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Synthesis, Biological and Biochemical Properties of New Anthracyclines Modified in the Aminosugar Moiety

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Summary. *New 4′-C-methyl analogues of daunorubicin, synthesized by the coupling reaction of daunomycinone with 1-chloroderivatives of protected 4-C-methyldaunosamine analogues, were chemically transformed to the corresponding doxorubicin analogues. Their cytotoxic effect against HeLa cells, ability to bind to DNA, and in vivo toxicity and antitumor activity were compared with those of daunorubicin, doxorubicin, and their 4′-O-methyl analogues. The cytotoxic effect of the new anthracyclines could be correlated with their ability to bind to DNA and with their toxicity in experimental animals; however, the antitumor effectiveness did not seem to be related to these parameters. In general all the compounds retained a remarkable antitumor activity at their optimal doses. The most active compound against P388 leukemia was 4′-O-methyldoxorubicin, which was also more active than doxorubicin against L1210 leukemia.*

Introduction

The synthesis of analogues of daunorubicin (DNR, *I*) and doxorubicin (DX, *II*) in which the amino sugar moiety is functionally and/or configurationally modified is of great pharmacological and practical interest. Particularly the C-4′ position can be modified, giving compounds endowed with more favorable pharmacological properties and/or a wider spectrum of activity. At molecular level, the biologic activity of the antitumor anthracyclines is thought to be related to their ability to form complexes with DNA, with consequent inhibition of the enzymes involved in DNA replication and transcription. The epimerization at C-4′ in DNR and DX did not decrease the ability to bind to DNA and to inhibit DNA and RNA synthesis [11, 14]. 4′-Epi-DX (*IV*), at present under extensive clinical evaluation, was as effective as DX in experimental tumors, less toxic and less cardiotoxic in experimental animals, with a consequent increase of the therapeutic index [8, 9, 12]. The 4′-O-methyl derivatives of DNR (*V*) and DX (*VI*) and of their 4′-epi analogues (*VII, VIII*) were shown to exert a high antitumor activity [10], 4′-O-methylDX (*VI*) being more active than DX against L1210 leukemia [9] and against human colon adenocarcinomas transplanted in nude mice [15].

It was therefore of interest to investigate further the effect of other modifications at the same position of the sugar moiety. We here report the synthesis, the biochemical properties, the cytotoxicity, and the antitumor activity of eight new anthracyclines bearing new C-4 branched-chain aminodeoxy sugars related to daunosamine (Fig. 1).

Materials and Methods

The melting points, obtained with a SMP-20 apparatus (Büchi), are uncorrected. Optical activity was measured with a Perkin-Elmer Model 151 at 589 nm and 23° C, 1-dm tubes being used. ^{1}H-NMR spectra were recorded at 60 MHz with Varian A-60-A or EM-360 spectrometers in $CDCl_3$ solution, using tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were recorded at 60 MHz with a Varian CFT-20 spectrometer. The mass spectra were recorded with Perkin-Elmer 270 and Varian MAT 311 mass spectrometers.

The method for determining the binding parameters from fluorescence quenching has been described previously [6, 9, 20]. The fluorescence study of the binding of the new anthracyclines to DNA were carried out using a Perkin-Elmer MPF 44A fluorescence spectrophotometer. The excitation wavelength was 470 nm and the emission was monitored at 592 nm. All the measurements were carried out at 20° C in 0.01 *M* Tris HCl (pH 7), 0.1 *M* NaCl, and 0.5 m*M* EDTA. The binding data were analyzed by the Scatchard method [18]. Calf thymus DNA was prepared as previously described [20].

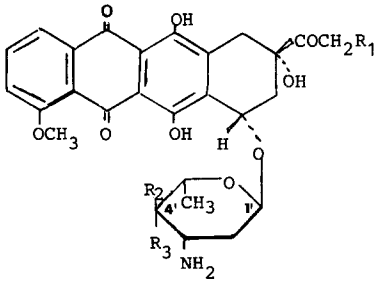
All the drugs were dissolved in distilled water immediately before use for in vitro and in vivo tests.

The colony inhibition test on HeLa cells in vitro was performed as previously described [13].

