indomethacin 0.01, N-α-tosyl-L-lysine chloromethyl ketone 0.1, glucose 2 mg/mL, and albumin 2 mg/mL. Respiratory medium (RM) pH 7.4 contained (in mM NaCl 132.5, KCl 5.4, $MgCl_2$ 1.2, $CaCl_2$ 1.0, NaH_2PO_4 1.0, Na_2HPO_4 5.0, indomethacin 0.01, glucose 2 mg/mL, and albumin 2 mg/mL.

Inhibitors. For the in vitro experiments the inhibitor, substituted imidazo[1,2-a]pyridines, and related analogues, 1–12, were dissolved and diluted in methanol. Aliquots were pipetted into the incubation media. The final methanol concentration was 1%, which had no effect on the enzyme or the gland preparations.

Methods. Determination of the H^+/K^+-ATPase Activity. Membrane vesicles (10 μg) and inhibitor were preincubated for 30 min in 2 mM Pipes–Tris (pH 7.4) and 10 mM KCl at 37 °C. An ATP solution was added to give a final concentration of 2 mM ATP and 2 mM $MgCl_2$. The ATPase activity was estimated as release of inorganic phosphate from ATP.[10] K^+-stimulated activity was obtained by subtracting the basal Mg^{2+} activity from the enzyme reaction in the presence of K^+ and Mg^{2+}. The effect of the highest concentration of each inhibitory compound on the recovery of a standard amount of inorganic phosphate was also determined.

Determination of Acid Formation in Gastric Glands. Acid formation was monitored by uptake of the weak base aminopyrine.[11] In a final volume of 2500 μL, 10 mg (wet weight) of glands were incubated in polypropylene vials. The incubation medium was RM containing 0.05 μCi [^{14}C]aminopyrine. Incubations were carried out at 37 °C in a shaker bath for 1 h and secretagoque activation by the addition of 10^{-4} M histamine.

Protein Determination. Protein was determined according to the method described by Bradford,[12] with use of the Bio-Rad Protein Assay kit. Bovine gammaglobulin was used as a standard.

Molecular Modeling. Computational Procedures. All molecular modeling was performed with use of an Evans and Sutherland PS350 tethered to a μVAXII serving as a host machine. Conformational analyses were conducted by using the SEARCH subroutine within SYBYL 3.4 using the search criteria and boundary conditions described in Table II.

Energy minimizations were conducted in MacroModel V1.5 by using the MM2 force field and applying the block diagonal Newton–Raphson algorithm. The energy is expressed in kJ/mol and the gradient first derivative root mean square (RMS) convergence criterion of the total energy of the molecule was ≤0.05 kJ/mol per Å (0.01 kcal/mol per Å). The minimum energy conformation and those conformations within 20 kJ/mol (~5 kcal/mol) of the minimum were recorded.

Interaction between SYBYL 3.4 and MacroModel V1.5 was accomplished via the INTERFACE program.[15]

Molecules were compared by using the FIT command within SYBYL as described.

Finally, the molecular database containing the compared molecules was ported to a Silicon Graphics 4D/240 acting as a server. Molecular volumes were generated from the logical combination of molecules by using the MVOLUME command within SYBYL 5.3. The total molecular volume of the active analogues, 1–5, 7–9, and 11, was obtained from the arithmetic sum of the molecular volume of each conformation for each active analogue described in Table III. Likewise, the total molecular volume of the inactive analogues, 6, 10, and 12, was obtained from the arithmetic sum of the molecular volume of each conformation for each inactive analogue described in Table III. The excluded and included molecular volumes were obtained by applying the difference operator to the arithmetic sums of the active and inactive molecular volumes described above, i.e. excluded volume = (inactive – active) volumes and included volume = (active–inactive) volumes.

Graphical representation of the molecular volumes was visualized and recorded by using SYBYL 5.3 on a Silicon Graphics 4D/120 workstation.

Acknowledgment. We thank Dr. John W. Clader of the Schering-Plough Corporation for developing and making available to us the INTERFACE program, and Dr. Arne Brandstrom of AB Hassle for providing the theoretical pK_a values for various substituted imidazo[1,2-a]pyridines and related analogues. We also thank Dr. Robert E. Carter of AB Hassle for his comments in reviewing the manuscript. We are especially grateful to Dr. Daniel M. Solomon for many valuable discussions throughout the course of this work, and for providing his expertise in photography.

Registry No. 1, 76081-98-6; 2, 91848-84-9; 3, 91848-94-1; 4, 130883-17-9; 5, 85542-20-7; 6, 85345-71-7; 7, 85333-23-9; 8, 85333-50-2; 9, 85333-46-6; 10, 121394-23-8; 11, 85332-63-4; 12, 121394-24-9; ATPase, 9000-83-3.

Supplementary Material Available: The data and Figures 1 and 3–6, describing the correlations between the in vitro biochemical activity and the in vivo gastric antisecretory activity of substituted imidazo[1,2-a]pyridines and related analogues, and SYBYL 5.3 MOL files for the conformations described in Table III (83 pages). Ordering information is given on any current masthead page.

Synthesis, Peroxidating Ability, and Antineoplastic Evaluation of 1-[(Aminoalkyl)amino]-4-hydroxy-10-imino-9-anthracenones

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A novel group of cytotoxic anthraquinone derivatives, 1-[(aminoalkyl)amino]-4-hydroxy-10-imino-9-anthracenones, has been synthesized. It has been shown that imino analogues of the anthracenediones exhibit diminished ability to generate oxygen radicals. The cytotoxic activity of iminoanthracenones obtained was lower than that of the related quinone carbonyl analogues. One of the obtained imino compounds showed a moderate antileukemic activity in vivo.

