Synthesis and Antitumor Activity of Isodoxorubicin Analogues

Jean-Claude Florent,† Gilbert Gaudel,† Claude Monneret,*,† Dieter Hoffmann,‡ and Hans-Peter Kraemer‡

Service de Chimie, CNRS, URA 1387, Institut Curie, Section de Biologie, 26 rue d'Ulm, 75231 Paris Cedex 05, France, and Research Laboratories of Behringwerke A.G., P.O. Box 1140, D-3550 Marburg, Germany

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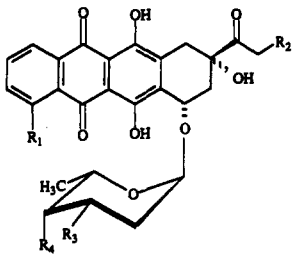
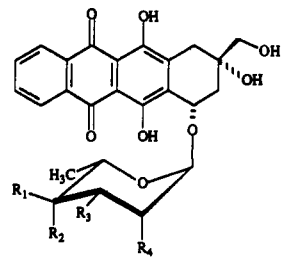
The synthesis and biological activity of the new 4-demethoxyanthracyclines **15**, **22**, and **23** are reported. They were obtained from synthetic 9-deacetyl-9-(hydroxymethyl)-4-demethoxydaunomycinone (isopropylidene derivative **9**) and from 4-azido- or 4-amino-2,4,6-trideoxy-L-*lyxo*-hexoses. Anthracycline **22** (hydrochloride salt), the most active compound in the series, was slightly more potent than doxorubicin in vitro against three cell lines (L1210, HT29, A549). It was found to exhibit similar antitumor activity in vivo (iv route) against L1210 leukemia, but was less active than doxorubicin against three human tumors in a subrenal capsule assay (LXF, A549, and HT29).

The anthracyclines, daunorubicin (**1**) and doxorubicin (**2**), are widely used in the treatment of a large variety of human cancers.[1] However, their clinical use was hampered by a number of problems, particularly a dose-related and irreversible cardiotoxicity. As a consequence, an extensive search for analogues with a better therapeutic index was actively pursued.[2-4] Among the large number of new anthracyclines which have been prepared, synthetic 4-demethoxy daunorubicin (**3**) has been claimed to be more potent and less toxic than the parent compound[5] along with other 4-demethoxy analogues.[6] As a result, **3** (or idarubicin) has undergone extensive clinical trials.[7]

A few years ago the synthesis of a new aglycon, 9-deacetyl-9-(hydroxymethyl)-4-demethoxydaunorubicinone, was simultaneously reported by us[8,9] and the Roche group[10] and, later, by Terashima[11] and Umezawa.[12] It has been shown that the corresponding glycosides of this aglycon with daunosamine or acosamine (**4** or **5**, respectively), displayed significant antitumor activity,[13] as well as the 2-deoxy-α-L-fucose[14] or 2′,6′-dideoxy-2′-iodo-α-L-*manno*-hexopyranoside glycosides **6** or **7**.[15]



	R_1	R_2	R_3	R_4
1	OCH_3	H	NH_2	OH
2	OCH_3	OH	NH_2	OH
3	H	H	NH_2	OH
8	OCH_3	OH	OH	NH_2



	R_1	R_2	R_3	R_4
4	H	OH	NH_2	H
5	OH	H	NH_2	H
6	H	OH	OH	H
7	OH	H	OH	I

For a better understanding of structure–activity relationships in this new series of anthracyclines, glycosides modified at both the C-3′ and C-4′ positions have been selected as new targets for synthesis and biological evaluation. The effect of such modifications in the sugar residue have recently been explored with daunomycinone and adriamycinone aglycons. As a result, 3′-deamino-3′-hydroxy-4′-deoxy-4′-aminodoxorubicin (or isodoxorubicin, **8**),[16] showed remarkable activity against leukemia and solid tumors and appeared to be less cardiotoxic than doxorubicin.

To our knowledge, contrary to these 4′-amino analogues, the 4′-azido- and 4′-morpholinoanthracyclines had not yet been reported, thus making a comparison of their activity an appealing study which is the subject of this paper.