The antitumor activity was evaluated according to the previously reported protocol for testing new anthracyclines [7]. Stage I studies were carried out on IP transplanted L1210 and P388 leukemias in hybrid (C57BL/6 × DBA/2) F1 (BDF1) mice (10^6 P388 leukemia cells or 10^5 L1210 leukemia cells/mouse). Stage II evaluation was performed in C3H/He mice bearing IV transplanted Gross leukemia (2×10^6 cells/mouse).

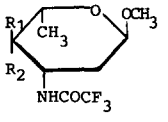
The drugs were administered IP or IV, in a volume of 10 ml/kg body weight on day 1 after tumor transplantation. The mice were supplied by the Charles River Breeding Laboratories, Calco, Italy. Each experimental group consisted of at least eight animals, with one exception (a group of four mice) due to lack of material. Toxicity was evaluated from the macroscopic autopsy findings, mainly from the reduction of spleen size.

Syntheses. The 4′-C-methyl and 4′-C-methyl-4′-O-methyl analogues of daunorubicin and doxorubicin (*IX–XVI*, reported in Fig. 1) are new anthracyclines obtained following a general scheme which includes the synthesis of the desired aminosugar in the appropriately protected form and the subsequent coupling reaction with daunomycinone (*XXIX*).

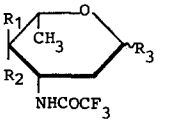


Compound	R_1	R_2	R_3
Daunorubicin (daunomycin) (DNR, *I*)	H	H	OH
Doxorubicin (adriamycin) (DX, *II*)	OH	H	OH
4′-Epi-DNR (*III*)	H	OH	H
4′-Epi-DX (*IV*)	OH	OH	H
4′-O-MethylDNR (*V*)	H	H	OCH_3
4′-O-MethylDX (*VI*)	OH	H	OCH_3
4′-Epi-4′-O-methylDNR (*VII*)	H	OCH_3	H
4′-Epi-4′-O-methylDX (*VIII*)	OH	OCH_3	H
4′-C-MethylDNR (*IX*)	H	CH_3	OH
4′-C-MethylDX (*X*)	OH	CH_3	OH
4′-Epi-4′-C-methylDNR (*XI*)	H	OH	CH_3
4′-Epi-4′-C-methylDX (*XII*)	OH	OH	CH_3
4′-C-Methyl-4′-O-methylDNR (*XIII*)	H	CH_3	OCH_3
4′-C-Methyl-4′-O-methylDX (*XIV*)	OH	CH_3	OCH_3
4′-Epi-4′-C-methyl-4′-O-methylDNR (*XV*)	H	OCH_3	CH_3
4′-Epi-4′-C-methyl-4′-O-methylDX (*XVI*)	OH	OCH_3	CH_3

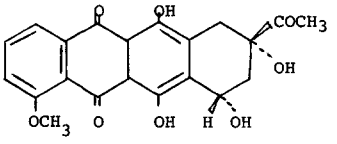
Fig. 1. The 4′-C-methyl and 4′-C-methyl-4′-O-methyl analogues of daunorubicin and doxorubicin



XVII: $R_1=CH_3$; $R_2=OH$
XVIII: $R_1=OH$; $R_2=CH_3$
XIX: $R_1=CH_3$; $R_2=OCH_3$
XX: $R_1=OCH_3$; $R_2=CH_3$



XXI: $R_1=CH_3$; $R_2=R_3=OH$
XXII: $R_1=R_3=OH$; $R_2=CH_3$
XXIII: $R_1=CH_3$; $R_2=OCH_3$; $R_3=OH$
XXIV: $R_1=OCH_3$; $R_2=CH_3$; $R_3=OH$
XXV: $R_1=CH_3$; $R_2=p-NO_2-C_6H_4-COO$; $R_3=Cl$
XXVI: $R_1=p-NO_2-C_6H_4-COO$; $R_2=CH_3$; $R_3=Cl$
XXVII: $R_1=CH_3$; $R_2=OCH_3$; $R_3=Cl$
XXVIII: $R_1=OCH_3$; $R_2=CH_3$; $R_3=Cl$



