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The effect of ionic strength on DNA-ligand unwinding angles for acridine and quinoline derivatives

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ABSTRACT

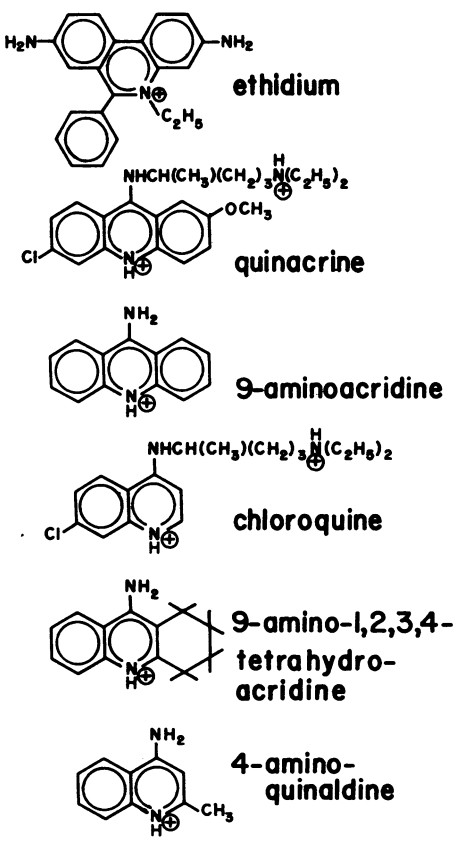
We have quantitatively examined the unwinding angles for the complexes of a related series of acridine and quinoline derivatives with DNA. Ethidium bromide was used as a control for determining superhelix densities at different ionic strengths. Relative to ethidium, 9-aminoacridine and quinacrine had an essentially constant unwinding angle of approximately 17° at all ionic strengths tested. The apparent unwinding angle for chloroquine and 9-amino-1,2,3,4-tetrahydroacridine was found to be ionic strength dependent, increasing with increasing ionic strength. This suggests that competitive nonintercalative binding at low ionic strengths causes an apparent lowering of the quinoline unwinding angle. This can also explain why 4-aminoquinaldine, examined at low ionic strength, gives a quite low apparent unwinding angle. Quinacrine along with chloroquine and 9-aminoacridine approaches a limiting value for their unwinding angle of approximately 17°. 4-aminoquinaldine and 9-amino-1,2,3,4-tetrahydroacridine could not be examined at an ionic strength above 0.03 because of their very low equilibrium binding constants.

INTRODUCTION

Acridine derivatives such as quinacrine, and quinoline derivatives such as quinine and chloroquine are among the most active known intercalating antimalarial drugs (2). We have been interested in the structural features which characterize the interaction of compounds of this type with DNA (3-5). A base pair in B-form DNA is wound by approximately 36° relative to the base pair immediately below it and intercalation decreases this winding angle (cf. reference 6). The most frequently analyzed intercalating compound, ethidium bromide, decreases the winding angle by 26°, so that the base pair above ethidium is wound only 10° relative to the base pair below ethidium (7,8). Unwinding angles reported for other compounds have generally been less than the value for ethidium. These lower unwinding angles could occur due to differences in the intercalated complex of the different ring systems and/or due to competitive binding by a nonintercalative binding mode (9).

The effect that the structure of the intercalating ligand has on the un-

winding angle in the DNA complex is largely unknown. As part of our continuing investigation on quinolines and acridines, we have determined the unwinding angles for the compounds shown in Figure 1 using the quantitative method developed by Vinograd and coworkers (10). At low salt concentrations, the amount of 4-aminoquinaldine (4AQ) and 9-amino-1,2,3,4-tetrahydroacridine (9ATHA) bound to DNA as determined by this method is greater than that predicted by the neighbor exclusion principle (11,12). A detailed analysis of the chloroquine and 9-aminoacridine (9AA) unwinding angles, reported here as a function of ionic strength, suggests that the unwinding angles of the quinoline derivatives at low salt are incorrect due to competitive nonintercalative binding of these compounds which have low apparent intercalation equilibrium constants. The acridine derivatives and chloroquine all approach a



1. Compound Structures.

limiting unwinding angle of approximately 17° at salt concentrations above 0.05M. This suggests that in the limit some structural feature of these compounds and not competitive nonintercalative binding, accounts for their low unwinding angle relative to ethidium.

MATERIALS AND METHODS

Chloroquine, quinacrine (Sigma) and ethidium bromide (Aldrich) were purified as previously described (5). Chloroquine, 4AQ and 9ATHA from Aldrich were found to be pure as indicated by ^{1}H and ^{13}C NMR, thin layer chromatography (TLC) on silica gel (solvent: chloroform/methanol/acetone, 2:1:2, V/V), and chemical analysis. 9-aminoacridine was recrystallized three times from methanol and analysis as above indicated that the recrystallized compound was pure. Calf thymus DNA, obtained from Worthington (Lot No. 36K890), was sonicated as described (5). This DNA sample had an A_{260}/A_{280} ratio of 1.82, and A_{260}/A_{230} ratio of 2.47 and a total hyperchromicity at 260 nm of 29%.

