[a]	3.1	146		NT[b]
8k	3.1	146	6.3	126		NT[b]
8l	25	>286(5)[a]	25	134	13	42
8m	13	>278(5)[a]	13	115	13	33
adriamycin	7.5	>254(4)[a]	7.5	116	7.5	31

[a] Numbers in parentheses indicate the number of 30-day survivors in a group of five mice. [b] NT: not tested.

compounds (Table 1, **8a** vs **7a**, **5a**). Also, the 7-hydroxy compound **8a** shows higher antitumor activity in vivo against murine P388 leukemia than **7a** and **5a** (Table 2). It is worth noting that the 7-hydroxy group is necessary for high antitumor activity in vivo. The enhancement of antitumor activity in vivo by chromophore hydroxylation also has been observed in other classes of acridones,[11-14] however, the role of the hydroxy group still remains unknown.

Thirteen compounds of the 7-hydroxy derivatives bearing side chains (Table 1, **8a–m**) were synthesized. The superior activities of the compounds containing $CH_2NH(CH_2)_mOH$ at C2 and $NH(CH_2)_nNRR'$ at C5 against P388 and P388/ADM leukemias are shown with a lower dose amount than adriamycin. It seems that the number of *m* and *n* units increases the activity according to the following pattern, 2 > 3 > 4; thus, **8d** is more active than **8e** and **8f**, and further, **8d** is a little more active than **8j** and **8k**. The compounds **8a**, **8l**, and **8m** need a slightly greater dose amount than adriamycin, but their activity against P388 and P388/ADM leukemias is excellent and almost all tested mice survived 30 days during the test using P388 leukemia.

These compounds also show superior activity against sarcoma 180 solid tumor; **8d**, **8e**, **8g**, and **8h** especially are more effective than adriamycin with nearly the same dose amount.

The pyrazoloacridone was designed to be a new type drug with no quinonimine-like structures and resistant to the enzymatic reduction. Now, in our laboratories, studies of pulse radiolysis of pyrazoloacridones as well as redox biochemistry using liver microsome are intended, and these results will be reported elsewhere.

Conclusions

Our results indicate that the substituted 6*H*-pyrazolo[4,5,1-*de*]acridin-6-ones constitute a novel class of antitumor agents. They show DNA intercalating ability, antiproliferative activity in vitro against Hela S_3 cells, and significant antitumor activity in vivo against P388 leukemia and sarcoma 180 solid tumor. Concerning the structure–activity relationships, the 7-hydroxy and the (aminoalkyl)amino and (hydroxyalkyl)amino side chains at C2 and C5 play an important role, as has been observed in other classes of DNA intercalating agents. Some of these show excellent antitumor activity in vivo with a lower dose amount than adriamycin, and they are also effective against P388/ADM leukemia.

Experimental Section

Melting points were determined with a Yanagimoto hot-stage microscope and are uncorrected. ^1H NMR spectra were recorded on a JEOL JNM-GX270 spectrometer using TMS as an internal

standard. Mictroanalytical results, indicated by atomic symbols, are within ±0.4% of the theoretical values and were recorded on a Yanaco MT-3 CHN corder. Chromatography was performed with E. Merck silica gel 60 (230–400 mesh) by the method of Still et al.[20] All the samples were dried at 40 °C in vacuo (0.5–1.0 mmHg) for 24 h. The water content of the final compounds was measured by Fischer titrations with a Kyoto Electronics MKA-3p moisture meter. The amount of sample for the measurement was 9–18 mg. The purity of the samples was checked by HPLC analysis, and the content of hydrogen chloride was determined by ion chromatography in addition to the combustion analysis.

