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Structure-Based Design of a New Bisintercalating Anthracycline Antibiotic

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A new bisintercalating anthracycline antibiotic, WP631, has been designed and synthesized. The rational design of the new compound was based upon the geometry of monomeric anthracyclines bound to DNA oligonucleotides observed in high-resolution crystal structures. Monomeric units of daunorubicin have been linked through their reactive 3′ NH_2 substituents on the daunosamine moieties to form the new bisanthracycline WP631. Viscosity studies confirmed that WP631 binds to DNA by bisintercalation. Differential scanning calorimetry and UV melting experiments were used to measure the ultratight binding of WP631 to DNA. The binding constant for the interaction of WP631 with herring sperm DNA was determined to be 2.7×10^{11} M^{-1} at 20 °C. The large, favorable binding free energy of -15.3 kcal mol^{-1} was found to result from a large, negative enthalpic contribution of -30.2 kcal mol^{-1}. A molecular model was generated that shows the favorable stereochemical fit of the linker in the DNA minor groove. The cytotoxicity of WP631 was compared to that of doxorubicin using MCF-7-sensitive and MCF-7/VP-16 MRP-mediated multidrug-resistant cell lines. These initial studies showed that while WP631 is slightly less cytotoxic than doxorubicin in the sensitive cell line, it appears to overcome MRP-mediated multidrug resistance and was much more cytotoxic against the MCF-7/VP-16 cell line than was doxorubicin. The design of new potential anticancer agents based on known structural principles was found to produce a compound with significantly increased DNA binding affinity and with interesting biological activity.

Introduction

The anthracycline antibiotics daunorubicin (daunomycin) and doxorubicin (Adriamycin) are among the most potent and clinically useful agents currently used in cancer chemotherapy. Although there have been intense efforts to synthesize more efficacious anthracyclines with fewer toxic side effects, these efforts have not succeeded,[1] and the parent compounds remain the best chemotherapeutic agents of the chemical class. The failure to obtain new, improved anthracycline antibiotics might be attributed, in part, to flaws in the design process. Early efforts in anthracycline synthesis were not guided by any known structural or mechanistic principles. In the last decade, however, the anthracylines have become perhaps the best understood of the DNA intercalators.[2−4] Over 22 high-resolution structures have now been reported for a variety of anthracyclines intercalated into DNA oligonucleotides of various sequences (see Table 1 in ref 2). The DNA sequence preference of daunorubicin binding to DNA has been characterized by high-resolution footprinting,[5] and the origin of its preferential binding to certain triplet sequences has been explored by computational chemistry.[6,7] The thermodynamics and kinetics of daunoru-

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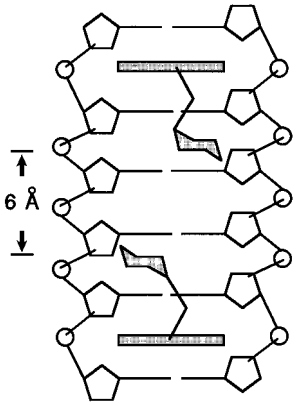

Figure 1. A schematic of the structure of monomeric anthracycline antibiotic molecules bound to a DNA hexanucleotide as revealed by high-resolution crystallographic structures. The daunosamine moieties of the drug monomers point toward the center of the hexanucleotide, placing the reactive 3′ NH_2 substituents within <7 Å of one another.

bicin binding to DNA have been thoroughly studied.[8−10] This wealth of detailed information provides a foundation for the rational design of new anthracyclines targeted toward DNA. The design, synthesis, and initial characterization of one such compound is described here.

The logic of the design of WP631, a bisanthracycline composed of two daunorubicin monomers, is based on the high-resolution crystallographic structures of anthracyclines bound to DNA hexanucleotides. In all of the structures reported for noncovalent complexes of anthracyclines bound to DNA, a 2:1 stoichiometry of drug to DNA duplex was observed. In all such complexes, each drug was observed to be intercalated at either end of the hexanucleotide, with the daunosamine moieties of the two drug molecules pointing toward one another, lining the minor groove. Figure 1 shows a schematic of the observed geometry. This arrangement brings the reactive NH_2 substituents of each drug molecule to within <7 Å of one another. The simple and obvious design strategy to emerge from these considerations is to link the two amine groups to form a bisanthracycline with the potential of bisintercalating into DNA. The linker must be designed to be of the appropriate length (approximately 7 Å) and must fit into the minor groove of DNA without steric hindrance. A compound designed and synthesized according to these specifications, WP631, is shown in Figure 2.

