(OCH_2, 5), 57.6 (1-cyclohexyl, s), 41.6 ($NHCH_2$, t), 36.3 (t), 32.6 (t), 26.1 (t), 23.7 (t), 22.3 (t); IR (thin film) 3340, 1445, 1053, 760, 702 cm^{-1}.

A 15% aqueous solution of $KMnO_4$ was slowly added to a stirred solution of 11 g of the arylcyclohexylamino alcohol, 75 mL of H_2O, and 20 mL of 5 N HCl at room temperature. The solution was examined periodically to determine if the pH was acidic, if there was an excess of $KMnO_4$, and if any starting material remained (via HPLC). After the addition of 24 g (0.15 mol) of $KMnO_4$ over 2 h, the reaction was quenched with 100 mL of isopropyl alcohol. Solid $NaHSO_3$ was then added with stirring until the brown color of the MnO_2 had been dissipated. The mixture was filtered then the filtrate was concentrated to a thick slurry by evaporation at 40 °C under vacuum. To form the methyl ester and to aid in the removal of water, 3 × 75 mL portions of anhydrous methanol were added to the highly acidic residue and then evaporated at 40 °C to dryness. The crude product was chromatographed on a 3 × 30 cm silica gel column (Woelm, 0.05–0.2 mm) using 300 mL of CH_2Cl_2, 300 mL of 5% CH_3OH in CH_2Cl_2, and 300 mL of 10% CH_3OH in CH_2Cl_2 for elution to give 4.2 g of the crude methyl ester. A diethyl ether solution of the product was washed with an aqueous NH_4OH solution and dried with Na_2SO_4, and then the HCl salt of the product was precipitated with HCl–ether. The final product (**2**) was recrystallized from methanol–ethyl acetate (2:75) to give fine colorless needles: mp 189–191 °C; ^1H NMR (HCl salt, $CDCl_3$) δ 7.2–7.8 (phenyl, 5), 3.37 (OCH_3, 3, s), 1.1–2.8 (br m); ^{13}C NMR (HCl salt, $CDCl_3$) δ 173.2 (C=O, s), 135.4 (1′-phenyl, s), 129.4 (phenyl, d), 129.0 (phenyl, d), 128.4 (phenyl, s), 64.6 (1′-cyclohexyl, s), 51.5 (OCH_3, q), 41.5 (t), 33.5 (t), 33.3 (t), 26.1 (5), 25.2 (t), 22.4 (t); IR (KBr disk) 1726 (C=O), 1272, 774 (phenyl), 706 cm^{-1} (phenyl); mass spectrum (70 eV), *m/e* (relative intensity), 289 (M^+, 3.7), 246 (42), 159 (26), 115 (38), 91 (100). Anal. ($C_{18}H_{28}ClNO_2$) C, H, N.

5-[*N*-(1′-Phenylcyclohexyl)amino]pentanoic Acid (1). Though small amounts of 1 were isolated from the later fractions of the chromatographic purification of **2**, the yields were typically 10–15% of that of the methyl ester. A more useful procedure was to reflux the HCl salt of II in H_2O for 22 h to give nearly quantitative conversion to the free acid: mp 214–215 °C (from acetone); IR (KBr disk) 1733 (C=O), 1170, 771 (phenyl), 698 cm^{-1} (phenyl); mass spectrum (70 eV), *m/e* (relative intensity), 275 (M^+, 12), 232 (100), 216 (2.5). 132 (23), 91 (26). Anal. ($C_{17}H_{26}ClNO_2$) C, H. N.

Quantitation and Identification of Phencyclidine Metabolites. A 9-kg dog was dosed at 5 mg/kg with phencyclidine hydrochloride and this dose was repeated 60 and 180 min after the initial injection. A total of 370 mL of urine was then collected over the next 30 h.

A 10-mL portion of the urine sample was mixed with 10 mL of 95% ethanol, and then the mixture was saturated with solid anhydrous K_2CO_3, which caused the mixture to separate into two layers. After the mixture was centrifuged, the top ethanol layer was transferred to a clean conical centrifuge tube and evaporated on a steam cone under a stream of nitrogen (recovery >90%). After the sealed tubed cooled, 0.5 mL of BF_3·CH_3OH was added and the sealed tube was heated to 100 °C for 10.0 min. After the mixture cooled 0.5 mL of H_2O was added, and 10 min later the mixture was made alkaline (pH ≃10) with the dropwise addition of concentrated NH_4OH and then extracted with 3 mL of diethyl ether. After the mixture was dried with Na_2SO_4, the ether extract was evaporated under a stream of nitrogen. The residue was then taken up in 100 μL of ethyl acetate for gas chromatographic analysis (1 μL used).

