Acknowledgment. We thank Hubertus Jahn and Wolfgang Uhl (Department of Chemical Research) for their excellent technical assistance in the synthetic work.

Registry No. **3**, 6298-19-7; **4** (R^1 = 4-Cl), 5900-58-3; **4** (R^1 = 5-Cl), 5202-89-1; **4** (R^1 = 4-CH_3), 18595-17-0; **4** (R^1 = 5-CH_3), 18595-16-9; **4** (R^1 = 5-Et), 2475-78-7; **4** (R^1 = 5-Br), 52727-57-8; **6a**, 1022-30-6; **6b**, 114368-11-5; **6c**, 118306-19-7; **6d**, 1214-92-2; **6e**, 118306-15-3; **6f**, 118306-17-5; **7a**, 28797-51-5; **7b**, 28797-50-4; **7c**, 120990-79-6; **7d**, 28797-53-7; **7e**, 120990-80-9; **7f**, 120990-81-0; **7** (R^1 = H, n = 0), 87571-90-2; **7** (R^1 = H, n = 1), 28797-48-0; **7** (R^1 = H, n = 2), 31265-81-3; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 100158-61-0; (±)-**8** (Z = CH_2, m = 2, R^3 = R^4 = C_2H_5, 2-position), 120990-82-1; (±)-**8** (Z = CH_2, m = 3, R^3 = R^4 = C_2H_5, 2-position), 120990-83-2; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 3-position), 120990-84-3; **8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 4-position), 116905-90-9; (±)-**8** (Z = bond, m = 1, R^3 = R^4 = C_2H_5, 2-position), 121053-95-0; (±)-**8** (Z = $(CH_2)_2$, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-85-4; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = CH_3, 2-position), 100158-60-9; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = C_2H_5, 2-position), 120990-86-5; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = C_4H_9, 2-position), 120990-87-6; (±)-**8** (Z = CH_2, m = 1, R^3 = C_2H_5, R^4 = H, 2-position), 120990-88-7; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = C_6H_{11}, 2-position), 121053-96-1; (±)-**8** (Z = CH_2, m = 1, R^3 = CH_3, R^4 = cycloheptyl, 2-position), 120990-89-8; (±)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_3H_7, 2-position), 120990-90-1; (±)-**8** (Z = CH_2, m = 1, NR^3R^4 = pyrrolidino, 2-position), 112282-40-3; (±)-**8** (Z = CH_2, m = 1, NR^3R^4 = piperidino, 2-position), 120990-91-2; (±)-**8** (Z = CH_2, m = 1, NR^3R^4 = morpholino, 2-position), 120990-92-3; (−)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-93-4; (−)-**8**·2L-tartrate (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-95-6; (+)-**8** (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-94-5; (+)-**8**·2D-tartrate (Z = CH_2, m = 1, R^3 = R^4 = C_2H_5, 2-position), 120990-96-7; **9**, 120990-62-7; **10**, 100158-38-1; **11**, 100158-14-3; **12**, 121011-73-2; **13**, 120990-63-8; **14**, 100158-44-9; **15**, 120990-64-9; **16**, 121054-51-1; **17**, 120990-65-0; **18**, 100158-13-2; **19**, 121011-74-3; **20**, 120990-66-1; **21**, 120990-67-2; **22**, 120990-68-3; **23**, 120990-69-4; **24**, 120990-70-7; **25**, 120990-71-8; **26**, 120990-72-9; **27**, 120990-73-0; (−)-**28**, 100158-38-1; (+) **29**, 121029-35-4; (+) **29**·2HBr, 120990-97-8; **30**, 114367-96-3; **31**, 120990-74-1; **32**, 120990-75-2; **33**, 120990-76-3; **34**, 120990-77-4; **35**, 120990-78-5; $ClCH_2COCl$, 79-04-9.

Synthesis of Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones as Potential Antileukemic Agents

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The synthesis of unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones bearing one "mitoxantrone side arm" and another (aminoalkyl)amino moiety has been described. These unsymmetrical anthracene-9,10-diones exhibit cytotoxic activity against L1210 leukemia cells and antitumor activity against P388 leukemia in mice.