Chemistry

The isopropylidene derivative **9** of 9-deacetyl-9-(hydroxymethyl)-4-demethoxydaunorubicinone was prepared as reported.[9] Methyl 4-azido-3-*O*-acetyl-2,4,6-trideoxy-α-L-*lyxo*-hexopyranoside (**10**),[16] used as starting material for the sugar moiety, quantitatively afforded the corresponding 1,3-di-*O*-acetyl analogue **11** by acetolysis. Therefore glycosyl bromide **12** prepared in situ and used in a double excess was coupled with **9** in an anhydrous dichloromethane solution in the presence of mercuric bromide, yellow mercuric oxide, and 4-Å molecular sieves. Anthracycline **13** was isolated as a crystalline compound in 96% yield based on the aglycon used, after flash chromatography. Selective hydrolysis of the acetal ring of **13** in a mild acidic medium led to **14** and its subsequent treatment with 1 N aqueous NaOH at 0 °C afforded **15** which crystallized from methanol (55% overall yield).

Glycosyl bromide **17** prepared in situ from 3-*O*-benzoyl-2,4,6-trideoxy-4-(trifluoroacetamido)-L-*lyxo*-hexopyranose (**16**)[17] was also coupled with **9** under the above conditions (vide supra) to give the glycoside **18** in 90% yield after flash chromatography. Successive deprotections under mild acidic conditions and in the presence of 1 N aqueous NaOH solution at 0 °C afforded **19** and then **20** (50% overall yield). Finally, treatment of **20** with 1 N aqueous NaOH solution at room temperature gave the amino compound **21** which was isolated as the crystalline hydrochloride **22** after addition of a methanolic solution of HCl.

Since 3′-deamino-3′-morpholino analogues are known to be highly potent anthracyclines,[18-21] treatment of **21** with 2,2′-oxybis(acetaldehyde) (prepared in situ from 1,4-anhydroerythritol)[22] and sodium cyanoborohydride was carried out to provide **23** in 65.5% yield.

† Institut Curie.
‡ Behringwerke AG.

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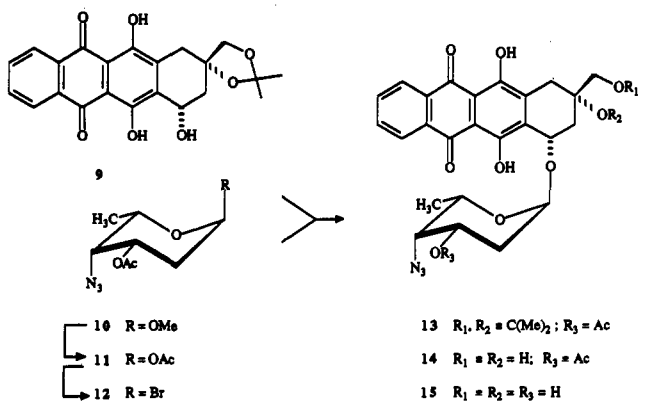
Scheme I



Results and Discussion

The effects of the new 4-azido- and 4-aminoanthracycline analogues on the growth of L1210 murine leukemia cells after a 1-h and 3-day incubation period at 37 °C are shown in Table I.

Compared to doxorubicin, compounds **15** and **23** were 5–6 times less potent against L1210 cells, whereas compound **20** was slightly less growth-inhibitory at equimolecular concentration. More interestingly, anthracycline **21** with the amino group at C-4′ tested as its hydrochloride **22** was twice as potent as doxorubicin after a 1-h incubation as well as after a 3-day exposure.

Such high potency observed for compound **22** was also demonstrated on the growth inhibition of two other cell lines, HT29 and A549, and is reported in Table II.

As indicated above (L1210 test), the anthracycline analogue **15** having an azido group at C-4′ was the least potent compound of the series, whereas **20** retained good cytotoxicity when compared to **22**. The morpholino derivative **23** was less potent than **22** against the HT29 and A549 cell lines.

Since hydrochloride **22** demonstrated high cytotoxicity in the different test systems, in vivo testing was undertaken and compound **22** showed about the same activity as doxorubicin againt the L1210 mouse leukemia, as depicted in Table III.

However, this compound was less active than doxorubicin against three human tumors tested in a subrenal capsule assay:[23] LXF, A549, and HT29 growing human tumors (SRCA) (Table IV).

In conclusion, anthracycline **22** was significantly cytotoxic in vitro and with no difference comparing continuous to short-time exposure. This compound showed similar activity as the doxorubicin against the L1210 mouse leukemia but was less active against three human tumors tested in a subrenal capsule assay.

The partial cross-resistance of the drug **22** with doxorubicin was demonstrated both in in vitro and in vivo assays against a L1210/AM cell line and a doxorubicin-resistant tumor (LXF/ADM), respectively (see Tables I and IV).

From these preliminary studies, it appears that **22** does not offer any advantage over compounds currently in the clinic except if a markedly reduced cardiotoxicity were displayed following further biological investigations.