A Model 900 Perkin-Elmer gas chromatograph was equipped with a 2 mm × 183 cm glass column packed with 3% OV-17 on 110–120 mesh Anachrom ABS support. The column was operated at 240 °C, the injector at 270 °C, and the manifold at 270 °C. Helium was used as the carrier (27 mL/min), and the column effluent was split equally between a flame-ionization detector (H_2 = 19 mL/min) and a rubidium bead nitrogen–phosphorus selective detector (H_2 = 2.3 mL/min). The latter detector was operated in the mode where both nitrogen- and phosphorus-containing compounds would be detected and the bead current setting of 6.8 was used. The response index of the metabolite peak on the two detectors was measured relative to caffeine as a standard as previously reported.[13]

For the purpose of quantification and identification, a known quantity of 1 in a blank sample was extracted and derivatized using the above procedure. Quantitations were based on the peak-height ratios of the standard and unknown. The retention time of the standard was found to be 5.8 min and the Kovat retention index was 2421. The detector response index of the standard was 0.14, which was typical of secondary amines (0.12–0.40).[13] The retention time, Kovat retention index, response index, and mass spectra of the metabolite peak obtained from the dog urine were also measured.

Pharmacology. The CNS stimulant effect of phencyclidine and the related compounds were measured using actometers and protocol similar to that previously reported for phencyclidine.[16] Groups of nine male Swiss mice were given the same dose of the test compound (8 mg/kg ip) in the same volume of water (0.01 mL/g of body weight). The locomotor activity of the individual mice was measured for 1 h before the injection and for 1 h after the injection. The difference in the locomotor activity (postinjection–preinjection) for the individual mice was then taken as a measure of the effect of the test compound on CNS activity. The control group receiving an injection of water was found to have an activity score of −1426 (Table I), indicating that the locomotor activity of the mice decreased in the second hour compared to the first hour when no drug was given.

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Synthesis and Preliminary Antitumor Evaluation of 5-Iminodoxorubicin

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Doxorubicin required protection as the *N*-(trifluoroacetyl) 14-*O*-*p*-anisyldiphenylmethyl ether in order to prevent extensive degradation of the α-hydroxy ketone side chain during ammonolysis of the quinone to give the stable 5-imino derivative. In the initial test against mouse leukemia P-388, 5-iminodoxorubicin showed efficacy (T/C = 173%) comparable to that of the analogue 5-iminodaunorubicin and of doxorubicin as the parent but inexplicably required at least 10 times the dose. The two imino compounds were indistinguishable by in vitro tests, which suggested weaker DNA interactions compared to doxorubicin and much weaker redox cycling with O_2 to generate free radicals.

The quinone moiety occurs in the structure of numerous anticancer agents and is often recognized as a key site of biochemical action, particularly[1-11] in the clinically important anthracyclines doxorubicin[12] (Adriamycin, 1) and

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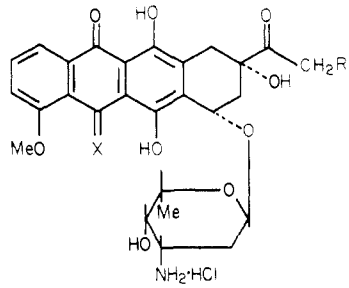
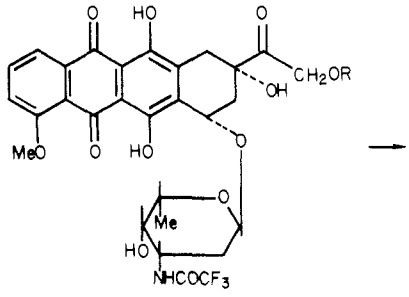
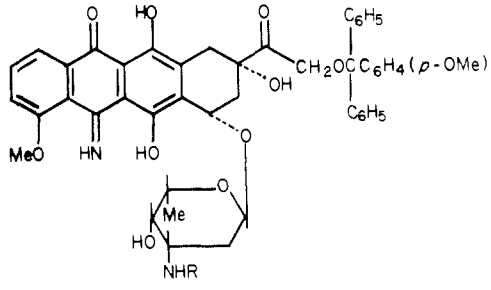


