Articles

DNA Binding by Antitumor Anthracene Derivatives

Timothy P. Wunz, Mary T. Craven, Michael D. Karol, G. Craig Hill, and William A. Remers*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, Arizona 85721.
Received December 8, 1988

The relative DNA binding strengths of bisantrene and nine new analogues were measured by spectrophotometric titration and melt transition temperature (T_m) techniques. Data from the spectrophotometric titrations could not be fit by simple Scatchard plots. However, they were fit by a McGhee–von Hippel equation over part of the binding range. The entire range of data was fit by a smoothing cubic spline function. The first derivative of this function gave, for each compound, a curve whose intercept provided a measure of relative binding strength. The ΔT_m values agreed qualitatively with the spectrophotometric titration results, although there was not a precise linear relationship. Determinations of macroscopic pK_as revealed that most of the compounds were dications at pH 7.0, but a few were mixtures of monocations and dications. No correlation was found between these binding studies and antitumor potencies in a clonogenic assay, which suggests that factors other than DNA binding can determine cytotoxicity for some of the analogues.

A variety of anthracene derivatives show significant activity in animal models and four of these compounds have undergone clinical testing. They are pseudourea (**1**),[1] ametantrone (**12**),[2] mitoxantrone (**13**),[3] and bisantrene (**2**)[4] (Figure 1). Among these compounds, mitoxantrone (Novantrone) is approved for clinical use. A new compound (**14**), containing the anthra[1,9-*cd*]pyrazol-6(2H)-one ring system, shows considerable promise as an antitumor agent.[5] We recently reported on a series of anthracene derivatives having at the 9- and 10-positions side chains containing basic nitrogen atoms.[6] Some of these compounds (e.g., **3**) were closely related to bisantrene, but others, including **4** and **5**, had much more flexible side chains. They were tested with a panel of cloned human tumor cells, wherein they showed a wide range of potencies for inhibition of cell growth. However, compounds **4** and **5** were very effective inhibitors: **4** was as potent as bisantrene and **5** even matched mitoxantrone in its ID_{50} value. Despite these encouraging activities, **4** and **5** were not very effective in inhibiting cultured P388 murine leukemia cells or fresh human tumor cells, and their activities against P388 and L1210 leukemias in mice were only marginal. Alkaline DNA elution studies using L1210 cells showed that bisantrene produced substantial DNA scission at cytoxic doses, whereas **4** did not.[6] Thus, it seemed possible that differences in the modes of antitumor action might be partly responsible for the greater activity of bisantrene.

In order to gain possible insight into the comparative activities of bisantrene and our 9,10-disubstituted anthracene analogues, we have determined their relative DNA binding strength. Correlations between antitumor potency and DNA binding strength are frequently attempted, although their success varies with the system.

Two widely used methods, spectrophotometric titration and melt transition temperature (T_m) were investigated. These methods already had been applied to bisantrene and two closely related analogues by Foye and co-workers.[7] However, they were not able to obtain a precise ΔT_m for bisantrene under their experimental conditions. There also was some concern about the statistical validity of the straight line they drew in a Scatchard plot of the spectrophotometric titration data on bisantrene. For these reasons, we began with a careful redetermination of bisantrene binding to DNA and then measured the binding of a number of our analogues.

Results and Discussion

Spectrophotometric Titrations. Data from the experiments on bisantrene (**2**) are plotted (Figure 2) as (bound drug/total base pairs)/free drug versus bound drug/total base pairs, with the McGhee–von Hippel notation of ν/c versus ν. It is evident that the resulting curve does not have the kind of linear portion that lends itself to a simple Scatchard analysis. The various analogues **3–7** (Figure 2) also gave plots in which there was no linear portion. Compounds **8** and **9** showed no appreciable DNA binding. The shapes of these curves suggested that they might be fitted by one of the McGhee–von Hippel equations. These equations take into account ligand site size and ligand–ligand cooperativity in addition to the intrinsic binding constant.[8] Adequate fits of the data were obtained

(1) Miller, M. W.; Amidon, R. W.; Tawney, P. O. *J. Am. Chem. Soc.* **1955**, *77*, 2845. Frei, E.; Luce, J. K.; Loo, T. L. *Cancer Chemother. Rep., Part 1* **1971**, *55*, 91. Wilson, W. L.; Weiss, A. J.; Andrews, N. C. *Cancer Chemother. Rep., Part 1* **1971**, *55*, 525.

