Dr. Atassi, EORTC) or 10^6 P388 leukemia cells. The drugs were administered by the same route as a single dose 24 h after the inoculation of the leuekmia cells (10 animals per dose, 200 μL, and 20 animals for the control). In the case of compounds 4 and 6, which are less soluble than others compounds, we could not administer the highest dose ($LD_0/2$) in this volume. Dead mice were counted each day at the same hour. The comparison of the mean of the median survival time of the controls (C) (9 ± 0.3 days for L1210 and 9.5 ± 0.6 days for P388) and of the treated (T) animals allows us to estimate the antitumor activity of the tested compounds. Antitumor efficiency is expressed in term of ILS (increase in life span) over controls: (T − C) × 100/C. Survivors are not included in the ILS determination.

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Registry No. **1a**, 72237-98-0; **1b**, 72237-99-1; **2**, 115464-61-4; **3**, 115464-63-6; **4**, 115464-65-8; **5**, 115464-66-9; **6**, 115464-68-1; **7**, 115464-70-5; 8, 115464-71-6; **9**, 115464-73-8; H_2NEt, 75-04-7; H_2NPr, 107-10-8; H_2NBu-*i*, 78-81-9; $H_2N(CH_2)_2CH(CH_3)_2$, 107-85-7; $H_2N(CH_2)_9CH_3$, 2016-57-1.

Supplementary Material Available: Figure 3 containing hydrophobic properties of compounds **3–6** (2 pages). Ordering information is given on any current masthead page.

Synthesis and Antineoplastic Evaluation of 1,4-Bis(aminoalkanamido)-9,10-anthracenediones

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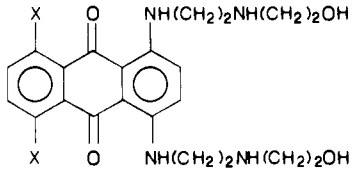
The effect of the replacement of amino groups, attached to the anthraquionone ring in [(aminoalkyl)amino]-anthraquinones, by an amido function on DNA binding, cytotoxicity, and antileukemic activity has been studied. The corresponding 1,4-bis(aminoalkanamido)-9,10-anthracenediones have been synthesized and examined. It has been concluded that such modification does not exclude the DNA binding and cytotoxicity of mentioned compounds but decreases or abolishes the in vivo antileukemic activity.

Among the anthraquinone derivatives with antitumor activity the 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)-amino]ethyl]amino]-9,10-anthracenedione (mitoxantrone, **1**) and its dideoxy analogue (ametantrone **2**) are the most promising as potential clinical drugs.[1-3] Mitoxantrone is



1: X = OH
2: X = H

active against a broad range of experimental tumors[3,4] and possibly less cardiotoxic than adriamycin.[3,5-9] At present, intercalative binding of mitoxantrone to DNA[1,9,10] is considered to be responsible for cytotoxic activity, but also metabolically generated free radical species[11] can contribute to the damage of DNA. However, the significantly lower rate of metabolic activation in comparison with anthracyclines may be correlated with the lower cardiotoxicity of this drug.[12] The encouraging properties of this compound have promoted many studies on structural modifications in order to obtain more active compounds and for a better understanding of molecular nature of their properties. Some of these studies have been directed toward developing new molecules with modified anthracenedione nuclei[13] and were based on the concept, previously applied to anthracyclines, that chromophore modifications might diminish cardiotoxicity by reducing the ability to mediate the electron transfer and thus decreasing the formation of reactive oxygen species.[17]

In our studies on the elucidation of the role of structural factors of natural and synthetic anthracenedione derivatives in their biological properties, attention has been drawn to the role of heteroatoms in 1,4-bis-substituted 9,10-anthracenediones side chains. In this paper we examine the influence of modification of properties of nitrogen atoms attached directly to the anthraquinone nucleus at positions 1 and 4. These heteroatoms exert significant effect on the anthraquinone moiety, influencing