Ametantrone, 1,4-bis[[2-[(2-hydroxyethyl)amino]-ethyl]amino]anthracene-9,10-dione (**1**) and its 5,8-dihydroxy analogue, mitoxantrone (**2**), have shown outstanding antineoplastic activity and are among the most promising anticancer agents.[1,2] These compounds are thought to exert their cytotoxic effect by an intercalation into DNA mechanism.[3,4] Properly designed amino-substituted side chains may stabilize the intercalated planar chromophore by interacting with the sugar and phosphate units of DNA and modify its conformation and function. Therefore, a number of analogues of **1** and **2** with various (aminoalkyl)amino side chains localized in different positions of the 9,10-anthracenedione ring were synthesized for structure–activity relationship evaluation.[2,5–9] One of the studied groups of anthracenediones are compounds with nonidentical side chains. It has been shown that the simplest compounds of that type, with one "mitoxantrone arm" at position 1 and a hydroxyl or amino group at position 4, exhibit considerable antitumor activity.[6,9] In another synthetic approach unsymmetrically 1,4-bis-substituted anthracenediones were obtained by a two step photolytic–thermolytic procedure, starting from 1,4-dimethoxyanthracene-9,10-dione.[10] However, the method applied allowed the synthesis of compounds within a limited range of structures of side chains. None of the obtained compounds exhibited any significant antileukemic activity although some of them were active in vitro.[11] Several unsymmetrical 1,4-diaminoanthraquinones have been also synthesized by Zielske,[12] but antineoplastic activity of these derivatives has not been evaluated.

In this report we describe the synthesis of a number of unsymmetrically 1,4-bis-substituted anthracene-9,10-diones with a "mitoxantrone side arm" at position 1 and with different alkylamino chains at position 4 (Figure 1; **5**, **6**, **8**, **10**, and **12**). These compounds contain the basic

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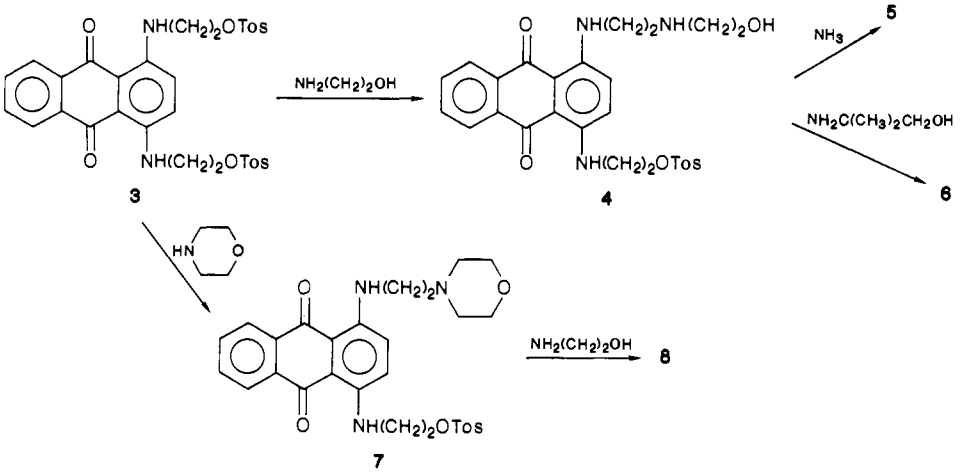
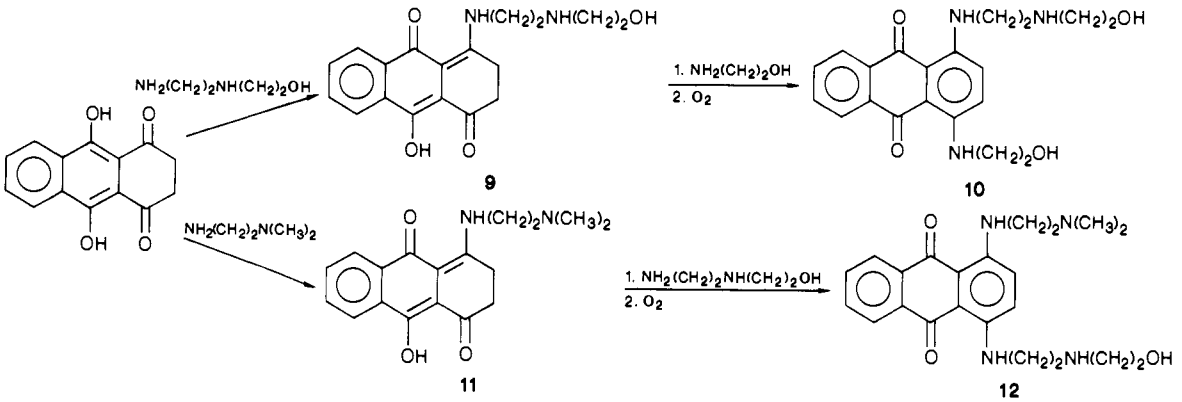
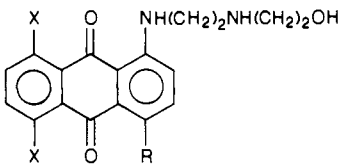
Scheme I