(2) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291. Cheng, C. C.; Zee-Cheng, R. K. Y. *Prog. Med. Chem.* **1983**, *20*, 83.

(3) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, F. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024. Wallace, R. E.; Murdock, K. C.; Angier, R. B.; Durr, F. E. *Cancer Res.* **1979**, *39*, 1570. Smith, I. E. *Cancer Treat. Rev.* **1983**, *10*, 103.

(4) Murdock, K. C.; Child, R. G.; Lin, Y.; Warren, D. J.; Fabio, P.; Lee, V. J.; Izzo, P. T.; Lang, S. A., Jr.; Angier, R. B.; Citarella, R. V.; Wallace, R. E.; Durr, F. E. *J. Med. Chem.* **1982**, *25*, 505. Alberts, D. S.; Mackel, C.; Pocelinko, R.; Salmon, S. E. *Cancer Res.* **1982**, *42*, 1170. Yap, H.; Yap, B.; Blumenschein, G. R.; Barnes, B. C.; Schell, F. C.; Bodey, G. P. *Cancer Res.* **1983**, *43*, 1402.

(5) Showalter, H. D. H.; Johnson, J. L.; Werbel, L. M.; Leopold, W. R.; Jackson, R. C.; Elslager, E. F. *J. Med. Chem.* **1984**, *27*, 255.

(6) Wunz, T. P.; Dorr, R. T.; Alberts, D. S.; Tunget, C. L.; Einspahr, J.; Milton, S.; Remers, W. A. *J. Med. Chem.* **1987**, *30*, 1313.

(7) Foye, W. O.; Karnik, P. S.; Sengupta, S. K. *Anti-Cancer Drug Des.* **1986**, *1*, 65.

0022-2623/90/1833-1549$02.50/0 © 1990 American Chemical Society

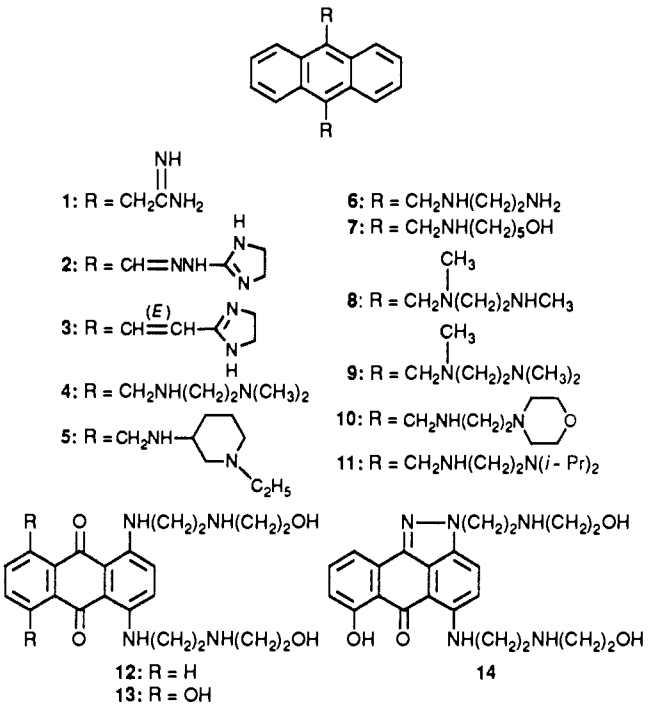


Figure 1. Structures of bisantrene and related compounds.