Among the most clinically useful intercalating agents against human malignancy are the anthracycline antibiotics daunorubicin (DR) and adriamycin (ADR) (1a and 1b, Chart I). Their utilization is, however, limited by undesired effects such as irreversible cardiotoxicity.[1] Several studies have suggested that anthracyclines cardiotoxicity may be associated with the mediation by anthraquinone drugs of the formation of reactive oxygen species and subsequent cellular lipid peroxidation.[2]

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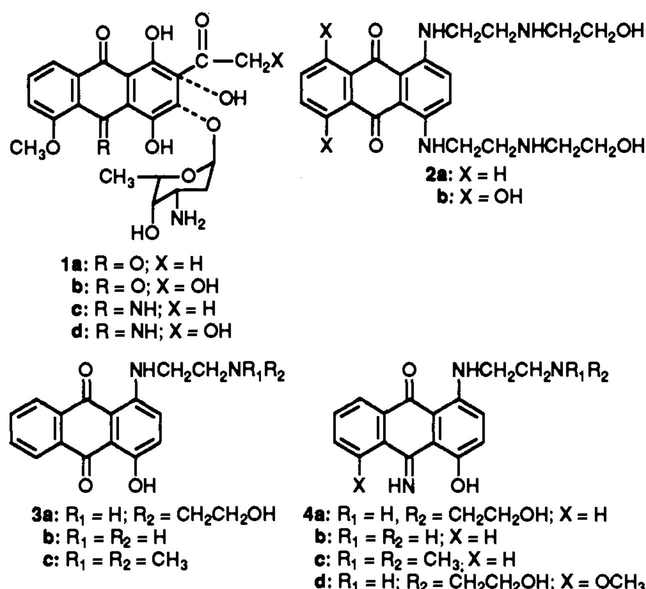
Chart I



Numerous semisynthetic derivatives of DR and ADR have been obtained with hope of finding a noncardiotoxic anthracycline retaining the broad-spectrum activity of the parent antibiotics. Among the numerous semisynthetic derivatives of anthracyclines obtained of special interest are imino analogues: 5-imino-DR (**1c**) and 5-imino-ADR (**1d**), which exhibited high antitumor activity and significantly reduced cardiotoxicity.[3a] The imino derivatives of native[4] as well as modified[5] anthracyclines also exhibit significantly lowered ability of oxygen-radical generation.

The synthetic class of anthraquinone anticancer agents, namely anthracenedione derivatives, has also been developed. 1,4-Bis[[(substituted-amino)alkyl]amino]-anthracenediones as mitoxantrone (**2a**) and ametantrone (**2b**), as well as 1-[(aminoalkyl)amino]-4-hydroxyanthraquinone (**3a**) have exhibited promising antitumor properties.[6a-c,f,7] Their imino analogues have not been obtained as yet.

The above findings, pointing to the favorable effects of amination of quinone carbonyl, prompted us to synthesize imino derivatives of some synthetic anthracenediones and to examine whether such modification leads to the similar, as in anthracyclines, effect of diminished capability to generate active oxygen species. We made an effort to synthesize the imino derivatives of two major groups of antitumor anthracenediones bearing two or one (aminoalkyl)amino side chains: 1,4-bis[(aminoalkyl)amino]-10-imino-9-anthracenones and 1-[(aminoalkyl)amino]-4-hydroxy-10-imino-9-anthracenones (**4a–c**). Moreover, the synthesis of 1-[(aminoalkyl)amino]-4-hydroxy-5-methoxy-10-imino-9-anthracenone (**4d**), structurally related to the anthraquinone moiety of 5-iminoanthracyclines, was also undertaken.

The structure of the side chains selected for the synthesis of compounds **4a–d**, with a basic terminal nitrogen atom, fulfills known requirements for the activity of 1,4-bis[(substituted-amino)alkyl]amino]anthraquinones.[6a,8] The obtained iminoanthraquinones were tested for their ability to stimulate oxygen-radical production. Also inhibitory activity against L1210 mouse leukemia cells in tissue culture and in vivo activity against P388 murine leukemia were examined.

Chemistry

The synthesis of iminoanthraquinones has been little studied.[3,5,9] The general procedure for the synthesis of these derivatives comprises the treatment of appropriate anthraquinones with methanol saturated with ammonia and a catalytic amount of *p*-toluenesulfonic acid at room or elevated temperature.

It is known that the presence in the anthraquinone molecule of OH or better of OH and OCH_3 groups peri to quinone favors the ammonolysis reaction, presumable by intramolecular hydrogen bonding with the resulting imino group.[3a,9a] In addition to the above requirement, we have found that the substrates for the ammonolysis could be only compounds with strongly chelated quinone C=O, evidenced by IR. Therefore, the compounds with free phenolic group(s), for example quinizarin,[9a] undergo ring amination, whereas 1,4-[9] and 1,8-dimethoxyanthraquinones[10] are unreactive in this reaction. We have observed that the treatment of 1,4-bis(alkylamino)-9,10-anthracenediones, as well as of their 5,8-$(OH)_2$ analogues, with ammonia did not lead to the corresponding iminoanthracenones. In the first case the failure to react with ammonia can be explained by rather weak chelation of C=O by peri NHR. However, the lack of reactivity of the latter class of compounds, with strongly chelating OH groups, indicates some unfavorable effect of amino groups adjacent to C=O, the mechanism of which at this stage of our investigation cannot be explained.