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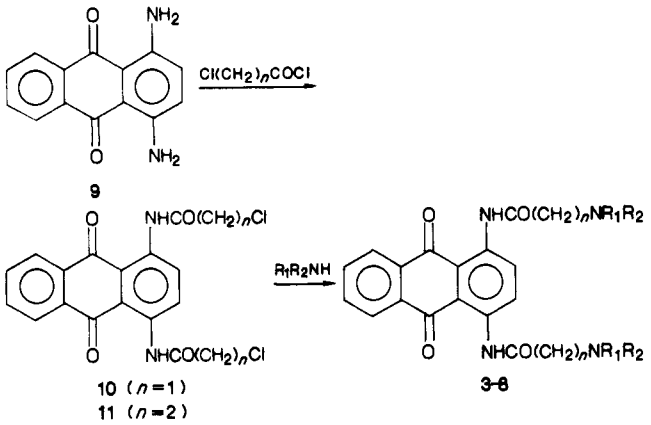
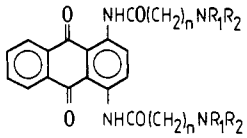
Scheme I



the electron density and thus affecting π-electron interaction with DNA bases and also electron-accepting ability of the quinonoid system, essential for the metabolic activation of the molecule. Therefore the heteroatoms bonded to the anthraquinone ring are important for the antileukemic activity and presumably the cardiotoxicity of the compounds.

We have synthesized and examined a series of 1,4-bis-substituted 9,10-anthracenediones in which the nitrogen atoms attached to the aromatic ring have lost their basic character as a result of amidation. This modification causes the strong diminishment of π-electron density in the quinone moiety.



3	n=1	R_1=H	R_2=CH_2CH_2OH	6	n=2	R_1=H	R_2=CH_3
4	n=2	R_1=H	R_2=CH_2CH_2OH	7	n=1	R_1=R_2=CH_3	
5	n=1	R_1=H	R_2=CH_3	8	n=2	R_1=R_2=CH_3	

The synthesized compounds were holding side chains with a basic terminal nitrogen atom and with one or two methylene groups between both nitrogen atoms. This structure of the side chain seems to be optimal, according to known requirements, for active [(aminoalkyl)amino]-9,10-anthracenediones.[1,18] The terminal nitrogen atoms of the side chains were substituted, like in active [(aminoalkyl)amino]anthraquinones,[1,2,19] by a hydroxyethyl, methyl, or dimethyl group.

The obtained compounds were examined for their in vitro cytotoxicity and selected substances were subjected to the determination of their in vivo antileukemic activity. Also, the ability of the studied compounds to interact with DNA was evaluated.

Some of (aminoalkanamido)anthraquinone derivatives were obtained previously,[2022] but in addition to the amido-linked side chains, other ring-attached functional groups increasing the π-electron density were also present in the molecule. Therefore these data did not allow any conclusions about the influence of the replacement of the amino group attached to the aromatic ring by the amido function on antitumor activity of the anthracenediones.

Chemistry

The synthetic procedure utilized for the preparation of compounds **3–8** is outlined in the Scheme I. Treatment of 1,4-diamino-9,10-anthracenedione (**9**) with an excess of chloroacetyl chloride or 2-chloropropionyl chloride in chloroform–dimethylacetamide or toluene solution, respectively, led to the corresponding 1,4-bis(ω-chloroalkanamido)-9,10-anthracenediones (**10** and **11**). Further condensation of **10** or **11** with the appropriate amines performed at 50–60 °C in chloroform–methanol or dimethylacetamide–methanol gave 1,4-bis[ω-(alkylamido)-alkanamido]-9,10-anthracenediones (**3–8**). The synthesis of **5–8** proceeded smoothly with good yields. However, in the reaction of 1,4-bis(ω-chloroalkanamido)-9,10-anthracenediones with 2-hydroxyethylamine, the substitution of the chlorine atom in the second ω-chloroalkanamido chain occurred very slowly, accompanied by simultaneous hydrolysis to 1-[(alkylamino)alkanamido]-4-amino- and -1,4-diamino-9,10-anthracenediones (**9**). This side reaction was especially marked during the condensation of 2-hydroxyethylamine with 1,4-bis(ω-chloroacetamido)-9,10-anthracenedione (**10**). The course of the reaction could be easily monitored by TLC because of great differences in the R_f values and various colors for the monosubstituted (purple) and disubstituted (yellow-orange) compounds. The structure of the byproducts was established by ^{1}H NMR, IR, and MS-FD spectral data as 1-[2-[(2-hydroxyethyl)amino]acetamido]-4-amino- and 1-[3-[(2-hydroxyethyl)amino]propionamido]-4-amino-9,10-anthracenedione for the reaction of **10** or **11** with 2-hydroxyethylamine, respectively.

