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Antitumor Anthracycline Antibiotics. Structure–Activity and Structure–Cardiotoxicity Relationships of Rubidazone Analogues

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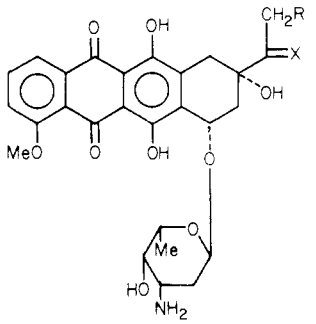
A series of rubidazone analogues (4–14) with varying phenyl group substituents was prepared. The effect of these compounds on inhibition of nucleic acid synthesis in cultured cells, on in vivo antitumor properties, and on cardiotoxicity was examined. Substituent effects on drug–DNA binding as indicated by DNA melting temperature measurements were also investigated. Substituent effects were essentially absent among the rubidazone analogues in in vivo and in vitro test systems which measure cytotoxic characteristics; however, the rubidazone analogues varied substantially in their cardiotoxic effects and this variation was closely correlated with the electronic character of the phenyl substituent.

Daunorubicin (**1**) and especially adriamycin (**2**) have become important drugs for cancer chemotherapy.[1-3] Not unexpectedly, their usefulness has generated considerable interest in developing analogues with improved properties.[1] Among these analogues, rubidazone (daunorubicin benzhydrazone, **3**) has received considerable attention. After



1, R = H; X = O (daunorubicin)
2, R = OH; X = O (adriamycin)
3, R = H; X = $NNHCOC_6H_5$ (rubidazone)

the first report of its activity in experimental systems,[4] clinical evaluation quickly showed it to be at least equivalent to daunorubicin in efficacy against leukemia and to have potentially advantageous pharmacological properties.[5-8] In particular, rubidazone was described as less toxic and easier to administer than daunorubicin.[8] Biochemical studies have not indicated, however, that rubidazone differs in any fundamental way from the parent antibiotic.[9-11]

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The favorable clinical reports on rubidazone and the very limited published reports on related structure–activity studies[12] led us to consider it as a starting point for our analogue study. We were further encouraged by its decreased cardiotoxicity relative to adriamycin[12,13] because the dose-limiting, cumulative cardiotoxic properties of the latter are well-known.[14] The inclusion of cardiotoxicity evaluation in this study was made possible by development of a reproducible screening system in the rat that is economical in cost and in required amount of drug.[13] Important cardiotoxicity models in the rabbit[15] and rhesus monkey[16] have also been developed, but they are not practical for primary screening. A mouse model using microscopically determined morphological criteria as the end point has also been proposed.[17a] The rat model employs as end point the characteristic electrocardiographic (ECG) changes that follow repeated administration of cardiotoxic anthracycline derivatives. These ECG effects are associated with impairment of heart mitochondrial function.[17b]

Rubidazone is an excellent candidate for a lead on which to base a quantitative structure–activity study because the benzhydrazone moiety is easily incorporated into daunorubicin and the benzoyl group can serve as a readily accessible carrier for systematic variation of substituents. We therefore decided to prepare a series of rubidazone analogues with phenyl group substituents that would provide varying electronic and partition properties. At the beginning of this work we were aware of the problems involved in finding a set of noncollinear aromatic substituents that would describe electronic and partition properties; we therefore chose the initial targets to give low collinearity with reasonable synthetic accessibility.[18,19] A σ vs. π plot of the substituents is shown in Figure 1. This plot is normalized against daunorubicin benzhydrazone (**3**).

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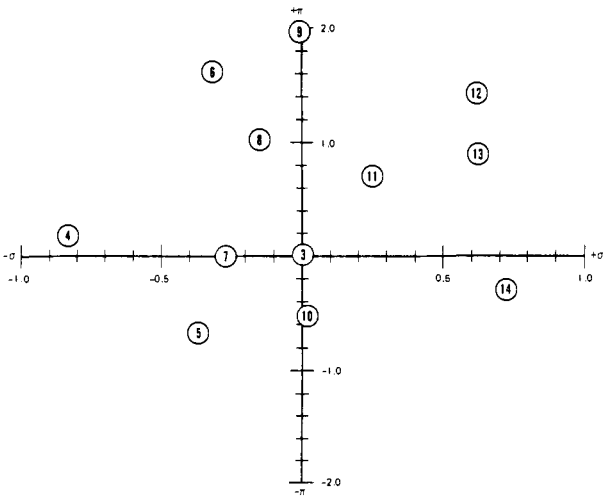


Figure 1. Plot of $\Delta\pi$ and $\Delta\sigma$ values for derivatives of daunorubicin and adriamycin. The plot is normalized against the lead compound, daunorubicin benzhydrazone (rubidazone, **3**), and represents changes in π and σ relative to it. Data from ref 23.

