Preparation and Evaluation of 2-Substituted Anthraquinones Based on the Anthracyclines

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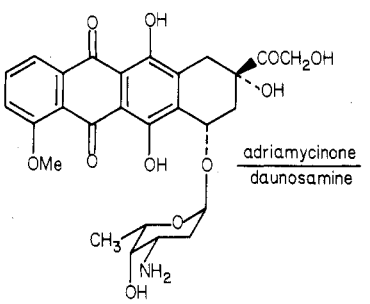
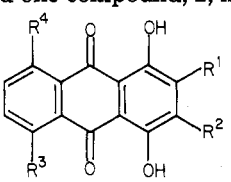
Anthraquinones substituted at the 2 position with a basic side chain were prepared, and their binding to DNA was evaluated. All compounds showed an intercalative mode of binding to DNA; 1,4-dihydroxy derivatives bound more strongly than 1-hydroxy or nonhydroxylated compounds. Greatest DNA-binding activity was found where there were five atoms between the anthraquinone ring and the basic nitrogen.

Doxorubicin (1) has a wide spectrum of antitumor ac-



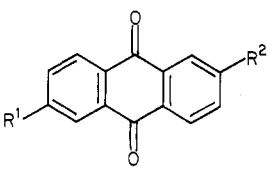
doxorubicin (1)

tivity[1] but is cardiotoxic and causes bone marrow depression.[2] Although alternative natural and semisynthetic derivatives have been sought[3-6] and synthetic routes developed,[7] less attention has been directed toward *totally synthetic* analogues. Doxorubicin could act either by direct[8] or indirect[9] action on DNA; in either case the anthraquinone unit is central to the action. Hence, anthraquinones with amino-substituted side chains have been synthesized,[10-13] and one compound, **2**, is in clinical trial.[14]



	R^1	R^2	R^3	R^4
2	H	H	$NH(CH_2)_2NH(CH_2)_2OH$	$NH(CH_2)_2NH(CH_2)_2OH$
3	$(CH_2)_2C_6H_{10}$-3-NH_2	H	OH	H
4	$CH_2N(CH_3)_2$	H	OH	H
5	$CH_2N(CH_3)_2$	$CH_2N(CH_3)_2$	H	H
6	$NH(CH_2)_3N(CH_3)_2$	H	H	H
7	$S(CH_2)_2N(CH_3)_2$	H	H	H
8	H	CH_2O-daunosamine	OCH_3	H

Generally these compounds have peri substituents, whereas doxorubicin can be regarded as having substituents at the 2 and 3 positions. Although 2-substituted anthraquinones are a closer analogy, few have been evaluated to date: **3–5** are stated to bind to DNA,[15] and **6** and **7** have been shown to inhibit cellular nucleic acid synthesis.[16] However, compounds with an aromatic ring in the side chain are inactive,[16,17] and **8** is inactive against P-388 lekemia "in vivo".[18] Biologically active, nonhydroxylated, 2-substituted anthraquinones are also known, for example, **9–11**



9, $R^1 = R^2 = SO_2NH(CH_2)_3N(C_4H_9)_2$
10, $R^1 = R^2 = O(CH_2)_2N(C_2H_5)_2$
11, $R^1 = R^2 = COO(CH_2)_3N(C_2H_5)_2$
12, $R^1 = R^2 = NHCO(CH_2)_2N(CH_3)_2$

are interferon inducers[19,20] and compounds such as **12** have antiprotozoal activity,[21] and 2-methylanthraquinone de-

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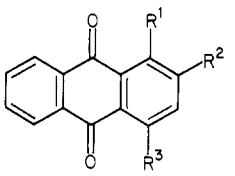
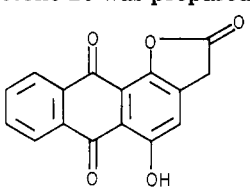
rivatives substituted with a good leaving group have been prepared, such that "in vivo" reduction will generate the reactive anthraquinone methide moiety.[22] Here the acetamide and carboxamide derivatives **13–24** (Table I) have been prepared and evaluated.

Syntheses. 1,4-Dihydroxy-9,10-dioxoanthracene-2-acetamide Hydrochlorides 13–17. 1,4-Dihydroxy-9,10-dioxoanthracene-2-acetic acid (**25**) was prepared by Mar-



25, $R^1 = R^3 = OH$; $R^2 = CH_2COOH$
27, $R^1 = OH$; $R^2 = COOH$; $R^3 = H$
28, $R^1 = NO_2$; $R^2 = CH_3$; $R^3 = H$

schalk reaction[23] of sodium oxoethanoate with the leuco derivative of 1,4-dihydroxyanthracene-9,10-dione. The intramolecular lactone **26** was prepared using DCCI and



26

reacted with the appropriate amines to give the free bases (yields between 30 and 70%), which were converted to their hydrochloride salts **13–17**.