Compounds **3–8** were purified by column chromatography (Sephadex LH-20). The structures of these compounds were supported by IR, ^{1}H NMR, and MS-FD spectral data and elemental analyses.

Biological Results and Discussion

All tested compounds caused significant inhibition of HeLa S_3 cell growth, comparable to that of ametantrone. The considerable inhibition of growth of more adequate model L1210 cells was also recorded, except for compounds **3** and **4**, which were inactive in this test. Thus, replacement of the amino group bonded directly to the anthraquinone ring by an amido function, resulting in diminishment of the electron-donating effect, does not generally abolish the cytotoxic activity and even can cause its increase (compounds **5**, **6**, and **8**).

Compounds with two methylene groups between the amido function and the basic nitrogen atom in the side chains are more active than ones having shorter alkyl moieties. These results extend the requirement of appropriate length of the alkyl moieties between both nitrogen atoms in the side arms of [(aminoalkyl)amino]-anthraquinones for optimal cytotoxic activity also to (aminoalkanamido)anthraquinones.

The compounds containing in the side chains methylamino or dimethylamino moieties (**5–8**) are distinctly more active. Poor or no activity of **3** and **4** with the ethanolamine moiety is contrary to the excellent activity of [(aminoalkyl)amino]anthraquinones having the same N-terminal residue (e.g., ametantrone and mitoxantrone).[2]

The determined ΔTm values for synthesized compounds in comparison with ametantrone (Table I) did not point

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Table I. Thermal Denaturation of Double-Helical DNA and Cells Growth Inhibition in the Presence of Tested Compounds

compd	ΔTm,[a] °C	EC_{50},[b] μg/mL L1210	EC_{50},[b] μg/mL HeLa S_3
2	14.9	1.77 (±0.47)	0.17 (±0.99)
3	8.3	>5	1.41 (±0.17)
4	8.8	>5	0.55 (±0.04)
5	9.0	0.79 (±0.04)	0.14 (±0.02)
6	nt[c]	1.28 (±0.23)	0.08 (±0.03)
7	10.0	2.26 (±0.33)	0.39 (±0.09)
8	15.3	0.36 (±0.09)	0.05 (±0.00)

[a] ΔTm = the difference between the melting temperature of DNA in the presence and absence of tested compounds. [b] EC_{50} = concentration of compound required to inhibit by 50% the growth of L1210 and HeLa S_3 cells. [c] Not tested.

Table II. Effect of (Aminoalkanamido)-9,10-anthracenedione Derivatives on Survival of Mice with P388 Murine Leukemia

compd	dose, mg/kg	% T/C[a]	tox D[b] Surv
2	25.00	200	6/6
	12.5	300	6/6
	6.25	210	6/6
	3.12	190	6/6
5	50.0	111	8/8
	25.0	100	8/8
	12.5	100	8/8
	6.25	111	8/8
	3.12	100	8/8
	1.5	100	8/8
6	40.0	toxic	8/8
	20.0	120	8/8
	10.0	130	8/8
	5.0	130	8/8
	2.5	120	8/8
	1.25	110	8/8
8	50.0	toxic	0/8
	25.0	toxic	6/8
	12.5	100	8/8
	6.25	100	8/8
	3.12	91	8/8
	1.6	91	8/8

[a] % T/C = ratio of medium survival time expressed as percent of untreated controls. [b] Tox D surv = survivor recorded on day 4 after day of first injection as a measure of drug toxicity.

to a clear-cut correlation between DNA binding and cytotoxicity with regard to L1210 leukemia. However, the most cytotoxic compound, 8, exhibited the highest ability to interact with DNA, comparable to that of ametantrone.

The compounds most cytotoxic against both L1210 and HeLa S_3 cells (5, 6, and 8) were evaluated in vivo for their antileukemic activity. The results are given in Table II. The obtained data allowed the following conclusions to be drawn. Regardless of the cytostatic activity in vitro of examined compounds higher than that of ametantrone, as well as their good ability to intercalate into DNA, they did not show any distinct antitumor activity in vivo. In higher doses they were toxic.