The failure in obtaining the desired imino analogues of 1,4-bis[[(substituted-amino)alkyl]amino]anthracenediones by the direct amination of corresponding N-protected derivatives of ametantrone and mitoxantrone prompted us to reverse the course of synthesis. However, the reaction of 9-iminoquinizarin as well as of its 2,3-dehydro derivative with 2-(dimethylamino)ethylamine, under the condition suitable for 1,4-amino chain substitution, also did not lead to the formation of the desired products. Only 1-[[2-(dimethylamino)ethyl]amino]-4-hydroxy-10-amino-9-

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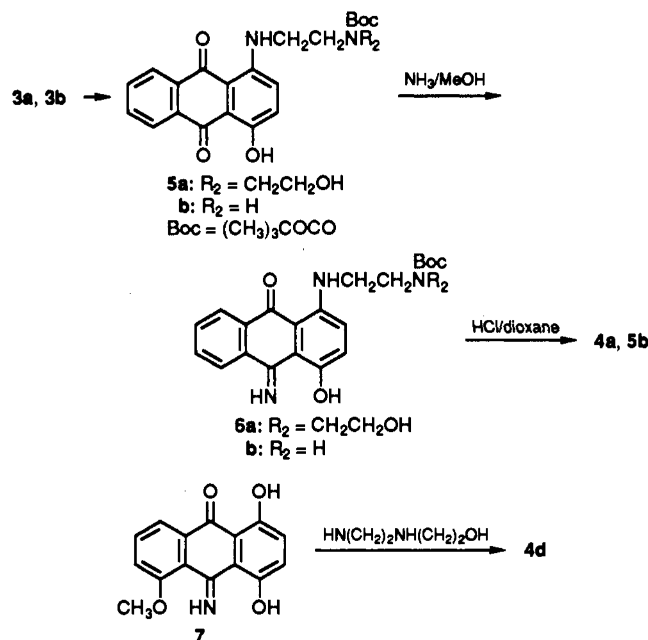
Scheme I



anthracenone (**4c**) and the corresponding 2,3-dehydro derivative could be isolated. These data indicate that =NH peri to hydroxyl group makes impossible the aromatic nucleophilic displacement of the hydroxyl by the amines.

The data obtained show that the ring amination of 1,4-bis[(aminoalkyl)amino]anthracenediones or introduction of (aminoalkyl)amino chains to iminoanthraquinone nucleus are procedures not suitable for the synthesis of imino analogues of compounds of ametantrone and mitoxantrone type.

The substrates in the synthesis of **4a–c** were unsymmetrically substituted 1-[(aminoalkyl)amino]-4-hydroxy-9,10-anthracenediones **3a–c**. With regard to the possibility of side reactions during aminations, N-terminal nitrogen atom of the side chain of **3a** and **3b** had to be protected. A base-stable *tert*-butoxycarbonyl group was found to be most suitable. Thus 3a and **3b** were transformed into appropriate *tert*-butoxycarbonyl derivatives **5a**[11] and **5b**, which were subject to further treatment with methanolic ammonia to give imino derivatives (**6a** and **6b**, respectively) in 50–60% yield (Scheme I). The deprotection performed with HCl/dioxane to yield **4a** and **4b** underwent smoothly without loss of the imino function. Although the iminoanthracenediones **6a** and **6b** have no OCH_3 groups stabilizing the imino function, their stability to acid conditions is similar to that of 5-iminoanthracyclines.[3a,b]

To obtain compound **4d** the different sequence of the reaction steps was applied, as a more advantageous way. At first, 5-methoxyquinizarin[12] was transformed into 5-methoxy-10-iminoquinizarin[9a] (**7**), followed by its reaction with 2-[(2-aminoethyl)amino]ethanol performed in *n*-butanol solution at elevated temperature. The ammonolysis of 5-methoxyquinizarin facilitated by the methoxy group, adjacent to the quinone carbonyl, occurred more easily than analogous reaction of **5a**, **5b**, and **3c**. However, in the comparison with the iminoanthracyclines literature,[3a,b] it can be said that to obtain iminoanthracenones more drastic conditions ought to be used. In accord with the literature

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Table I. NADH Oxidation by Iminoanthraquinone Derivatives and Their Parent Compounds[a]

compd	NADH oxidn μmol/min	compd	NADH oxidn μmol/min
4a	19.3	**4c**	17.7
3a	28.9	**3c**	25.7
4b	20.9	ametantrone	28.9
3b	25.7		

[a] Tested compounds were used as hydrochlorides. The reaction mixture (1.0 mL) contained 0.05 M TRIS-HCl buffer (pH = 7.2), 100 μM tested compound, and 1.0 unit/mL NADH dehydrogenase.

data,[9a] the structure of **7** was assigned as 10-imino isomer as a consequence of the presence of the OCH_3 group adjacent to the =NH. Anthracenediones bearing an "ametantrone arm" are particularly susceptible to the cyclization to form hexahydronaphtoquinoxalines.[13] Therefore, the presence of uncyclized (aminoalkyl)amino arm in **4d** was additionally evidenced by its converting to the *N-tert*-butoxycarbonyl derivative.

As a result of **3c**, **5a**, **5b**, and 5-methoxyquinizarin ammonolysis, the desired products (**4c**, **6a**, **6b**, and **7**) were obtained, but this reaction was also accompanied by side-product formation, especially under longer reaction time. Therefore, the amination was not performed to the complete disappearance of the substrates, which after isolation could be again treated with ammonia. Blue with violet reflection compounds **4a–d** were purified by column chromatography (**4a**, **4b**, and **4d** on Sephadex LH-20, **4c** on silica gel). Side products of ammonolysis reaction were not identified.

The structures of **4a–d** were evidenced by the analyses in accord with the presence of three nitrogen atoms (elemental analysis and MS-FD spectra data) by ^1H NMR and IR spectra and from the shift in UV–visible absorption spectrum to a longer wavelength. The ^1H NMR spectra in Me_2SO-d_6 solution showed, in relation to the substrates, two new H-bonded protons at about δ 13.2 attributed to the hydroxyl group chelated with peri =NH group and at about δ 9.0 assigned to the imino function. Both signals were broad singlets. The protons at C-2 and C-3 were different from those of the parent compounds and an increase of the distance between these protons has been observed, also.