It is noteworthy that glycosides 4-demethoxy-9-deacetyl-9-(hydroxymethyl)daunomycinone **6**, **7**, and **22**, as well as those previously reported from the Roche group,[24] were highly potent antitumor compounds but with reduced efficiency. Since our investigations have shown that the three anthracyclines are less active than doxorubicin or inactive against solid tumors,[25] the presence of a 9-hydroxymethyl side chain appears to give these derivatives a pharmacological profile closer to that of daunorubicin than to that of doxorubicin.

Experimental Section

Melting points (Kofler hot stage microscope) are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer, calibrated against polystyrene film, and were expressed in cm^{-1}. ^1H NMR spectra at 270 MHz were obtained on a Bruker HX 270 in $CDCl_3$. Chemical shifts are expressed in ppm downfield from internal Me_4Si with the notations indicating the multiplicity of the signal (s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet). The coupling constants are expressed as J values in units of hertz. Mass spectra (DCI/NH_3) were recorded on a Nermag R 1010.

Merck silica gel 60 no. 9385 was used for column (flash) chromatography. Analytical thin-layer chromatographies were performed on Merck silica gel 60 F_{254}.

Ether solvents (THF and ether) were dried over sodium benzophenone and distilled, and dry CH_2Cl_2 was distilled over CaH_2.

Microanalyses were performed by the "Laboratoire de Microanalyse du CNRS", Gif-sur-Yvette and Lyon.

1,3-Di-*O*-acetyl-4-azido-2,4,6-trideoxy-L-*lyxo*-hexopyranose (11). To a solution of **10**[16] (900 mg, 3.9 mmol) in AcOH (5 mL) and Ac_2O (5 mL) was added 0.5 mL of 5% concentrated H_2SO_4 in Ac_2O. After being stirred for 2.5 h at room temperature, the reaction mixture was extracted with Et_2O, and the organic phases were washed with a saturated aqueous $NaHCO_3$ solution with water and dried over Na_2SO_4. After evaporation under reduced pressure, a solid was obtained, which crystallized from hexane (790 mg, 99%): mp 48–50 °C; $[\alpha]^{20}_D$ −69° (c = 0.2, $CHCl_3$); IR (KBr, cm^{-1}) 2110 (N_3), 1722 and 1073 (ester); MS (DCI) m/z 275 $(M + NH_4)^+$, 215 $[M + NH_4 - 60]^+$; ^1H NMR δ 6.09 (d, 1 H, J = 3 Hz, 1-H), 5.27 (m, 1 H, J = 10, J' = 5, J'' = 5 Hz, 3-H), 3.04 (d, J = 6.5 Hz, 5-H), 3.75 (dd, 1 H, J = 5, J' = 2 Hz, 4-H), 2.20 (m, 1 H, J = 14, J' = 10, J'' = 3 Hz, 2a-H), 2.09 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 1.88 (m, 1 H, J = 14, J' = 5, J'' < 0.5 Hz, 2e-H), 1.19 (d, 1 H, J = 6.5 Hz, 6-CH_3). Anal. ($C_{10}H_{15}N_3O_5$) C, H, N.

(*S*)-*cis*-1-*O*-(3-*O*-Acetyl-4-azido-2,4,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-3-(hydroxymethyl)-3,13-*O*-isopropylidene-6,11-naphthacenedione (13). A mixture of the bromide **12**, prepared in situ from **11** (180 mg, 0.7 mmol) [**11** was dissolved in benzene (20 mL) and distilled to ca. 10 mL before a dry hydrogen bromide was passed into the benzene solution; after being stirred for 5 min at room temperature, the solution was evaporated under reduced pressure, and the remaining acid was removed by codistillation (Θ <40 °C) with benzene (three 10-mL portions)],[26,27] aglycon **9** (140 mg, 0.35 mmol), yellow mercuric oxide (870 mg, 4 mmol), mercuric bromide (230 mg, 0.68 mmol), and 4-Å molecular sieves (1.5 g) in anhydrous dichloromethane (150 mL) was stirred overnight at room temperature. After filtration, the filtrate was diluted with CH_2Cl_2 (100 mL) and washed with water, and the organic layer was concentrated under reduced pressure. Chromatography of the residue with hexane–EtOAc (4:1) as eluent afforded 200 mg (96 % yield) of crystalline compound: mp 143–145 °C dec; $[\alpha]^{20}_D$ +148° (c = 1, chloroform); IR ($CHCl_3$, cm^{-1}) 2100 (N_3), 1725 (CO), and 1680 (chelated quinone); MS (DCI) m/z 611 $(M + NH_4)^+$, 414, 396, 233, 215, 155, 132; ^1H NMR δ 13.50 (s, 1 H, OH phenol), 13.25 (s, 1 H, OH phenol), 8.25 (m, 2 H, arom H), 7.75 (m, 2 H, arom H), 5.20 (m, 1 H, J = 10, J' = 4, J'' = 3 Hz, 3′-H), 5.05 (bs, 1 H, $W_{1/2}$ = 7 Hz, 1-H), 5.00 (t, 1 H, J = J' = 3 Hz, 1′-H), 4.27 (q, 1 H, J = 6.5 Hz, 5′-H), 3.89 (d, 1 H, J = 9 Hz, 13a-H), 3.82 (d, 1 H, J = 9 Hz, 13b-H), 3.78 (m, 1 H, J = 3, J' = 0.5 Hz, 4′-H), 3.24 (d, 1 H, J = 18 Hz, 4a-H), 2.82 (d, 1 H, J = 18 Hz, 4b-H), 2.03 (s, 3 H, OAc), 1.46 (s, 6 H, CMe_2), 1.32 (d, 1 H, J = 6.5 Hz, 6′-CH_3). Anal. ($C_{30}H_{31}N_3O_{10}$) C, H, N.