The conclusion can be made that for the antileukemic activity in vivo 1,4-bis-substituted anthracenedione derivatives should contain basic nitrogen atoms attached to the aromatic ring. However, this requirement is not indispensable for cytotoxic activity and for the ability to interact with DNA, providing that the side arms are of appropriate structure.

Experimental Section

Melting points determined on a Boetius PHMK 05 apparatus were uncorrected. Elemental analyses were performed by the Department of Elemental Microanalyses, Polish Academy of Science, Warsaw; the obtained results were within 0.4% of theoretical values. IR spectra were recorded on a UR 10 Zeiss spectrometer in KBr pellets; ^{1}H NMR spectra were recorded on Varian 90 MHz and Varian XL-300 (for compounds 10) spectrometers, with tetramethylsilane as the internal standard. Molecular weights were determined by mass spectrometry (field desorption technique) on a Varian Mat 711 instrument. The instrumental conditions were as follows: wire heating current 5–20 mA, ion source temperature 70–100 °C, accelerating voltage 4–6 kV. Column chromatography was performed on MN silica gel (35–70 mesh, Merck) and on Sephadex LH-20 (Pharmacia). The following TLC solvent systems were used: (A) benzene–acetone (2:1), (B) the same solvents (5:1), (C) 1-butanol–pyridine–acetic acid–water (8:2:3:5).

1,4-Bis(chloroacetamido)-9,10-anthracenedione (10). A sample of 1.2 g (5 mmol) of **9** in 15 mL of dimethylacetamide was cooled to 0 °C, and then 5 mL (60 mmol) of chloroacethyl chloride in 150 mL of chloroform was slowly added with vigorous stirring. The reaction mixture was further stirred for 1 h at room temperature and next evaporated in vacuo to a smaller amount. The product was precipitated by treatment of ethyl ether and then the filtrate washed carefully with ethyl ether. The crude product was purified by means of column chromatography (silica gel, eluent chloroform, 1 L) and next was crystallized from chloroform–ethyl ether to afford 1.3 g (yield 70%) of **10** as yellow-brown needles melting at 282–285 °C dec: ^{1}H NMR (Me_2SO-d_6) δ 4.58 (s, 4 H, $COCH_2$), 7.95 (m, 2 H, C-6, C-7), 8.24 (m, 2 H, C-5, C-8), 8.98 (s, 2 H, C-2, C-3), 12.8 (s, 2 H, NHCO, exchangeable with D_2O); IR 1595, 1650, 1695 cm^{-1}; MS-FD, m/z (relative intensity) 390 ([M − 1]$^+$, 100), 391 ([M]$^+$, 58), 392 ([M + 1]$^+$, 80). Anal. ($C_{18}H_{12}Cl_2N_2O_4$) C, H, N.

1,4-Bis(3-chloropropionamido)-9,10-anthracenedione (11). A sample of 120 mg (0.5 mmol) of **9** in 2 mL of toluene was cooled to 0 °C, and then 0.5 mL (5 mmol) of 3-chloropropionyl chloride in 5 mL of the same solvent and 0.07 mL (0.5 mmol) of NEt_3 were added. The reaction mixture was stirred for 15 h and additionally refluxed for 1 h. The progress of the reaction was monitored by TLC (solvent systems A and B). Then the solid was filtered off and washed with toluene very carefully. The filtrate was extracted with 3% NaCl solution and next was concentrated to a small volume in vacuo. The product (red-orange) was precipitated with ethyl ether–hexane: yield 310 mg (73%); mp (subl) 176–177 °C; ^{1}H NMR ($CDCl_3$) δ 3.0 (t, J = 6 Hz, 4 H, $COCH_2$), 3.9 (t, J = 6 Hz, CH_2Cl), 7.8 (m, 2 H, C-6, C-7), 8.3 (m, 2 H, C-5, C-8), 9.2 (m, 2 H, C-2, C-3), 12.8 (m, 2 H, CONH, exchangeable with D_2O); IR 1600, 1650, 1710 cm^{-1}; MS-FD, m/z (relative intensity) 419 ([M]$^+$, 100), 384 ([M −35]$^+$, 80), 348 ([M − 71]$^+$, 20). Anal. ($C_{20}H_{16}Cl_2N_2O_4$) C, H, N.