Scheme II





1: R = $NH(CH_2)_2NH(CH_2)_2OH$, X = H
2: R = $NH(CH_2)_2NH(CH_2)_2OH$, X = OH
5: R = $NH(CH_2)_2NH_2$, X = H
6: R = $NH(CH_2)_2NHC(CH_3)_2CH_2OH$, X = H
8: R = $NH(CH_2)_2N$ O, X = H
10: R = $NH(CH_2)_2OH$, X = H
12: R = $NH(CH_2)_2N(CH_3)_2$, X = H

Figure 1.

moiety $-NHCH_2CH_2N<$ attached to the aromatic ring, which is believed to be requested for the antitumor activity.[2]

Compounds **5**, **6**, and **8** were obtained by the modified method applied previously for the synthesis of bis-symmetrically substituted anthracenediones.[13] In the first step of the reaction 1,4-bis[2-[(*p*-toluenesulfonyl)oxy]ethyl]-amino]anthracene-9,10-dione (**3**) reacted with appropriate amines to give **4** or **7**. The obtained intermediate monotosylates were submitted to the second step aminolysis to yield final products **5**, **6**, and **8** (Scheme I).

The described method has no special limitations as concerns the structural types of side chains and therefore is a general one for the synthesis of 1,4-bis-unsymmetrically substituted [(aminoalkyl)amino]anthracene-9,10-diones. An additional advantage of the presented way is that the general substrate **3** already contains part of the side chain optimal for antineoplastic activity. This allows one to use for the reaction rather simple, and thus more readily available, amines.

Compounds **10** and **12** were obtained in a two-step "one-pot" reaction of leucoquinizarin with sequentially added amines[9] (Scheme II). This alternative method was applicable for the above compounds, because these products could be isolable from the rather complex reaction mixture.

The synthesized 1,4-unsymmetrically disubstituted anthracene-9,10-diones, were tested for their cytotoxicity against leukemia L1210 cells and antitumor acivity against leukemia P388 in mice (Table I).

Chemistry

In the first step of the synthesis of **5** and **6**, the reaction of **3** with ethanolamine was performed (Scheme I); then the obtained intermediate **4** was further treated with gaseous ammonia or 2-amino-2-methylpropanol, to give **5** and **6**, respectively. We have found that this sequence of applied amines gave purer products. On the contrary, in the case of the synthesis of **8** (Scheme I) better yield was obtained, when **3** was transformed into **7** in the reaction with morpholine, and then with ethanolamine to give **8**. The reaction of converting **3** into **4** or **7** was carried out by heating **3** in acetonitrile with 3-fold excess of the appropriate amines. The crude **4** and **7** were purified by means of column chromatography (Sephadex LH-20 for **4**, silica gel for **7**). Further reaction of **4** and **7** with an excess of the corresponding amine was performed under reflux in the same solvent as above. The course of both reactions was readily monitored by TLC, because of great differences in R_f between the substrates and products. The

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final products **5** and **6** were purified on a Sephadex LH-20 column; **8**, on silica gel column chromatography.

Compounds **10** and **12** were obtained in a two-step "one-pot" reaction of leucoquinizarin with sequentially added appropriate amines at temperatures lower than 50 °C (Scheme II). In the first step, leucoquinizarin reacted with a two molar excess of the respective amine [2-(2-aminoethyl)ethanolamine or *N,N*-dimethylethylenediamine] in ethanol as solvent. Treatment of the formed **9** and **11** with the other amines led to the respective dihydro intermediates, which were oxidized with air in a methanol suspension to **10** and **12** and finally purified by column chromatography on Sephadex LH-20; compound **10** can also be purified on silica gel. The structures of the intermediates **4** and **7** as well as of the products **5**, **6**, **8**, **10**, and **12** were supported by ^{1}H NMR spectra and mass determination (FD-MS technique). The structures of the end products were also confirmed by elemental analysis and characterized by the UV–vis spectra.