1-Hydroxy-9,10-dioxoanthracene-2-carboxamide Hydrochlorides 18–21. 2-Methyl-1-nitroanthracene-9,10-dione (from 2-methylanthracene-9,10-dione[24]) was oxidized to 1-nitro-9,10-dioxoanthracene-2-carboxylic acid.[25] Borohydride reduction,[26] followed by diazotization and hydrolysis, gave **27**. The free bases of **18–21** were then prepared in 25–80% yields from the appropriate amines using DCCI and *N*-hydroxysuccinimide in dry THF and converted to their hydrochloride salts.

9,10-Dioxoanthracene-2-carboxamide Hydrochlorides 22–24. Chromic acid oxidation of 2-methylanthracene-9,10-dione gave the parent acid. This was then converted to the acid chloride and reacted with the appropriate amines to give the free bases, which were converted to the salts **22–24**.

DNA-Binding Studies. All compounds showed bathochromic and hypsochromic shifts on binding to DNA with a clear isosbestic point, in the visible region of the spectrum (studies on **13–17** and **22–24** were carried out at pH 7.0, whereas those on **18–21** were carried out at pH 5.8 since the phenolic group of the latter compounds has a pK_a of 7.2). For **13–17** the shifts in λ_{max} were between 21 and 29 nm (with decreases in extinction of between 25 and 35%), for **18–21** the shifts were between 4 and 6 nm (with decreases of 14–30%), and for **22–24** the shifts were between 2 and 3.5 nm (with a 15–21% decrease in extinction). The bathochromic shift expected on basification of phenols is not seen with **13–21** in the presence of DNA (Table I). All these changes, particularly for **13–17**, are suggestive of an intercalative mode of binding.[27,28] Only **13–17** showed sufficient fluorescence for evaluation of the effects of DNA binding on their fluorescence properties. All showed between 78 and 90% reduction in fluorescence intensity (compared to 92% for doxorubicin) on binding to DNA. When maximally bound, the degrees of polarization of fluorescence for **13–17** (when irradiated with polarized light) were 0.33, 0.31, 0.28, 0.27, and 0.21, respectively, compared to 0.04–0.06 for free drug. The reduction in fluorescence and increase in polarization are consistent with intercalation into DNA. With all compounds, the T_m of DNA was increased in the presence of drug (Table I). Doxorubicin under the same conditions gives a ΔT_m of 14.75 °C: hence, the affinity of binding is as follows: doxorubicin >>> dihydroxy compounds ≫ monohydroxy compounds > anthraquinones with no hydroxy group. Data from spectrophotometric titration of drug with DNA were analyzed by a nonlinear regression program for their fit to a one-site or two-site binding model.[28] The dihydroxy compounds had the highest affinity for DNA (Table I), but the values were, at best, only half that for doxorubicin, with *n* values consistent with an intercalative mode of binding.

Antitumor Testing. The compounds prepared were evaluated, as their hydrochloride salts, for activity against P-388 leukemia in mice, and the results to date are given in Table II. One compound, **17**, shows moderate activity (T/C = 125%).

Conclusions

The changes in the spectral properties of compounds **13–24** on interaction with DNA and the effects of these compounds on the thermal denaturation of DNA are consistent with an intercalative mode of interaction. The 1,4-dihydroxyanthraquinones **13–17** have a greater affinity for DNA than do the 1-hydroxy and nonhydroxylated compounds **18–24**, and there is greatest activity where there are five atoms between the ring system and the basic nitrogen. The nature of the alkyl substituents on the nitrogen does not appear to be important. All the compounds prepared have a lower affinity for DNA than does doxorubicin. The retention of DNA-binding properties in the 1,4-dihydroxy-2-substituted-anthraquinones provides a further lead for the development of totally synthetic anthraquinones based on the DNA-binding properties of doxorubicin, since the group is amenable to synthetic manipulation. Enhancement of the binding to DNA is necessary; this might be achieved by multiple substitution (e.g., at the 2 and 3 or the 2 and 6 positions of hydroxyanthraquinones) or, more likely, by preparation of bis-intercalating analogues.

Experimental Section

Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. All hydrochloride salts were hygroscopic and were stored over P_2O_5.

1,4-Dihydroxy-9,10-dioxoanthracene-2-acetic Acid (25). Oxygen-free nitrogen was bubbled through 10%, w/v, sodium carbonate in freshly boiled distilled water (800 mL) containing a small amount of sodium dithionite. Leuco-1,4-dihydroxyanthracene-9,10-dione (20 g, 0.082 mol) was added and dissolved by heating to 90 °C. A solution of oxoethanoic acid (glyoxylic acid) dihydrate (6.6 g, 0.052 mol) in freshly boiled and cooled water (50 mL) was made just alkaline and added to the reaction mixture