XXIX

Fig. 2. Structures of sugar derivatives

The *N*-trifluoroacetyl methylglycosides of the new aminosugar (*XVII–XX*), having L-*lyxo* and L-*arabino* configurations and reported in Fig. 2, were prepared as previously described [5]. Acid hydrolysis (20% aqueous acetic acid, 100° C for 2 h) of the methylglycosides *XVII–XX* gave almost quantitatively the corresponding hexoses *XXI* (m.p. 181–182° C; $[\alpha]_D$-127°, c 1.0 in CH_3OH), *XXII* (m.p. 110–111° C; $[\alpha]_D$-83°, c 1 in CH_3OH), *XXIII* (syrup; $[\alpha]_D$-95°, c 1.1 in $CHCl_3$), and *XXIV* (syrup; $[\alpha]_D$-70°, c 1.0 in $CHCl_3$). Treatment of *XXI* and *XXII* in methylene chloride with *p*-nitrobenzoyl chloride in the presence of triethylamine and 4-dimethylaminopyridine (45° C for 90 min) gave the corresponding 1,4-di-O-*p*-nitrobenzoylderivatives (m.p. 169–170° C and 151–152° C, respectively), which were treated with dry hydrogen chloride in anhydrous methylene chloride (30 min at 0° C). After filtering the precipitate of *p*-nitrobenzoic acid and solvent evaporation under nitrogen and anhydrous conditions, the corresponding *1*-chloroderivatives *XXV* and *XXVI* were obtained as white foams and directly used for the coupling reaction. Analogously the 1-O-*p*-nitrobenzoates of compounds *XXIII* and *XXIV* (m.p. 180–181° C and 175–176° C, respectively), obtained by the standard procedure (*p*-nitrobenzoyl chloride in pyridine, 10 h, room temperature), were converted into the corresponding *1*-chloroderivatives *XXVII* and *XXVIII*.

The coupling reaction of 2 mmol daunomicinone (*XXIX*) with 2 mmol 2,3,6-trideoxy-4-C-methyl-4-O-*p*-nitrobenzoyl-3-trifluoroacetamido-L-*lyxo*hexopyranosyl chloride (*XXV*) in

Table 1. Physical and chemical data of 4′-C-methylDNR and DX analogues as hydrochlorides

Compound	Yield (%)	Empirical formula	Mol. wt. (calcd)	M.p. (° C) (dec.)	$[\alpha]_D$ c. 0.05 in CH_3OH
4′-C-MethylDNR (*IX*)	57[a]	$C_{28}H_{31}NO_{10} \cdot HCl$	578	162–163	+ 320°
4′-C-MethylDX (*X*)	86[b]	$C_{28}H_{31}NO_{11} \cdot HCl$	594	185–186	+ 285°
4′-Epi-4′-C-methylDNR (*XI*)	50[a]	$C_{28}H_{31}NO_{10} \cdot HCl$	578	187–188	+ 285°
4′-Epi-4′-C-methylDX (*XII*)	60[b]	$C_{28}H_{31}NO_{11} \cdot HCl$	594	169–170	+ 250°
4′-C-Methyl-4′-O-methylDNR (*XIII*)	38[a]	$C_{29}H_{33}NO_{10} \cdot HCl$	592	178–180	+ 285°
4′-C-Methyl-4′-O-methylDX (*XIV*)	70[b]	$C_{29}H_{33}NO_{11} \cdot HCl$	608	185–186	+ 291°
4′-Epi-4′-C-methyl-4′-O-methylDNR (*XV*)	40[a]	$C_{29}H_{33}NO_{10} \cdot HCl$	592	187–188	+ 251°
4′-Epi-4′-C-methyl-4′-O-methylDX (*XVI*)	60[b]	$C_{29}H_{33}NO_{11} \cdot HCl$	608	190–191	+ 272°

[a] Overall yield calculated on daunomycinone (*XXIX*)
[b] From the corresponding DNR analogue

Table 2. Comparison of the new anthracyclines (*IX–XVI*) with DNR (*I*) and DX (*II*) by TLC[a]

Compound	Rf value
DNR (*I*)	0.34
4′-C-MethylDNR (*IX*)	0.36
4′-Epi-4′-C-methylDNR (*XI*)	0.44
4′-C-Methyl-4′-O-methylDNR (*XIII*)	0.55
4′-Epi-4′-C-methyl-4′-O-methylDNR (*XV*)	0.60
DX (*II*)	0.22
4′-C-MethylDX (*X*)	0.24
4′-Epi-4′-C-methylDX (*XII*)	0.29
4′-C-Methyl-4′-O-methylDX (*XIV*)	0.39
4′-Epi-4′-C-methyl-4′-O-methylDX (*XVI*)	0.45