Compounds **4a–d** appeared to be single regioisomeric imines, in accord with the results of Acton et al.[3a,b] with 5-imino derivatives of ADR and DR, as well as with the data for 9-iminoquinizarin.[9a]

Biological Results

The capacity of the examined compounds to stimulate NADH oxidation have been determined. The results obtained indicate that the oxygen-radical production by 1-[(aminoalkyl)amino]-4-hydroxy-10-imino-9-anthracenones paralleled those obtained for the corresponding 5-iminoanthracyclines.[4] As is presented in Table I, all examined iminoanthracenones were less effective in stimulating NADH oxidation catalyzed by NADH dehydrogenase (by 65–80%) than the corresponding parent compounds.

It is difficult to say to what extent such decrease of peroxidating activity affects the cardiotoxic properties of the compounds. It could be concluded that the decrease of the ability to undergo a redox cycle and consequently to initiate a free-radical cascade is not a specific feature only for 5-iminoanthracyclines but is exhibited also by synthetic anthracenediones.

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Table II. Activity of Iminoanthraquinone Derivatives and Related Anthraquinones against L1210 Leukemia Cells and P388 Murine Leukemia

compd[a]	EC_{50},[b] g/mL	P388 murine leukemia[c] dose, mg/kg	% T/C[d]	tox D[e] surv
4a	1.43 (±0.3)	80, 40, 20	160	7/7
		10	150	7/7
		5	130	7/7
		2.5	120	7/7
4b	0.89 (±0.18)	80	55	5/6
		40	118	6/6
		20	109	6/6
		10	118	6/6
		5	109	6/6
		2.5	100	6/6
4c	2.21 (±0.18)	80	125	7/7
		40, 20	100	7/7
		10. 5, 2,5	92	7/7
4d	2.80 (±0.37)		nt[f]	
3a	0.27 (±0.03)	50	127	7/7
		25	117	7/7
		12.5	109	7/7
		6.25, 3.12, 1.56	100	7/7
3b	0.24 (±0.04)		nt[f]	
3c	0.33 (±0.04)	60, 30	117	7/7
		15	109	7/7
		7.5	100	7/7
		3.78, 1.87	91	7/7
ametantrone	0.21 (±0.04)	25	200	6/6
		12.5	300	6/6
		6.25	210	6/6
		3.12	190	6/6

[a] Tested compounds were used as hydrochlorides. [b] EC_{50} = concentration of compound required to inhibit by 50% the growth of L1210 cells. [c] Treatment schedule QD1-5. [d] T/C = ratio of medium survival time expressed as percent of untreated controls. [e] Tox D surv = survivor recorded on day 4 after day of first injectin as a measure of drug toxicity. [f] Not tested.

The obtained iminoanthraquinone derivatives were also tested for their inhibitory activity against L1210 mouse leukemia cells in tissue culture and in vivo against P388 murine leukemia. For comparison the cytotoxicity of the parent compounds was also examined. Ametantrone was used in these tests as a reference. The obtained data are given in Table II.

The imino compounds were less cytotoxic than the parent compounds and ametantrone. Compound **4b**, containing an ethylenediamine residue attached to the anthraquinone nucleus at position 1, is the most active, while **4d**, probably because of the bulky methoxy group at C-5, exhibited poor cytotoxicity. The evaluation of the in vivo antileukemic activity showed that only **4a**, holding an "ametantrone arm" at position 1, is moderately active (% T/C was 160 at a dose 20 mg/kg). It should be noticed that our evaluation of the in vivo activity of **3a** (triple tests with carefully purified sample) demonstrated a T/C = 127% (at a dose of 50 mg/kg), which is discordant to the data published in the literature (% T/C was 342 at a dose 10 mg/kg).[6e,f] Taking into account our results with **3a**, it could be concluded that the replacement of the quinone carbonyl by the imino group (compound **4a**) does not diminish the antileukemic activity. However, the cytotoxic and antileukemic activity exhibited by some of the obtained iminoanthracenones indicate that the amination of the quinone carbonyl, diminishing the ability to generate the oxygen radicals, does not lead, as a rule, to the loss of antitumor activity.

Experimental Section

Melting points, determined on a Boetius PHMK 05 apparatus, were uncorrected. Elemental analyses were performed by the Laboratory of Elemental Microanalyses, University of Camerino; the obtained results were within 0.4% of the theoretical values, except where specified otherwise. A Varian Techtron Model 635 spectrophotometer was used for UV spectral determination and for measurements of NADH oxidation. IR spectra were recorded on a UR Zeiss spectrometer in KBr pellets; ^{1}H NMR spectra were taken on a Varian 90-MHz spectrometer in Me_2SO-d_6, with reference to 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, 5–18 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) $CHCl_3$–MeOH (5:1), (B) AcOEt–MeOH–H_2O (4:1:1), (C) 1-BuOH–Py–AcOH–H_2O (8:2:3:5), (D) toluene–acetone (3:1). Compounds **3a**, **3c**, **4c**, and **4d** were transformed into hydrochlorides by addition of HCl/ethyl ether to their cold chloroform solutions; separated salts were purificated on Sephadex LH-20 columns (eluent MeOH).