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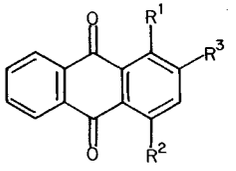
Table I. DNA-Binding Properties of 13–24



no.	R^1	R^2	R^3	increase in λ_{max}, nm, on basification: absence of DNA	increase in λ_{max}, nm, on basification: presence of DNA	ΔT_m,[b] °C	K,[c] $M^{-1} \times 10^{-4}$	n[c]	Kn, $M^{-1} \times 10^{-5}$
13	OH	OH	$CH_2CONH(CH_2)_2N(CH_3)_2 \cdot HCl$	51	0	6.7	28.9	0.26	0.75
14	OH	OH	$CH_2CONH(CH_2)_2N(C_2H_5)_2 \cdot HCl$	70	0	7.3	52.9	0.19	1.00
15	OH	OH	$CH_2CONH(CH_2)_3N(CH_3)_2 \cdot HCl$	57	0	7.1	25.9	0.25	0.65
16	OH	OH	$CH_2CONH(CH_2)_3N(C_2H_5)_2 \cdot HCl$	60	10	6.1	12.7	0.23	0.29
17	OH	OH	$CH_2CONHCH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot HCl$	67	7	5.2	30.8	0.16	0.49
18	OH	H	$CONH(CH_2)_2N(C_2H_5)_2 \cdot HCl$	86	7	2.8	4.6	0.20	0.091
19	OH	H	$CONH(CH_2)_3N(CH_3)_2 \cdot HCl$	92	4	4.8	(7.8)	(0.12)	0.094
20	OH	H	$CONH(CH_2)_3N(C_2H_5)_2 \cdot HCl$	88	0	3.0	9.2	0.14	0.129
21	OH	H	$CONHCH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot HCl$	86	0	3.5	7.8	0.13	0.101
22	H	H	$CONH(CH_2)_2N(C_2H_5)_2 \cdot HCl$	na[d]	na	2.5			
23	H	H	$CONH(CH_2)_3N(CH_3)_2 \cdot HCl$	na	na	2.5	12.0	0.15	0.18
24	H	H	$CONHCH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot HCl$	na	na	2.6	5.9	0.19	0.11

[a] For compounds 13–17, the spectra at pH 7.0 and 9.35 were compared; for compounds 18–21, the values were 5.8 and 7.8. [b] ΔT_m is the T_m of the DNA in the presence of drug minus the T_m of the DNA in the absence of the drug; the value is the mean of two determinations. [c] K and n are the values where the fit to the two-site model is not significantly better than the fit to the one-site model; where K and n are given in parentheses, these are the values for the strongest site in the two-site model, this model giving a significantly better fit to the data than the one-site model (F test). [d] na = not applicable.

Table II. In Vivo Activity against Leukemia P-388[a]

no.	optimal dose, mg kg^{-1}	% T/C
13	100	118
14	100	106
15	50	106
16	50	108
17	40	125
19	50	118
21	5	106
22	200	119
23	200	115
24	200	127

[a] For screening procedure, see Instruction booklet 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, 1978 (revised 1980).

over 30 min. After a further 2 h at 90 °C, the mixture was cooled and oxygenated for 1 h and then acidified. The precipitate was filtered, washed extensively with cold water, and then exhaustively extracted with successive portions (3 × 600 mL) of hot 2%, w/v, sodium bicarbonate solution. The sodium salt was salted out using solid sodium chloride, filtered, and dissolved in water, and the solution was acidified. The product was removed by filtration, washed with water, and dried at 50 °C. The filtrate was also acidified and treated in a similar manner to yield a second cruder product. The product was crystallized from methanol as orange-brown crystals: yield 19.2 g (78%); mp 200 °C; IR (KBr) 3000 (OH), 1710 (acid CO), 1620 (quinone CO), 1585 and 1430 cm^{-1} (aromatic); UV (C_2H_5OH) λ_{max} 206 nm (ϵ 21 980), 252 (36 137), 463 (8598), 486 (9553); ^1H NMR (Me_2SO-d_6) δ 13.4 (1 H, s, replaceable with D_2O, C_1OH), 12.9 (1 H, s, replaceable with D_2O, C_4OH), 8.3–8.1 (2 H, m, 5-CH and 8-CH), 7.9–7.7 (2 H, m, 6-CH and 7-CH), 7.2 (1 H, s, 3-CH), 3.6 (2 H, s, benzyl CH_2); EIMS, m/e 298 (M^+). Anal. ($C_{18}H_{10}O_6$) C, H.

Intramolecular Lactone of 1,4-Dihydroxy-9,10-dioxoanthracene-2-acetic Acid (26). 1,4-Dihydroxy-9,10-dioxoanthracene-2-acetic acid (**25**; 2.0 g, 0.068 mol) was dissolved in dry tetrahydrofuran (150 mL). *N,N'*-Dicyclohexylcarbodiimide (2.0 g, 0.01 mol) in dry tetrahydrofuran (50 mL) was added, and the mixture was stirred at room temperature for 3.5 h. After the mixture was filtered, the filtrate was evaporated to dryness in vacuo, and the product was washed with 2%, w/v, sodium bicarbonate (3 × 300 mL) and then with water, dried at 60 °C, and crystallized from glacial acetic acid: yield 2.2 g (75%); mp 245–248 °C; IR (KBr) 3320 (OH), 3080 (aromatic), 2930 (intramolecular H-bonded OH), 2850 (CH), 1815 (γ-lactone), 1660 and 1630 (quinone CO), 1585 (aromatic) cm^{-1}; UV (C_2H_5OH) λ_{max} 208 nm (ϵ 30 661), 250 (34 612), 282 (16 887), 384 (5330); ^1H NMR ($CDCl_3$) δ 13.1 (1 H, s, replaceable with D_2O), 8.25–8.05 (2 H, m, 5-CH and 8-CH), 7.85–7.65 (2 H, m, 6-CH and 7-CH), 7.2 (1 H, s, 3-CH), 4.1 (2 H, s, benzyl CH_2); high-resolution MS, m/e 280.2390 (M^+) ($C_{16}H_8O_6$ requires 280.2392, 2 ppm error).