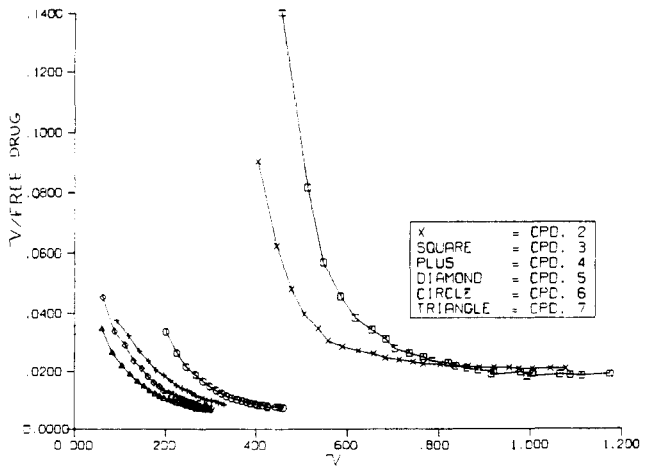


Figure 2. Scatchard plots of bisantrene and five analogues.

by using the equations $\nu/c = K(1 - n\nu)[(2\omega - 1)(1 - n\nu) + (\nu - R)/2(\omega - 1)(1 - n\nu)]^{n-1}[1 - (n - 1)\nu + R/2(1 - n)]^2$, where ω is a cooperativity factor derived from the curve fitting process, and $R = \{[1 - (n - 1)\nu]^2 + 4\omega\nu(1 - n\nu)\}^{1/2}$. The complete sets of spectrophotometric data were truncated for use in the calculations. Data in the range of 65–100% binding was chosen, except for bisantrene, which gave a horizontal line at less than 76% binding. At very low drug/DNA ratios the experimental error is great and some values appeared at >100% binding, which cannot be. In these cases, the first point at lower than 100% binding was used. Binding values below 65% (76% for bisantrene) involve so many drug molecules per base pair that they bind nonspecifically in modes such as stacking on top of each other. Figure 3 shows the curve obtained for **6**, a representative example. Similar curves for the other compounds are given in the microfilm edition and are available as supplementary material (Figures 7–11). The results in Table I show that the apparent binding constants for bisantrene (**2**) and **3** are significantly greater than those of the other compounds. Furthermore **2** and **3** have slightly smaller binding sites than the others.

Although the McGhee–von Hippel equation fit the spectrophotometric binding in a significant portion of the binding range, it could not fit all of the data for any compound, for the reasons given above. We thought that if a function could be found that fit all of the data, additional insight into the DNA binding process might be obtained. For this purpose, we made a plot of the binding data as bound drug/total base pairs versus total drug/total base pairs. This kind of plot does not have the large errors inherent in the term (bound drug/total base pairs)/free drug at high percent binding. It also does not have the same variable, bound drug/total base pairs, in both the abscissa and ordinate. On the other hand, it does not yield the apparent binding constant and binding-site size that are obtained from Scatchard plots. Fitting a curve to the

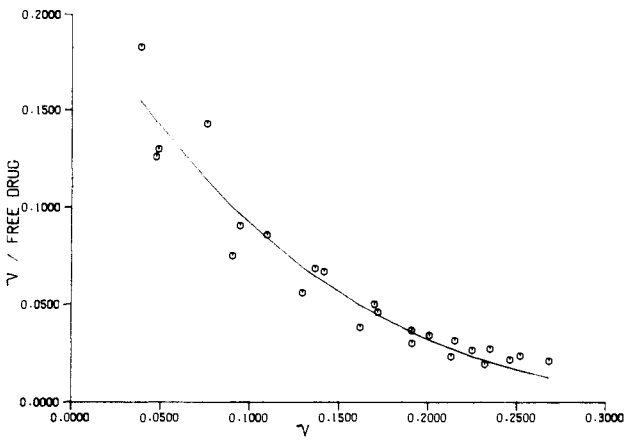


Figure 3. McGhee–von Hippel equation applied to the data of **6**.

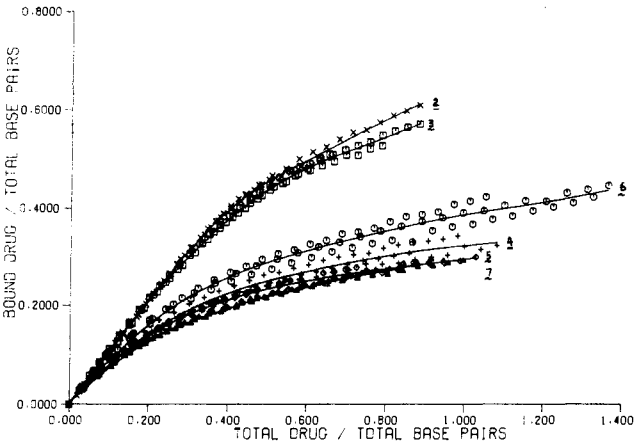


Figure 4. Plots of curves for a smoothing cubic spline function with the data for compounds **2**–**7**. Each set of data represents three independent determinations.