[a] Silica gel 60 F_{254}-precoated plates (E. Merck, Darmstadt, FRG); solvent, chloroform-methanol-acetic acid and water (80:20:7:3, by volume)

methylene chloride (15 ml) was performed at room temperature for 30 min as previously described [2], using silver trifluoromethanesulfonate as catalyst. Silica gel column chromatography, using a 95 : 5 chloroform : acetone mixture as eluent, gave 1.3 mmol (65% yield) of pure 4′C-methyl-4-O-*p*-nitrobenzoyl-*N*-trifluoroacetylDNR: m.p. 172–173° C; $[\alpha]_D$ + 420° (c 0.05, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) 1.60 (s, CH_3-C-4′), 2.48 (s, CH_3CO), 4.02 (s, OCH_3), 5.21 (broad s, C-7H), 5.50 (broad s, C-1′-H), 13.11 and 13.92 δ (two s, phenolic OH).

Analogously, the coupling reaction of daunomicinone (*XXIX*) with the *1*-chloroderivatives *XXVI, XXVII,* and *XXVIII* gave, respectively, 4′-epi-4′-C-methyl-4′-O-*p*-nitrobenzoyl-*N*-trifluoroacetylDNR (m.p. 155–156° C; ^{1}H-NMR: CH_3-C-4′ s 1.56 δ), 4′-C-methyl-4′-O-methyl-*N*-trifluoroacetyl-DNR (m.p. 91–92° C; ^{1}H-NMR: CH_3-C-4′ s at 1.19 δ and CH_3O-C-4′ s 3.45 δ) and its 4′-epianalogue (m.p. 75–76° C; ^{1}H-NMR: CH_3-4′ s 1.13 δ, and CH_3-O-C-4′ 3.25 δ). In the coupling products, the α-configuration on the glycosidic linkage was assigned on the basis of their C-1′-H-NMR signals which appear as broad singlet (W_H = 6.5 Hz) at 5.40–5.60 δ ($CDCl_3$); this is characteristic for the equatorial anomeric proton of 2-deoxy-glycopyranosides of anthracyclinones. Mild alkaline treatment of the protected glycosides (0.1–0.2 *N* aqueous sodium hydroxide, 1–2 h at 10–20° C, under nitrogen) gave respectively 4′-C-methyl- (*IX*), 4′-epi-4′-C-methyl- (*XI*), 4′-C-methyl-4′-O-methyl- (*XIII*), and 4′-epi-4′-C-methyl-4′-O-methyl- (*XV*) daunorubicin analogues, isolated as the hydrochlorides.

The corresponding doxorubicin analogues, *X, XII, XIV,* and *XVI* were obtained as the hydrochlorides via 14-bromo-derivatives, according to a procedure already described for the chemical transformation of daunorubicin (*I*) to doxorubicin (*II*) [1].

Some physical and chemical data of the new 4′-C-methyl analogues of DNR and DX (*IX–XVI*) are reported in Table 1, and their TLC behavior is shown in Table 2.

All the new compounds described in this paper gave correct micro-analyses and exhibited spectroscopic characteristics that are in agreement with the assigned structures.

Results

The new anthracyclines (*IX–XVI*) synthesized according to the procedure described above were obtained in pure form with reasonably good yields (Table 1).

The cytotoxic effect of the new anthracyclines under study against HeLa cells in vitro and their binding affinity for calf thymus DNA (in Table 3) were compared with the corresponding data for DNR and DX.

The substitution of the C-4′ hydrogen atom with a methyl group in DNR (*I*) and DX (*II*) afforded the less cytotoxic 4′-C-methyl derivatives *IX* and *X*, while the corresponding 4′-epianalogues *XI* and *XII* were found to be more cytotoxic than the parent compounds *III* and *IV* as well as more so than DNR and DX.

The introduction of two methyl groups at the 4′ position of DNR (*I*) and 4′-epi-DX (*IV*), giving *XIII* and *XVI,* respectively, brought about a decrease of cytotoxicity, while in the case of DX (*II*) and 4′-epi-DNR (*III*) the corresponding 4′-C-methyl-4′-O-methyl derivatives *XIV* and *XV* were found to be more cytotoxic than the parent compounds.