Because 1-[(2-aminoethyl)amino]-9,10-anthracenediones are particularly susceptible to cyclization to form hexahydronaphthoquinoxalines,[14] we have evidenced the presence of alkylamino groups in **5** by converting it to the *N,N*-diacetyl derivative by the procedure used for the acetylation of alkylamino groups of [(aminoalkyl)amino]anthracene-9,10-diones.[14,15] Additionally, the UV–vis spectra of **5** appeared to be typical of 1,4-bis(alkylamino)anthraquinones.[14]

Biological Activity and Discussion

The obtained unsymmetrical 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones were tested for cytotoxic activity against L1210 leukemia cells and for antitumor activity against P388 murine leukemia. The results are presented in Table I. The tested compounds exhibit various growth-inhibitory activities with regard to L1210 mouse leukemia in vitro. Thus **6** and **12** showed ED_{50} in fewfold lower doses than ametantrone (**1**), **5**, and **8** comparable to that of **1**, whereas **10** was several times less active than **1**.

The tested compounds exhibited antitumor activity in vivo, in dose levels higher in relation to those of **1** and **2**. Compound **5** exhibited the best T/C value, comparable with that of **1**, and was selected for further biological studies.

Compounds **5**, **6**, **8**, and **12** bear side chains, which fulfill the requirements for cytotoxic activity (the presence of a basic nitrogen atom in the center of the chain and the appropriate distance between the two nitrogen atoms).[2,8] In agreement with these statements also, the respective symmetrically substituted compounds showed marked cytotoxicity.[8,16] On the other hand, the presence of (hydroxyethyl)amino function at position 4 (compound **10**) decreases the antitumor activity both in vitro and in vivo. It is known that the respective symmetrical 1,4-bis[(2-hydroxyethyl)amino]anthracene-9,10-dione is totally inactive.[8]

Unsymmetrical 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones synthesized up to now[11] were inactive in vivo, which was probably due to the unproper structure of side arms.

It can be concluded that identical side chains on the 1,4-diaminoanthracene-9,10-diones is not an essential factor in antitumor activity of these compounds. The activity depends primarily on the presence in the side chains of defined pharmacophoric moieties[2] regardless of whether they are in symmetrical or unsymmetrical anthracenediones derivatives.

Table I. Cytotoxic and Antitumor Activities of Tested Unsymmetrical 1,4-Bis[(aminoalkyl)amino]anthracenene-9,10-diones[a] against L1210 Leukemia Cells and P388 Murine Leukemia

compd (NSC no.)	L1210 leukemia cells,[b] ED_{50}, μg/mL	P388 murine leukemia[c] dose, mg/kg	% T/C	tox D surv
ametantrone (1) (287513)	2.54 ± 0.11	25.00	200	6/6
		12.5	300	6/6
		6.25	240	6/6
		3.12	200	6/6
mitoxantrone (2) (279836)		12.00	118	6/6
		6.00	192	6/6
		3.00	288	6/6
		1.5	201	6/6
		0.75	209	6/6
5	2.38 ± 0.86	40.00	80	5/7
		20.00	300	7/7
		10.00	300	7/7
		5.00	200	7/7
		2.5	180	7/7
		1.25	170	7/7
6 (613391)	1.48 ± 0.24	80.00	153	6/6
		40.00	182	6/6
		20.00	163	6/6
		10.00	151	6/6
		5.00	147	6/6
8 (613393)	2.36 ± 0.15	80.00	153	6/6
		40.00	147	6/6
		20.00	135	6/6
		10.00	132	6/6
		5.00	125	6/6
10 (613390)	12.29 ± 0.29	80.00	144	6/6
		40.00	127	6/6
		20.00	142	6/6
		10.00	125	6/6
		5.00	115	6/6
12 (613392)	0.64 ± 0.29	80.00	192	6/6
		40.00	192	6/6
		20.00	173	6/6
		10.00	151	6/6
		5.00	144	6/6

[a] Used as hydrochlorides. [b] Data obtained in our laboratory (see Experimental Section). ED_{50} = concentration of compound required to inhibit by 50% the growth of L1210 leukemia cells. ED_{50} values represent means ±SEM from at least three experiments carried out in triplicate. [c] Data for **2**, **6**, **8**, **10**, and **12** are the results of screening performed under the auspices of the Development Therapeuties Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Data for **1** and **5** were obtained in our laboratory. CDF_1 mice were injected ip with 10^6 P388 lymphotic leukemia cells on day 0 and treated ip on days 1 and 5 with the drug dose specified.[18] % T/C = the ratio of medium survival time expressed as percent of untreated controls. tox D surv = survivor recorded on day 4 after day of first injection as a measure of drug toxicity.