The potential advantages of a bisintercalator over its monomeric counterpart are many.[11] First, DNA binding affinity should be greatly enhanced. The binding constant for the bisintercalator should be roughly the *square* of the binding constant for the monomer. The biological activity of the anthracyclines has been correlated with their DNA binding affinity,[12] so the potential of WP631 as an improved chemotherapeutic agent is great. Second, the site size of a bisintercalator is increased relative to the monomer. Increased site size can potentially lead to increased sequence selectivity.[13] Daunorubicin binds preferentially to triplets of the type 5′(A/T)CG and 5′(A/T)GC, where the notation (A/T) means that either A or T can occupy the position.[5] The bisanthracycline WP631 potentially will occupy 6 bp instead of 3, with a concomitant increase in potential sequence selectivity. Six base pairs define the specific cleavage sites of many restriction enzymes, so the larger

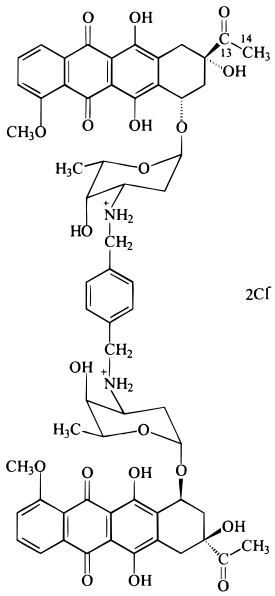

Figure 2. Structure of WP631, a newly synthesized bisanthracycline in which two daunorubicin molecules are linked through their 3′ NH_2 substituents by a *p*-xylene linker.

site size of WP631 could impart a binding specificity on par with such enzymes.

Bisanthracyclines have been synthesized before.[14−16] In these cases, monomers were linked through either the C13 or C14 positions, a strategy we believe to have led to a design flaw. The C13 and C14 positions are labeled in one of the monomeric units in Figure 2. High-resolution structures show that substituents near these positions may participate in specific molecular interactions within the drug−DNA complex. Attachment of a bulky linker at those positions might sterically hinder the binding of the monomeric units. Linkage through the NH_2 groups is likely to be less invasive and should be stereochemically preferred. Indeed, the linker might provide additional favorable interactions within the minor groove to further enhance DNA binding and specificity. Daunorubicin alone is a polyfunctional molecule, with both an intercalating domain and a groove binding domain.[2] An appropriately designed linker might serve to expand and enhance the groove binding domain, in contrast to linkers attached at C13 or C14, which would likely not fit properly into the minor groove.

Reported here is the synthesis and initial characterization of the DNA binding properties and biological activity of the novel bisanthracycline WP631. WP631 binds to DNA by bisintercalation, as judged by viscosity studies and molecular models, and has greatly enhanced DNA binding affinity. The biological activity of WP631 shows outstanding promise. WP631 appears to overcome MRP-mediated multidrug resistance in cultured cells, while retaining activity near that observed for doxorubicin in sensitive cells.

Results and Discussion

WP631 (Figure 2) is a rationally designed bisanthracycline that is intended to bisintercalate into DNA, with the concomitant advantages of enhanced DNA binding affinity and sequence selectivity over its monomeric counterpart, daunorubicin. These advantages will potentially make WP631 an improved anticancer agent,

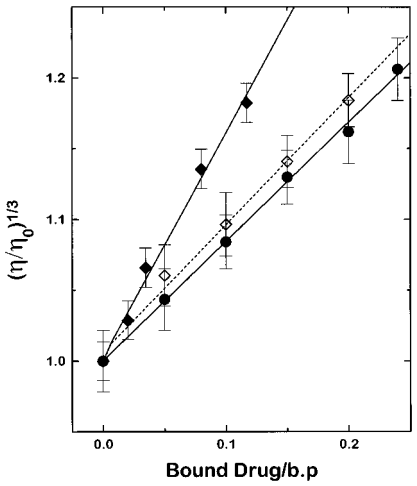

Figure 3. Viscosity studies of WP631−DNA interaction. The cubed root of the relative viscosity (η/η_0) is shown as a function of the ratio of bound drug per DNA base pair: solid circles, ethidium bromide; open diamonds, daunorubicin; solid diamonds, WP631. Linear least squares fits to these data yielded the following slopes: ethidium, 0.84 ± 0.02; daunorubicin, 0.89 ± 0.04; WP631, 1.58 ± 0.08.

since the anthracycline antibiotics upon which it is based have been successfully used in cancer chemotherapy for over three decades. The initial characterization of the DNA binding and biological activity of WP631 will be described.