The normalization places the adriamycin analogues **10** and **13** 0.52 π units lower, reflecting the difference between measured log P values for **1** and **2**.

The physical and biological properties of the compounds studied are presented in Table I. The compounds are arranged in order of increasing σ_p. Table I includes two adriamycin benzhydrazones that are not strictly analogues of rubidazone. However, the very close chemical and biological relationship of adriamycin and daunorubicin made the synthesis of corresponding analogues of interest. In this paper we discuss the effect of substituents on inhibition of nucleic acid synthesis in cultured cells, on in vivo antitumor properties, and on cardiotoxicity. Attempts to determine substituent effects on drug–DNA binding, as indicated by DNA melting temperature measurements (ΔT_m), are also described.

Results and Discussion

It has been well documented that optimum antitumor properties are correlated with a high degree of thermal stabilization of helical DNA (T_m) among anthracycline drugs.[20] The ΔT_m values for the daunorubicin-derived rubidazone analogues did not vary from that of daunorubicin by more than 0.8 °C (Table I). Because rubidazone is reported to revert to daunorubicin and 13-dihydrodaunorubicin very rapidly in man,[21] the uniformity of the ΔT_m results suggested that hydrolysis was occurring during the ΔT_m experiments. Spectral hydrolysis–rate determinations (pH 7.4 at 50 °C in the absence of DNA) on **3**, **4**, and **11**, which were selected for a wide range of σ, were as expected, and in each case the rate was more than sufficient to fully cleave the derivatives under the conditions of the ΔT_m experiments (50–100 °C at pH 7.0 for ca. 3 h). Table I reports half-lives ($t_{1/2}$) for the three compounds. Interestingly, adriamycin benzhydrazones **10** and **13** showed ΔT_m values substantially less than that of adriamycin, suggesting that intact benzhydrazones bind more poorly than the parent drug and that adriamycin derivatives have a slower hydrolysis rate. Although the ΔT_m values gave no information on DNA binding, they established that hydrolysis of daunorubicin benzhydrazones is little affected by the presence of DNA. This is consistent with our model of the DNA–daunorubicin complex,[1] in which the 13-carbonyl region is sterically free to interact with the aqueous environment.

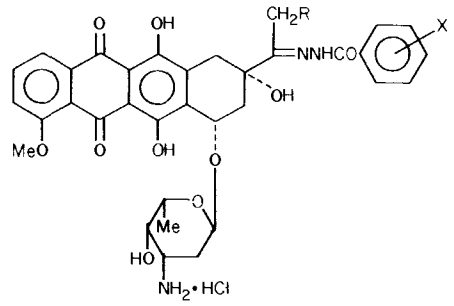
All of the compounds inhibited DNA and RNA synthesis in cultured L1210 cells. The data in Table I show that substituent variation does not significantly affect this biological parameter. The most electronegative and electropositive substituents yielded compounds with essentially identical values that did not differ appreciably from those of rubidazone itself. At least a several-fold loss of potency relative to the parent antibiotic was evident in each case, however. Under the conditions of this assay (4 h at 37 °C), hydrolysis could occur to a large extent for some of the compounds (e.g., for **11**). However, this factor does not appear to influence the biological activity.