Experimental Section

Melting points were determined with a Kofler hot plate apparatus and are uncorrected. Elemental analyses were performed by the Department of Elemental Microanalyses, Polish Academy of Sciences, Warsaw. The obtained results were within 0.4% of theoretical values. ^{1}H NMR spectra were recorded on a Varian 90-MHz spectrometer using tetramethylsilane as internal standard. A Beckman spectrometer was used for UV spectral determinations. Molecular weights were determined by mass spectrometry (FD) on a Varian MAT 711 instrument. The instrumental conditions were the following: wire heating current 5–20 mA, ion source temperature 70–100 °C, accelerating voltage 4–6 kV. Column chromatography was performed with silica gel 60 (Merck, 70–230 mesh) and on Sephadex LH-20 (Pharmacia). The following solvent systems were used: (I) *n*-butanol/pyridine/acetic acid/water = 8:2:3:5 v/v; II, III, IV, and V, chloroform/methanol = 1:1, 4:1, 8:1, and 25:1 v/v, respectively.

1-[[2-[(*p*-Toluenesulfonyl)oxy]ethyl]amino]-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione (4). A solution of **3** (0.63 g, 1 mmol) and 0.2 mL (3.3 mmol) of eth-

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anolamine in acetonitrile (15 mL) was refluxed for 80–120 min. The reaction progress was followed by TLC using solvent systems I and II. After removal of the solvent, the residue was dissolved in chloroform and washed several times with water. The crude product was purified by column chromatography on silica gel (70–230 mesh) with solvent system III: yield 130 mg (25%); mp 115–116 °C; MS-FD, m/z (relative intensity, %) 524 [$(M + 1)^+$, 100]; ^{1}H NMR ($CDCl_3$) δ 2.2 (s, 3 H, CH_3), 3.3 (m, 4 H, CH_2NHCH_2), 3.7 (m, 4 H, $ArNHCH_2$), 4.0 (m, 4 H, CH_2OH and CH_2OSO_2), 7.1 (s, 2 H, H-2,3 and m, 2 H aromatics), 7.5 (m, 2 H, aromatics), 7.8 (m, 4 H, H-6, 7, H-5, 8), 10.3 (broad s, 2 H, ArNH, vanished by addition of D_2O). Anal. ($C_{27}H_{29}N_3O_6S$) C, H, N.

1-[[2-[(*p*-Toluenesulfonyl)oxy]ethyl]amino]-4-[(morpholinoethyl)amino]anthracene-9,10-dione (7). The reaction of **3** (0.63 g, 1 mmol) with 0.25 mL (2.8 mmol) of morpholine in acetonitrile (12 mL) was carried out by refluxing this solution for about 3 h. The course of the reaction was controlled by TLC using solvent system III. Then, the reaction mixture was worked up as described for compound **4**, with solvent system IV as eluent in column chromatography: yield 325 mg (60%); mp 94–95 °C; MS-FD, m/z (relative intensity, %) 550 [$(M + 1)^+$, 100]; ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3 H, CH_3), 2.6 [m, 6 H, $CH_2N(CH_2)_2$], 3.5 [m, 4 H, $O(CH_2)_2$], 3.8 (m, 4 H, $ArNHCH_2$), 4.2 (t, 2 H, CH_2OSO_2), 7.1 (s, 2 H, H-2, 3, and m, 2 H, aromatics), 7.7 (m, 4 H, H-6, 7, and 2 H aromatics), 8.3 (m, 2 H, H-5, 8), 11.3 (broad s, 2 H, ArNH, vanished by addition of D_2O). Anal. ($C_{29}H_{31}N_3O_6S$) C, H, N.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-[(aminoethyl)amino]anthracene-9,10-dione (5). A solution of **4** (0.52 g, 1 mmol) in methanolic ammonia (25 mL) saturated at 0 °C was heated in a sealed tube at 70 °C for 2.5 h. After cooling, the solution was evaporated and the residue was dissolved three times in chloroform and evaporated to remove traces of ammonia. The crude product was purified by chromatography on Sephadex LH-20, in solvent system II. The product, dissolved in a small amount of chloroform/methanol mixture, was precipitated by ethyl ether/hexane. The solid was separated and dried in vacuo: yield of **5** 0.25 g (70%); mp 104–105 °C; MS-FD, m/z (relative intensity, %) 368 [$(M)^+$, 100], 369 [$(M + 1)^+$, 63]; ^{1}H NMR (**5**·2HCl; D_2O) δ 3.3 (m, 6 H, CH_2NH_2 and CH_2NHCH_2), 3.5 (m, 4 H, $ArNHCH_2$), 4.0 (m, 2 H, CH_2OH), 6.6 (s, 2 H, H-2, 3), 7.4 (m, 2 H, H-6, 7), 7.7 (m, 2 H, H-5, 8); UV–vis λ_{max} (H_2O) 625 nm (10 000) and 582 nm (10 000). An analytical sample of **5** was prepared as its dihydrochloride by addition of HCl/ethyl ether to the cold chloroform solution of **5** and two crystallizations of the solid from methanol/ethyl ether. mp 187–190 °C. Anal. ($C_{20}H_{24}N_4O_3$·2HCl·2H_2O) C, H, N.