1,4-Bis[2-[(2-hydroxyethyl)amino]acetamido]-9,10-anthracenedione Dihydrochloride (3). To a suspension of 195 mg (0.5 mmol) of **10** in 16 mL of dimethylacetamide was added a solution of 0.36 mL (6 mmol) of 2-hydroxyethylamine in 12 mL of methanol. The reaction mixture was heated for 2 h at 60 °C and the course of the reaction was monitored by TLC (solvent system C). After cooling, 30 mL of water was added, and the byproducts 1-[2-[(2-hydroxyethyl)amino]ethanamido]-4-amino-9,10-anthracenedione and 1,4-diamino-9,10-anthracenedione] were removed by extraction with benzene. The water layer was saturated with NaCl and extracted several times with 1-butanol. The water was removed from the 1-butanol layer in vacuo and the yellow-orange product was precipitated by addition of hydrogen chloride–ethyl ether and purified by column chromatography (Sephadex LH-20, eluent methanol): yield 103 mg (43%); mp 240–242 °C dec; ^{1}H NMR (as free base, Me_2SO-d_6) δ 3.2 (t, 4 H, $NHCH_2$), 3.8 (t, J = 6 Hz, 4 H, CH_2O), 4.25 (s, 4 H, $COCH_2$), 7.9 (m, 2 H, C-6, C-7), 8.25 (m, 2 H, C-5, C-8), 8.95 (s, 2 H, C-2, C-3), 12.5 (s, 1 H, NHCO, exchangeable with D_2O); IR 1595, 1640, 1710 cm^{-1}; MS-FD, m/z (relative intensity) 441 ([M + 1]$^+$, 100), 442 ([M + 2]$^+$, 24). Anal. ($C_{22}H_{24}N_4O_6 \cdot 2HCl \cdot H_2O$) C, H, N.

1-[2-[(2-Hydroxyethyl)amino]acetamido]-4-amino-9,10-anthracenedione was isolated from the benzene layer (see above) by means of column chromatography (Sephadex LH-20, eluent methanol–chloroform 1:1): mp 263–265 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.2 (t, 2 H, $NHCH_2$), 3.8 (t, J = 6 Hz, 2 H, CH_2O), 4.2 (s, 2 H, $COCH_2$), 7.3 (d, J = 9 Hz, 1 H, C-3), 7.8 (m, 2 H, C-6, C-7), 8.2 (m, 2 H, C-5, C-8), 8.5 (d, J = 9 Hz, 1 H, C-2), 8.4 and 9.3 (br, OH and NH_2, exchangeable with D_2O); IR 1590, 1620, 1705 cm^{-1}; MS-FD, m/z (relative intensity) 339 ([M]$^+$, 100), 340 ([M$^+$ 1]$^+$, 65).

1,4-Bis[3-[(2-hydroxyethyl)amino]propionamido]-9,10-anthracenedione (4). The solution of 210 mg (0.5 mmol) of 11 and 0.6 mL (10 mmol) of 2-hydroxyethylamine in 10 mL of methanol and 4 mL of toluene was heated for 1 h at 60 °C. The course of the reaction was controlled by TLC (solvent systems B and C). After removal of the solvents in vacuo, the residue was dissolved in chloroform and washed with 3% NaCl solution. The crude product was purified by column chromatography (Sepahdex LH-20, eluent methanol–chloroform 1:1). The yellow-orange product (220 mg, 80% yield) was treated with hydrochloride–ethyl ether to form its dihydrochloride: mp 243–245 °C; ^{1}H NMR (D_2O) δ 2.8 (m, 4 H, $COCH_2$), 3.4 (m, 8 H, CH_2NHCH_2), 3.9 (t, J = 3.5 Hz, 4 H, CH_2O), 7.5 (m, 4 H, C-5, C-6, C-7, C-8), 8.3 (s, 2 H, C-2, C-3); IR 1600, 1650, 1710 cm^{-1}; MS-FD, m/z (relative intensity) 469 ([M + 1]$^+$, 100), 408 ([M − 60]$^+$, 60), 348 ([M − 120]$^+$, 40). Anal. ($C_{24}H_{28}N_4O_6 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