Table II presents in vivo antitumor data for the rubidazone analogues at a range of doses sufficiently wide to encompass toxicity and inactivity. The data show that the optimum dose for all analogues, including rubidazone, is between 2 and 8 mg/kg. However, these differences are probably not significant because of the inherently high variability of this test. The specific data presented in Table II for the rubidazone analogues were obtained in a single test. The data for rubidazone, daunorubicin, and adriamycin are averages based on a large number of tests; the standard deviations given with these averages indicate the reliability of any given single set of data. Because of this high variability, the variation in optimum dose and T/C value found among the rubidazone analogues does not represent a significant difference. Discrimination among these compounds would require further extensive comparative tests. Based on this concept and on additional test data from isolated experiments with some of these compounds, we believe that all the analogues must be regarded as equivalent in in vivo activity. The higher potency of daunorubicin and adriamycin is clearly shown, as is the superior efficacy of adriamycin over daunorubicin. In this test, rubidazone is equal to adriamycin in efficacy but only at a fourfold larger dose.

It was disappointing to find that substituent effects were essentially absent among rubidazone analogues in in vivo and in vitro test systems measuring cytotoxic characteristics. It was therefore quite interesting to find that the rubidazone analogues varied substantially in their cardiotoxic effects in the rat, as is shown in Table I. The minimum cumulative cardiotoxic dose (MCCD) varied from 12 mg/kg for 4-dimethylaminorubidazone (**4**) to 48 mg/kg for 4-chloro derivative **11**. It should be noted that the reproducibility of the rat cardiotoxicity screen is excellent. For example, in five experiments the MCCD of adriamycin was never less than 10 mg/kg or more than 12 mg/kg. In both experiments conducted with rubidazone the MCCD was found to be 24 mg/kg. The fourfold difference in cardiotoxicity in a series of compounds that are essentially equal in potency and efficacy as antitumor drugs is, therefore, of high biological significance. Even more interesting was the finding that cardiotoxicity was clearly associated with the electronic character of the substituents on the phenyl ring. Therefore, we investigated the quantitative structure–cardiotoxicity relationships of the compounds in Table I. Multiparameter linear regression analysis was done using the substituent parameters and log P as presented in Table I. In addition, $\Sigma\sigma$, the sum of σ_m and σ_p, was also investigated. Substituent parameters are from the compilation published by Hansch et al.[23] Compound **6** was excluded from the analysis since a specific MCCD was not determined owing to the insufficient supply. The most significant single parameter was σ_p. Equation 1 correlates the MCCD data. No other parameter from Table I added to eq 1 was significant at the 95% level. Equation 1 indicates that electron–

$$\log \text{MCCD} = 0.43\ (\pm 0.23)\ \sigma_p + 1.6\ (\pm 0.30) \qquad (1)$$
$$n = 11;\ r = 0.77;\ s = 0.12;\ F = 13\ (p < 0.01)$$

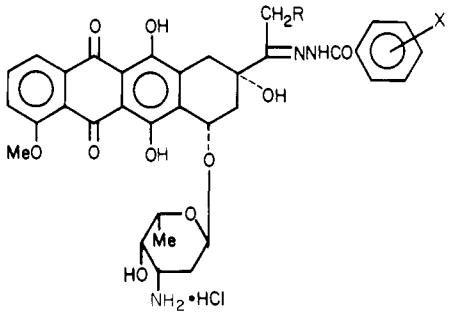
Table I. Physical and Biological Data for Rubidazone Analogues