The O-methylation at the 4′ position in DNR (*I*) and in its analogues *IX* and *XI*, affording, respectively, *V, XIII,* and *XV,* did not significantly affect the cytotoxicity; in contrast, the 4′-O-methylDX analogues *VI, VIII,* and *XIV* were found to be more cytotoxic than the parent compounds *II, IV,* and *X*. The 4′-O-methylation of the 4′-epianalogues *III* and *XII* brought about a significant reduction of the cytotoxicity.

As regards binding to DNA, in agreement with previously reported data [20] DX showed the highest affinity, and all the chemical modifications considered here caused a reduction of the affinity, which was particularly evident in the case of 4′-epi-DNR analogues. In Fig. 3 we have plotted the ID_{50} in HeLa cells in vitro vs. the binding affinity to DNA ($2n \times K_{app}$). In general there was an inverse relation between these parameters, except for 4′-epi-4′-C-methylDNR (*XI*) and its

Table 3. Cytotoxicity and DNA binding parameters of analogues of DNR and DX modified at the C-4′ position

Compound	Concentration (ng/ml) required for 50% inhibition on HeLa cells[a]	Affinity parameters for calf thymus DNA ($2n \times K_{app} \times 10^{-5}$)[b]
DNR (*I*)	10.0 (8.8–12.5)	14.5
DX (*II*)	10.0 (7–12.5)	22.2
4′-Epi-DNR (*III*)	10.0	
4′-Epi-DX (*IV*)	8.5	
4′-O-MethylDNR (*V*)	11.5 (9, 14)	10.4
4′-O-MethylDX (*VI*)	5.0 (5, 5)	12.9
4′-Epi-4′-O-methylDNR (*VII*)	38.0	6.0
4′-Epi-4′-O-methylDX (*VIII*)	7.4	16.0
4′-C-MethylDNR (*IX*)	35.0	8.7
4′-C-MethylDX (*X*)	16.5 (15, 18)	10.2
4′-Epi-4′-C-methylDNR (*XI*)	3.4 (2.8, 4)	4.5
4′-Epi-4′-C-methylDX (*XII*)	1.8 (0.6, 3)	14.2
4′-C-Methyl-4′-O-methylDNR (*XIII*)	27.0 (20, 34)	7.0
4′-C-Methyl-4′-O-methylDX (*XIV*)	5.0	18.0
4′-Epi-4′-C-methyl-4′-O-methylDNR (*XV*)	3.2 (2.5, 4)	7.0
4′-Epi-4′-C-methyl-4′-O-methylDX (*XVI*)	27.0	7.2

[a] HeLa cells were exposed to drugs for 24 h. In most cases, the data represent the average of two to seven experiments; in parentheses, data (,) obtained in individual experiments, or range (–)

[b] K_{app} (the apparent binding constant) and n (the apparent number of binding sites per nucleotide) were determined from Scatchard plot (r/m vs. r), where r is the molar ratio of bound antibiotic per nucleotide and m is the molar concentration of free antibiotic. In this plot, K_{app} is the negative of the slope and n is the intercept of the linear region of the binding curve with the horizontal axis. These values of K_{app} and n refer in all cases to an almost linear region of the binding isotherm at small values of r. Each value represents an average of at least three independent determinations

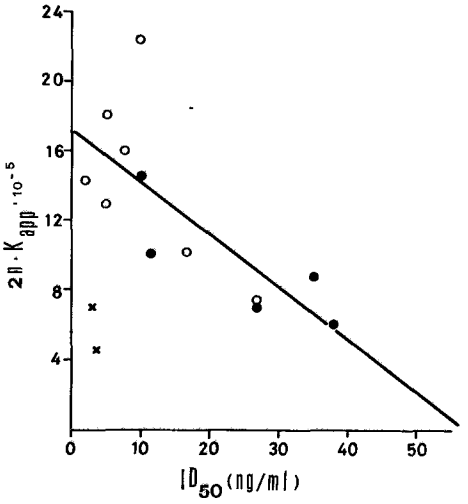


Fig. 3. Relationship between cytotoxicity and ability to bind to DNA of anthracycline derivatives. *Abscissa:* ID_{50} on HeLa cells (ng/ml); *ordinate:* $2n \cdot K_{app} \cdot 10^{-5}$. ●, DNR and its derivatives; ○, DX and its derivatives; ×, 4′-epi-4′-C-methylDNR and 4′-epi-4′-C-methyl-4′-O-methylDNR, which were not considered for the calculations of the linear regression. The correlation coefficient was = −0.76; intercept = 17.1; slope = −0.302