The *N,N*-diacetyl derivative of compound **5** was obtained by treatment of its methanol solution with acetic anhydride and pyridine for 1 h at room temperature. The ^{1}H NMR spectrum ([D_5]pyridine) confirmed the presence of two acetyl groups at δ 2.25 [s, 3 H, $-N(CH_2CH_2OH)COCH_3$] and 2,1 (s, 3 H, $-NHCOCH_3$).

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-[[2-[(1,1-dimethyl-2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione (6). The reaction of 4 (0.52 g, 1 mmol) with 1 mL (11 mmol) of 2-amino-2-methyl-1-propanol, in acetonitrile (15 mL) and chloroform (6 mL) as solvents, was carried out by heating this solution at 70 °C for 8 h. The reaction progress was followed by TLC using solvent system III. After removal of the solvent, the residue was dissolved in chloroform and then washed several times with water. The crude product was purified as described for compound **5**: yield 0.29 g (67%); mp 79–80 °C; MS-FD, m/z (relative intensity, %) 440 [$(M)^+$, 100]; ^{1}H NMR (**6**·2HCl, D_2O) δ 1.4 [s, 6 H, $C(CH_3)_2$], 3.3 (m, 6 H, CH_2NH and CH_2NHCH_2), 3.5 (m, 4 H, $ArNHCH_2$), 4.0 (m, 4 H, CH_2OH), 6.6 (s, 2 H, H-2, 3), 7.6 (m, 4 H, H-5, 6, 7, 8); UV–vis λ_{max} (H_2O) 625 nm (11 000) and 585 nm (11 000). Anal. ($C_{24}H_{32}N_4O_4$) C, H, N.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-[(2-morpholinoethyl)amino]anthracene-9,10-dione (8). The reaction of **7** (0.55 g, 1 mmol) with 1.2 mL (20 mmol) of ethanolamine in acetonitrile (8 mL) as solvent was carried out by heating of this solution. The reaction progress was followed by TLC using solvent system II. The reaction mixture was worked up as described for compound **5**. The purification of the crude product was performed on column chromatography (4 × 30 cm) with 35–70 mesh silica gel, which was eluted subsequently with solvent systems I, II, and V. The product was precipitated from the chloroform solution by treatment with ethyl ether/hexane: yield 0.35 g (80%); mp 107–108 °C; MS-FD, m/z (relative intensity, %) 438 [$(M)^+$, 100]; ^{1}H NMR ($CDCl_3$) δ 2.6 [m, 6 H, $CH_2N(CH_2)_2$], 3,0 (m, 4 H, CH_2NHCH_2), 3.5 [m, 4 H, $O(CH_2)_2$], 3.8 (m, 6 H, CH_2OH and $ArNHCH_2$), 7.2 (s, 2 H, H-2, 3), 7.7 (m, 2 H, H-6, 7), 8.35 (m, 2 H, H-5, 8), 11.2 (m, 2 H, ArNH, vanished by addition of D_2O); UV–vis λ_{max} (H_2O) 630–632 nm (10 000) and 587 nm (10 000). An analytical sample of **8** was prepared as its dihydrochloride by addition of HCl/ethyl ether to the cold chloroform solution of **8** and two crystallizations of the solid from methanol/ethyl ether. mp 181–184 °C. Anal. ($C_{24}H_{30}N_4O_4$·2HCl·H_2O) C, H, N.