WP631 Bisintercalates into DNA. In the absence of high-resolution structural data, hydrodynamic studies, especially viscosity, provide the most reliable means of inferring the binding mode of agents that interact with DNA.[17] Figure 3 shows the results of viscosity experiments that verify that WP631 binds to DNA by bisintercalation. The theory of Cohen and Eisenberg[18] predicts that, for monointercalation, plots of the cubed root of the relative viscosity ($(\eta/\eta_0)^{1/3}$) versus the binding ratio (bound drug/DNA bp) ought to have a slope of 1.0. Figure 3 shows that the proven monointercalators ethidium and daunorubicin, run as controls in the experiment, both show viscosity changes in excellent agreement with the theory. For bisintercalators, the slope is expected to be twice that observed for monointercalators, an expectation that has been verified for a variety of bisintercalating compounds.[11] The slope observed for WP631 in Figure 3 is nearly double that observed for daunorubicin, consistent with a bisintercalative binding mode. Such a binding mode is stereochemically reasonable, as was determined by constructing a molecular model of WP631 bound to a DNA hexanucleotide (figure not shown at the suggestion of a reviewer). The model was constructed using HyperChem software (v. 4.5, Hypercube, Inc.) by using the coordinates of the high-resolution structure reported by Wang *et al.*[19] for a 2:1 daunorubicin−DNA complex. The sequence of the hexanucleotide in that study was d(G·CTAG·C)$_2$, where the two equivalent intercalation sites are indicated. WP631 was built in the complex by attaching the *p*-xylene linker to the daunorubicin amines within the minor groove. The geometry of the drug was then optimized using the MM+ force field in HyperChem while constraining the DNA conformation to that observed in the crystal structure. In the resultant complex, the *p*-xylene linker was found to fit without steric hindrance in the minor groove and, most importantly, to be of the appropriate length to connect to the two amine without any radical change in the structure of the intercalated monomeric units. The exact orientation of the aromatic ring of the linker was found to be flexible and might be free to rotate within the minor groove.

Ultratight Binding of WP631 to DNA. The binding affinity of a bisintercalator for DNA ought to approximately equal the square of the binding constant of the corresponding monomer. Since daunorubicin binds to DNA with a binding constant of 10^7 M^{-1} under the ionic conditions used here, a binding constant of 10^{14} M^{-1} is expected for WP631. Traditional spectrophotometric methods for measuring affinity fail for such ultratight binding, but optical melting studies or differential scanning calorimetry provides reliable alternatives for the accurate determination of binding constants.[21−23] UV melting studies were used to determine the binding constant for the interaction of WP631 with herring sperm DNA in BPE buffer (16 mM total Na^+). In the absence of WP631, the T_m of herring sperm DNA was found to be 67.2 °C. In the presence of 10 μM WP631, a concentration sufficient to saturate the DNA lattice, the T_m was elevated to 93.5 °C. McGhee[22] has shown that the shift in T_m is a function of the ligand binding constant and site size. Assuming no interaction of ligand with single-stranded DNA, McGhee derived the equation

$$(1/T_m^{\,0} - 1/T_m) = (\Delta H_m/R)\ln(1 + KL)^{1/n}$$

where $T_m^{\,0}$ is the melting temperature of the DNA alone, T_m is the melting temperature in the presence of saturating amounts of ligand, ΔH_m is the enthalpy of DNA melting (per bp), R is the gas constant, K is the ligand binding constant at T_m, L is the free ligand concentration (approximated at the T_m by the total ligand concentration), and n is the ligand site size. A value of $\Delta H_m = 7.0 \pm 0.3$ kcal mol^{-1} for herring sperm DNA, determined by separate differential scanning calorimetry experiments, was used. From the experimentally determined increase in T_m observed for WP631, a value of $K = 8.8 \times 10^6$ M^{-1} (at 93.5 °C) was computed, assuming $n = 6$ bp. Correction of this value to lower temperatures requires knowledge of the binding enthalpy, which was determined by differential scanning calorimetry.