Materials. Cytochrome *c* (type VI from horse heart), NADH (grade III), and cardiac NADH dehydrogenase were obtained from the Sigma Chemical Corp., St. Louis, MO. Stock solutions were prepared just prior to use.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-hydroxy-9,10-anthracenedione (3a) was obtained according to the literature method (ref 6e): mp 139–140 °C (lit.[6e] 138–140 °C); ^{1}H NMR δ 2.7 (t, J = 6 Hz, 2 H, NCH_2 or CH_2N), 2.9 (t, J = 6 Hz, CH_2N or NCH_2), 3.5 (m, 4 H, CH_2O, $ArNCH_2$), 4.6 (m, 1 H, OH exchangeable with D_2O), 7.37 (d, J = 10 Hz, 1 H, H-2), 7.55 (d, J = 10 Hz, 1 H, H-3), 7.9 (m, 2 H, H-6, H-7), 8.28 (m, 2 H, H-5, H-8), 10.4 (m, 1 H, ArNH); IR 1580, 1610, 1635 cm^{-1}; UV–vis (MeOH) λ_{max} 225 nm (ε 45.036), 245 (50.584), 550 (14.360), 588 (12.400).

1-[[2-[(2-Hydroxyethyl)(*tert*-butoxycarbonyl)amino]ethyl]amino]-4-hydroxy-9,10-anthracenedione (5a) was obtained by the treatment of **3a** with di-*tert*-butyl dicarbonate.[11] The crude product was purified by means of column chromatography (silica gel, eluent $CHCl_3$–MeOH 10:1) and next was crystallized from $CHCl_3$–MeOH–hexane to afford **5a** as violet needles melting at 130 °C: ^{1}H NMR δ 1.4 (s, 9 H, $OC(CH_3)_3$), 3.25 (m, 2 H, CH_2O), 3.55 (m, 6 H, $ArNCH_2$, CH_2NCH_2), 4.75 (m, 1 H, OH exchangeable with D_2O), 7.2 (t, J = 10 Hz, 1 H, H-2), 7.45 (dd, J = 10, 4 Hz, 1 H, H-3), 7.8 (m, 2 H, H-6, H-7), 8.2 (d, J = 8 Hz, 2 H, H-5, H-8), 10.2 (t, 1 H, ArNH), 13.6 (s, 1 H, ArOH exchangeable with D_2O); MS-FD m/z (relative intensity) 426 ([M]$^+$, 100). Anal. ($C_{23}H_{36}N_6O_6$) C, H, N.

1-[[2-[(2-Hydroxyethyl)(*tert*-butoxycarbonyl)amino]ethyl]amino]-4-hydroxy-10-imino-9-anthracenone (6a). A mixture of 420 mg (1 mmol) of **5a**, 10 mL of methanolic ammonia (saturated at 0 °C), and a catalytic amount of *p*-toluenesulfonic acid was stirred in a sealed tube at 75 °C for 2 h and next at room temperature for about 3 days. During this time the reaction mixture was gradually transformed into a blue solution. The course of the reaction was controlled by TLC (solvent system A). Then the solvent was evaporated, and the residue was chromatographed (silica gel, eluent $CHCl_3$–MeOH, 15:1) to yield 260 mg (60%) of **6a** as a blue solid with violet reflection. An analytical sample was prepared by precipitation from chloroform solution with petroleum ether–hexane: mp 124–125 °C, ^{1}H NMR δ 1.3 (s, 9 H, $OC(CH_3)_3$), 3.25 (m, 2 H, $ArNCH_2$), 3.75 (m, 2 H, CH_2O), 4.7 (m, 1 H, OH exchangeable with D_2O), 7.0 (dd, J = 6.2, 4 Hz, 1 H, H-2), 7.6 (d, J = 10 Hz, 1 H, H-3), 7.85 (m, 2 H, H-6, H-7), 8.4 (m, 1 H, H-8), 8.5 (m, 1 H, H-5), 9.2 (m, 1 H, =NH exchangeable with D_2O), 12.0 (t, 1 H, ArNH), 13.3 (br s, 1 H, ArOH exchangeable with D_2O); MS-FD m/z (relative intensity) 425 ([M]$^+$, 100). Anal. ($C_{23}H_{27}N_3O_5$) C, H, N.

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-hydroxy-10-imino-9-anthracenone (4a). **6a** (210 mg) in a small amount of MeOH was treated with dioxane/HCl for 2 h. Next, to the reaction mixture was added ethyl ether, and the resultant solid was separated. The crude product was purified on a Sephadex LH-20 column (eluent MeOH) to afford 170 mg (95%) of **4a**·HCl as a blue solid with violet reflection. For conversion into the free base, **4a**·HCl was dissolved in the carbonate buffer (pH = 9) and **4a** was extracted with 1-BuOH; the butanol layers were evaporated, and the residue was purified Sephadex LH-20 column

(eluent MeOH–$CHCl_3$ 1:1): mp 159–160 °C; ^1H NMR δ 2.6 (t, J = 5.6 Hz, 2 H, NCH_2 or CH_2N), 2.9 (t, J = 6.1 Hz, 2 H, CH_2N or NCH_2), 3.5 (t, J = 5.6 Hz, 2 H, $ArNCH_2$), 3.6 (q, J = 6 Hz, 2 H, CH_2O), 4.5 (m, 1 H, OH exchangeable with D_2O), 7.0 (d, J = 10 Hz, 1 H, H-2), 7.55 (d, J = 10 Hz, 1 H, H-3), 7.8 (m, 2 H, H-6, H-7), 8.4 (m, 1 H, H-8), 8.5 (m, 1 H, H-5), 9.15 (m, 1 H, =NH exchangeable with D_2O), 12.0 (t, 1 H, ArNH), 13.3 (br s, 1 H, ArOH exchangeable with D_2O); IR 1565, 1589, 1615, 1640, 1655 cm^{-1}; UV–vis (MeOH) λ_{max} 242 nm (ε 28.530), 255 (24.730), 260 (25.540), 320 (5.870), 563 (11.086), 608 (17.830); MS-FD m/z (relative intensity) 325 ($[M]^+$, 100). Anal. ($C_{18}H_{19}N_3O_3\cdot H_2O$) C, H, N.