1, R = OH; X = O
2, R = H; X = O
3, R = H; X = NH
4, R = OH; X = NH



5, R = H
6, R = $C(C_6H_5)_2C_6H_4$-p-OMe



7, R = $COCF_3$
8, R = H

daunorubicin[12] (2). Despite that, 5-iminodaunorubicin[13] (3) is apparently the only quinone-modified analogue in the entire anthracycline series. Interestingly, 3 was significantly less potent than 1 and 2 in causing cardiotoxic effects in certain tests,[13,14] yet it retained antitumor activity comparable to 1 and 2 in the mouse screen.[13] Analogues with lower cardiotoxicity are widely sought,[15] because the risk of heart failure from this cumulative, dose-dependent side effect limits the use of 1 and 2. Other needed improvements are[15] lowered acute toxicity, better efficacy against the unusually broad spectrum of tumors that respond to 1 (in particular) and 2, and activity against tumors that do not respond to 1 or 2.

Although the mechanism of anthracycline cardiotoxicity has not been established and the potential for 3 to show reduced cardiotoxicity allied with antitumor efficacy has not been tested in the clinic, it is tempting to seek a connection between the reduced cardiotoxic effects observed[13,14] with 3 and its demonstrated[4,16,17] reduction in capacity to generate free radicals through cyclic reduction–reoxidation of the quinoid structure. Along with further evaluation of 3, it is clear that other quinone-modified anthracyclines should be synthesized and studied.

A logical target was 5-iminodoxorubicin (4). Preliminary attempts showed that 4 can not be synthesized directly from 1 by the method for 5-iminodaunorubicin (3), which is obtained in one step by treating 2 with cold methanolic ammonia. Similar treatment of 1 gave extensive decomposition, with only a trace of desired 4 detectable as a violet spot on thin-layer chromatography (TLC) of the reaction mixture.

The increased lability of doxorubicin (1) relative to 2 is often underestimated. This effect in 1 is largely because of the α-hydroxyketone side chain, which can tautomerize to an α-hydroxy aldehyde, followed by a retro-aldol process, loss of the side chain, and aromatization of the A ring with elimination of the sugar.[18] Consequently, blocking of the 14-OH is required before a number of common chemical methods, including those for the synthesis of 4, can be applied to 1. Availability of efficient methods for blocking the functional groups of 1 is of general importance, but, surprisingly, there are few detailed procedures for preparing derivatives directly from 1 in satisfactory yield.

Synthesis. For synthesis of 4, the 14-*O*-(MeO)Tr group (14-*O*-*p*-anisyldiphenylmethyl) appeared attractive as a base-stable protecting group that could be cleaved with mild acid.[19,20] However, 1 (the hydrochloride salt) was insoluble in pyridine, the normal methoxytritylation solvent, and the suspension underwent essentially no reaction with excess (MeO)TrCl (*p*-anisyldiphenylmethyl chloride). When 1 was solubilized by adding dimethylformamide (DMF) to the pyridine, tritylation occurred at the sugar NH_2 as well as at the 14-OH, judging from the appearance in the TLC of two major spots presumably for the 14-*O*-(MeO)Tr and *N*,14-*O*-[(MeO)Tr]$_2$ derivatives. If the pyridine present in the DMF was limited to an equivalent

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Table I. Comparison of Biological Test Data

compd	act. vs. leukemia P-388 in mice,[a] relative survival time after dosing q4d 5,9,13: % T/C, mg/kg		inhibn of synth in leukemia L1210 cells[b]: ED_{50}, μM		ΔT_m of isolated helical DNA in soln,[c] °C	augmentation of microsomal O_2 consumption rel to doxorubicin,[d] %
			DNA	RNA		
doxorubicin (1)	125 ± 20 (16) 162 ± 22 (8.0) 144 ± 14 (4.0) 130 ± 13 (2.0) 123 ± 10 (1.0)	53 tests[e]	1.5	0.7	13.4	100
daunorubicin (2)	123 ± 15 (16.0) 129 ± 8 (8.0) 126 ± 11 (4.0) 123 ± 13 (2.0)	45 tests[e]	1.0	0.3	11.2	109
5-iminodaunorubicin (3)	126 ± 3 (24) 116 ± 14 (12) 124 ± 1 (6.0) 130 ± 6 (3.0) 126 ± 8 (1.5)	2 tests[f]	1.6	1.3	6.2	8.2
	140 (16) 180 (9.6) 186 (5.76) 177 (3.46)	1 test				
	157 (9.0) 161 (5.4) 150 (3.24) 137 (1.94)	1 test				
5-iminodoxorubicin (4)	173 (50) 155 (25) 132 (12.5) 122 (6.25)	1 test	2.0	2.1	6.9	7.5