***N*-[2-(Dimethylamino)ethyl]-1,4-dihydroxy-9,10-dioxoanthracene-2-acetamide Hydrochloride (13).** A mixture of 2-(dimethylamino)ethylamine (1.056 g, 0.012 mol) and the intramolecular lactone **26** (1.12 g, 0.004 mol) in sodium-dried toluene (100 mL) was heated under reflux for 1 h. The reaction mixture was cooled and evaporated to dryness in vacuo, and the excess amine was removed by steam distillation in vacuo. The residue was shaken with concentrated hydrochloric acid (2 mL), and the hydrochloride salt was dissolved in hot water (100 mL). The solution was filtered, and the filtrate was just basified and then extracted with ethyl acetate (3 × 100 mL). The organic layers were combined, backwashed with water, dried over anhydrous sodium sulfate, and evaporated to dryness in vacuo. After crystallization from chloroform, the free base was dissolved in the minimum quantity of dry ethyl acetate, dry hydrogen chloride was passed through the solution, and the hydrochloride salt was quickly removed by filtration: yield 50 mg (40%); mp 100 °C; IR (KBr) 3290 (H-bonded OH), 3090 (aromatic), 2950 (intramolecular H-bonded OH), 2905 (CH), 2790 (NCH_3), 1650 (>NCO); UV (H_2O) λ_{max} 463 nm (ϵ 7331), 482 (7140); ^1H NMR ($CDCl_3$) δ

12.95 (1 H, s, replaceable with D_2O, C_1OH), 12.4 (1 H, s, replaceable with D_2O, C_4OH), 8.4–8.2 (2 H, m, 5-CH and 8-CH), 7.82–7.7 (2 H, m, 6-CH and 7-CH), 7.2 (1 H, s, 3-CH), 6.55–6.4 (1 H, br s, replaceable with D_2O, NH), 3.62 (1 H, s, benzyl CH_2), 3.4–3.2 (2 H, q, CH_2), 2.45–2.3 (2 H, t, CH_2), 2.2 (6 H, s, CH_3); CIMS (ammonia), m/e 371 (M^+ +3), 370 (M^+ +2), 369 (M^+ +1). Anal. ($C_{20}H_{20}N_2O_5$·HCl) C, H, N, Cl.

Compounds **14–17** were prepared in an analogues manner in yields of 70, 56, 52, and 32%, respectively.

14: mp 77–79 °C; IR (KBr) 3440 (OH), 3320 (H-bonded OH), 3100 (aromatic), 3000 (OH), 2960 (CH), 2840 (NCH_2) 1665 (>NCO), 1645 (quinone CO), 1605 (aromatic), 1560^{-1} (>NCO); UV (H_2O) λ_{max} nm (ϵ 6640), 484 (6444); ^1H NMR ($CDCl_3$) δ 13.1 (1 H, s, replaceable with D_2O, C_1OH), 12.4 (1 H, s, replaceable with D_2O, C_4OH), 8.35–8.2 (2 H, m, 5-CH and 8-CH), 7.85–7.7 (2 H, m, 6-CH and 7-CH), 7.29 (1 H, s, 3-CH), 6.8–6.5 (1 H, s, replaceable with D_2O, NH), 3.62 (1 H, s, benzyl CH_2), 3.4–3.2 (2 H, q, CH_2), 2.6–2.38 (6 H, q, NCH_2), 1.3–0.88 (6 H, t, CH_3); CIMS (ammonia), m/e 399 (M^+ +3), 398 (M^+ +2), 397 (M^+ +1). Anal. ($C_{22}H_{24}N_2O_5$·HCl) C, H, N, Cl.

15: mp 112–115 °C; IR (KBr) 3290 (OH), 3050 (aromatic), 2950 (H-bonded OH), 2910 (CH), 1648 (CONH), 1630 (quinone CO), 1588 (aromatic), 1550 (CONH) cm^{-1}; UV (H_2O) λ_{max} 463 nm (ϵ 6280), 482 (6050); ^1H NMR ($CDCl_3$) δ 13.1 (1 H, s, replaceable with D_2O, C_1OH), 12.5 (1 H, s, replaceable with D_2O, C_4OH), 8.35–8.25 (2 H, m, 5-CH and 8-CH), 7.85–7.7 (2 H, m, 6-CH and 7-CH), 7.28 (1 H, s, 3-CH), 7.4–7.2 (1 H, br s, replaceable with D_2O, NH), 3.61 (2 H, s, benzyl CH_2), 3.42–3.2 (2 H, q, CH_2), 2.4–2.25 (2 H, t, NCH_2), 2.11 (6 H, s, CH_3), 1.75–1.55 (2 H, q, CH_2); CIMS (ammonia), m/e 384 (M^+ +2), 383 (M^+ +1). Anal. ($C_{21}H_{22}N_2O_5$·HCl) C, H, N, Cl.