DSC Determination of the Binding Enthalpy. Figure 4 shows the results of differential scanning calorimetry experiments using herring sperm DNA in the presence and absence of saturating amounts of WP631. The area under the curves shown in Figure 4 provides a model-free estimate of the enthalpy of melting of the DNA alone and the DNA−WP631 complex. By Hess's law, these data may be used to determine the enthalpy of WP631 binding to DNA. The equilibria to be considered, along with the experimentally determined enthalpy values, are as follows:

duplex ⇄ 2(single strand)

$$\Delta H_1 = 7.0 \pm 0.3 \text{ kcal mol}^{-1}$$

WP631−duplex ⇄ 2(single strand) + WP631

$$\Delta H_2 = 11.4 \pm 1.0 \text{ kcal mol}^{-1}$$

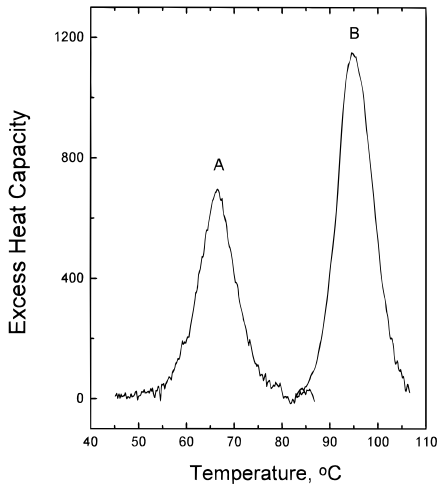

Figure 4. Results of differential scanning calorimetry studies of the melting of herring sperm DNA alone (A) or in the presence of near saturating amounts of WP631 (B). Excess heat capacity (cal mol^{-1} $°C^{-1}$) is plotted as a function of temperature.

Combining these two reactions, the binding reaction and its enthalpy may be obtained:

$$\text{WP631}-\text{duplex} \rightleftarrows \text{duplex} + \text{WP631}$$
$$\Delta H_3 = \Delta H_2 - \Delta H_1$$

The enthalpy ΔH_3 needs to be corrected for the amount of WP631 bound to the DNA, and the sign changed, to get the binding enthalpy,

$$\Delta H_b = -\Delta H_3/(\text{mol of WP631/mol of bp})$$

From five determinations, a value of $\Delta H_b = -30.2 \pm 2.6$ kcal mol^{-1} was obtained for the association of WP631 with DNA.

Complete Thermodynamic Profile for the Binding of WP631 to DNA. ΔH_b may be used, assuming that it is constant with temperature, to calculate the binding constant at 20 °C by application of the standard van't Hoff equation, yielding a value of 2.7×10^{11} M^{-1}. In BPE buffer at 20 °C, the binding constant for the interaction of daunorubicin with herring sperm DNA is 1.6×10^7 M^{-1} (Leng and Chaires, unpublished data), so the binding constant of WP631 indeed approaches the value expected for a bisintercalator. The magnitude of the WP631 binding constant approaches that observed for the binding of many regulatory proteins for their specific DNA binding sites. Knowledge of the binding constant and the binding enthalpy allows us to construct the complete thermodynamic profile for the binding of WP631 to DNA. The free energy is obtained from the standard relation $\Delta G^0 = -RT \ln K$, yielding a value of −15.3 kcal mol^{-1} at 20 °C. The entropy may be evaluated from the equation $-T\Delta S = \Delta G - \Delta H$, yielding $\Delta S = -51$ cal mol^{-1} K^{-1} at 20 °C. The thermodynamic profile indicates that the large, favorable binding free energy of −15.3 kcal mol^{-1} is derived from the large negative enthalpic contribution of −30.2 kcal mol^{-1}. Binding is opposed by an unfavorable entropic contribution of $T\Delta S = -14.9$ kcal mol^{-1} at 20 °C.

Comparison with Daunorubicin Binding. Under comparable ionic conditions, the thermodynamic profile for daunorubicin binding to herring sperm DNA is $\Delta G^0 = -9.9$ kcal mol^{-1}, $\Delta H = -10.8$ kcal mol^{-1}, and $\Delta S = -3$ cal mol^{-1} K^{-1} (Leng and Chaires, unpublished data). Comparison shows that while the DNA binding of both daunorubicin and WP631 is driven by the enthalpy contribution, WP631 shows a comparatively larger unfavorable entropic contribution. Two plausible contributions to this behavior could be WP631 effects on DNA structure and losses of conformational freedom in WP631. The bisintercalation of WP631 could result in a proportionally larger increase of the stiffness of the DNA helix relative to daunorubicin, and thus a greater unfavorable entropic cost for the loss of DNA conformational freedom. In addition, preliminary molecular modeling studies have indicated that there is considerable conformational freedom in WP631, with free rotation about many of the bonds in the linker. An unfavorable entropic contribution might come from the restriction of this conformational freedom upon bisintercalation, an effect in addition to the general entropic cost for bimolecular complex formation resulting from the loss of translational and rotational freedom of the reacting partners.