Table I. McGhee–von Hippel Analysis of the DNA Binding by Bisantrene and Analogues[a]

compd	correlation coefficient R	K_{app}[b]	n[c]	ω[d]
bisantrene (**2**)	0.967	9.03	0.9	0.11
3	0.973	8.29	1.2	0.18
4	0.702	0.100	1.5	0.14
5	0.956	0.0605	1.3	0.15
6	0.962	0.207	1.5	0.14
7	0.941	0.0532	1.5	0.13

[a] Data were collected in the 65–100% binding range, except for bisantrene, whose data was in the range 76–100%. For bisantrene only, data beyond 76% gave a horizontal line in Scatchard plots. [b] Apparent binding constant in μmol^{-1}. [c] Number of base pairs per binding site. [d] Parameter for binding cooperativity.

(8) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.

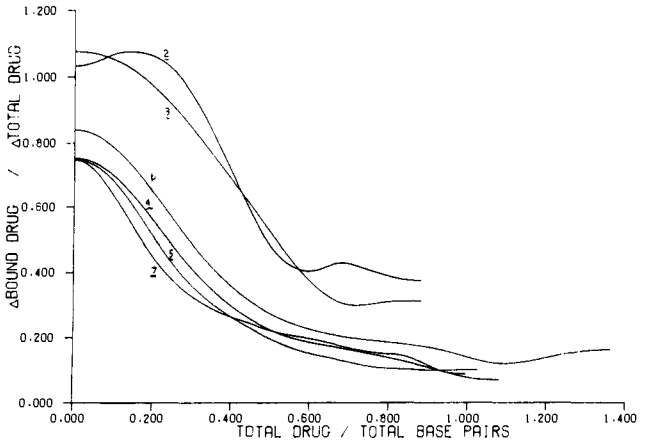


Figure 5. Plot of the first derivative of bound drug/total base pairs versus total drug/total base pairs for compounds **2–7**.

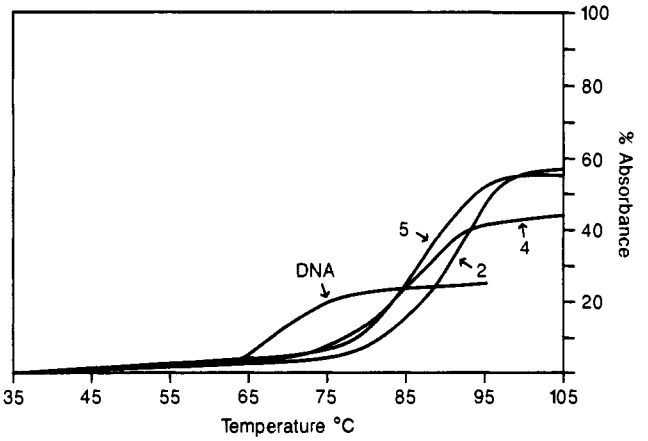


Figure 6. Melt transition temperature curves for compounds **2**, **4**, and **5** and calf thymus DNA.

Table II. Correlations among DNA Binding Strength by Spectrophotometric Titration, Melt Transition Temperature, and Antitumor Potency in Cell Culture

	spectrophotometric titration			
compd	McGhee–von Hippel K_{app}, μmol^{-1}	cubic spline relative binding	ΔT_m[a], °C	antitumour potency:[b] ID_{50}
2	9.03	1.03	19	2.40
3	8.29	1.08	>20	un[d]
4	0.100	0.75	12	1.60
5	0.0605	0.75	16	0.03
6	0.270	0.75	14	un[d]
7	0.0532	0.84	13	74.9
8	no binding[c]		3	4.14
9	no binding		1	3.42
10	not measured		5	5.76
11	not measured		9	0.70

[a] ΔT_m values are the differences between T_m for calf thymus DNA with drug and without (T_m = 71 °C for DNA alone). [b] Data taken from ref 6. They represent activity against human ovarian cancer cells in clonogenic assay. [c] Nearly horizontal lines were found in Scratchard plots. [d] Results where it was not possible to obtain an ID_{50} are denoted by un.