[a] Reference 25. Mice injected ip with leukemia cells on day 0 were treated ip on days 5, 9, and 13 with the specified dose. Doses that produce T/C values (survival time of treated/control mice) ≥ 120% are defined as active. [b] Procedure the same as in ref 26, except for addition of 1% Me_2SO. We are indebted to D. L. Taylor for these and the ΔT_m data. [c] Procedure same as in ref 26, except that the 0.010 M phosphate buffer was at pH 7.0 and contained EDTA at 10^{-5} M plus 5% Me_2SO to solubilize compounds. [d] Data from ref 17. [e] Data received from NCI, 1977–1980, and consistent over that period. [f] Data from ref 13; the data from single tests at more closely spaced doses of 3 were received from NCI subsequently.

of the HCl liberated in the methoxytritylation reaction [in order to minimize release of HCl from the sugar amine hydrochloride and avoid *N*-methoxytritylation], there was considerable glycoside cleavage, presumably acid catalyzed, after workup. Consequently, **1** was first protected by N-trifluoroacetylation. Conversion of **1** in situ to the free base and treatment with *S*-ethyl trifluorothioacetate[21] afforded *N*-(trifluoroacetyl)doxorubicin[22] (**5**) in 84% yield. Previously, the only direct[23] (as opposed to indirect[24] from **2**) synthesis of **5** gave a 44% yield after treating the isolated free base of **1** with trifluoroacetic anhydride and chromatographic purification. A pyridine solution of **5** then underwent methoxytritylation smoothly, without heating and without requiring chromatographic purification,[20] to yield **6** (95%). Amination of **6** to give the 5-imino derivative **7** occurred with cold methanolic ammonia under the conditions for the synthesis of **3**, except that it was necessary first to dissolve the methoxytrityl compound **6** in dichloromethane. The amination was accompanied in small degree (5–10%) by cleavage of the *N*-(trifluoroacetyl) blocking group to give 14-*O*-(methoxytrityl)-5-iminodoxorubicin (**8**), but the extensive decomposition seen in attempts to aminate **1** was significantly reduced. Rather than separate **7** and **8**, it was efficient to treat the mixture in dioxane–methanol (2:1) with cold dilute sodium hydroxide to complete the conversion to **8**, which was isolated chromatographically in 31% yield. Deblocking of the 14-*O*-(methoxytrityl) ether with 80% acetic acid gave 5-iminodoxorubicin (**4**) in 81% yield. As expected from the acid stability[13] of **3**, there was no loss of the imino function. The overall yield from **1** was 21%. The ^1H NMR spectrum of **4** showed three H-bonded protons at δ 15.80 (sharp singlet, 11-OH), 13.48 (broad singlet of 6-OH, coupled with =NH), and 9.55 (broad singlet, =NH, weakly H bonded to 4-OCH_3). These were nearly identical with those which were found to be characteristic of the 5-imino structure of **3** and which explained formation of the stable imino derivative exclusively at the 5 position. The structure of **4** was further confirmed by ultraviolet and mass spectra analogous to those of **3**.

The acid stability of the imino function in **4** was further demonstrated by treatment with 0.1 M hydrochloric acid at 100 °C for 5 min, which gave complete glycoside cleavage but considerable 5-iminoadriamycinone among the decomposition products.