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione (10). To a suspension of leucoquinizarin (0.6 g, 2.5 mmol) in 50 mL of methanol was added dropwise 2-(2-aminoethyl)ethanolamine (1 mL, 9 mmol) in 10 mL of methanol, under nitrogen, with cooling and stirring. The reaction mixture was heated at 50–55 °C for 1 h. Then ethanolamine (0.3 mL, 5 mmol) was added and the reaction mixture heated at 50–55 °C for 2 h. Dry air was then bubbled through the mixture while the temperature was maintained at 50 °C for 3 h. The volume of the reaction mixture was reduced to 10 mL in vacuo, and 150 mL of petroleum ether was added. The mixture was then allowed to stand overnight at room temperature. The crude product was purified by column chromatography (silica gel) in solvent system II. Collection of the major blue fraction (400 mL) gave, after evaporation and crystallization from methanol/ethyl ether, 0.2 g of **10** (22%): mp 89–101 °C dec; MS-FD, m/z (relative intensity, %) 369 [$(M)^+$, 100]; ^{1}H NMR (**10**·HCl in D_2O) δ 3.25 (m, 4 H, CH_2NCH_2), 3.7 (m, 4 H, $ArNHCH_2$), 3.95 (m, 4 H, CH_2O), 6.18 (s, 2 H, H-2, 3), 7.12 (m, 2 H, H-6, 7), 7.3 (m, 2 H, H-5, 8); UV–vis λ_{max} (H_2O) 625 nm (11 800) and 580 nm (9800). Anal. ($C_{20}H_{23}N_3O_4$) C, H, N.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-[[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (12). A mixture of leucoquinizarin (1,4,9,10-tetrahydroxyanthracene) (0.3 g, 1.25 mmol) and 30 mL of methanol was treated with *N,N*-dimethylethylenediamine (0.25 mL, 2.5 mmol) in 5 mL of methanol under nitrogen with cooling and stirring. When a homogeneous paste was obtained, the reaction mixture was heated at 50–55 °C for 1 h. The reaction mixture was cooled to room temperature, treated with 2-[(2-aminoethyl)amino]ethanol (0.5 mL, 4.5 mmol), and heated at 50–55 °C for 1 h. After air oxidation the reaction mixture was reduced to a volume of 5 mL in vacuo. Petroleum ether (100 mL, bp 35–60 °C) was added, and stirring was continued for an additional 2 h. The resulting product was dissolved in 1-butanol and extracted twice with water. The 1-butanol layer was concentrated in vacuo to 10 mL, and **12** was precipitated by the addition of ethyl ether. Chromatographic purification of the crude product (Sephadex LH-20, solvent system II) and recrystallization from methanol/ethyl ether led to pure **12** (70 mg, 15%): mp 112–115 °C dec; MS-FD, m/z (relative intensity, %) 396 [$(M)^+$, 100]; ^{1}H NMR (**12**·2HCl, D_2O) δ 2.98 [s, 6 H, $(CH_3)_2N$], 3.3 [m, 6 H, CH_2NCH_2 and $CH_2N(CH_3)_2$], 3.5 (m, 4 H, $ArNHCH_2$), 4.1 (m, 2 H, CH_2O), 6.58 (s, 2 H, H-2, 3), 7.6 (m, 4 H, H-6, 7, H-5, 8); UV–vis λ_{max} (H_2O) 625 nm (11 600) and 580 nm (9400). Anal. ($C_{22}H_{28}N_4O_3$) C, H, N.

Biological Tests. The mouse L1210 leukemic cells (RPMI, U.S.) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum and penicillin (100 000 units/L) plus streptomycin (100 mg/L) in controled air–5% CO_2 humidified atmosphere at 37 °C. L1210 mouse leukemic cells were seeded at a density of 0.05×10^6 cells/mL. The tested compounds solubilized in 50% ethanol were added to the cell suspension at four different concentrations. The cytotoxic activities (the ED_{50} values) of the tested compounds were defined as their in vitro concentrations causing 50% inhibition of 48-h growth (measured by cell protein content determination).[17]

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The antitumor activity in vivo of examined compounds was assayed as described in the legend to Table I.