Table 4. Activity of 4′-O-methyl analogues of DNR and DX on ascitic leukemias in mice

Compound	Dose[a]	P388 leukemia		L1210 leukemia	
		MST[b]	Toxic deaths	MST	Toxic deaths
DNR (*I*)	2.9	169	0/10		
	4.4	169	2/10		
DX (*II*)	6.6	241	0/10	175	0/10
	10.0	>545	0/10	187	3/10
4′-O-MethylDNR (*V*)	2.9	156	0/10		
	4.4	191	3/10	287	1/10
4′-O-MethylDX (*VI*)	2.9	277	0/10		
	4.4	287		312	0/10
	6.6	231		275	1/20
4′-Epi-4′-O-methylDNR (*VII*)	20.0	174	0/9		
	40.0	34	9/9		
4′-Epi-4′-O-methylDX (*VIII*)	10.0			187	0/10
	15.0			181	2/10

[a] Treatment IP on day 1 (mg/kg body weight)

[b] Median survival time expressed as percentage of untreated controls

4′-O-methyl derivative (*XV*), which exerted a cytotoxic activity much higher than was expected on the basis of their ability to bind to DNA. As the cytotoxicity of anthracyclines depends not only on biochemical, but also on pharmacokinetic properties [17], assuming that cell uptake could be related to some extent to the lipid/water partition coefficient [4], we found that the partition coefficient of compounds *XI* and *XV* was actually higher than that of DNR and DX (F. Zunino et al. 1982, unpublished work).

However, other mechanisms could be behind this surprisingly high cytotoxicity. Excluding these two compounds, the correlation coefficient between the two variables considered was = −0.76. Another compound rather outstanding in this study was DX itself, which was not so highly cytotoxic as one would expect on the basis of its ability to bind to DNA, which was extremely high in these experiments. In this regard it must be observed that the superiority of DX over DNR as regards binding to DNA is not a constant finding [3].

Table 5. Activity of 4′-C-methyl analogues of DNR and DX on experimental leukemias in mice

Compound	P388 leukemia			Gross leukemia		
	Dose[a]	MST[b]	Toxic deaths	Dose[c]	MST	Toxic deaths
DNR (*I*)	2.9	175	0/40	15.0	166	0/10
	4.4	180	3/39	22.5	200	2/10
DX (*II*)	6.6	193	0/30	13.0	192	0/17
	10.0	227	1/28	16.9	212	0/17
4′-C-MethylDNR (*IX*)	20.0	155	0/10			
	40.0	60	10/10			
4′-C-MethylDX (*X*)	7.7	172	0/10	21.9	183	0/18
	10.0	233	2/20	28.4	200	1/8
4′-Epi-4′-C-methylDNR (*XI*)	0.44	163	0/10	2.5	183	0/10
	0.66	158	1/20	3.1	200	3/10
4′-Epi-4′-C-methylDX (*XII*)	2.0	156	0/10			
	4.0	106	10/10			

[a] Treatment IP on day 1 (mg/kg body weight)
[b] Median survival time expressed as percentage of untreated controls
[c] Treatment IV on day 1 (mg/kg body weight)

Table 6. Activity of 4′-O-methyl-4′-C-methyl analogues of DNR and DX on experimental leukemias in mice

Compound	P388 leukemia			Gross leukemia		
	Dose[a]	MST[b]	Toxic deaths	Dose[c]	MST	Toxic deaths
DNR (*I*)	2.9	163	0/48	15.0	175	1/8
	4.4	161	3/47	22.5	233	0/9
DX (*II*)	6.6	201	0/28	13.0	200	0/20
	10.0	241	5/28	16.9	235	0/20
4′-C-Methyl-4′-O-methylDNR (*XIII*)	33.7	150	0/26	18.0	116	0/10
	50.5	137	1/5	36.0	150	0/4
4′-C-Methyl-4′-O-methylDX (*XIV*)	6.6	172	0/10	13.0	208	0/18
	10.0	202	2/17	16.9	183	5/10
4′-Epi-4′-C-methyl-4′-O-methylDNR (*XV*)	22.5[d]	150	0/10			
4′-Epi-4′-C-methyl-4′-O-methylDX (*XVI*)	10.0[d]	205	0/8			