Biological Results

The data in Table I show that in several preliminary tests the properties of **4** were closely comparable to those of **3**, except for a 10-fold (or more) decrease in antitumor potency (reflected in the higher optimum dose) as distinguished from antitumor efficacy (% T/C or survival time of treated mice relative to controls).[25] Thus, the efficacy

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of 4 (T/C = 173%) is comparable to that of 3 (T/C values up to 186%) when 3 is tested in a series of closely spaced doses (the optimum dose of 3 apparently was not observed in the usual series of dose doublings; 1.5 to 24 mg/kg, best T/C = 130%). However, 4 required an optimum dose of 50 mg/kg (the top dose in the single test so far) compared to 5–6 mg/kg of 3. Similarly, 4 is comparable to 1 (T/C = 162% at 8 mg/kg) in efficacy but at sixfold higher doses. Further tests of 4 will be required to determine if 4 shows toxic side effects typical of the anthracyclines and at what dose levels. The relative potency of therapeutic and toxic effects is the critical factor in determining whether a new analogue offers an advantage for possible clinical use.

The imino compounds 3 and 4 were indistinguishable in their inhibition[26] of incorporation of radiolabeled DNA–RNA precursors in L1210 cells and were not significantly different from 1 and 2. The stabilization of helical DNA toward thermal denaturation[26] by 3 and 4 (ΔT_m = 6.2, 6.9 °C) was only half that observed with 1 and 2. Augmentation of oxygen consumption[4] by various anthracyclines and quinoid agents in the presence of rat liver microsomes and flavoenzymes is a measure of the oxygen cycling and free-radical generating mechanism that has been proposed, involving the quinone structure, and which has been associated with cardiotoxicity. The stimulation of O_2 consumption observed with 3[17] and 4 was only 7.5–8.2% of the stimulation observed with 1. This markedly weaker effect can be correlated in the case of 3, with the decreased radical formation observed as decreased DNA nicking by reductively activated drug, and with the poorer tendency of 3 to undergo redox cycling that was observed electrochemically.[16]

Clearly, further testing is needed to evaluate the potential of both 3 and 4. At present, 5-iminodaunorubicin appears to be superior to the doxorubicin analogue 4 because of the weaker antitumor potency of 4. However, the unpredictable variation in potency between 3 and 4 (despite their similarity by other criteria) suggests there may be other differences and illustrates the need to gather extensive data using different types of tests before concluding any evaluation of active analogues.

Experimental Section

General Methods. Solutions in organic solvents were dried over sodium sulfate and filtered. Evaporations were carried out in vacuo on a rotary evaporator. Melting points are uncorrected. IR spectra in Nujol mull were recorded on a Perkin-Elmer Model 137 spectrometer. Proton nuclear magnetic resonance (NMR) was determined with Varian EM390 and XL-100 spectrometers on solutions as noted with Me_4Si (δ 0.0) internal reference, and the signals are described as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad); we thank Dr. K. F. Kuhlmann and L. Garver for the spectra. UV–visible spectra of 4 were obtained on a Perkin-Elmer Model 575 recording spectrometer, for which we thank D. L. Taylor. The mass spectrum of 4 was recorded on an LKB Model 9000 spectrometer at 12 eV; we thank Dr. D. W. Thomas for the spectrum and interpretation. Thin-layer chromatography (TLC) was carried out on 2 × 8 in. glass plates coated with 0.25-mm layers of silica gel GF; R_f values are given for products purified to homogeneity.

***N*-(Trifluoroacetyl)doxorubicin (5).**[22] A stirred suspension of 2.98 g (5.14 mmol) of adriamycin hydrochloride (1) in 100 mL of $CHCl_3$–CH_3OH (1:1) cooled in an ice bath was treated with 10.3 mL of 0.5 M methanolic $NaOCH_3$ dropwise, followed by 3.3 mL (25.7 mmol) of *S*-ethyl trifluorothioacetate. The solution was stirred at 23 °C in the dark for 5 h. Additional 0.5 M methanolic $NaOCH_3$ (1.0 mL) and *S*-ethyl trifluorothioacetate (0.66 mL, 5.1 mmol) were added, and stirring was continued for 17 h. After evaporation of the reaction mixture, the residue was dissolved in 25 mL of $CHCl_3$–CH_3OH (1:1), diluted with 15 mL of toluene, and reevaporated. A solution of the residue in 500 mL of $CHCl_3$–CH_3OH (9:1) was washed with 50 mL of 0.1 M citric acid and NaCl solution (3 × 100 mL), dried, and evaporated. Trituration of the residue with 40 mL of CH_2Cl_2 afforded 2.76 g (84%) of 5: mp 172–174 °C (lit.[24] 174–176 °C), unchanged upon recrystallization from $CHCl_3$; IR 2.90 (OH, NH), 5.82 (C=O), 6.20, 6.32 μm (H-bonded quinone); 90-MHz NMR ($CDCl_3$–CD_3OD 6:1) δ 7.93 (d, 1, J = 8 Hz, H-1), 7.74 (t, 1, J = 8 Hz, H-2), 7.37 (d, 1, J = 8 Hz, H-3), 5.48 (br s, 1, H-1′), 5.20 (br s, 1, H-7), 4.75 (s, 2, H-14), 4.05 (s, 3, OCH_3), 4.0–4.3 (m, 2, H-3′, H-5′), 3.62 (m, 1, H-4′), 3.18 (d, 1, J = 19.5 Hz, H-10B), 2.93 (d, 1, J = 19.5 Hz, H-10A), 2.22 (m, 2, H-8), 1.87 (m, 2, H-2′), 1.29 (d, 3, J = 6.5 Hz, CH_3-5′); TLC ($CHCl_3$–CH_3OH, 19:1) R_f 0.2.