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Antimalarials. 16. Synthesis of 2-Substituted Analogues of 8-[(4-Amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy]quinoline as Candidate Antimalarials

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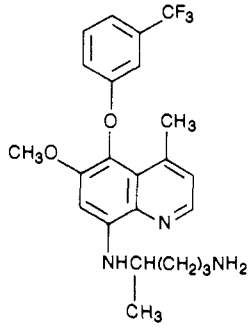
A series of 2-substituted analogues of the exceptional drug 8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy]quinoline (I) were prepared and evaluated for both suppressive and prophylactic antimalarial activity. The preparation of analogues of compound I was of interest due to the high level of both blood and tissue schizonticidal activity demonstrated by this compound. One analogue, **8a**, was found to be both more active and less toxic than the parent compound I. In addition, three analogues of example **8a** were prepared. Although two of the three analogues showed significant antimalarial activity, both were inferior to compound **8a**.

In a preceding paper in this series,[1] we reported the preparation of a series of 5-(aryloxy)-4-methylprimaquine analogues. Several of these compounds surprisingly were found to be highly active in both the suppressive and radical curative antimalarial screens. Example I, the 5-

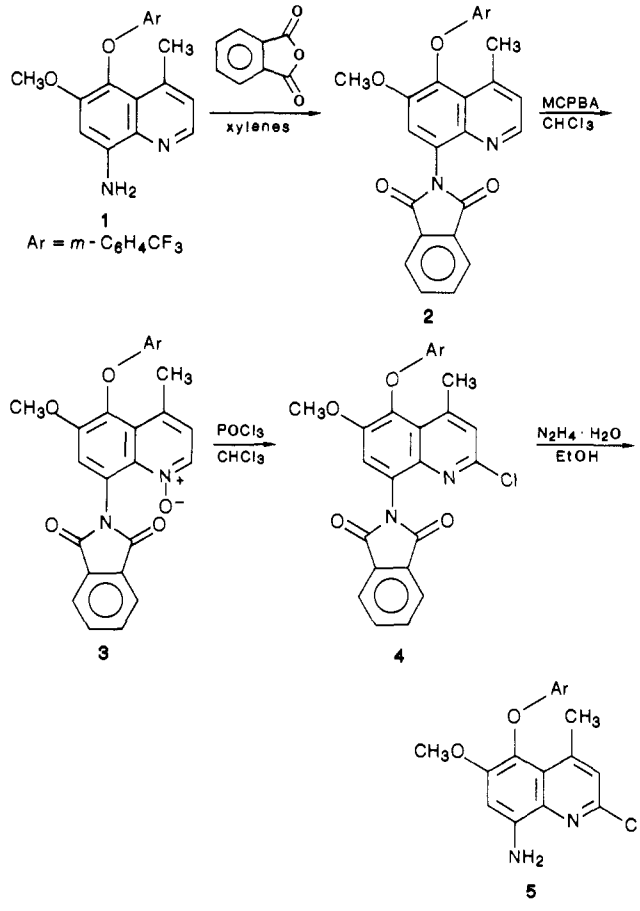


I

[3-(trifluoromethyl)phenoxy] analogue, was selected for preclinical studies which showed that, although the compound was more active than primaquine, it was also more toxic, especially with respect to methemoglobin formation. On the basis of a report[2] that a 2-methoxy substituent in a pamaquine analogue led to a decrease in toxicity, we felt it would be desirable to prepare selected examples of I bearing a 2-alkoxy group.

Chemistry. The key intermediate in the preparations of the four 2-substituted analogues of I (**8a–d**) was the 2-chloroquinoline **5**. Attempts to prepare this intermediate via the procedure used earlier by Talati and co-workers,[3] who prepared a similar analogue, failed. The approach used in the current work is shown in Scheme I. The previously described[1] 8-aminoquinoline **1** was protected as the phthalimide **2** and then converted to the *N*-oxide **3** with *m*-chloroperbenzoic acid in chloroform.[4] Treatment

Scheme I



of compound **3** with excess phosphorous oxychloride afforded the 2-chloroquinoline **4**, which was deprotected with excess hydrazine hydrate to afford the requisite quinoline **5**. The intermediate 2-substituted quinolines **6a–c** were prepared by treating **5** with the appropriate nucleophile in dimethylformamide as shown in Scheme II. Side-chain introduction was accomplished by alkylating the 8-aminoquinolines with 4-iodo-1-phthalimidopentane in acetonitrile. Diisopropylamine was utilized as the acid acceptor except in the preparation of intermediate **7c**, where sodium bicarbonate was used. Removal of the

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