1-[[2-[(*tert*-Butoxycarbonyl)amino]ethyl]amino]-4-hydroxy-9,10-anthracenedione (5b). A solution of 1.6 mL of ethyldiamine in 6 mL of *n*-BuOH was added to a stirred suspension of 2.9 g (12 mmol) of quinizarin in 32 mL of *n*-BuOH. The resulting suspension was heated with stirring, under N_2 on an oil bath at 90 °C. Progress of the reaction was monitored by TLC (solvent system B): **5b** (violet), $R_f \approx 0.3$; the byproduct (hexahydronaphto[2,3-*f*]quinoxaline-7,10-dione) (violet), $R_f \sim 0.8$. After about 40 min the reaction mixture was cooled and petroleum ether–hexane was added. The resulting solid was filtered, washed with petroleum ether, and dried. This crude material was subject to treatment with di-*t*-butyl dicarbonate. The obtained product was purified by means of column chromatography (silica gel, eluent $CHCl_3$–MeOH, 90:1) to yield 2.75 g (60%) of **5b**: mp 146–147 °C; ^1H NMR δ 1.35 (s, 9 H, $OC(CH_3)_3$), 3.2 (q, J = 5.8 Hz, 2 H, CH_2NCO), 3.45 (q, J = 6.3 Hz, 2 H, $ArNCH_2$), 7.0 (t, J = 6 Hz, 1 H, NHCO, exchangeable with D_2O), 7.3 (d, J = 10 Hz, 1 H, H-2), 7.5 (d, J = 10 Hz, 1 H, H-3), 7.9 (m, 2 H, H-6, H-7), 8.2 (dd, J = 7.8, 1.2 Hz, H-5, H-8), 10.2 (t, 1 H, ArNH), 13.6 (s, 1 H, ArOH, exchangeable with D_2O; MS-FD m/z (relative intensity) 382 ($[M]^+$, 100). Anal. ($C_{21}H_{22}N_2O_5$) C, H, N.

1-[(2-Aminoethyl)amino]-4-hydroxy-9,10-anthracenedione hydrochloride (3b) was obtained by treatment of **5b** with HCl/dioxane. Purification of **3b** was carried out on a Sephadex LH-20 column (eluent MeOH): mp 220–221 °C; ^1H NMR δ 3.0 (t, 2 H, CH_2N), 3.7 (q, 2 H, $ArNCH_2$), 7.4 (d, 1 H, H-2), 7.6 (d, 1 H, H-3), 7.9 (m, 2 H, H-6, H-7), 8.2 (m, 2 H, NH_2, exchangeable with D_2O), 8.3 (m, 2 H, H-5, H-8), 10.2 (t, 1 H, ArNH); MS-FD m/z (relative intensity) 282 ($[M]^+$, 100); IR 1600, 1615, 1635 cm^{-1}; UV–vis (MeOH) λ_{max} 245 nm (ε 24.850), 545 (9.660), 580 (7.810). Anal. ($C_{16}H_{14}N_2O_3\cdot HCl\cdot H_2O$) C, H, N.

1-[[2-[(*tert*-Butoxycarbonyl)amino]ethyl]amino]-4-hydroxy-10-imino-9-anthracenone (6b). The reaction of **5b** with methanolic ammonia was performed as described for **6a**; time of the reaction: 2 h at 70 °C, 7 days at room temperature. The crude product was applied to a chromatography column (silica gel, eluent $CHCl_3$–MeOH 20:1) to afford **6b** (50% yield) as a blue-violet powder; mp 178–179 °C; ^1H NMR δ 1.4 (s, 9 H, $OC(CH_3)_3$), 3.2 (q, J = 5.4 Hz, 2 H, CH_2NCO), 3.6 (q, J = 6 Hz, 2 H, $ArNCH_2$), 7.0 (d, J = 10 Hz, 1 H, H-2), 7.1 (t, 1 H, NHCO, exchangeable with D_2O), 7.5 (d, J = 10 Hz, 1 H, H-3), 7.8 (m, 2 H, H-6, H-7), 8.4 (dd, J = 4.1, 1.7 Hz, 1 H, H-8), 8.5 (dd, J = 4.5, 2 Hz, 1 H, H-5), 9.2 (m, 1 H, =NH exchangeable with D_2O), 12.0 (t, 1 H, ArNH), 13.3 (br s, 1 H, ArOH exchangeable with D_2O); MS-FD m/z (relative intensity) 382 ($[M]^+$, 100). Anal. ($C_{21}H_{23}N_3O_4$) C, H, N.

1-[(2-Aminoethyl)amino]-4-hydroxy-10-imino-9-anthracenone (4b). Compound **6b** was transformed into **4b** in the same manner as that described for **4a**: mp 197–198 °C; ^1H NMR δ 3.1 (t, J = 6.8 Hz, 2 H, CH_2N), 3.9 (q, J = 6.8 Hz, 2 H, $ArNCH_2$), 7.05 (d, J = 10 Hz, 1 H, H-2), 7.6 (d, J = 10 Hz, 1 H, H-3), 7.9 (m, 2 H, H-6, H-7), 8.2 (m, 2 H, NH_2 exchangeable with D_2O), 8.4 (dd, J = 7, 2 Hz, 1 H, H-8), 8.55 (dd, J = 7, 2 Hz, 1 H, H-5), 9.4 (m, 1 H, =NH exchangeable with D_2O), 11.8 (t, 1 H, ArNH), 13.5 (br s, 1 H, ArOH exchangeable with D_2O); IR 1570, 1580, 1615, 1650 cm^{-1}; UV–vis (MeOH) λ_{max} 235 nm (ε 21.430), 252 (20.530), 257 (20.530), 383 (6.250), 563 (12.500), 605 (18.730); MS-FD m/z (relative intensity) 281 ($[M]^+$, 100). Anal. ($C_{16}H_{15}N_3O_2\cdot 4H_2O$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-4-hydroxy-9,10-anthracenedione (3c) was obtained according to literature procedure (ref 6e): mp 118–120 °C (in accord with lit.[6e]); ^1H NMR δ 2.25 (s, 6 H, CH_3), 2.55 (t, J = 7 Hz, 2 H, CH_2N), 3.5 (q, J = 6.8 Hz, 2 H, $ArNCH_2$), 7.35 (dd, J = 10, 3 Hz, 1 H, H-2), 7.5 (dd, J = 10, 2.8 Hz, 1 H, H-3), 7.9 (m, 2 H, H-6, H-7), 8.3 (m, 2 H, H-5, H-8), 10.4 (t, 1 H, ArNH); IR 1570, 1605, 1635 cm^{-1}; UV–vis (MeOH) λ_{max} 248 nm (ε 26.740), 550 (11.240), 590 (9.460).