14-*O*-(*p*-Anisyldiphenylmethyl)-*N*-(trifluoroacetyl)doxorubicin (6). A solution of 2.76 g (4.32 mmol) of 5 and 5.35 g (17.3 mmol) of *p*-anisylchlorodiphenylmethane in 40 mL of dry pyridine was stirred at 23 °C in the dark for 19 h. Another 1.33 g (4.3 mmol) of *p*-anisylchlorodiphenylmethane was added, and stirring was continued for 23 h longer. The reaction mixture was diluted with 5 mL of CH_3OH, stirred at 23 °C for 1 h, and then poured into 400 mL of cold H_2O. The mixture was extracted with CH_2Cl_2 (3 × 40 mL); the combined extracts were washed with NaCl solution (3 × 200 mL), dried, and evaporated. The residue was thrice dissolved in 15 mL of toluene and evaporated. A solution of the gummy residue in 5 mL of CH_2Cl_2 was stirred, and 75 mL of Et_2O was added dropwise, followed by 75 mL of petroleum ether (35–60 °C). The resulting precipitate was triturated in the mixture for 1.5 h, collected, washed with Et_2O–petroleum ether (35–60 °C) (1:1; 5 × 20 mL), and dried to afford 3.75 g (95%) of 6 as an amorphous solid:[20] IR 2.86 (OH, NH), 5.80 (C=O), 6.20, 6.34 μm (H-bonded quinone); 90-MHz NMR ($CDCl_3$) δ 13.87 (s, 1, OH-6), 13.05 (s, 1, OH-11), 7.92 (d, 1, J = 8 Hz, H-1), 7.69 (t, 1, J = 8 Hz, H-2), 6.7–7.6 (m, 15, H-3 and Ar H's of trityl), 5.38 (br s, 1, H-1′), 5.03 (br s, 1, H-7), 4.46 (s, 2, H-14), 3.9–4.2 (m, 2, H-3′, H-5′), 3.98 (s, 3, 4-OCH_3), 3.78 (s, 3, trityl OCH_3), 3.53 (m, 1, H-4′), 2.90 (d, 1, J = 19 Hz, H-10B), 2.67 (d, 1, J = 19 Hz, H-10A), 2.10 (br s, 2, H-8), 1.82 (m, 2, H-2′), 1.07 (d, 3, J = 6.5 Hz, CH_3-5′); TLC ($CHCl_3$–CH_3OH, 19:1) R_f 0.5.