(*S*)-*cis*-1-*O*-(3-*O*-Acetyl-4-azido-2,4,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-3-(hydroxymethyl)-6,11-naphthacenedione (14). A solution of **13** (150 mg, 0.25 mmol) in a mixture of Me_2CO and MeOH (6

Scheme II

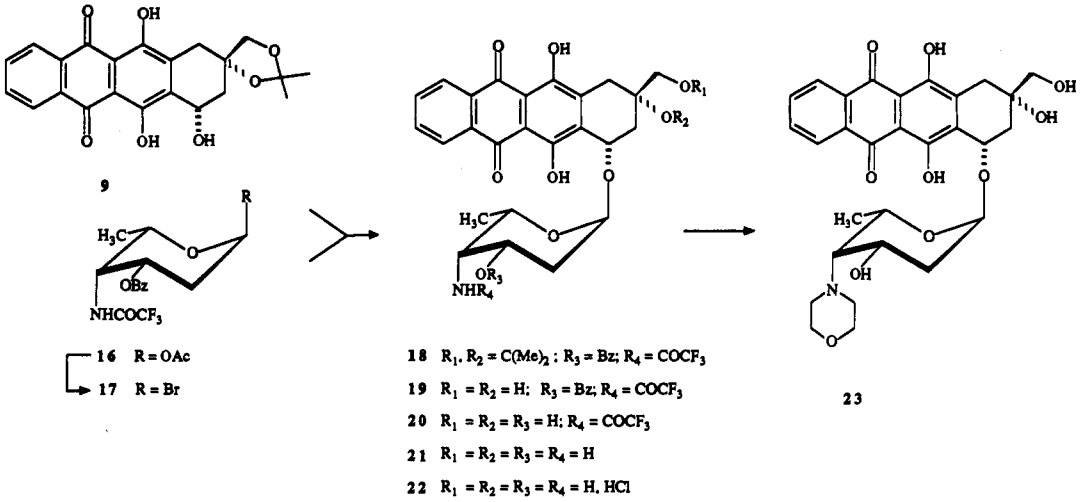


Table I. Growth Inhibition of L1210 Leukemia Cells in Vitro by New Anthracycline Analogues

	IC_{50} (μg/mL)[a]				
	1-h exposure[b]		3-day exposure[c]	degree of	degree of cross-
compd	L1210	L1210	L1210/ADM[d]	resistance[e]	resistance[f]
doxorubicin	0.04	0.02	0.90	45	100
15	0.26	0.13			
20	0.0064	0.015			
22	0.022	0.009	0.10	11	24
23	0.21	0.15			

[a] Drug concentration that inhibited cell growth by 50% compared to untreated control cultures. [b] Stem cell assay. [c] MTT reduction. [d] Cell line made resistant by repeated treatment of the parent cell line with stepwise increasing amounts of duxorubicin. [e] Degree of resistance (DR) = IC_{50} L1210/ADM/IC_{50} L1210. [f] Degree of cross-resistance (DCR) = 100 × DR of doxorubicin/DR of compound 22.

Table II. Growth Inhibition of HT29 and A549 Cell Lines in Vitro by New Anthracycline Analogues.

	IC_{50} (μg/mL)[b,c]	
compd	HT 29[a]	A 549[a]
doxorubicin	0.06	0.05
	0.02[d]	0.02[d]
15	0.46	0.24
20	0.026	0.011
22	0.01	0.009
23	0.13	0.054

[a] MTT reduction. [b] The drug concentration that inhibited cell growth by 50% compared to untreated control cultures. [c] Incubation time: 3 days. [d] Incubation time: 7 days.