			aromatic substituent constants[a]						log P^b	pK_a^c of Ar-COOH	hydrolysis rate,[d] $t_{1/2}$, min	ΔT_m	nucleic acid synth, inhibn[e] (ED_{50}, μM)		cardiotoxicity		
															MCCD		MCCD/MC-CD_{adria}
no.	R	X	π	σ_p	σ_m	F	R	MR					DNA	RNA	mg/kg	μM	
4	H	4-NMe_2	0.18	−0.83	0.0	0.10	−0.92	1.66	1.22	4.97	554	10.7	2.6	2.5	12	16	0.8
5	H	4-OH	−0.67	−0.37	0.0	0.29	−0.64	0.38	0.37	4.52		11.5	3.6	1.9	20	29	1.5
6	H	4-*n*-OBu	1.61	−0.32	0.0	0.25	−0.55	1.81	2.65	4.23		10.2	3.2	1.4	⩾48	⩾64	⩾3.4
7	H	4-OMe	−0.02	−0.27	0.0	0.26	−0.51	0.89	1.02	4.47		10.9	3.1	1.6	24	34	1.8
8	H	4-Et	1.02	−0.15	0.0	−0.05	−0.10	1.1	2.06	4.35		10.5	2.6	1.1	20	28	1.5
9	H	4-C_6H_5	1.96	−0.01	0.0	0.08	−0.08	2.64	3.00	3.97		10.3	1.9	1.0	40	53	2.8
3	H	H	0.0	0.0	0.0	0.0	0.0	0.2	1.04	4.19	324	11.7	2.2	1.2	24	35	1.8
10	OH	H	0.0	0.0	0.0	0.0	0.0	0.2	0.52	4.19		8.9	14	8.8	32	46	2.4
11	H	4-Cl	0.71	0.23	0.0	0.41	−0.15	0.7	1.75	4.03	37	11.4	1.9	1.0	48	67	3.5
12	H	3,4-Cl_2	1.42	0.23	0.37	0.82	−0.30	1.2	2.46	3.62		11.0	2.1	1.7	40	53	2.8
13	OH	3,4-Cl_2	1.42	0.23	0.37	0.82	−0.30	1.2	1.94	3.62		5.3	6.6	3.6	20	26	1.4
14	H	3-NO_2	−0.28	0.0	0.71	0.67	0.16	0.84	0.76	3.46		10.5	3.2	2.3	32	44	2.3
1	daunorubicin								0.82			11.0	0.48 ± 0.09	0.23 ± 0.07	14	25	1.3
2	adriamycin								0.30			13.4	1.5	0.67	11 (10–12)	19	1.0

[a] Data from ref 23 for substituents on the aromatic ring. [b] Experimental log P values for 1–3 provided by Dr. Robert Engle of NCI. Remaining values were calculated according to the principles cited in ref 23 and 24. [c] Values from ref 25. [d] Method described in the Experimental Section. [e] Cultured L1210 cells. The method of ref 26 was used except that drugs were initially dissolved in a volume of Me_2SO that resulted in a final Me_2SO concentration of 1% in the assay medium. This modification greatly aided solubilization of poorly soluble compounds and did not affect assay results, according to extensive control experiments.

Table II. Activity of Rubidazone Analogues against P-388 Lymphocytic Leukemia in the Mouse[a]



			T/C at indicated dose, mg/kg							
no.	R	X	16	8	4	2	1	0.5	0.25	0.125
4	H	4-NMe_2	89	136	172	181	154	154	111	94
5	H	4-OH	111	181	161	160	145	157	111	115
6	H	4-*n*-OBu	54	72	127	179	152	151	143	115
7	H	4-OMe	59	183	211	174	158	136	129	119
8	H	4-Et	68	93	175	175	154	148	139	111
9	H	4-C_6H_5	63	98	190	163	157	138	134	107
3	H	H	112 ± 64[b]	164 ± 62	192 ± 39	170 ± 18	152 ± 14	140 ± 11	133 ± 15	
10	OH	H	128	201	172	154	154	126	99	103
11	H	4-Cl	63	109	211	163	145	139	145	110
12	H	3,4-Cl_2	54	81	163	175	166	157	137	117
13	OH	3,4-Cl_2	72	109	202	154	172	143	125	116
14	H	3-NO_2	72	202	190	172	146	130	130	125
1	daunorubicin				83 ± 26[c]	112 ± 36	162 ± 20	167 ± 17	152 ± 12	
2	adriamycin				77 ± 15[d]	149 ± 71	193 ± 40	187 ± 40	170 ± 19	

[a] Assays performed by the Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute. BDF or CDF mice are injected ip with 10^6 P-388 lymphocytic leukemia cells on day 0 and treated ip on days 1-9 with the specified drug dose. T/C is the ratio of the average survival time of treated mice to that of untreated controls in percent. The average survival time of untreated controls is approximately 11 days. Values of T/C < 85 indicate drug toxicity. [b] $n = 21$. [c] $n = 19$. [d] $n = 17$.