[a] Treatment IP on day 1 (mg/kg body weight)
[b] Median survival time expressed as percentage of untreated controls; average data from several experiments
[c] Treatment IV on day 1 (mg/kg body weight)
[d] Maximal dose tested

The antitumor effect of the compounds was tested in mice bearing P388 or L1210 ascitic leukemia, treated IP on day 1 after the tumor inoculation with at least three dose levels/compound. We report here only the results obtained at the optimal dose (dose which exerts the maximum antitumor effect without giving toxic side-effects) and at the lowest toxic dose investigated. Data on the effects of 4′-O-methyl analogues against P388 leukemia are shown in Table 4. 4′-O-MethylDNR was as active as DNR. In agreement with the in vitro data, its 4′-epi derivative was significantly less potent but at the optimal dose it was as active as the parent compound. As previously described, 4′-O-methylDX was more toxic than DX, equally active against P388 leukemia, but more active against L1210 leukemia at the optimal dose. Its 4′-epi derivative, when tested against L1210 leukemia, showed lower potency and an activity comparable to that of DX at the optimal dose. Within this series of derivatives, therefore, 4′-epi derivatives are less potent than the parent compounds, and at the optimal dose they are endowed with antitumor activity of the same order of magnitude as that of DNR and DX. The data on the antitumor activity of 4′-C-methyl analogues of DNR and DX are shown in Table 5. In agreement with the cytotoxicity data reported above, 4′-epi-4′-C-methyl derivatives were more toxic than 4′-C-methylDNR and 4′-C-methylDX. 4′-Epi-4′-C-methylDNR was about six times more potent than DNR, whether it was administered IP or IV; its antileukemic activity at the optimal dose was comparable to that of DNR.

Antitumor activity data on the compounds bearing two methyl groups at position 4′ are shown in Table 6. While the introduction of two methyl groups in the aminosugar of DNR markedly reduced the potency, such a reduction was not observed in the case of the DX analogues. 4′-C-Meth-

yl-4′-O-methylDX was as potent as DX when given IP to mice bearing the P388 ascitic leukemia, and more potent than DX when given IV to mice bearing Gross leukemia. The 4′-epi analogues of this group preserved their antitumor activity against P388 leukemia.

Discussion

It has been shown previously [16] that cytotoxicity of a set of 10 antitumor anthracycline analogues could be expressed as a function of DNA damage, inhibition of thymidine incorporation, and drug retention. In this paper we present data showing that the cytotoxic effect of new anthracyclines could be linearly related to their ability to bind to DNA, thus providing further evidence that this mechanism is one of the major contributors to the biologic activity of this class of compounds. As previously observed [7], cytotoxicity correlated well with the potency, and also with the toxicity in experimental animals, as evidenced by the lowest dose giving toxic effects in tumor-bearing mice. In fact, among these derivatives, except for 4′-epi-4′-C-methyl-4′-O-methylDNR there was a good relation between ID_{50} in vitro and MxTD in P388 leukemia-bearing mice treated IP. However, antitumor effectiveness did not seem to be related to these parameters.

In general, all these compounds at their optimal dose exerted an antitumor activity against P388 leukemia that was not markedly different from that of their parent compounds (DNR or DX). Similar data were also obtained when the drugs were given IV to mice bearing Gross leukemia: all the new derivatives tested retained a good antitumor activity. Also within this group, the DX analogues appear to be more effective than DNR analogues in prolonging survival of mice bearing P388 leukemia, confirming a higher selectivity of compounds having the hydroxyacetyl side-chain for the inhibition of growth of this type of tumor cells in vivo. The most active compound against P388 leukemia was 4′-O-methylDX, which is also more active than DX against L1210 leukemia and colon adenocarcinomas, and is less cardiotoxic [9, 15]. This compound will therefore be further investigated in different experimental systems.

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