1-[3-[(2-Hydroxyethyl)amino]propionamido]-4-amino-9,10-anthracenedione was isolated on a Sephadex LH-20 column (see above): mp 225–228 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.5 (m, 2 H, $COCH_2$), 2.8 (m, 4 H, CH_2NHCH_2), 3.6 (m, 2 H, CH_2OH), 7.3 (d, J = 9 Hz, 1 H, C-3), 7.7 (m, 2 H, C-6, C-7), 8.2 (m, 2 H, C-5, C-8), 8.6 (d, J = 9 Hz, 1 H, C-2), 12.3 (m, 1 H, NHCO exchangeable with D_2O) and two signals (δ 5.5, 8.4) exchangeable with D_2O (NH_2 and OH); IR 1600, 1650, 1700 cm^{-1}; MS-FD; m/z (relative intensity) 354 ([M]$^+$, 100), 293 ([M − 60]$^+$, 15).

1,4-Bis[2-(methylamino)acetamido]-9,10-anthracenedione Dihydrochloride (5). To 195 mg (0.5 mmol) of 10 slurried with 25 mL of dimethylacetamide were added 670 mg (10 mmol) of methylamine hydrochloride and 1.4 mL (10 mmol) of NEt_3 in 90 mL of methanol. The reaction mixture was heated in a selected tube for 3 h at 60 °C and then was worked up as described for 4. The crude, yellow-brown product was purified as its dihydrochloride by column chromatography (Sephadex LH-20, eluent methanol) and next was crystallized from methanol–ethyl ether to afford 112 mg (50% yield) of 5, which melted at 190–195 °C dec: ^{1}H NMR (as free base, $CDCl_3$) δ 2.4 (s, 6 H, NCH_3), 3.2 (s, 4 H, $COCH_2$), 6.9 (m, 1 H, NH exchangeable with D_2O), 7.8 (m, 2 H, C-6, C-7), 8.35 (m, 2 H, C-5, C-8), 9.3 (s, 2 H, C-2, C-3), 12.9 (s, 2 H, NHCO exchangeable with D_2O); IR 1590, 1650, 1720 cm^{-1}; MS-FD, m/z (relative intensity) 380 ([M]$^+$, 100). Anal. ($C_{20}H_{20}N_4O_4 \cdot 2HCl$) C, H, N.

1,4-Bis[3-(methylamino)propionamido]-9,10-anthracenedione Dihydrochloride (6). To 210 mg (0.5 mmol) of 11, in 2 mL of toluene, were added 610 mg (10 mmol) of methylamine hydrochloride and 1.4 mL (10 mmol) of NEt_3 in 5 mL of methanol. The reaction mixture was heated in a selected tube for 40 min at 60 °C and then was worked up as described for 4 to yield 170 mg (70%) of the yellow-orange product 6: mp 233–235 °C dec; ^{1}H NMR (as free base, $CDCl_3$) δ 2.6 (m, 10 H, CH_3 and $COCH_2$), 2.9 (m, 4 H, CH_2N), 6.8 (m, 1 H, NH exchangeable with D_2O), 7.5 (m, 2 H, C-6, C-7), 8.0 (m, 2 H, C-5, C-8), 8.5 (s, C-2, C-3), 12.3 (br s, NHCO exchangeable with D_2O); IR 1600, 1650, 1705; MS-FD, m/z (relative intensity 408 ([M]$^+$, 100). Anal. ($C_{22}H_{24}N_4O_4 \cdot 2HCl$) C, H, N.

1,4-Bis[2-(dimethylamino)acetamido]-9,10-anthracenedione Dihydrochloride (7). The reaction of 195 mg (0.5 mmol) of 10 with 800 mg (10 mmol) of dimethylamine hydrochloride was performed for 5 h in the same manner as described for 5. Then the reaction mixture was worked up as described for 5 to afford 145 mg (60%) of 7: mp 273–275 °C dec; ^{1}H NMR (as free base, $CDCl_3$) δ 2.5 (s, 6 H, $N(CH_3)_2$), 3.2 (s, 4 H, $COCH_2$), 7.8 (m, 2 H, C-6, C-7), 8.35 (m, 2 H, C-5, C-8), 9.2 (s, 2 H, C-2, C-3), 13.5 (s, 2 H, NHCO exchangeable with D_2O); IR 1595, 1650, 1720 cm^{-1}; MS-FD, m/z (relative intensity) 408 ([M]$^+$, 100). Anal. ($C_{22}H_{24}N_4O_4 \cdot 2HCl$) C, H, N.