Table 1. *In Vitro* Evaluation of the Cytotoxicity of WP631 and Doxorubicin

	ID_{50} (μM)	
cell line	doxorubicin	WP631
MCF-7	0.9 ± 0.5	4.8 ± 2.5
MCF-7/VP-16	14.2 ± 0.8	2.5 ± 3.4
RI[a]	16	0.5

[a] RI is the ratio ID_{50}(MCF-7/VP-16)/ID_{50}(MCF-7).

Cytotoxicity of WP631. The biological activity of WP631 was studied by its *in vitro* cytotoxicity against MCF-7 and MCF-7/VP-16 cell lines. The former is a sensitive human breast carcinoma cell line, while the latter is its multidrug-resistant counterpart. The results are shown in Table 1. In comparison with doxorubicin, WP631 is slightly less toxic toward the sensitive MCF-7 cells, by a factor of 4−5. Against the multidrug-resistant MCF-7/VP-16 cell line, however, WP631 is considerably more cytotoxic than is doxorubicin, by a factor of 6. The ratio of the cytotoxicities for the two cell lines (RI = ID_{50}(MCF-7/VP-16)/ID_{50}(MCF-7)) was found to be 16 and 0.5 for doxorubicin and WP631, respectively. The exciting implication of this finding is that WP631 can overcome multidrug resistance in these cell lines. These studies indicate promising biological activity for WP631, and a potentially improved anticancer activity as was intended in the design.

Comparison with Previously Synthesized Bisanthracyclines. WP631 appears to offer several advantages over previously synthesized bisintercalating anthracyclines.[11,14−16] In these earlier attempts, monomers were linked through the C13 and C14 positions. The primary advantage of WP631 over these earlier designs is that it expands the inherent groove binding domain of daunorubicin, creating a molecule with distinctive intercalating and groove binding functionalities. Molecular modeling studies have shown that the linkers attached to the C13 and C14 positions do not fit into the DNA minor groove and consequently do not themselves add any favorable interactions with the DNA. In contrast, WP631 was designed and built to include intercalating and groove binding moieties that will both contribute to DNA binding affinity and speci-

ficity. The biological activity of previously synthesized bisanthracyclines was judged to be disappointing,[16] in spite of increases in the DNA affinity and of the lifetimes of the complexes. In contrast, WP631 shows exciting biological activity, with an unexpected ability to apparently overcome multidrug resistance in the initial studies.

Implications for Rational Drug Design. The rational, structure-based design of new DNA binding agents is an area of great current interest. Small synthetic molecules that could bind to DNA with high affinity and sequence specificity would be valuable tools for molecular biology and of potential medical use as chemotherapeutic agents against a variety of infections and diseases. WP631 represents a promising new compound resulting from a novel approach to the structure-based design of DNA binding agents. Previous design efforts have focused largely on groove binding agents, whereas WP631 represents an attempt to combine the intercalation and groove binding motifs to yield a molecule that exploits the advantages of each binding mode. Groove binding agents, as exemplified by netropsin and distamycin, in general bind selectively to runs of AT base pairs, in part because of the steric hindrance resulting from the protrusion of the N2 amino group of guanine into the minor groove. The "lexitropsins" were perhaps the first structure-based DNA binding agents to be synthesized and were designed to incorporate the reading of GC base pairs into the groove binding motif.[24−26] More recently, impressive progress for the recognition of DNA by groove binding pyrrole−imidazole polyamides has been achieved,[27,28] an approach that exploits the side-by-side groove binding structural motif first described by Pelton and Wemmer.[29] Such agents bind to DNA with sequence-specific binding constants of 10^8−10^{10} M^{-1}.[28] The strategy used to construct WP631 is complementary to these designs based on the groove binding mode. Intercalators in general preferentially bind to CpG or GpC sites,[30] a property that might be exploited in the design of new agents. Linking GC specific intercalators by tethers that bind in the minor groove can expand the repertoire of available design elements. WP631 shows the promise of such an approach. While the sequence specificity of WP631 is not yet known, its affinity for DNA surpasses that of the polyamides[28] and its biological activity shows outstanding promise in the initial studies described here.

Summary

The design, synthesis, and initial characterization of WP631, a novel bisintercalating bisanthracycline, is described. The structure of WP631 is based on the proximity and orientation of daunorubicin molecules bound to DNA hexanucleotides observed in high-resolution crystal structures. As intended in the design, WP631 was found to bisintercalate into DNA with ultratight binding affinity. WP631 appears to overcome a clinically relevant MRP-mediated multidrug resistance in a human breast carcinoma cell line, a promising biological activity showing the potential of this rationally designed compound.