Table III. Activity against iv-Inoculated L1210 Mouse Leukemia

compd	dose,[a] mg/kg	schedule[b]	% T/C[c]
doxorubicin	8.1	3 iv	148
22	3.1	3 iv	133

[a] A dose giving the highest value of T/C. [b] On days 1, 5, and 9. [c] Ratio of the mean survival time of treated animals to untreated controls.

mL, 1:1) was stirred for 10 h at 50 °C in the presence of AcOH (24 mL) and H_2O (3 mL). After extraction with EtOAc, purification by flash chromatography (CH_2Cl_2–MeOH, 95:5) afforded 100 mg of a crystalline residue. Recrystallization from EtOAc gave 14 (80 mg, 57%): mp 210 °C dec; $[\alpha]^{20}_D$ +136° (c = 0.1, chloroform); MS (DCI) m/z 571 $(M + NH_4)^+$, 356, 233, 215 (base peak), 138; ^1H NMR δ 13.42 (s, 1 H), 13.17 (s, 1 H, OH phenol), 8.25 (m, 2 H) and 7.76 (m, 2 H, arom H), 5.50 (m, 1 H, $W_{1/2} \simeq 7$ Hz, 1-H), 5.20 (m, 1 H, $W_{1/2} \simeq 8$ Hz, 1′-H), 5.07 (m, 1 H, $J = 12$, $J' = J'' = 3$ Hz, 3′-H), 4.20 (q, 1 H, $J = 6.5$, $J' \leq 0.5$ Hz, 5′-H), 3.71 (d, 1 H, $J = 11$ Hz, 13a-H), 3.50 (d, 1 H, $J = 11$ Hz, 13b-H), 3.20 (d, 1 H, $J = 18$ Hz, 4a-H), 2.55 (d, 1 H, $J = 18$ Hz, 4b-H), 2.02 (s, 3 H, OAc), 1.36 (d, 1 H, $J = 6.5$ Hz, 6′-CH_3), 2.15–1.80 (m, 4 H, 2-H and 2′-H). Anal. ($C_{27}H_{27}N_3O_{10}$) C, H, N.

Table IV. Activity of 22 against Intrarenally Growing Human Tumors[23]

	tumors			
compd	LXF	A 549	HT 29	LXF/ADM
doxorubicin	c	b	b	b
22	b	a	a	a

[a] Tumor area of treated group > 50% of control group. [b] Tumor area of treated group > 10% < 50% of control group. [c] Tumor area of treated group < 10% of control group.

(*S*)-*cis*-1-*O*-(4-Azido-2,4,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-3-(hydroxymethyl)-6,11-naphthacenedione (15). To a cooled solution (0 °C) of 14 (60 mg, 0.11 mmol) in CH_2Cl_2 (25 mL) and MeOH (3 mL) was added 1 mL of 0.25 N NaOH. After being stirred at the same temperature for 3 h, the reaction mixture was neutralized by dropwise addition of AcOH. As soon as the red color was obtained, a saturated aqueous solution of NaCl (20 mL) was added and the reaction mixture was extracted with EtOAc. The organic phase was separated off, washed with H_2O, and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give 15 (55 mg, 98%) as a solid recrystallized from MeOH: mp 241–242 °C; $[\alpha]^{20}_D$ +83° (c = 0.02, MeOH); MS (DCI) m/z 529 $(M + NH_4)^+$, 374, 356, 338, 321, 275; IR ($CHCl_3$, cm^{-1}) 2100 (N_3); ^1H NMR δ 13.40 (s, 1 H, OH phenol), 12.40 (s, 1 H, OH phenol), 8.22 (m, 2 H, arom H) and 7.74 (m, 2 H) (arom H), 5.45 (m, 1 H, $W_{1/2} \simeq 7$ Hz, 1-H), 5.17 (dd, 1 H, $J = J' = 3$ Hz, 1′-H), 4.13 (q, 1 H, $J = 6.5$ Hz, 5′-H), 3.98 (m, 1 H, 3′-H), 3.65 (d, 1 H, $J = 10$ Hz, 13a-H), 3.47 (d, 1 H, $J = 10$ Hz, 13b-H), 3.58 (br s, 1 H, 4′-H), 3.16 (d, 1 H, $J = 18$ Hz, 4a-H), 2.53 (d, 1 H, $J = 18$ Hz, 4b-H), 2.10–1.60 (m, 4 H, 2′-H and 2-H), 1.38 (d, 3 H, $J = 6.5$ Hz, 6′-CH_3). Anal. ($C_{25}H_{25}N_3O_9$) C, H, N.