16: mp 80–82 °C; IR (KBr) 3440 (OH), 3220 (OH), 3100 (intramolecular H-bonded OH), 2960 (CH), 2845 (CH), 2845 (CH), 1660 (CN<), 1645 (quinone CO), 1605 (aromatic), 1560 (CONC) cm^{-1}; UV (H_2O) λ_{max} 462 nm (ϵ 6524); ^1H NMR ($CDCl_3$) δ 12.9 (1 H, s, replaceable with D_2O, C_1OH), 12.35 (1 H, s, replaceable with D_2O, C_4OH), 8.38–8.22 (2 H, m, 5-CH and 8-CH), 7.88–7.75 (2 H, m, 6-CH and 8-CH), 7.88–7.75 (2 H, m, 6-CH and 7-CH), 7.65–7.40 (1 H, br s, replaced with D_2O, NH), 7.31 (1 H, s, 3-CH), 3.6 (2 H, s, benzyl CH_2), 3.45–3.25 (2 H, q, CH_2), 2.6–2.35 (6 H, m, NCH_2), 1.75–1.50 (2 H, m, CH_2), 1.06–0.89 (6 H, t, CH_3); CIMS (isobutane), m/e 413 (M^+ +3), 412 (M^+ +2), 411 (M^+ +1). Anal. ($C_{23}H_{26}N_2O_5$·HCl) C, H, N, Cl.

17: mp 92–95 °C; IR (KBr) 3260 (OH), 3050 (aromatic), 2950 (intramolecular H-bonded OH), 2905 (saturated CH), 2790 (NCH_2), 1650 (secondary amide), 1630 (quinone CO), 1590 (aromatic), 1550 (>NCO) cm^{-1}; UV (H_2O) λ_{max} 436 nm (ϵ 7304), 478 (6540), ^1H NMR ($CDCl_3$) δ 12.9 (1 H, s, replaceable with D_2O, C_1OH), 12.35 (1 H, s, replaceable with D_2O, C_4OH), 8.33–8.2 (2 H, m, 5-CH and 8-CH), 7.82–7.7 (2 H, m, 6-CH and 7-CH), 7.28 (1 H, s, 3-CH), 4.1–3.85 (1 H, br s, replaceable with D_2O, NH), 3.57 (2 H, 2, benzyl CH_2), 2.6–2.38 (7 H, m, N-CH_2 and methine), 1.5–1.4 (4 H, m, CH_2), 1.15–0.9 (6 H, m, CH_3); CIMS (ammonia), m/e 440 (M^+ +2), 439 (M^+ +1), 438 (M^+). Anal. ($C_{25}H_{30}N_2O_5$·HCl·2H_2O) C, H, N, Cl.

1-Nitro-9,10-dioxoanthracene-2-carboxylic Acid. 2-Methyl-1-nitroanthracene-9,10-dione[24] (3 g, 0.0112 mol) and 80%, w/w, sulfuric acid (25.7 g) were heated to 55 °C and chromium trioxide (3.89 g, 0.039 mol) was added with stirring over 0.5 h. The temperature was raised to 65 °C, and 96%, w/w, sulfuric acid (17.7 g) was added over 7 h. The mixture was heated at 70–75 °C for 4 h, diluted with water (4.5 mL), and filtered while still hot. The residue was washed with water and then digested in 5% aqueous sodium hydroxide solution (9 mL) at 90 °C for 1 h. After the mixture was filtered, the product was precipitated from the filtrate by acidification with 75%, w/w, sulfuric acid. The product was filtered, washed free of acid with warm water, dried at 60 °C, and crystallized from glacial acetic acid: yield 1.73 g (52%); mp 286–288 °C (lit.[25] 284–288 °C); IR (KBr) 1710 (acid CO), 1675 (quinone CO), 1590 (aromatic), and 1320 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.4–7.9 (m, aromatic CH); EIMS, m/e 297 (M^+). Anal. ($C_{15}H_7NO_6$) C, H, N.

1-Amino-9,10-dioxoanthracene-2-carboxylic Acid. An aqueous solution (10 mL) of $NaBH_4$ (0.70 g, 0.0185 mol) was added to 1-nitro-9,10-dioxoanthracene-2-carboxylic acid (6.0 g, 0.0202 mol) in aqueous 1%, w/w, ammonia (400 mL) at 25 °C. The mixture was stirred for 4 h, diluted with water (100 mL), and carefully acidified, and the product was collected by filtration: yield 5.29 g (98%); mp 292 °C after recrystallization from DMF (lit.[26] 291 °C); IR (KBr) 3410 (H-bonded OH), 1750 (acid CO), 1660 (quinone CO), 1585 (aromatic) cm^{-1}; EIMS, m/e 267 (M^+).