Experimental Section

Synthesis of WP631. Daunorubicin hydrochloride (282 mg, 0.5 mmol) was dissolved in a mixture of *N*,*N*-dimethylformamide (DMF) and CH_2Cl_2 (1:1 v/v; 6 mL). Then, Na_2CO_3 (100 mg) and α,α-dibromo-*p*-xylene (68.7 mg, 0.26 mmol) were added. The reaction mixture was stirred at room temperature for 20−28 h. The progress of the reaction was monitored by TLC (chloroform:methanol:NH_4OH[aq], 86:13:1). After the reaction was complete, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and poured into water (100 mL). The organic layer was separated and washed with water until neutral pH, dried over anhydrous Na_2SO_4, and evaporated under diminished pressure. The crude product was purified by column chromatography (Silicagel 60, 230−400 mesh; Merck), eluted with $CHCl_3$, and then eluted with $CHCl_3$:MeOH at v/v ratios of 98:2 and 95:5. The final product was isolated as the free amine (WP630) and precipitated from CH_2Cl_2:hexane to give a red solid (178 mg, 0.153 mmol) in 61.5% yield. Anal. ($C_{62}H_{64}N_2O_{20}\cdot H_2O$) C, H; N: calcd, 2.38; found, 2.28. ^{1}H-NMR ($CDCl_3$): δ 13.95 (s, 2H, OH), 8.01 (d, 2H, $J_{3,2}$ = 7.7 Hz, H-3), 7.77 (app t, 2H, $J_{2,3}$ = 7.7 Hz, H-2), 7.38 (d, 2H, $J_{1,2}$ = 8.5 Hz, H-1), 7.18 (s, 4H, *p*-xylene), 5.50 (d, 2H, $J_{1',2'a}$ = 3.4 Hz, H-1′), 5.28 (bs, 2H, H-7), 4.66 (bs, 2H, 9-OH), 4.07 (s, 6H, OCH_3), 4.05 (q, 2H, $J_{5',6'}$ = 6.25 Hz, H-5′), 3.77 (d, 2H, J = 12.78 Hz, CH_2 from *p*-xylene), 3.63 (m, 4H, CH_2 from *p*-xylene and H-4′), 3.22 (d, 2H, $J_{10e,10a}$ = 18.8 Hz, H-10e), 2.96 (d, 2H, $J_{10a,10e}$ = 18.8 Hz, H-10a), 2.98−2.93 (m, 2H, H-3′), 2.41 (s, 6H, H-14), 2.37 (d, 2H, $J_{8a,8e}$ = 14.7 Hz, H-8e), 2.09 (dd, 2H, $J_{8a,8e}$ = 14.9 Hz, $J_{7,8a}$ = 4.1 Hz, H-8a), 1.76 (td, 2H, $J_{2'a,3'}$ = 13.0 Hz, $J_{1',2'a}$ = 3.8 Hz, H-2′a), 1.68 (dd, 2H, $J_{2'e,3'}$ = 4.7 Hz, $J_{2'a,2'e}$ = 13.2 Hz, H-2′e), 1.37 (d, 6H, $J_{5',6'}$ = 6.65 Hz, H-6′).

The free amine WP630 was suspended in methanol (2 mL). Then 1 N dry HCl in MeOH was added (to pH 4), followed by an excess of diethyl ether to precipitate the hydrochloride of WP630. The red solid was washed with ether until neutral pH and then dried to give analytically pure WP631 (170 mg, 0.138 mmol, 55.3% yield calculated from daunorubicin). Mp: 160−170 °C dec. $[\alpha]_D^{29}$: 184.7° (*c* 0.05, $CHCl_3$:CH_3OH, 1:1). Anal. ($C_{62}H_{65}N_2O_{20}\cdot 2HCl\cdot 4H_2O$) C, H, Cl, N.

Viscosity Studies. Relative viscosity studies were conducted exactly as described previously.[17] Data were cast into a plot of $(\eta/\eta_0)^{1/3}$ versus ratio of *bound* drug per DNA base pair, in accord with the theory of Cohen and Eisenberg.[18] Ethidium bromide and daunorubicin were run as controls for these experiments.

UV Melting Studies. Ultraviolet DNA melting curves were determined using a Cary 3E UV/visible spectrophotometer (Varian, Inc., Palo Alto, CA), equipped with a thermoelectric temperature controller. Sonicated herring sperm DNA at a concentration near 20 μM bp in BPE buffer (2 mM NaH_2PO_4, 6 mM Na_2HPO_4, 1 mM Na_2EDTA, pH 7.0) was used for melting studies. Samples were heated at a rate of 1 °C min^{-1}, while the absorbance at 260 nm was continuously monitored. Primary data were transferred to the graphics program Origin (Microcal, Inc., Northampton, MA) for plotting and analysis.