(*S*)-*cis*-1-*O*-(3-*O*-Benzoyl-2,4,6-trideoxy-4-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,-12-tetrahydroxy-3-(hydroxymethyl)-3,13-*O*-isopropylidene-6,11-naphthacenedione (18). To a solution of bromo sugar 17 (prepared from 16, 1.3 g (3.34 mmol) in C_6H_6 (30 mL) by bubbling dry gaseous HBr into the solution for 5 min at room temperature and evaporation of solvent, followed by three coevaporations with 10 mL of benzene) in anhydrous CH_2Cl_2 (250 mL) were added HgO (3.24 g, 15 mmol), $HgBr_2$ (1.12 g, 3.11 mmol), 4-Å molecular sieves (4.8 g), and then aglycon 9 (400 mg, 1 mmol). After being stirred at room temperature for 18 h, the reaction mixture was filtered on a path of Celite. Extraction in the usual manner with dichloromethane followed by flash chromatography with toluene–acetone–acetic acid (95:4.8:0.2) gave 700 mg (94%) of 18 which crystallized from acetone–hexane: mp 127–128 °C; $[\alpha]^{20}_D$ + 67.5° (c = 0.04, chloroform); MS (DCI) m/z 743 $(M + NH_4)^+$, 379; IR ($CHCl_3$, cm^{-1}) 3420 (NH), 1725 (CO ester), 1670

(CO amide), 1625 and 1590 (chelated quinone); ^{1}H NMR δ 13.69 (s, OH phenol), 12.92 (s, OH phenol), 8.27 (m, 2 H), 7.76 (m, 5 H, arom H), 7.50 (m, 2 H, arom H), 6.52 (d, 1 H, J = 10 Hz, NH), 5.56 (m, 1 H, $W_{1/2} \simeq 7$ Hz, 1-H), 5.43 (m, 1 H, J = 10, J' = J'' = 3 Hz, 3'-H), 5.05 (t, 1 H, J = J' = 4 Hz, 1'-H), 4.60 (q, 1 H, J = 6.5 Hz, 5'-H), 4.52 (m, 1 H, 4'-H), 3.92 (d, 1 H, J = 9 Hz, 13a-H), 3.85 (d, 1 H, J = 9 Hz, 13b-H), 3.29 (d, 1 H, J = 18 Hz, 4a-H), 2.89 (d, 1 H, J = 18 Hz, 4b-H), 2.33–2.04 (m, 4 H, 2-H and 2'-H), 1.50 (s, 6 H, CMe_2), 1.24 (d, 3 H, J = 6.5 Hz, 6'-CH_3). Anal. ($C_{37}H_{34}NO_{11}F_3$) C, H, N.

(*S*)-*cis*-1-*O*-(3-*O*-Benzoyl-2,4,6-trideoxy-4-(trifluoroacetamido)-α-L-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-3-(hydroxymethyl)-6,11-naphthacenedione (19). A solution of 18 (700 mg) in a mixture of MeOH (45 mL), AcOH (42 mL), and H_2O (6 mL) was stirred at 45 °C for 18 h. Extraction with EtOAc and subsequent flash chromatography gave 350 mg of recovered starting material 18 and 330 mg of 19. An additional amount of 19 (130 mg, overall yield 70%) was obtained by similar treatment of the recovered 350 mg of 18. Compound 19 was crystallized from MeOH: mp 149–152 °C; $[\alpha]^{20}_D$ +140° (c = 0.0062 chloroform); MS (DCI) m/z 703 (M + NH_4)$^+$, 365, and 325; ^{1}H NMR δ 13.46 (s, OH phenol), 13.15 (s, OH phenol), 8.21 (m, 2 H, arom H), 7.73 (m, 7 H, arom H), 6.49 (d, 1 H, J = 10 Hz, NH), 5.57 (m, 1 H, $W_{1/2} \simeq 8$ Hz, 1'-H), 4.47 (m, 2 H, 5'-H and 4'-H), 3.71 (d, 1 H, J = 12 Hz, 13a-H), 3.49 (d, 1 H, J = 12 Hz, 13b-H), 3.23 (d, 1 H, J = 18 Hz, 4a-H), 2.60 (d, 1 H, J = 18 Hz, 4b-H), 2.61–2.50 (m, 2 H, 2-H), 2.31–1.76 (m, 2 H, 2'-H), 1.29 (d, 3 H, J = 6 Hz, 6'-CH_3). Anal. ($C_{34}H_{30}NO_{11}F_3$) C, H, N.