1-Hydroxy-9,10-dioxoanthracene-2-carboxylic Acid (27). 1-Amino-9,10-dioxoanthracene-2-carboxylic acid (12 g, 0.045 mol) was added quickly to a solution of sodium nitrite (3.36 g, 0.049 mol) in 90% H_2SO_4 (72 mL), and the mixture was heated at 50–60 °C for 0.5 h until the water-soluble diazo intermediate was formed. Water (4 mL) was added, and the mixture was heated at 95–100 °C for 2 h to effect N_2 evolution. The mixture was cooled and added to excess ice/water, and the precipitate was removed by filtration, washed with water, dried at 60 °C, and crystallized from glacial acetic acid: yield 10.5 g (87%); IR (KBr) 1700 (acid CO), 1665 and 1630 (quinone CO), 1590 (aromatic) cm^{-1}; EIMS, m/e 268 (M^+). Anal. ($C_{15}H_8O_5$) C, H.

***N*-[2-(Diethylamino)ethyl]-1-hydroxy-9,10-dioxoanthracene-2-carboxamide Hydrochloride (18).** A solution of 1-hydroxy-9,10-dioxoanthracene-2-carboxylic acid (1.072 g, 0.004 mol) in dry THF (150 mL) was stirred at 0 °C with exclusion of light, and *N*-hydroxysuccinimide (0.912 g, 0.008 mol) and *N*,*N*′-dicyclohexylcarbodiimide (0.9 g, 0.004 mol), dissolved in the minimum quantity of dry THF, were added. After 20 min, it was allowed to warm to room temperature; after an additional 0.5 h, 2-(diethylamino)ethylamine (1.4 g, 0.012 mol) in dry THF (5 mL) was added, and the mixture was stirred for 62 h. After the mixture was filtered, the filtrate was evaporated to dryness in vacuo, and the free base was extracted as with **13** earlier and crystallized from methanol, and the hydrochloride salt was prepared: yield 700 mg (48%); mp 180 °C; IR (KBr) 3400 (OH), 3060 (aromatic), 2955 (CN), 1650 and 1630 (quinone CO), 1600 (aromatic), 1540 (CON<) cm^{-1}; UV (pH 5.8 buffer) λ_{max} 406 nm (ϵ 6000); ^1H NMR ($CDCl_3$) δ 12.9 (1 H, s, replaceable with D_2O, C_1OH), 8.75–8.58 (1 H, d, 3-CH), 8.4–8.25 (2 H, p, 5-CH and 8-CH), 7.95–7.78 (3 H, m, 4-CH, 6-CH, 7-CH), 3.7–3.5 (2 H, m, CH_2), 3.2–3.0 (1 H, br s, replaceable, slowly with D_2O, NH), 2.8–2.5 (6 H, m, NCH_2), 1.15–0.9 (6 H, t, CH_3); CIMS (ammonia), m/e 368 (M^+ +2), 367 (M^+ +1). Anal. ($C_{21}H_{22}N_2O_4$·HCl) C, H, N, Cl.

Compounds **19–21** were prepared in an analogous manner in yields of 52, 25, and 82%, respectively.

19: mp 85–90 °C; IR (KBr) 3400 (OH), 3055 (aromatic) 2940 (CH), 1660 and 1635 (quinone CO), 1595 (aromatic), 1540 (CON<) cm^{-1}; UV (pH 5.8 buffer) 404 nm (ϵ 5340); ^1H NMR ($CDCl_3$) δ 12.85 (1 H, s, replaceable with D_2O, C_1OH), 8.65–8.57 (1 H, d, 3-CH), 8.4–8.25 (2 H, m, 5-CH and 8-CH), 7.95–7.77 (3 H, m, 4-CH, 6-CH, and 7-CH), 3.2–3.05 (1 H, br s, replaceable slowly with D_2O, NH), 3.70–3.45 (2 H, m, CH_2), 2.5–2.28 (8 p, NCH_2 and CH_3), 1.9–1.75 (2 H, t, CH_2); CIMS (ammonia), m/e 354 (M^+ +2), 353 (M^+ +1). Anal. ($C_{20}H_{20}N_2O_4$·HCl) C, H, N, Cl.

20: mp 80–85 °C; IR (KBr) 3400 (OH), 3060 (aromatic), 2940 (CH), 1660 and 1635 (quinone CO), 1595 (aromatic), 1540 (CON<) cm^{-1}; UV (pH 5.8 buffer) λ_{max} 404 nm (ϵ 4980); ^1H NMR ($CDCl_3$) δ 12.82 (1 H, s, replaceable with D_2O, C_1OH), 8.65–8.58 (1 H, d, 3-CH), 8.4–8.25 (2 H, m, 5-CH and 8-CH), 7.95–7.78 (3 H, m, 4-CH, 6-CH, 7-CH), 3.6–3.4 (1 H, br s, replaceable slowly with D_2O, NH), 3.2–2.9 (2 H, m, CH_2), 2.7–2.4 (6 H, m, NCH_2), 1.75–1.5 (2 H, m, CH_2), 1.10–0.95 (6 H, t, CH_3); CIMS (ammonia), m/e 382 (M^+ +2), 381 (M^+ +1). Anal. ($C_{22}H_{24}N_2O_4$·HCl) C, H, N, Cl.