Table III. Squared Correlation Matrix for Variables of Equation 2

	π	σ_p	σ_m	F	R	MR
π	1.0	0.220	0.002	0.045	0.034	0.558
σ_p		1.0	0.147	0.239	0.531	0.010
σ_m			1.0	0.634	0.110	0.001
F				1.0	0.000	0.003
R					1.0	0.025
MR						1.0

withdrawing groups on the phenyl ring result in less cardiotoxic rubidazone analogues. Equation 1 includes the adriamycin phenylhydrazones in Table I (**10**, **13**). When these compounds were dropped from the regression analysis, the result was eq 2, which includes only the rubidazone analogues. This equation has almost the same

$$\log \text{MCCD} = 0.53\ (\pm 0.07)\ \sigma_p + 1.63\ (\pm 0.19) \quad (2)$$
$$n = 9;\ r = 0.94;\ s = 0.065;\ F = 57.8\ (p < 0.01)$$

slope as eq 1 and an identical intercept. Figure 2 is a plot of the MCCD data vs. σ_p, with the regression line of eq 2 indicated (see Table III).

Since the recent work[23] factoring σ into field (F) and resonance (R) parameters enables a separation of electronic effects into resonance and nonresonance components, we used these parameters in our analysis. These substituent parameters are also given in Table I. Equation 3 represents a statistically significant ($p < 0.01$) equation found for R; no significant relationship was found for F and none of the other parameters in Table I was significant at the 95% level. Given the high collinearity of σ_p and R for our

$$\log \text{MCCD} = 0.36\ (\pm 0.13)\ R + 1.65\ (\pm 0.05) \quad (3)$$
$$n = 11;\ r = 0.67;\ s = 0.14;\ F = 7.2\ (p < 0.01)$$

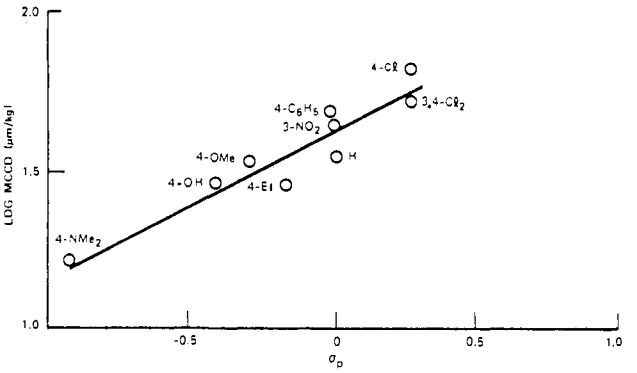


Figure 2. Plot of σ_p vs. log MCCD. The correlation equation is log MCCD = 0.53 (±0.07) σ_p + 1.63 (±0.19).

substituent set, this is an expected result. However, this result is meaningful in terms of formulating a chemical rationale for the observed biological data.

Table I reports pK_a values for the benzoic acids from which the rubidazone analogues were prepared. Not unexpectedly, because σ and pK_a are related characteristics, the MCCD generally increases with increasing acid strength. However, two exceptions to this generalization are obvious. Compound **6**, the 4-butoxy analogue, is derived from an acid with about the same pK_a as benzoic acid but is clearly less cardiotoxic than rubidazone. This may be a lipophilicity effect and further study will be required to clarify this point. Compounds **12**–**14**, all of which are derived from stronger acids than *p*-chlorobenzoic acid (the precursor to the least cardiotoxic analogue, **11**), all contain meta substituents. Although the number of examples is very small, the negative influence of meta substituents on cardiotoxicity is evident. The association of pK_a with cardiotoxicity may be of more practical value