Table III. Data on Spectrophotometric Titrations

		$\epsilon \times 10^{-3}$	
compd	wavelength, nm	free	bound
2	410	14.93	8.03
3	415	13.67	7.60
4	393	10.20	3.03
5	394	11.96	3.54
6	394	10.20	3.97
7	394	12.29	3.63
9	395	12.30	11.34

data plotted by a new method proved to be very difficult. It was possible, however, to make an excellent fit to the data for each compound by a smoothing cubic spline function. An IMSL routine[9] was used for this purpose. These fits are shown in Figure 4. Although apparent binding constants cannot be derived from these curves, intercepts on the ordinate of plots made from the first derivative of the function afford a relative ranking of the DNA binding affinities of the compounds. These relative affinities correspond to the situation in which the first drug molecule binds to the DNA. Thus, there are no cooperativity or binding-site size effects from other molecules. As shown in Figure 5, there is a significant difference between the binding of bisantrene and **3** and the other compounds. The correlation between relative binding strengths given by the McGhee–von Hippel treatment and the alternative approach (Table II) is not linear. There is, however, qualitative agreement in that the two strongest binders, bisantrene and **3**, are clearly set apart from the other compounds by both treatments. It must be emphasized that these two methods for analyzing the data do not give the same kind of result. Thus, the intercept in first derivative plot in the spline method gives relative binding affinities when no other drug molecules are present, whereas the McGhee–von Hippel analysis gives apparent binding constants in a range of concentrations of bound and unbound drug molecules. Results from the spline method should be valuable for comparison with relative binding energies obtained from molecular mechanics calculations wherein cooperativity and site-size effects are usually neglected.

Melt Transition Temperatures. Foye and co-workers were unable to obtain an accurate ΔT_m for the complex formed between bisantrene and DNA, because a plateau in the curve was not obtained before 100 °C.[7] However, by changing the buffer to 0.010 M phosphate at pH 7.0, we were able to decrease ΔT_m values for calf thymus DNA by about 10 °C. This permitted accurate values to be measured for complexes with bisantrene and seven of the eight analogues given in Table II. Compound **3** appeared to bind more strongly than bisantrene, but a ΔT_m value could not be obtained because the curve did not reach a plateau by 100 °C. As shown in Figure 6, the curves for DNA alone and in its complexes with bisantrene (**2**), **4**, and **5** are well shaped and the ΔT_m determinations are easy. The other analogues gave similarly good curves, except for **3** (microfilm edition, Figures 12 and 13). There is a wide range of ΔT_m values among the compounds in Table II, but they appear to lie in two different groups: those with relatively high values (9–20 °C) and those with relatively low values (1–5 °C). Bisantrene and **3** have higher ΔT_m values than the other compounds, but the differences are not as great as those found in spectrophotometric titrations (Table III). The reason why the ΔT_m and K_{app} do not match well for compounds **2**, **3**, and **4–7** is not obvious, but it might involve the 11-fold difference in ionic strength between the two methods (0.01 for the former and 0.11 for the latter).

Extents of Protonation. The foregoing DNA binding studies were all measured at pH 7.0. In order to interpret them properly, it is necessary to know the extent of pro-

(9) International Mathematical and Statistical Libraries, Inc., Computer Subroutines Libraries in Mathematics and Statistics, April, 1977. Sixth floor, GNB Building, 7500 Bellaire Blvd., Houston, TX 77035.

Table IV. pK_a Values for Amino Groups in the Compounds

compd	pK_{a_1}	pK_{a_2}	pK_{a_3}	pK_{a_4}
2	6.80	---[b]		
3	>8.5[a]	>10[a]		
4	3.90	5.35	8.40	9.40
5	2.40	3.00	5.20	9.15
7	8.00	>9[a]		
9	3.10	4.20	8.45	9.45
10	3.85	6.30	7.50	8.50

[a] Insolubility at higher pH values rendered the pK_as inaccurate. [b] Complete insolubility made it impossible to determine this pK_a.

tonation of the various amino groups in the molecules at this pH. Consequently, we titrated each compound and determined the macroscopic pK_a values, which are listed in Table IV. Reduced solubility at higher pH prevented precise measurements of a few of the pK_as and bisantrene was so insoluble above pH 6.8 that its higher pK_a could not be determined. Nevertheless, the states of ionization of the compounds at pH 7.0 are usually obvious. Compounds **3**, **4**, **7**, **9** and **10** are dications, whereas **6** is a mixture of about 75% monocation and 25% dication. Bisantrene (**2**) can only be estimated, but from the lower pK_a of 6.8 it should be more than 50% monocation with the rest dication.