21: mp 157–159 °C; IR (KBr) 3400 (OH), 3060 (aromatic), 2940 (CH), 1660 and 1630 (quinone CO), 1591 (aromatic), 1540 (CON<) cm^{-1}; UV (buffer at pH 5.8) λ_{max} 406 nm (ϵ 5875); ^1H NMR ($CDCl_3$) δ 12.7 (1 H, s, replaceable with D_2O, C_1OH), 8.62–8.50 (1 H, d, 3-CH), 8.40–8.15 (2 H, m, 5-CH, 8-CH), 7.90–7.75 (3 H, m, 4-CH, 6-CH, 7-CH), 4.45–4.10 (1 H br s, replaceable slowly with D_2O, NH), 2.80–2.46 (7 H, m, NCH_2 and methine), 1.75–1.55 (4 H, m, CH_2), 1.40–0.95 (9 H, m, CH_3); MS, m/e 408 (M^+). Anal. ($C_{24}H_{28}N_2O_4$·HCl) C, H, N, Cl.

***N*-[2-(Diethylamino)ethyl]-9,10-dioxoanthracene-2-carboxamide Hydrochloride (22).** 9,10-Dioxoanthracene-2-carboxylic acid, prepared by chromic acid oxidation of 2-methylanthraquinone, was converted to its acid chloride with redistilled thionyl chloride in THF. This acid chloride (1.08 g, 0.005 mol) was heated under reflux (2 h) with 2-(diethylamino)ethylamine (0.58 g, 0.005 mol) in dry THF (150 mL). The mixture was cooled and evaporated to dryness in vacuo, and the

free base was isolated as for 13. The product was crystallized from acetone, and the hydrochloride was prepared: yield 0.98 g (70%); mp 105 °C; UV (H_2O) λ_{max} 331 nm (ϵ 5720); ^1H NMR ($CDCl_3$) δ 8.56 (1 H, s, 1-CH), 8.4–8.2 (4 H, m, 3-CH, 4-CH, 5-CH, 8-CH), 7.85–7.7 (2 H, m, 6-CH, 7-CH), 3.62–3.45 (2 H, q, CH_2), 2.76–2.5 (6 H, m, NCH_2), 1.15–1.10 (6 H, t, CH_3); CIMS (ammonia), m/e 352 (M^+ +2), 351 (M^+ +1). Anal. ($C_{21}H_{22}N_2O_3$) C, H, N, Cl.

Compounds 23 and 24 were prepared in an analogous manner in yields of 69 and 73%, respectively.

23: mp 100–102 °C; UV (H_2O) 331 nm (ϵ 5293); ^1H NMR ($CDCl_3$) δ 8.7 (1 H, s, 1-CH), 8.5–8.38 (4 H, m, 3-CH, 5-CH, 8-CH), 7.96–7.85 (2 H, m, 6-CH, 7-CH), 3.8–3.6 (2 H, q, CH_2), 2.75–2.62 (2 H, t, CH_2), 2.5 (6 H, s, CH_3), 2.45–2.25 (1 H, br s, replaceable with D_2O, NH), 1.95–1.8 (2 H, t, CH_2); MS, m/e 336 (M^+). Anal. ($C_{20}H_{20}N_2O_3$·HCl) C, H, N, Cl.

24: mp 90–93 °C; UV (H_2O) λ_{max} 330 nm (ϵ 5687); ^1H NMR ($CDCl_3$) δ 8.55 (1 H, s, 1-CH) 8.4–8.2 (4 H, m, 3-CH, 4-CH, 5-CH, 8-CH), 7.88–7.74 (2 H, m, 6-CH, 7-CH), 4.32–4.1 (1 H br s, replaceable with D_2O, NH), 2.68–2.4 (7 H, NCH_2 and methine), 1.75–1.6 (4 H, m, CH_2), 1.36–1.28 (3 H, d, CH_3), 1.1–0.92 (6 H, t, CH_3); CIMS (ammonia), m/e 394 (M^+ +2), 393 (M^+ +1). Anal. ($C_{24}H_{28}N_2O_3$·HCl) C, H, N, Cl.

DNA Binding. Solutions were prepared in pH 7.0, 0.05 M NaCl, 0.008 M Tris-Cl buffer and for compounds 18–21 also in pH 5.8, Sorensen's citrate II buffer. Calf thymus DNA (Sigma Type I) in buffer (1 mg mL^{-1}) was assayed using the figure $\epsilon(P)_{260}$ = 6600. All compounds obeyed Beer–Lambert's law over the concentration range used. All glassware was silanized before use.

(a) Determination of Spectral Shifts. Six solutions of drug (2.5×10^{-5} M) and DNA were prepared with DNA/drug ratios of 0, 1, 2, 5, 10, and 15. The spectra for each drug were recorded superimposed.