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) experiments utilized a Microcal MC2 instrument (Microcal, Inc., Northampton, MA) along with its DA2 software (July 1986 version) for data acquisition and analysis. Sonicated herring sperm DNA at a concentration of 1 mM bp in BPE buffer was used for all experiments. A scan rate of 1 °C min^{-1} was used. Primary data were corrected by substraction of a buffer−buffer baseline, normalized to the concentration of DNA base pairs, and further baseline-corrected using the Cp(0) software option. Baseline-corrected, normalized data were transferred to Origin graphics software for integration and plotting. Samples for DSC of DNA + WP631 were prepared by weighing appropriate amounts of solid WP631 and dissolving the solid directly into 2 mL of 1 mM DNA solution. Any undissolved drug was removed by low-speed centrifugation. The exact amount of WP631 bound to the DNA was determined spectrophotometrically.

***In Vitro* Cytotoxicity against MCF-7 and MCF-7/VP-16 Cell Lines.** *In vitro* drug cytotoxicities against human breast carcinoma wild-type MCF-7 and MRP-resistant MCF-7/VP-16 cells were assessed by using the MTT reduction assay, as previously reported.[20] The MTT dye was obtained from Sigma Chemical Co. (St. Louis, MO). Cells were plated in 96-well microassay culture plates (10^4 cells/well) and grown overnight at 37 °C in a 5% CO_2 incubator. Drugs were then

added to the wells to achieve a final drug concentration ranging from 0.1 to 50 μg/mL. Four wells were used for each concentration. Control wells were prepared by adding appropriate volumes of calcium- and magnesium-free PBS (pH 7.4). Wells containing culture medium without cells were used as blanks. The plates were incubated at 37 °C in a 5% CO_2 incubator for 72 h. Upon completion of the incubation, 20 μL of stock MTT dye solution (5 mg/mL) was added to each well. After a 4 h incubation, 100 μL of buffer containing 50% *N*,*N*-dimethylformamide and 20% SDS was added to solubilize the MTT formazan. Complete solubilization was achieved by placing the plate in a mechanical shaker for 30 min at room temperature. The optical density of each well was then measured with a microplate spectrophotometer at a wavelength of 570 nm. The percent cell viability was calculated by the following equation:

$$\% \text{ cell viability} = (\text{OD treated wells}/\text{OD control wells}) \times 100$$

where OD is the mean optical density from four determinations. The percent cell viability values were plotted against the drug concentrations used, and the ID_{50} was calculated from the curve. Cytotoxicity experiments were repeated at least three times.