1-[[2-(Dimethylamino)ethyl]amino]-4-hydroxy-10-imino-9-anthracenone (4c). The reaction of **3c** with methanolic ammonia was carried out as described for **6a** (time of reaction 18 days at room temperature). The crude product was applied to a column of silica gel which was eluted with $CHCl_3$–MeOH (5:1) and then $CHCl_3$–MeOH (1:1). The yield of **4c** was 70%: mp 176–177 °C; ^1H NMR δ 2.27 (s, 6 H, CH_3), 2.58 (t, J = 8 Hz, 2 H, CH_2N), 3.32 (q, J = 7.8 Hz, 2 H, $ArNCH_2$), 7.0 (d, J = 10 Hz, 1 H, H-2), 7.5 (d, J = 10 Hz, 1 H, H-3), 7.8 (m, 2 H, H-6, H-7), 8.4 (dd, J = 7, 2 Hz, 1 H, H-8), 8.52 (dd, J = 6, 2 Hz, 1 H, H-5), 9.3 (m, 1 H, =NH exchangeable with D_2O), 12.1 (t, 1 H, ArNH), 13.3 (br s, 1 H, ArOH exchangeable with D_2O); IR 1570, 1580, 1615, 1660 cm^{-1}; UV–vis (MeOH) λ_{max} 232 nm (ε 19.200), 258 (17.710), 320 (4.920), 563 (9.600); MS-FD m/z relative intensity 309 ($[M]^+$, 100). Anal. ($C_{18}H_{19}N_3O_2\cdot 3H_2O$) C, H, N.

1,4-Dihydroxy-5-methoxy-10-imino-9-anthracenone (7). The reaction of 2.7 g (10 mmol) of 1,4-dihydroxy-5-methoxy-9,10-anthracenedione[12] with methanolic ammonia and *p*-toluenesulfonic acid as catalyst was performed at 45 °C during 35 min; the yellow reaction mixture was gradually transformed to red-violet. The course of the reaction was controlled by TLC (solvent systems A and D). The purification of crude product on silica gel column (eluent $CHCl_3$–MeOH 20:1) yielded 2.43 g (90%) of **7**. An analytical sample was crystallized from MeOH–ethyl ether: mp 267–268 °C; ^1H NMR 4.1 (s, 3 H, OCH_3), 7.1 (d, J = 9 Hz, 1 H, H-3 or H-2), 7.2 (d, J = 9 Hz, 1 H, H-2 or H-3), 7.65 (d, J = 7.6 Hz, 1 H, H-6), 7.7 (t, J = 8 Hz, 1 H, H-7), 8.1 (dd, J = 7, 1 Hz, H-8), 10.0 (m, 1 H, =NH exchangeable with D_2O); IR 1570, 1600–1610 cm^{-1}; MS-FD m/z (relative intensity) 269 ($[M]^+$, 100).

1-[[2-[(2-Hydroxyethyl)amino]ethyl]amino]-4-hydroxy-5-methoxy-10-imino-9-anthracenone (4d). The solution of 540 mg (2 mmol) of **7** and 14 mL of 2-[(2-aminoethyl)amino]ethanol in 20 mL of 1-BuOH was heated at 85 °C for about 50 min. During this time the color of the reaction mixture was transformed from red-violet to blue. The course of the reaction was monitored by TLC (solvent systems B and C). Then, the reaction mixture was diluted with 1-BuOH and washed several times with H_2O. After removal of 1-BuOH in vacuo, **4d** was isolated by means of a column (Sephadex LH-20; eluent $CHCl_3$–MeOH 1:1) to yield 285 mg (40%) of a dark blue solid. An analytical sample was obtained by precipitation with ethyl ether–hexane of a **4d**-chloroform solution: mp 185–186 °C; ^1H NMR δ 2.7 (t, J = 5 Hz, 2 H, NCH_2 or CH_2N), 3.05 (t, J = 5.5 Hz, 2 H, CH_2N or NCH_2), 3.6 (t, J = 5 Hz, 2 H, $ArNCH_2$), 3.75 (q, J = 6 Hz, 2 H, CH_2O), 4.1 (s, 3 H, OCH_3), 5.0 (m, 1 H, OH exchangeable with D_2O), 7.0 (d, J = 10 Hz, 1 H, H-2), 7.5 (m, 2 H, H-3, H-6), 7.75 (t, J = 8 Hz, 1 H, H-7), 8.1 (d, J = 6.5 Hz, 1 H, H-8), 9.6 (m, 1 H, =NH exchangeable with D_2O), 11.75 (t, 1 H, ArNH), 14.3 (br s, 1 H, ArOH exchangeable with D_2O); IR 1605, 1655 cm^{-1}; UV–vis (MeOH) λ_{max} 243 nm (ε 18.340), 383 (5.920), 574 (11.600), 610 (15.150); MS-FD m/z relative intensity 355 ($[M]^+$, 100). Anal. ($C_{19}H_{21}N_3O_4\cdot 2H_2O$) C, H, N.