There are some interesting differences among the macroscopic pK_a values of these compounds. For example, pK_{a_3} of **5** is much lower than that of **4** and this difference results in the former being mostly a monocation at pH 7.0. Both compounds, however, have two tertiary amino groups and two secondary amino groups. The difference might be caused by steric hindrance or conformational effects. Compounds **9** and **10** resemble **4** in pH profiles, although the pK_{a_3} and pK_{a_4} values of **10** are lower, possibly because they involve the morpholino groups. (Macroscopic pK_as probably include contributions from two or more amino groups.) As expected from its imidazoline rings, **3** is highly basic. Substituting such rings with hydrazine groups decreases the basicity, as found in **2**, insofar as it could be measured.

Structure–Activity Relationships. Table II compares the relative binding strengths by spectrophotometric titration and ΔT_m for bisantrene and nine analogues with their relative potencies in a clonogenic antitumor assay. Although there is not a nice linear correlation between ΔT_m and either of the two spectrophotometric measurements of binding strengths, there is qualitative agreement between them. Thus, compounds with relatively high ΔT_m values show good binding by spectrophotometric titration, whereas those with low ΔT_m values do not bind appreciably according to spectrophotometric titration. There are no simple correlations between the extent of protonation of compounds (Table IV) and their DNA binding strengths. Compound, **2**, which is less than 50% dication at pH 7.0, is one of the two strongest binders and **5**, which is about 25% dication at this pH, binds more strongly than many of the compounds that are fully dications. Evidently the electrostatic interactions with DNA do not dominate the binding energies. Other factors, including van der Waals forces and hydrogen bonds must make significant contributions. For example, preliminary molecular mechanics studies on the binding of **2** to DNA fragments show that it can make as many as five hydrogen bonds,[10] which is more than any of the other compounds can. The surprising result in Table II is that compounds such as **3** and **6** that bind strongly to DNA have poor antitumor activity, whereas compounds such as **8** and **9** that bind very poorly to DNA have significant antitumor activity. One factor in the unexpectedly low activity of **3** and **6** might be poor cell penetration, although they are not that different in structure from the other analogues. A possible explanation, and one that also fits the good activity of compounds that are poor DNA binders, is that metabolic activation of **8** and **9** is involved. Another possibility is differences in mode of action. Our previous work established that bisantrene behaved differently than analogue **4** toward DNA, in that only bisantrene caused strand cleavage.[6]

(10) Wunz, T. P.; Remers, W. A., unpublished data.

Conclusions

The binding of bisantrene and a set of analogues to DNA has been studied by two different methods. Both methods show significant differences in the relative binding strengths of these compounds, although many of them are rather closely related in structure. These differences can not be explained by the extents of protonation at pH 7.0. Other factors, such as van der Waals forces and hydrogen bonding, must be important. Bisantrene is known to be an intercalating agent.[7,11-14] The analogues probably intercalate as well, although they must have less planar side chains (except for **3**). Compounds **8** and **9**, with branched chains, are the poorest binders, indicating that steric hindrance to binding is important. Thus the binding process is complicated and dependent on many structural factors. A molecular mechanics study on the DNA binding of bisantrene and certain analogues is in progress.

The lack of correlation of DNA binding strength with antitumor activity was unfortunate, but this result has been observed for other DNA binding agents. Factors such as cellular uptake and metabolism of the compounds must strongly affect their potency. Possibly the high potency of **5** involves a combination of good solubility, relatively high lipophilicity, and a low proportion of dication at pH 7.0. The superior antitumor activity of bisantrene in animal models might reflect its high DNA binding strength, but a greater ability to cleave DNA probably is important.