14-*O*-(*p*-Anisyldiphenylmethyl)-5-iminodoxorubicin (8). A stirred solution of 300 mL of methanolic ammonia (saturated at 0 °C) in an ice bath was treated with a solution of 3.73 g (4.09 mmol) of 6 in 75 mL of CH_2Cl_2. The cold solution was stirred briefly (1 h), stored at 0–5 °C for 26 h, and evaporated. The violet residue was twice dissolved in 75 mL of CH_2Cl_2–CH_3OH (9:1) and the solution was evaporated. A solution of the residue in 100 mL of CH_2Cl_2 was filtered through Celite and evaporated to afford 3.70 g of a mixture of 14-*O*-(*p*-anisyldiphenylmethyl)-5-imino-*N*-(trifluoroacetyl)doxorubicin (7) and 8. To a stirred solution of the above mixture in 200 mL of dioxane–CH_3OH (2:1) cooled in an ice bath was added 200 mL of 0.2 N NaOH dropwise. After stirring at 0 °C under nitrogen for 6 h, the dark blue solution was adjusted to pH 8.0 with 1.0 N AcOH added dropwise. The aqueous mixture was extracted with $CHCl_3$–CH_3OH (19:1; 6 × 100 mL), and the combined extracts were washed with 200 mL of saturated NaCl, dried, filtered through Celite, and evaporated. The residue (2.82 g) in 15 mL of $CHCl_3$ was applied to a column (3.0 × 57 cm) of silica gel (Mallinckrodt SilicAR CC-7, 200–325 mesh) which was eluted with $CHCl_3$ (200 mL) and then $CHCl_3$–CH_3OH (98:2, 500 mL; 96:4, 500 mL; 94:6, 1500 mL; 92:8, 1500 mL; 90:10, 1500 mL). After collection (25 mL fractions, monitored by TLC) of 3.55 L of initial eluate, a 1.05-L fraction was evaporated to yield 1.08 g (31%) of 8 as an amorphous solid: IR 2.98 (OH, NH), 5.68 (C=O), 6.22 μm (H-bonded quinone); 90-MHz NMR ($CDCl_3$–CD_3OD, 3:1) δ 7.88 (d, 1, J = 8 Hz, H-1), 6.7–7.7 (m, 16, H-2, H-3 and Ar H's of trityl), 5.50 (br s, 1, H-1′), 5.10 (br s, 1, H-7), 4.47 (s, 2, H-14), 4.02 (s, 3,4-OCH_3), 3.9-4.1 (m, 1, H-5′), 3.80 (s, 3, trityl OCH_3), 3.45 (br s, 1, H-4′), 2.97 (m, 1, H-3′), 2.80 (br s, 2, H-10), 2.01 (m, 2, H-8), 1.75 (m, 2, H-2′), 1.10 (d, 3, J = 6.5

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Hz, CH_3-5′); TLC ($CHCl_3$-CH_3OH, 4:1) R_f 0.3. Anal. ($C_{47}H_{46}N_2O_{11}$·0.8$CHCl_3$) C, H, Cl, N.

5-Iminodoxorubicin Hydrochloride (4). A solution of 1.073 g (1.32 mmol) of **8** in 70 mL of 80% AcOH was stirred at 23 °C in the dark for 5 h, frozen, and lyophilized. The residue was dissolved in 100 mL of $CHCl_3$–CH_3OH (1:1), the solution was stirred, and 14.3 mL (1.32 mmol) of 0.092 M methanolic hydrogen chloride was added dropwise, followed by 200 mL of Et_2O. The resulting precipitate was collected and washed with $CHCl_3$ (3 × 25 mL) and with Et_3O (3 × 50 mL) to afford 0.642 g (82%) of hydrochloride **4**: IR 2.90 (OH, NH), 5.78 (C=O), 6.30 μm (H-bonded quinone); 100-MHz NMR (Me_2SO-d_6) δ 15.80 (s, 1, OH-11), 13.48 (br s, 1, OH-6), 9.55 (br s, 1, NH), 8.00 (d, 1, J = 8 Hz, H-1), 7.93 (br s, 3, NH_3^+), 7.78 (t, 1, J = 8 Hz, H-2), 7.56 (d, 1, J = 8 Hz, H-3), 5.46 (br s, 1, H-1′), 4.94 (br s, 1, H-7), 4.59 (s, 2, H-14), 4.19 (m, 1, H-5′), 4.09 (s, 3, OCH_3), 3.59 (br s, 1, H-4′), 3.38 (m, 1, H-3′), 2.84 (br s, 2, H-10), 2.07 (br s, 2, H-8), 1.72 (m, 2, H-2′), 1.15 (d, 3, J = 6.5 Hz, CH_3-5′); UV–vis λ_{max} (CH_3OH) 221 nm (ε 30 200), 233 sh (25 500), 252 (31 600), 305 (7200), 335 sh (4500), 360 sh (4050), 520 sh (8900), 551 (16 700), 592 (19 600); MS [as the $(Me_3Si)_6$ derivative], m/e 959 (M – CH_3), 944 (M – $2CH_3$); TLC ($CHCl_3$–CH_3OH–2 N AcOH, 40:10:1) R_f 0.11; HPLC 4.6 × 250 mm Altex Ultrasphere Octyl 5 μm, 0.01 M H_3PO_4–CH_3OH (30:70), 1.0 mL/min, UV at 254 nm, retention time 3.9 (5.3%, 5-iminoadriamycinone), 7.9 (0.3%), 15.6 (0.3%), and 25.7 min (94.1%, 5-iminodoxorubicin). Anal. ($C_{27}H_{30}N_2O_{10}$·HCl·H_2O) C, H, Cl, N.