(*S*)-*cis*-1-*O*-(2,4,6-Trideoxy-4-(trifluoroacetamido)-α-L-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-6,11-naphthacenedione (20). To a solution of 19 (690 mg) in a mixture of CH_2Cl_2 (120 mL) and MeOH (160 mL) at 0 °C was added 6 mL of 1 N NaOH. After being stirred for 6 h at 0 °C, the reaction mixture was neutralized by dropwise addition of AcOH until the coloration turned red. To this mixture was added 15 mL of saturated aqueous NaCl, and then extraction with CH_2Cl_2 as usual followed by evaporation afforded a residue (700 mg) which crystallized from MeOH, giving 340 mg of 20. The mother liquors, evaporated and purified by flash chromatography (CH_2Cl_2/MeOH, 95:5), gave 110 mg of additional 20: overall yield 70%; mp 206 °C; $[\alpha]^{20}_D$ +78° (c 0.05, dioxane); MS (DCI) m/z 599 [M + NH_4]$^+$, 582 (M + H)$^+$; ^{1}H NMR δ 6.41 (d, 1 H, J = 9 Hz, NH), 5.50 (d, 1 H, J = 3 Hz, 1-H), 5.23 (m, 1 H, $W_{1/2} \simeq 8$ Hz, 1'-H), 4.31 (q, 1 H, J = 6.5, J' < 0.5 Hz, 5'-H), 4.14 (m, 2 H, 3'-H and 4'-H), 3.71 (d, 1 H, J = 12 Hz, 13a-H), 3.50 (d, 1 H, J = 12 Hz, 13b-H), 3.21 (d, 1 H, J = 18 Hz, 4a-H), 2.58 (d, 1 H, J = 18 Hz, 4b-H), 2.50 (d, 1 H, J = 14 Hz, 2a-H), 1.82 (dd, 1 H, J = 14, J' = 3 Hz, 2b-H), 2.01 (dd, 1 H, J = 14, J' = 4 Hz, 2'e-H), 1.62 (m, 1 H, 2'a-H), 1.26 (d, 3 H, J = 6.5 Hz, 6'-CH_3). Anal. ($C_{27}H_{26}O_{10}NF_3$) C, H, N.

(*S*)-*cis*-1-*O*-(4-Amino-2,4,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-6,11-naphthacenedione (21) and Its Hydrochloride (22). To a solution of 20 (250 mg, 0.43 mmol) in THF (10 mL) was added NaOH (40 mL, 0.1 N). The reaction mixture was stirred for 5 h at 0 °C, and then an aqueous solution of 5 N HCl was added until pH 4. Addition of a saturated aqueous $NaHCO_3$ solution until pH 8 was followed by extraction with CH_2Cl_2. The organic layers were washed with a saturated NaCl solution and then dried (Na_2SO_4). Concentration under reduced pressure led to 21 as a crystalline compound (150 mg, 72%): mp 178–180 °C; $[\alpha]^{20}_D$ +10° (c 0.04, THF); IR (KBr, cm^{-1}) 3350, 1625, 1590 (quinone); MS (DCI) m/z 486 (M + H)$^+$, 356, 338, 148, 130, 94; ^{1}H NMR δ 8.34 (d, 2 H) and 7.72 (d, 2 H) (arom), 5.70 (s, 1 H, 1-H), 5.34 (br s, 1 H, 1'-H), 4.62 (dd, 1 H, J = 6.5, J' = 3 Hz, 3'-H), 4.33 (m, 1 H, 4'-H), 3.95 (q, J = 6 Hz, 5'-H), 3.41 (d, 1 H, J = 18 Hz, 13a-H), 3.17 (d, 1 H, J = 18 Hz, 13b-H), 3.04 (s, 1 H, OH), 2.75 (d, 1 H, J = 14 Hz, 4-H_a), 2.35 (dd, 1 H, J = 14, J' = 4 Hz, 4-H_b), 2.20 (m, 2 H, 2-H), 1.45 (d, 3 H, J = 6 Hz, 6'-CH_3).

Preparation of 22. A solution of 21 (100 mg) in $CHCl_3$ (9 mL) and MeOH (1 mL) was cooled to 0 °C, and then a methanolic solution of dry HCl was added until pH 3. After evaporation under reduced pressure, a residue was obtained. The latter was dissolved in MeOH (1 mL), and then dry Et_2O was added. The precipitate formed was filtered to give 80 mg of 22: mp 185–190 °C. $[\alpha]^{20}_D$ −67° (c 0.03, MeOH). Anal. ($C_{25}H_{28}NO_9Cl$) C, H, N, Cl.