5-Bromo-2-(bromomethyl)-7-methoxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one (4a). A mixture of **3a** (18.7 g, 54.5 mmol),[17] *N*-bromosuccinimide (15 g, 84.3 mmol), and benzoyl peroxide (5 g, 20.7 mmol) in 3 L of CCl_4 was heated under reflux for 2 h, to the mixture was added 5 g (28.1 mmol) of *N*-bromosuccinimide, and the mixture was further heated for 13 h. After cooling, the precipitate was purified by chromatography on silica gel (eluent: $CHCl_3$/acetone, 100/1) followed by recrystallization from $CHCl_3$/CCl_4 to afford **4a** as yellow crystals (14.5 g, 63%): mp 251–252 °C; ^{1}H NMR (DMSO-d_6) δ 3.95 (s, 3H), 5.22 (s, 2H), 7.12 (dd, 1H, *J* = 1.1, 8.3 Hz), 7.74 (dd, 1H, *J* = 1.2, 8.2 Hz), 7.82 (t, 1H, *J* = 8.2 Hz), 7.84 (d, 1H, *J* = 8.2 Hz), 8.21 (d, 1H, *J* = 8.2 Hz). Anal. ($C_{16}H_{10}Br_2N_2O_2$) C, H, N.

5-Bromo-2-(bromomethyl)-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one (4b). The compound **4b** was obtained from **3b** (1 g, 3.19 mmol) by a procedure similar to that described above, as yellow crystals (0.71 g, 56%): mp 204–205 °C; ^{1}H NMR ($CDCl_3$) δ 4.93 (s, 2H), 7.48 (dt, 1H, *J* = 1.1, 8.1 Hz), 7.81 (d, 1H, *J* = 8.4 Hz), 7.83 (dt, 1H, *J* = 1.5, 8.2 Hz), 8.05 (d, 1H, *J* = 8.4 Hz), 8.22 (dd, 1H, *J* = 1.1, 8.2 Hz), 8.50 (dd, 1H, *J* = 1.5, 8.1 Hz). Anal. ($C_{15}H_8Br_2N_2O$) C, H, N.

5-[(2-Aminoethyl)amino]-2-[[(2-aminoethyl)amino]methyl]-7-methoxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one Hydrochloride (5a). A mixture of **4a** (100 mg, 0.24 mmol) and 2 mL of 1,2-diaminoethane in 10 mL of $CHCl_3$ was heated under reflux for 1 h. To the mixture was added 10 mL of MeOH, and then the mixture was further heated for 1 h. After cooling, the solvent was evaporated and the residue was dissolved in MeOH and treated with HCl in *i*-PrOH. The precipitate was collected and recrystallized from aqueous MeOH to afford **5a** as yellow crystals (100 mg, 79%): mp 254–256 °C; ^{1}H NMR (DMSO-d_6) δ 3.12 (m, 2H), 3.30–3.48 (m, 4H), 3.83 (m, 2H), 3.93 (s, 3H), 4.72 (s, 2H), 7.09 (dd, 1H, *J* = 1.2, 5.6 Hz), 7.12 (d, 1H, *J* = 9.3 Hz), 7.81–7.83 (m, 2H), 8.29 (d, 1H, *J* = 9.1 Hz). Anal. ($C_{20}H_{24}N_6O_2$·3HCl·2.4H_2O) C, H, N. Water content: calcd 8.10%, found 8.42%.

Compound **5b** was prepared by a procedure similar to that described above, and the result is shown in Table 1.

5-Bromo-2-(bromomethyl)-7-hydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one (6). A mixture of **4a** (16.3 g, 38.6 mmol) and 600 mL of 25% HBr in AcOH was heated at 50 °C for 12 h. The solution was poured into water, and the precipitate was collected and washed with *i*-PrOH to afford **6** as a yellow solid (15.4 g, 98%): mp 217–219 °C; ^{1}H NMR ($CDCl_3$) δ 4.90 (s, 2H), 6.90 (dd, 1H, *J* = 1.5, 8.1 Hz), 7.59 (dd, 1 H, *J* = 1.2, 8.1 Hz), 7.67 (t, 1 H, *J* = 8.1 Hz), 7.78 (d, 1 H, *J* = 8.3 Hz), 8.03 (d, 1 H, *J* = 8.3 Hz), 13.02 (s, 1 H). Anal. ($C_{15}H_8Br_2N_2O_2$) C, H, N.