Experimental Section

Syntheses and properties of the compounds used in this study are described in ref 6. All measurements were recorded on a Perkin-Elmer Lambda 3A UV–visible spectrophotometer, equipped with a Model C570-0710 temperature programmer for melt transition temperature experiments. Calf thymus DNA containing less than 3% protein was obtained from Sigma Chemical Co. All DNA concentrations are expressed as moles of base pairs per liter.

Spectrophotometric Titrations. The buffer used for the titrations was 0.1 M NaCl, 0.01 M sodium phosphate, 0.001 M EDTA, at pH 7.0. A stock solution of approximately 2×10^{-4} M drug was prepared. A fresh 5×10^{-4} M solution of calf thymus DNA was prepared for each titration. The DNA concentration was determined spectrophotometrically with an $\epsilon_{260} = 6600\ M^{-1}$ nucleotide[15] and then expressed as moles of base pairs per liter. The wavelengths used and free and bound drug extinction coefficients are given in Table III. Extinction coefficients for the free and bound drug were determined from Beer's law plots of absorbances obtained by adding five 100-μL portions of drug solution to 3 mL of buffer or stock DNA solution. There was always at least a 10:1 ratio of base pairs/drug, assuring that all the drug is bound to the DNA.

(11) Bates, R. B.; Roberts, S. A.; Siahaan, T. J.; Gnanasambandan, T.; Yalkowsky, S. H. *Acta Crystallogr.* **1986**, *C42*, 186.
(12) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.
(13) Denny, W. A.; Wakelin, L. P. G. *Anti-Cancer Drug Des.* **1987**, *2*, 71.
(14) Lown, J. W.; Hanstock, C. C.; Bradley, R. D.; Scraba, D. G. *Mol. Pharmacol.* **1984**, *25*, 178.
(15) Bresloff, J. L.; Crothers, D. M. *Biochemistry* **1981**, *20*, 3547.

The spectrophotometric titrations were carried out by adding 50-μL portions (total of 26) of drug solution to 2.5 mL of approximately 1×10^{-4} M DNA solution and after 5 min measuring the absorbance at the wavelength used for the drug. The amount of free and bound drug in solution after each addition was calculated by the method of Muller and Crothers.[16] Data that was in the range of 65–100% bound, except for bisantrene, was used to analyze the drug binding by the McGhee–von Hippel equation. Each experiment was run at least three times and the data is a composite of three runs. For most compounds, there was little variation between runs; however, solubility problems were encountered with bisantrene. They were overcome by dissolving the bisantrene in 0.01 M sodium phosphate and 0.001 M EDTA at pH 7, without the NaCl. Addition of small volumes of this solution to the DNA solution made little difference in the total ionic strength (~0.11 M), but as larger volumes were added it fell, reaching a minimum of ~0.07 M when a total of 1.3 mL of bisantrene solution was added.

Melt Transition Temperatures. The buffer for these experiments was 0.01 M Na_3PO_4, 0.001 M EDTA, at pH 7.0. Into both the sample and reference cuvette were placed 3 mL of 5×10^{-5} M calf thymus DNA solution and the appropriate amount of drug solution to provide a ratio of five base pairs per drug molecule. The sample cuvette was heated from 25 to 110 °C at 1 °C per minute, while the absorbance at 260 nm was monitored.

Calculations. Curve fitting of the McGhee–von Hippel equation to spectrophotometric titration data was accomplished by use of the program FUNFIT[17] and the smoothing cubic spline function was obtained from IMSL routines,[9] both on a VAX computer.

pK_a Determinations. Solutions were prepared by dissolving 0.2 mmol of each compound as free base or dihydrochloride in sufficient 0.0392 N HCl to give 1 mequiv of acid beyond that required to protonate all amino groups. These solutions were stirred and titrated with 0.0242 N NaOH while the pH was measured on a Sargent-Welch Model IP pH meter. Data were graphed and pK_a values were determined from points on the curve where 0.5 equiv of base per each functional group had been added. Insolubility at higher pH values prevented accurate determinations of pK_a values for **3** and **7**; however, they clearly were dications at pH 7.0. Bisantrene (**2**) was so insoluble that it was titrated in very dilute solution. One milliequivalent of it was dissolved in 10 mL of 0.00392 N HCl and titrated with 0.00242 N NaOH. At the point where there was somewhat more monocation than dication present, bisantrene precipitated and could not be titrated further. Results of these titrations are given in Table IV.