(*S*)-*cis*-1-*O*-(4-Morpholino-2,4,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4,6,11-hexahydro-1,3,5,12-tetrahydroxy-3-(hydroxymethyl)-6,11-naphthacenedione (23). A solution of 1,4-anhydroerythritol (62 mg, 0.3 mmol) in H_2O was stirred at room temperature for 12 h with $NaIO_4$ (60 mg). The reaction mixture was neutralized by addition of dry $NaHCO_3$, diluted with $CHCl_3$, and filtered. The filtrate was then added to a solution of 21 (20 mg, 0.04 mmol) in CH_3CN containing $NaBH_3CN$ (15 mg dissolved in 5 mL of H_2O). After 15 min of stirring, the reaction mixture was made basic by addition of aqueous $NaHCO_3$ and extracted with EtOAc as usual. After purification by flash chromatography (CH_2Cl_2/MeOH, 90:10), 15 mg of 23 was obtained (65.5% yield): mp 90 °C (hexane); $[\alpha]^{20}_D$ + 60° (c 0.03, THF); IR (KBr, cm^{-1}) 3415 (OH), 1624, and 1590 (quinone); MS (DCI) m/z 556 (M + H)$^+$, 228, 200; ^{1}H NMR δ 8.29 (m, 2 H) and 7.68 (m, 2 H) (arom), 5.83 (br s, $W_{1/2} \simeq 7$ Hz, 1-H), 5.43 (dd, 1 H, 1'-H), 4.62 (q, 1 H, J = 6.5 Hz, 5'-H), 4.45 (m, 1 H, 3'-H), 4.02 (m, 2 H, 13-H), 3.70 (br s, 4 H, morph), 3.48 (d, 1 H, J = 18 Hz, 4-H_a), 3.25 (d, 1 H, J = 18 Hz, 4-H_b), 3.10–2.90 (m, 4 H, CH_2N), 2.60–2.17 (m, 4 H, 2-H and 2'-H), 1.58 (d, 3 H, 6'-CH_3). Anal. ($C_{29}H_{33}NO_{10}$) C, H, N.

Effect on Stem Cells of L1210 Leukemia. The assay was performed according to the procedure of Hamburger and Salmon with some modifications described below.[23]

Conditioned medium was replaced by McCoy 5A. The number of cells plated was reduced to 5×10^2 cells/plate, due to the high plating efficiency of the tumor cell lines.

Cells were incubated with various concentrations of the test substance for 1 h at 37 °C. Thereafter, the cells were washed twice with McCoy 5A and finally plated in an agar upper layer according to the method of Hamburger and Salmon.

In addition, parallel experiments were performed using a continuous incubation with various concentrations of the test substance by admixing the substance to the upper layer prior to plating.

Plates were stored in an incubator with 5% CO_2, 20% O_2, and 95% relative humidity for 5–7 days at 37 °C. After this time, colonies with a diameter >60 μm were counted using an inverted microscope.

Results were expressed as percentage of the number of colonies formed from treated cells over an untreated control. The coefficient of variation of repeated experiments was less than 15%.

From the dose–response curves, we evaluated the IC_{50} values for the continuous and 1-h exposures (Table I).

Proliferation Assay (MTT Reduction). Exponentially growing L1210, A549 or HT29 tumor cells at a density of 5×10^3/mL in RPMI were incubated in a 96-well microtiter plate for 72 h (37 °C, 5% CO_2, 95% relative humidity) with various concentrations of each test substance. Control consisted of cells exposed to fresh medium only. Quadruplicate wells were prepared for each drug concentration and for control. After 65 h, 50 μL MTT (52.5 mg/mL in PBS) were added. The MTT will be reduced by viable cells to a red insoluble formazan dye. After an additional 7–24-h incubation (depending on the cells used), the supernatant medium was carefully removed. The formazan dye was solubilized by adding 100 μL of DMSO to each well, followed by gentle shaking. The extinction was measured for each well using a Miltiscan photometer 340 CC, Fa. Flow, at 492 nm.

Results were expressed as the ratio of the extinction after incubation with test substances over that of control. The coefficient of variation for replicate experiments was less than 15%.

In Vivo Cross-Resistance Assay. The human tumor used for testing the in vivo cross resistance with doxorubicin was obtained by treatment of subcutaneously growing LXF tumors with doxorubicin (4 mg/kg, 3 × iv). After tumors had reached a size of about 1/3 cm in diameter, they were resected, cut in small pieces (ca. 5 mm^3), and again transplated in nude mice. This procedure was repeated several times until the tumors (LXF/

ADXR) showed a diminished sensitivity in the subrenal capsule assay against adriamycin in comparison to the original tumor (LXF).

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