(b) Effect of pH on the Drug λ_{max} in the Presence and Absence of DNA. Solutions were prepared containing just drug (2.5×10^{-5} M) and drug and DNA (2.5×10^{-5} and 3.75×10^{-4} M, respectively) in pH 7.0 and in pH 9.3 buffer (pH 5.8 and 7.8 for 18–21). The spectra for each compound were recorded superimposed.

(c) Spectrophotometric Titration. Aliquots (4×40, 12×20, and 7×100 μL) of DNA solution (2.5×10^{-3} M) were added sequentially to three samples of drug solution (5×10^{-5} M) in pH 7.0 buffer (3.0 mL) at 25 °C (pH 5.8 buffer for compounds 18–21), and the absorbance at the λ_{max} of the unbound drug was determined after a 5-min equilibration against a blank of buffer treated in an identical manner. The binding parameters K_1 and n_1 were then determined for a one-site binding model and, if necessary, for a two-site binding model by nonlinear regression of absorbance (as the dependent variable) against cumulative volume of DNA added (as independent variable) by the method previously reported.[28]

(d) Spectrofluorimetric Studies. For compounds 13–17, aliquots of DNA solution were added sequentially to three samples of drug solution (2.5×10^{-6} M in pH 7.0 buffer) at 25 °C, and the fluorescence polarization was determined at the λ_{max} of emission for the unbound drug when irradiated with polarized light at the λ_{max} of excitation for the unbound drug. The usual correction was applied to account for spurious polarization due to the instrument. The fluorescence polarization titrations were continued until ratios of DNA to drug were reached at which the drug is fully bound. The change (percent) in fluorescence was also calculated at these DNA/drug ratios. Additionally, polarization values at DNA/drug ratios of 50, 100, and 150 were determined.

(e) Thermal Denaturation. Two solutions with an identical concentration of DNA (about 15×10^{-5} M) were prepared in 0.018 M NaCl, 0.003 M Tris-chloride buffer at pH 7.0, one just containing DNA and the other also containing drug (about 1.5×10^{-5} M) such that the ratio of drug to DNA was precisely 1:10. The absorbance of each solution was measured at 260 nm, as the temperature was raised from 55 to 105 °C (0.25 °C min^{-1}), against a blank containing GMP, the absorbance of which was exactly matched to the starting absorbance of the sample.

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Use of Adenine Nucleotide Derivatives to Assess the Potential of Exo-Active-Site-Directed Reagents as Species- or Isozyme-Specific Enzyme Inactivators. 3.[1] Synthesis of Adenosine 5′-Triphosphate Derivatives with N^6- or 8-Substituents Bearing Iodoacetyl Groups

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Several series of N^6- or 8-substituted derivatives of adenosine 5′-triphosphate (ATP) were synthesized. N^6-(ω-Aminoalkyl) derivatives of adenosine 5′-monophosphate (AMP) were converted into their ω-*N*-carbobenzyloxy derivatives, and these were converted, via the 2′,3′-*O*-carbonyl derivatives of their 5′-phosphorimidazolidates, into the corresponding ATP derivatives. Hydrogenolytic removal of the carbobenzyloxy groups, followed by iodoacetylation of the ω-amino groups with *N*-(iodoacetoxy)succinimide, gave N^6-R-ATP, where R = $(CH_2)_nNHCOCH_2I$ (n = 2–8) or $(CH_2)_nCON(CH_3)(CH_2)_mN(CH_3)CO(CH_2)_nNHCOCH_2I$ ($n = m = 3$; $n = 3$, $m = 4$; $n = 4$, $m = 3$; $n = m = 4$). Condensation of N^6-(ω-aminoalkyl) derivatives of AMP with *N*-hydroxysuccinimide esters of ω-[*N*-(carbobenzyloxy)amino] carboxylic acids gave N^6-$(CH_2)_nNHCO(CH_2)_mNH$-Cbz derivatives of AMP which, upon conversion to the corresponding derivatives of ATP, followed by removal of the carbobenzyloxy group and iodoacetylation, as described above, gave N^6-$(CH_2)_nNHCO(CH_2)_mNHCOCH_2I$-ATP derivatives ($n = 3$, $m = 5$ or 6; $n = 4$, $m = 5$; $n = 6$, m = 1–6). The same sequence of reactions starting with N^6-[ω-(methylamino)alkyl] derivatives of N^6-CH_3-AMP gave N^6-CH_3, N^6-$(CH_2)_nN(CH_3)CO(CH_2)_mNHCOCH_2I$ derivatives of ATP ($n = 4$, $m = 3$, 5 or 6; $n = 6$, $m = 5$ or 6). Reaction of α,ω-diaminoalkanes with 8-Br-ATP gave 8-$NH(CH_2)_nNH_2$ derivatives of ATP, which upon iodoacetylation gave 8-$NH(CH_2)_nNHCOCH_2I$ derivatives of ATP (n = 2, 4, 6, or 8). Substrate and inhibitor properties indicated that the ATP derivatives are potential exo-ATP-site-directed inactivators of hexokinases, adenylate kinases, and pyruvate kinases.

It is now recognized that enzymes which catalyze the same transformation, whether they be derived from different species or are isozymes characteristic of different tissues of the same species, exhibit structural dissimilarities

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