Acknowledgment. This investigation was supported in part by Grant CA 37798 awarded by the National Cancer Institute, DHHS.

Supplementary Material Available: Plots of the McGhee–von Hippel equation for **4**, **5**, **7**, **3**, and **2** (Figures 7–11) and melt transition temperature curves for **6–8** and **9–11** (Figures 12 and 13) (7 pages). Ordering information is given on any current masthead page.

(16) Muller, W.; Crothers, D. M. *J. Mol. Biol.* **1968**, *35*, 251.

(17) Veng-Pederson, P. *J. Pharmacokin, Biopharm.* **1977**, *5*, 513.

Synthesis and Structure–Activity Relationships of 6-Substituted 2′,3′-Dideoxypurine Nucleosides as Potential Anti-Human Immunodeficiency Virus Agents

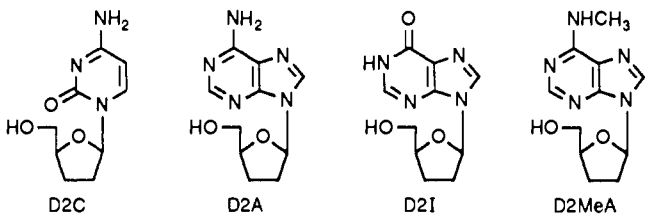
Chung K. Chu,*,† Giliyar V. Ullas,† Lak S. Jeong,† Soon K. Ahn,† Bogdan Doboszewski,† Zhi X. Lin,† J. Warren Beach,† and Raymond F. Schinazi*,‡

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The University of Georgia, Athens, Georgia 30602, and Veterans Administration Medical Center and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30033. *Received August 22, 1989*

In order to study the structure–activity relationships of 2′,3′-dideoxypurine nucleosides as potential anti-HIV agents, various 6-substituted purine analogues have been synthesized and examined in virus-infected and uninfected human peripheral blood mononuclear cells. N^6-methyl-2′,3′-dideoxyadenosine (D2MeA, **7a**) was initially synthesized from adenosine via 2′,3′-*O*-bisxanthate **3**. As extension of this reaction to other N^6-substituted compounds failed, a total synthetic method utilizing 2′,3′-dideoxyribose derivative **9** was used for the synthesis of other purine nucleosides. An acid-stable derivative of N^6-methyl-2′,3′-dideoxyadenosine, 2′-fluoroarabinofuranosyl analogue **32** (D2MeFA), has been synthesized from the appropriate carbohydrate **24** by condensation with N^6-methyladenine **23**. Among these compounds, N^6-methyl derivative (D2MeA) **7a** proved to be one of the most potent antiviral agents. The order of potency for the 6-substituted compounds was NHMe > NH_2 > Cl ≈ $N(Me)_2$ > SMe > OH ≈ NHEt > SH > NHBn ≈ H. The results suggest that a bulk tolerance effect at the 6-position of the 2′,3′-dideoxypurine nucleoside may dictate the antiviral activity of these compounds. Acid-stable analogue **32** (D2MeFA) was found to be 20-fold less potent than the parent compound. Both D2MeA and D2MeFA were resistant to calf intestine adenosine deaminase. The presence of a fluorine atom in the carbohydrate moiety greatly increased stability to acid, making D2MeFA a potential orally active antiviral agent that could be useful for the treatment of retroviral infections in humans.

Certain dideoxyncleosides exhibit potent antiviral activities against human immunodeficiency viruses (HIV) in vitro. 2′,3′-dideoxycytidine (D2C),[1] 2′,3′-dideoxyadenosine (D2A),[1] and 2′,3′-dideoxyinosine (D2I)[2] (Chart I) are currently undergoing clinical trials in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. The exact mechanism by which these nucleosides suppress the replication of HIV is not fully understood. It is reported that 2′,3′-dideoxynucleosides as their triphosphates inhibit the HIV reverse transcriptase and can cause chain termination of DNA.[1,3-6]

Chart I



† University of Georgia.
‡ Emory University School of Medicine.

0022-2623/90/1833-1553$02.50/0 © 1990 American Chemical Society