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References

(1) Weiss, R. B. The Anthracyclines: Will We Ever Find A Better Doxorubicin? *Sem. Oncol.* **1992**, *19*, 670−686.
(2) Chaires, J. B. Molecular Recognition of DNA by Daunorubicin. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Chaires, J. B., Eds.; JAI Press: Greenwich, CT, 1996; Vol. 2, pp 141−167.
(3) Priebe, W., Ed. *Anthracycline Antibiotics: New Analogues, Methods of Delivery and Mechanisms of Action*; ACS Symposium Series; American Chemical Society: Washington, DC, 1995.
(4) Lown, J. W., Ed. *Anthracycline and Anthracenedione Based Anticancer Agents*; Elsevier: Amsterdam, The Netherlands, 1988.
(5) Chaires, J. B.; Herrera, J. E.; Waring, M. J. Preferential Binding of Daunomycin to 5′(A/T)CG and 5′(A/T)GC Sequences by Footprinting Titration Experiments. *Biochemistry* **1990**, *29*, 6145−6153.
(6) Chen, K.-X.; Gresh, N.; Pullman, B. A Theoretical Investigation on the Sequence Selective Binding of Daunomycin to Double-Stranded Polynucleotides. *J. Biomol. Struc. Dynam.* **1987**, *3*, 445−466.
(7) Pullman, B. Sequence Specificity in the Binding of Anti-tumor Anthracyclines to DNA: A Success of Theory. *Anti-Cancer Drug Des.* **1991**, *6*, 95−105.
(8) Fritzsche, H.; Berg, H. Analysis of Equilibrium, Kinetic and Structural Data of Anthracycline-DNA Interaction. *Gazz. Chim. Ital.* **1987**, *117*, 331−352.
(9) Chaires, J. B. Biophysical Chemistry of the Daunomycin-DNA Interaction. *Biophys. Chem.* **1990**, *35*, 191−202.
(10) Chaires, J. B. Molecular Recognition of DNA by Daunorubicin; In *Anthracycline Antibiotics: New Analogues, Methods of Delivery and Mechanisms of Action*; Priebe, W., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, 1993; pp 156−157.
(11) Wakelin, L. P. G. Polyfunctional DNA Intercalating Agents. *Med. Res. Rev.* **1986**, *6*, 275−340.
(12) Valentini, L.; Nicolella, V.; Vannini, E.; Menozzi, M.; Penco, S.; Arcamone, F. Association of Anthracycline Derivatives with DNA: A Fluorescence Study. *Il Farmaco* **1985**, *40*, 377−390.
(13) Dervan, P. Design of Sequence-Specific DNA-Binding Molecules. *Science* **1986**, *232*, 464−471.
(14) Henry, D. W.; Tong, G. L. U.S. Patent 4,112,217, 1978.
(15) Phillips, D. R.; Brownlee, R. T. C.; Reiss, J. A.; Scourides, P. A. Bis-daunomycin Hydrazones: Interactions with DNA. *Invest. New Drugs* **1992**, *10*, 79−88.
(16) Skorobogaty, A.; Bownlee, R. T. C.; Chandler, C. J.; Kyratzis, I.; Phillips, D. R.; Reiss, J. A.; Trist, H. The DNA-Association and Biological Activity of A New Bis(14-thiadaunomycin). *Anti-Cancer Drug Des.* **1988**, *3*, 41−56.
(17) Suh, D.; Chaires, J. B. Criteria for the Mode of Binding of DNA Binding Agents. *Bioorg. Med. Chem.* **1995**, *3*, 723−728.
(18) Cohen, G.; Eisenberg, H. Viscosity and Sedimentation Study of Sonicated DNA-Proflavine Complexes. *Biopolymers* **1969**, *8*, 45−55.
(19) Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Rich, A. Interactions between an Anthracycline Antibiotic and DNA: Molecular Structure of Daunomycin Complexed to d(CpGpTpApCpGp) at 1.2−Å Resolution. *Biochemistry* **1987**, *26*, 1152−1163.
(20) Green, L. M.; Reade, J. L.; Ware, C. F. Rapid Colormetric Assay for Cell Viability Application to the Quantitation of Cytotoxic and Growth Inhibitory Lymphokines. *J. Immunol. Meth.* **1984**, *70*, 257−268.
(21) Crothers, D. M. Statistical Thermodynamics of Nucleic Acid Melting Transitions with Coupled Binding Equilibria. *Biopolymers* **1971**, *10*, 2147−2160.
(22) McGhee, J. D. Theoretical Calculations of the Helix-Coil Transition of DNA in the Presence of Large, Cooperatively Binding Ligands. *Biopolymers* **1976**, *15*, 1345−1375.
(23) Brandts, J. F.; Lin, L.-N. Study of Strong to Ultratight Protein Interactions using Differential Scanning Calorimetry. *Biochemistry* **1990**, *29*, 6927−6940.
(24) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. Binding of An Antitumor Drug to DNA Netropsin and C-G-C-G-A-A-T-T-^{Br}C-C-G-C-G. *J. Mol. Biol.* **1985**, *183*, 553−563.
(25) Lown, J. W. Lexitropsins: Rational Design of DNA Sequence Reading Agents as Novel Anti-cancer Agents and Potential Cellular Probes. *Anti-Cancer Drug Des.* **1988**, *3*, 25−40.
(26) Lown, J. W. Targeting the DNA Minor Groove for Control of Biological Function: Progress, Challenges, and Prospects. *CHEMTRACTS−Org. Chem.* **1993**, *6*, 205−237.
(27) Mrksich, M.; Wade, W. S.; Dwyer, T. J.; Geierstanger, B. H.; Wemmer, D. E.; Dervan, P. B. Antiparallel Side-by-Side Dimeric Motif for Sequence-Specific Recognition in the Minor Groove of DNA by the Designed Peptide 1-Methylimidazole-2-Carboxamide Netropsin. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7586−7590.
(28) Trauger, J. W.; Baird, E. E.; Dervan, P. B. Recognition of DNA by Designed Ligands at Subnanomolar Concentrations. *Nature* **1996**, *382*, 559−561.
(29) Pelton, J. G.; Wemmer, D. E. Structural Characterization of a 2:1 Distamycin A-d(CGCAAATTGGC) Complex by Two-Dimensional NMR. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5723−5727.
(30) Wilson, W. D. Reversible Interactions of Nucleic Acids with Small Molecules. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M., Gait, M. J., Eds.; IRL Press: Oxford, 1990; pp 297−336.

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