Table IV. Isolation and Characterization of Benzhydrazones

no.	formula	yield, %	mp, °C dec	reaction conditions (per mmol of 1·HCl or 2·HCl) MeOH vol, mL	hydrazide, mmol	time, days	isolation procedure
3	$C_{34}H_{35}N_3O_{10}\cdot HCl$	82	245-248	10	2	5	A
4	$C_{36}H_{40}N_4O_{10}\cdot HCl\cdot 2H_2O$	86	187-189	20	2[a]	3	B
5	$C_{34}H_{35}N_3O_{11}\cdot HCl$	62	245-247	85	1	7	A
6	$C_{38}H_{43}N_3O_{11}\cdot HCl\cdot {}^2/_3H_2O$	76	177-180	10	2[b]	4	C
7	$C_{35}H_{37}N_3O_{11}\cdot HCl$	86	205-207	10	3	3	D
8	$C_{36}H_{39}N_3O_{10}\cdot HCl$	83	245-247	10	2[c]	5	A
9	$C_{40}H_{39}N_3O_{10}\cdot HCl\cdot H_2O$	81	205-208	38	2[d]	4	C
10	$C_{34}H_{35}N_3O_{11}\cdot HCl$	87	204-207	25[g]	5	7	E
11	$C_{34}H_{34}ClN_3O_{10}\cdot HCl$	79	247-250	20	3	3	A
12	$C_{34}H_{33}Cl_2N_3O_{10}\cdot HCl$	82	249-251	20	2[e]	3	F
13	$C_{34}H_{33}Cl_2N_3O_{11}\cdot HCl$	94	203-206	165[f]	2	9[f]	F
14	$C_{34}H_{34}N_4O_{12}\cdot HCl$	86	247-249	25	2	3	A

[a] Reference 27. [b] Reference 28. [c] A mixture of methyl 4-ethylbenzoate[29] and 3.0 equiv of hydrazine hydrate was refluxed for 16 h and then diluted with water. Recrystallization of the product from benzene afforded 4-ethylbenzhydrazide, mp 89.5-90.5 °C. [d] Reference 30. [e] Reference 31. [f] After 3 days the reaction mixture was concentrated to 10 mL and stirred for an additional 6 days. [g] MeOH-H_2O (4:1).

than the association of σ with cardiotoxicity; σ is a characteristic primarily applicable to aromatic systems, whereas pK_a encompasses aliphatic acids as well. Thus, pK_a may provide a more general criterion on which to base selections of additional acylhydrazones for cardiotoxicity evaluation.

The cardiotoxicity section of Table I presents ratios of the MCCD of experimental drugs to the MCCD of adriamycin, based on micromolar concentrations. This value provides a direct comparison of the cardiotoxicity of analogues to that of adriamycin. Compound 4 is the only analogue that is more cardiotoxic than adriamycin. Both adriamycin benzhydrazones (10 and 13) were less cardiotoxic than adriamycin. The smaller improvement seen with adriamycin 3,4-dichlorobenzhydrazone (13 relative to 10) is presumably due to the meta effect found with the daunorubicin benzhydrazones. The least cardiotoxic analogues (6 and 11) are about 3.5 times less so than adriamycin. This suggests a reduction in cardiotoxic properties that is significant in compounds that retain antitumor efficacy. Whether a particular compound will show a useful separation of cardiotoxicity from efficacy in human therapy is difficult to say. At present we can only compare cardiotoxicity in a rat ECG model with antitumor activity in a mouse mortality test where loss in potency would seem to counteract the advantage. No doubt further studies are merited.

Only speculations are possible on the mechanism of substituent effects on cardiotoxicity, but it seems paradoxical that rapidly hydrolyzing derivatives should be less cardiotoxic than those that are more stable. The substituent effect could be related to its influence on the reductive metabolism at the 13 position. Daunorubicin is known to be rapidly reduced to 13-dihydrodaunorubicin[20] and electron-withdrawing substituents would be expected to influence the susceptibility of the 13-carbon to reductive attack. Of possible significance is the clinical observation by Benjamin et al.[21] that rubidazone, although very rapidly hydrolyzed in man, nevertheless gives a much higher ratio of daunorubicin to dihydrodaunorubicin in serum blood levels than does daunorubicin itself.

Regardless of the mechanism, this work has revealed a relationship between structure and cardiotoxicity that may permit separation of antitumor from cardiotoxic activities. We are following up this correlation from the standpoint of substituent constant and pK_a of parent acid and are also attempting to further delineate the relationship of lipophilicity to cardiotoxicity among rubidazone analogues.

Experimental Section

Melting points were taken on a Fisher-Johns apparatus and are not corrected. Ultraviolet spectra (MeOH) were routinely obtained for targets on a Cary 14 recording spectrophotometer. Reaction progress and product purity were monitored by TLC on silica gel, using $CHCl_3$-MeOH-H_2O (20:10:1-60:10:1) for development. By multiple development, this system can detect 1-2% residual daunorubicin.