5-[(2-Aminoethyl)amino]-2-[[(2-aminoethyl)amino]methyl]-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one Hydrochloride (7a). A mixture of **4b** (100 mg, 0.26 mmol) and 2 mL of 1,2-diaminoethane in 10 mL of $CHCl_3$ was heated under reflux for 1.5 h. After cooling, the solvent was evaporated and the residue was dissolved in MeOH and treated with HCl in *i*-PrOH. The precipitate was collected and recrystallized from aqueous MeOH to afford **7a** as yellow crystals (51 mg, 40%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.10–3.48 (m, 6H), 3.87 (m, 2H), 4.77 (s, 2H), 7.22 (d, 1H, *J* = 9.0 Hz), 7.60 (dt, 1H, *J* = 0.9, 7.7 Hz), 7.98 (dt, 1H, *J* = 1.3, 7.6 Hz), 8.29 (dd, 1H, *J* = 1.0, 7.7 Hz), 8.41 (dd, 1H, *J* = 1.2, 7.6 Hz), 8.44 (d, 1H, *J* = 8.9 Hz). Anal. ($C_{19}H_{22}N_6O$·3.3HCl·H_2O) C, H, N. Water content: calcd 3.68%, found 3.43%.

Compound **7b** was prepared by a procedure similar to that described above, and the result is shown in Table 1.

5-[(2-Aminoethyl)amino]-2-[[(2-aminoethyl)amino]methyl]-7-hydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one Hydrochloride (8a). Method A. A suspension of **5a** (92 mg, 0.17 mmol) in 1.8 mL of concentrated HCl was heated under reflux for 11 h. Concentrated HCl was evaporated, and the residue was recrystallized from aqueous MeOH to afford **8a** as yellow crystals (85 mg, 96%): mp 240–242 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (m, 2H), 3.28–3.47 (m, 4H), 3.88 (m, 2H), 4.76 (s, 2H), 6.89 (dd, 1H, *J* = 1.0, 8.1 Hz), 7.22 (d, 1H, *J* = 9.3 Hz), 7.67 (dd, 1H, *J* = 0.9, 8.2 Hz), 7.81 (t, 1H, *J* = 8.2 Hz), 8.45 (d, 1H, *J* = 9.2 Hz), 13.71 (s, 1H). Anal. ($C_{19}H_{22}N_6O_2$·3HCl·2.1H_2O) C, H, N. Water content: calcd 7.36%, found 7.61%.

Compound **8b** was prepared from **5b** by a procedure similar to that described above, and the result is shown in Table 1.

5-[(2-Aminoethyl)amino]-2-[[(2-hydroxyethyl)amino]methyl]-7-hydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one Hydrochloride (8d). Method B. A mixture of **6** (500 mg, 1.23 mmol), 1.5 g of ethanolamine, 100 mL of $CHCl_3$, and 20 mL of MeOH was stirred at room temperature for 24 h. After water was added, the mixture was partitioned and the organic layer was washed with saturated brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel (eluent: $CHCl_3$/MeOH, 9/1), providing 5-bromo-2-[[(2-hydroxyethyl)amino]methyl]-7-hydroxy-6*H*-pyrazolo[5,4,1-*de*]acridin-6-one (345 mg, 75%).[21] A mixture of this compound (150 mg) and 1 mL of ethylenediamine was heated at 70 °C for 1 h. The mixture was condensed in vacuo, and the residue was recrystallized from a mixture of dilute HCl, MeOH, and *i*-PrOH to afford **8d** as yellow crystals (144 mg, 59% from **6**): mp 287–289 °C; ^{1}H NMR (DMSO-d_6) δ 3.07–3.13 (m, 2H), 3.19 (t, 2H, *J* = 5.2 Hz), 3.76 (t, 2H, *J* = 5.0 Hz), 3.88 (m, 2H), 4.70 (s, 2H), 6.89 (dd, 1H, *J* = 0.9, 8.2 Hz), 7.23 (d, 1H, *J* = 8.9 Hz), 7.66 (dd, 1H, *J* = 0.9, 8.2 Hz), 7.81 (t, 1H, *J* = 8.2 Hz), 8.41 (d, 1H, *J* = 8.9 Hz), 13.73 (s, 1H). Anal. ($C_{19}H_{21}N_5O_3$·2HCl·0.7H_2O) C, H, N. Water content: calcd 2.78%, found 2.61%.