1,4-Bis[3-(dimethylamino)propionamido]-9,10-anthracenedione Dihydrochloride (8). The reaction of 210 mg (0.5 mmol) of 11 with 800 mg (10 mmol) of dimethylamine hydrochloride was performed as described for 6 and next was worked up as for 4 to provide 200 mg (80%) of 8 (yellow-orange): mp 262–264 °C; ^{1}H NMR (as free base, $CDCl_3$) δ 2.4 (s, 12 H, CH_3), 2.8 (m, 8 H, $COCH_2$ and CH_2N), 7.7 (m, 2 H, C-6, C-7), 8.2 (m, 2 H, C-5, C-8), 9.0 (s, 2 H, C-2, C-3), 12.5 (br s, 2 H, NHCO exchangeable with D_2O); IR 1590, 1650, 1710 cm^{-1}; MS-FD, m/z (relative intensity) 436 ([M]$^+$, 100). Anal. ($C_{24}H_{28}N_4O_4 \cdot 2HCl$) C, H, N.

Biological Tests. In Vitro Cytotoxicity Evaluation. The mouse L1210 leukemia cells (RPMI, USA) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum and penicillin (1000.000 units/L) plus streptomycin (100 mg/L) in controlled air–5% CO_2 humidified atmosphere at 37 °C. L1210 mouse leukemia cells were seeded at a density of 5×10^4 cells/mL. The tested compounds after dissolving in 50% ethanol were added, at four different concentrations, to the cell suspensions. The cytotoxic activity (EC_{50} value) of the tested compounds was defined as their in vitro concentrations causing 50% growth inhibition after 48 h, measured by cells protein contents.[23] The determination of protein synthesis inhibition on HeLa S_3 in tissue culture was performed according to ref 24. The tested compounds were dissolved in water, and 10-μL aliquots of these solutions were added to 4 mL of the incubation medium. The concentration of the drugs was in the range 0.005–5 μg/mL. The cytotoxic activity (the EC_{50} value) of the tested compounds was defined as the concentration that inhibited the increment of the cellular protein in cultured HeLa cells by 50% and was determined from dose–response curves.

In Vivo Antileukemic Evaluation. BDF_1 mice were injected ip with 10^6 P388 lymphotic leukemia cells on day 0 and treated ip on days 1–5 in accordance with the protocols described by the National Cancer Institute.[25] The mean survival time (MST) for each treatment group (eight mice) was calculated and the percent of T/C was determined by using the following formula: % T/C = [(MST treated)/(MST control)] × 100.

DNA Binding. For determination of DNA melting temperature[26] 0.01 M phosphate buffer, pH 6, containing 10^{-5} M EDTA and 5% DMSO was used. Drug to DNA concentrations were 1:20 μg. The 260-nm DNA peak was monitored on a Varian-Techtron UV–vis spectrophotometer (Model 635). The cells were heated by circulating ethylene glycol–water from a Haake PG11 controlled-temperature bath heated at a constant rate of 2 °C/min. The ΔTm values were obtained by plotting absorbance against temperature and taking the midpoint of the curve between the high- and low-temperature constant-absorbance regions. The melting temperature of uncomplexed DNA (calf thymus DNA, type II, Sigma) under these conditions was 71 °C.

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Registry No. 3, 115290-21-6; 3 (free base), 115290-22-7; 4, 115290-23-8; 4·2HCl, 115290-25-0; 5, 115290-26-1; 5 (free base), 115290-27-2; 6, 115290-28-3; 6 (free base), 115290-29-4; 7, 115290-30-7; 7 (free base), 115290-31-8; 8, 115290-32-9; 8 (free base), 115290-33-0; 9, 128-95-0; 10, 43182-24-7; 11, 115290-34-1; $ClCH_2COCl$, 79-04-9; $Cl(CH_2)_2COCl$, 625-36-5; 2-hydroxyethylamine, 141-43-5; methylamine hydrochloride, 593-51-1; dimethylamine hydrochloride, 506-59-2; 1-[2-[(2-hydroxyethyl)amino]ethanamido]-4-amino-9,10-anthracenedione, 102650-27-1; 1-[3-[(2-hydroxyethyl)amino]propionamido]-4-amino-9,10-anthracenedione, 115290-24-9.

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