The *N-tert*-butoxycarbonyl derivative of **4d** was obtained by the treatment of this compound with di-*tert*-butyl dicarbonate; the ^1H NMR spectrum confirmed the presence of a $OC(CH_3)_3$ group at δ 1.3 (s, 9 H).

Biological Tests. In Vitro Cytotoxicity Evaluation. The mouse L1210 leukemia cells (RPMI) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum and penicillin (100.000 units/L) plus streptomycin (100 mg/L) in a controlled (air–5% CO_2), humidified atmosphere at 37 °C. L1210 mouse leukemia cells were seeded at density 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 EC_{50} values) of the tested compounds were defined as their in vitro concentrations causing 50% inhibition of 48-h growth, measured by cells' protein-content determination.[14]

In Vivo Antileukemic Evaluation. BDF1 mice were injected ip with 10^6 P388 lymphotic leukemia cells on day 0 and treated

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ip on days 1–5 in accordance with the protocols described by the National Cancer Institute.[15] The mean survival time (MST) for each treatment group (eight mice/group) was calculated and the percent T/C determined by using the following formula:

$$\% \ T/C = \frac{\text{MST treated}}{\text{MST control}} \times 100$$

NADH dehydrogenase activity was determined with cytochrome *c* as the electron acceptor.[16] It was examined by following cytochrome *c* reduction at 550 nm with an extinction coefficient for cytochrome *c* (reduced minus oxidized) of 19600. The reaction mixture (1.0 mL) contained 0.05 M TRIS-HCl buffer (pH = 7.2), 50 μM cytochrome *c*, 100 μM NADH, and 1.0 unit/mL NADH dehydrogenase. Enzymatic activity has been expressed in units, where 1 unit of activity is the amount of NADH dehydrogenase capable of reducing 1 μM of cytochrome *c* per min at pH 7.2 at 25 °C. NADH oxidation was measured at 340 nm with an extinction coefficient of 6.22 mM^{-1} cm^{-1}. The 1-mL reaction mixture contained 0.05 M TRIS-HCl buffer (pH = 7.2), 100 μM tested compound, 100 μM NADH, and 1 unit/mL NADH dehydrogenase. NADH consumption was initiated by the addition of enzyme.

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Registry No. **3a**, 69895-67-6; **3b**, 131042-03-0; **3c**, 69895-69-8; **4a**, 131042-04-1; **4b**, 131042-05-2; **4c**, 131042-06-3; **4d**, 131042-07-4; **4d** (Boc deriv.), 131042-11-0; **5a**, 86991-03-9; **5b**, 131042-08-5; **6a**, 131042-09-6; **6b**, 131042-10-9; **7**, 96502-06-6; quinizarin, 81-64-1; 1,4-dihydroxy-5-methoxy-9,10-anthracenedione, 64831-67-0.

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Synthesis and Biological Activity of Bay-Region Metabolites of a Cyclopenta-Fused Polycyclic Aromatic Hydrocarbon: Benz[*j*]aceanthrylene

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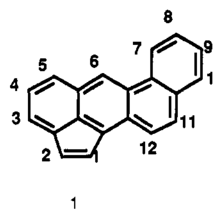
The possibility of bay-region activation of the cyclopenta PAH (polycyclic aromatic hydrocarbon with a peripherally fused cyclopenta ring) benz[*j*]aceanthrylene (1) was investigated by synthesis and bioassay of the bay-region metabolites *trans*-9,10-dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene (4), *trans*-9,10-dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrobenz[*j*]aceanthrylene (2), and 9,10-dihydrobenz[*j*]aceanthrylene 9,10-oxide (3). The known 1,2-dihydrobenz[*j*]aceanthrylene-9,10-dione (5) was obtained by published methods; however, the direct route to target dihydrodiol 4, dehydrogenation of the saturated five-membered ring of 5 followed by $NaBH_4$ reduction, gave a poor yield of 4 contaminated with tetrahydrogenated products. Acceptable yields of 4 were obtained by reduction of 5 to the corresponding tetrahydro diol, diacetylation of the diol, and dehydrogenation of the five-membered ring followed by base-catalyzed deacetylation to 4. *anti*-Diol epoxide 2 was generated by *m*-chloroperoxybenzoic acid oxidation of 4. Oxide 3 was synthesized by treatment of the monotosylate of 4 with NaOH in monoglyme. Diol epoxide 2 was an active mutagen in *Salmonella typhimurium* strain TA98 in the absence of metabolic activation, 3 showed marginal activity, while 3 and 4 were mutagenic with metabolic activation. These results coupled with previous studies support activation of benz[*j*]aceanthrylene via both 2 and cyclopenta ring epoxidation.

Introduction

Polycyclic aromatic hydrocarbons with peripherally fused cyclopenta rings (cyclopenta PAH) are environmental contaminants[1,2] and potential carcinogens.[3–7] Biological activity has been observed for a number of cyclopenta PAH that also contain a bay region.[6,7] Of these compounds, benz[*j*]aceanthrylene (1) is a potent mutagen,[8,9] cell-transforming agent,[6] and tumor initiator[7] in rodents. The activity of 1 is dependent upon microsomal metabolism and for this reason activation is expected to proceed via arene oxide formation. Although epoxidation of the cyclopenta ring is known to be an activation pathway[2] for biologically active cyclopenta PAH, including 1, molecular orbital correlations[10] and metabolism studies[6,8] on 1 also indicate that bay-region diol epoxide 2 and 9,10-oxide 3 are potentially active metabolites, suggesting multiple activation pathways. The importance of the cy-



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