Compounds **8c**, **8e–k**, and **8m** were prepared by a procedure similar to that described above, and the results are shown in Table 1.

2-[[Bis(2-hydroxyethyl)amino]methyl]-5-[[2-(dimethylamino)ethyl]amino]-7-hydroxy-6*H*-[4,5,1-*de*]acridin-6-one Hydrochloride (8l). Method A. A mixture of **4a** (200 mg, 0.47 mmol) and 0.25 g of diethanolamine in 27 mL of $CHCl_3$ was stirred at room temperature for 12 h. The precipitate was collected (176 mg)[22] and heated with 3 mL of *N*,*N*-dimethylethylenediamine at 70 °C for 1 h. The mixture was condensed in vacuo, the residue was triturated with *i*-PrOH, and the solvent was filtered off. The filtrate (159 mg)[22] was heated with 12 mL of concentrated HCl under reflux for 14 h. Then, by a procedure similar to that described in the synthesis of **8a**, **8l** was obtained as yellow crystals (167 mg, 66% from **4a**): mp 243–245 °C; ^{1}H NMR (DMSO-d_6) δ 2.86 (s, 6H), 3.36–3.43 (m, 6H), 3.91 (t, 4H, *J* = 4.9 Hz), 4.04 (m, 2H), 4.96 (s, 2H), 6.90 (dd, 1H, *J* = 0.9, 8.2 Hz), 7.27 (d, 1H, *J* = 9.2 Hz), 7.67 (dd, 1H, *J* = 0.9, 8.2 Hz), 7.81 (t, 1H, *J* = 8.2 Hz), 8.42 (d, 1H, *J* = 9.2 Hz), 13.66 (s, 1H). Anal. ($C_{23}H_{29}N_5O_4$·2HCl·1.8H_2O) C, H, N. Water content: calcd 5.95%, found 5.87%.

Biological Evaluation Procedures. Ethidium Fluorescence Assay. The relative affinity of the drugs to DNA was determined by the reduction in fluorescence of an ethidium–DNA complex in the presence of drugs as previously described.[18]

In Vitro Antiproliferative Activity. Hela S_3 cells were precultured in the culture medium supplemented with 10% fetal bovine serum for 24 h in 96-well microplates (Nunc, Roskilde, Denmark). Drugs were added to the plates in serial dilutions (*n* = 3), and the plates were incubated for another 72 h. The antiproliferative activity of the drugs was evaluated according to the neutral red dye-uptake method as previously described.[23]

Evaluation of Antitumor Activity. The antitumor activities of the drugs against ip-inoculated P388 cells and their adriamycin resistant line, P388/ADM cells, were evaluated by T/C (%), where [T] was mean survival days in a treated group and [C] was that in a control group by 60 days of observation. T/C (%) values > 130 are considered indicative of significant activity. The antitumor activity of the drugs against sc-inoculated sarcoma 180 cells were determined as previously described.[23] The criterion for effectiveness includes a treated versus control (T/C) value <50%.

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Supplementary Material Available: ^{1}H NMR data for final compounds (2 pages). Ordering information is given on any current masthead page.

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