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Synthesis and Antitumor Activity of Cysteinyl-3,4-dihydroxyphenylalanines and Related Compounds

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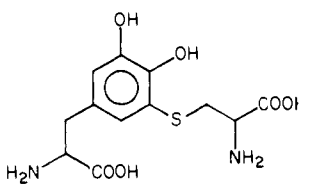
The natural catecholic amino acid 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine (1) was selectively toxic to a variety of human tumor cell lines in culture and exhibited antitumor activity against L1210 leukemia and B-16 melanoma in mice at doses which were not toxic to the host. Structural analogues of 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine, including several new compounds, were synthesized and tested for growth inhibition of cultured cells of human neuroblastoma YT-nu and Chinese hamster fibroblast Don-6. Some were also examined for antitumor activity against L1210 and B-16 in vivo. 4-*S*-Cysteinylcatechols and 2- and 4-*S*-cysteinylphenols, which cannot be prepared by conventional methods, were synthesized by the reaction of catechols and phenols with cystine in boiling aqueous HBr. 5-*S*-Cysteinyl- and 2-*S*-Cysteinyl-3,4-dihydroxyphenylalanine (1 and 2), L-3,4-dihydroxyphenylalanine (L-Dopa), and 2- and 4-*S*-cysteinylphenol (14 and 15) were toxic to the YT-nu cell line only, while 4-*S*-cysteinylcatechol (6), 3-*S*-cysteinyl-5-methylcatechol (8), 5-*S*-cysteaminyldopamine (9), and 4-methylcatechol were strongly toxic to both cell lines. Compounds 1 (1000 mg/kg), 6 (500 mg/kg), and 8 (400 mg/kg) increased the life span of L1210-bearing mice by 50, 50, and 43%, respectively, and compounds 1 and 8 were marginally effective against B-16 melanoma as well. Compound 9 was too toxic to show any activity. There was a good correlation between the cytotoxicity and the in vivo activity.

The catecholic amino acid 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine (5-*S*-cysteinyl-Dopa, 1) is the chief building stone of pheomelanins, yellow to reddish-brown melanins.[1] High levels of this amino acid have been found in the urine of patients with melanoma metastases.[2] Recently, Wick et al. showed that L-3,4-dihydroxyphenylalanine (L-Dopa) is toxic to melanoma cells in vitro[3] and that its analogues exhibit antitumor activity in several experimental tumor systems.[4-6] Later, we found that 1 is much more toxic to a variety of human tumor cell lines in culture than is L-Dopa and possesses antitumor activity against murine L1210 leukemia and B-16 melanoma with no untoward effects on the host.[7] It was suggested that the mechanism of action of these catechols may involve oxidation to an *o*-benzoquinone with subsequent sulfhydryl scavenging and inhibition of enzymes essential for DNA synthesis.[6,7]



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In an attempt to obtain antitumor agents that are more effective than 1 and to elucidate the mechanism of action of the catechols, we have synthesized structural analogues of 5-*S*-cysteinyl-Dopa (1) and tested these compounds (1–16, Chart I) for growth inhibition of cultured cells of human neuroblastoma YT-nu and Chinese hamster fibroblast Don-6. Some of the compounds (1, 6, 8, 9, and 15) were also examined for their effects on the life span of mice bearing L1210 leukemia or B-16 melanoma.

Chemistry. Physical constants and spectral data for the compounds prepared for this work are summarized in Table I. Among them, compounds 6, 9, and 14–16 had not been previously described and compounds 1–4 were obtained in the crystalline form for the first time.

The simplest method for the attachment of a thioether group to the C-3 position of a catechol involves the oxi-

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