Melting temperatures (T_m) of drug-DNA complexes were determined in 0.01 M, pH 7, phosphate buffer containing 5×10^{-5} M DNA, 5×10^{-6} M drug, 10^{-5} M EDTA, and 5% Me_2SO. The drug was weighed and dissolved in Me_2SO. The drug solution was diluted with buffer to 10% Me_2SO concentration and then diluted with DNA in buffer containing the EDTA. Calf thymus DNA was obtained from Worthington or Miles Laboratories; its concentration was determined spectroscopically, assuming a molar extinction coefficient of 6800 M^{-1} cm^{-1} at 258 nm. Melting curves were determined at 259 nm in a GCA/McPherson 700 recording spectrophotometer fitted with jacketed cell holders. The cells were heated by circulating ethylene glycol from a Lauda K2/R bath electronically controlled and programmed at a constant temperature rate increase of 18 °C/h. Cell temperature was monitored by a thermocouple inserted in the cell holder. Absorbance and temperature were automatically recorded every 30 s for up to four simultaneous samples. The T_m was determined on the PROPHET[22] computer system by digitizing temperature and absorbance curves. Corrections for volume expansion, temperature lag between cell and holder, and baseline drift were made automatically from previously determined correction curves. Selection of the midpoint (T_m) value of the corrected temperature/absorbance curve was made by the computer following assignment of the high-temperature leveling point by the operator. Under these conditions, average values of T_m for DNA from Worthington and Miles are 62.5 ± 0.5 and 64.5 ± 0.5 °C, respectively.

Hydrolysis rate studies were performed at drug concentrations of 1×10^{-5} M in pH 7.4, 0.01 M phosphate buffer at 50 °C. The spectrum of pure drugs was compared with that of daunorubicin to select the wavelength showing the greatest change upon hydrolysis. The selected wavelength for each drug (345, 259, and 259 nm for 4, 3, and 11, respectively) was monitored continuously for 3 h at 50 °C. The resulting absorbance curve was digitized into the PHOPHET computer.[22] Baseline curves (buffer only) were digitized and subtracted from the absorbance curve to correct for slight baseline drift. The resulting corrected data were fit to eq

$$A = \Sigma A_0 + ce^{-kt} \quad (4)$$

4 where A was the observed absorbance and ΣA_0 was the absorbance of 10^{-5} M solutions of daunorubicin and the arylhydrazone at 50 °C. The $t_{1/2}$ values shown in Table I were computed from the resulting fitted data. Multiple regression analysis was done on the PROPHET[22] system using a stepwise

addition of variables at the 95% level.

General Procedure for Benzhydrazones. A methanol solution of daunorubicin hydrochloride (1) [or adriamycin hydrochloride (2)] (1 mmol) and a benzhydrazide (1–5 mmol) was stirred at room temperature in the dark for several days. Individual benzhydrazones were isolated and characterized, as noted in Table IV, with the following variations.

A. The product, which had precipitated from the reaction mixture, was collected and washed with small portions of MeOH.

B. The reaction mixture was concentrated to 1 mL and then diluted with 40 mL of CH_3CN added dropwise. The resulting precipitate was reprecipitated from MeOH–CH_3CN (1:40).

C. The reaction mixture was concentrated to 5 mL and then diluted with 50 mL of CH_3CN added dropwise. The resulting precipitate was reprecipitated from MeOH–CH_3CN (1:10).

D. The reaction mixture was diluted with 50 mL of CH_3CN added dropwise. The resulting precipitate was collected and washed with small portions of MeOH–CH_3CN (1:5).

E. The reaction mixture was evaporated. The residue was dissolved in 50 mL of absolute EtOH–C_6H_6 (4:1) and reevaporated three times. A solution of the residue in 20 mL of MeOH was diluted with 60 mL of CH_3CN added dropwise. The resulting precipitate was collected and washed with small portions of MeOH–CH_3CN (1:3).

F. The reaction mixture was diluted with 100 mL of CH_3CN added dropwise. The resulting precipitate was collected and washed with MeOH–CH_3CN (1:5).

The samples were dried at room temperature (0.1 mmHg) overnight. Elemental analyses for C, H, N, and Cl were within ±0.4% of theoretical values for all benzhydrazones reported in Table IV.

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