The method used to prepare Colicinogenic factor E_1 (Col E_1) plasmid DNA was as described (5). Purified Col E_1 DNA had an A_{260nm}/A_{280nm} ratio of 1.89 and an A_{260nm}/A_{230nm} ratio of 2.33. Col E_1 DNA concentrations were determined utilizing an extinction coefficient of 6550 M^{-1} cm^{-1} at 260 nm. Agarose (0.9%) horizontal slab gel electrophoresis (13) revealed the presence of only a single component.

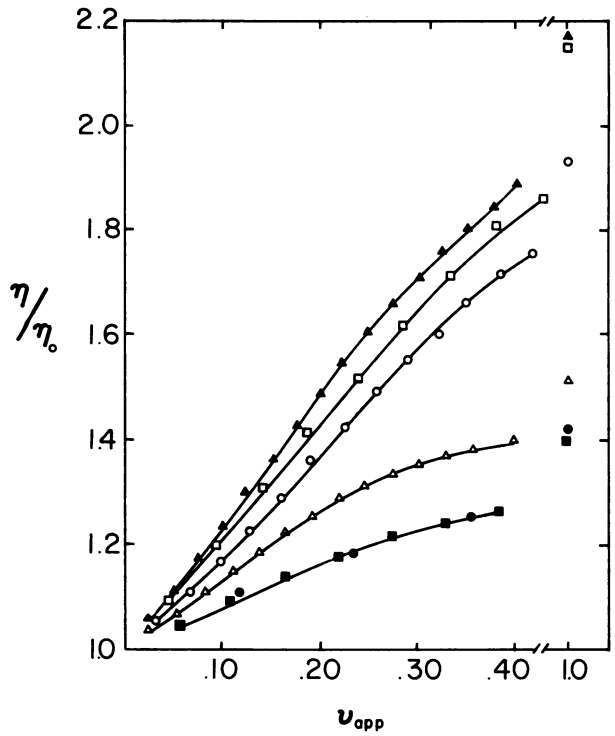
Viscometric titrations were conducted with electronic timing as described previously (5). We have found electronic timing to be essential in these quantitative investigations.

All solutions were prepared in PIPES buffer: 0.01 M 1,4-piperazinediethane sulfonic acid / 10^{-3} M EDTA adjusted to pH 7.0 with NaOH. To obtain high ionic strengths, NaCl was added to this basic buffer before pH adjustment.

RESULTS

SONICATED DNA.

Viscometric titrations with sonicated DNA and the compounds shown in Figure 1 are plotted in Figure 2. All compounds increase viscosity as expected for intercalating compounds. At the highest ratio of ligand to DNA (ν_{app}=1.0) the intercalation sites for quinacrine, 9AA, and ethidium are essentially saturated, under these conditions, and their η/η_o values are constant. Although some non-intercalative binding of these compounds no doubt occurs after saturation of the intercalation sites, this type binding has a



2. Viscometric titration of sonicated calf thymus DNA by ethidium (○), quinacrine (▲), chloroquine (△), 9-aminoacridine (□), 4-aminoquinaldine (●), 9-amino-1,2,3,4-tetrahydroacridine (■) conducted at 25°C in PIPES buffer as described in the experimental section. The reduced specific viscosity ratio (η/η_0 where $\eta = \eta_{sp}/C$) is plotted as a function of moles of drug added per mole of DNA base pairs (ν). η_0 is the reduced specific viscosity of DNA solution alone.

negligible effect on linear DNA viscosity. The η/η_0 values for chloroquine, 4AQ, and TH9AA are still slowly increasing even at this high ratio (ν_{app}=1.0) and it is impossible to reach a limiting value of η/η_0 for these compounds in a viscometric titration. For ethidium, quinacrine, and 9AA ν_{app} is essentially equal to ν (actual moles of ligand bound/mole of DNA-base pairs) for ν_{app} values less than 0.3. This is not true for the three weaker binding quinoline derivatives shown in Figure 2. Saturation of intercalation binding sites and exact quantitation of binding of these compounds are quite difficult due to competition from nonintercalative binding modes as will be shown below.

CLOSED CIRCULAR SUPERHELICAL DNA.

Vinograd and coworkers (10) have suggested that quantitative measurements of unwinding of closed circular superhelical DNA should be analyzed from several viscometric titrations at varying DNA concentrations. The results are then analyzed with the following equation:

$$C_T' = \nu N_T' + C_F' \qquad (1)$$

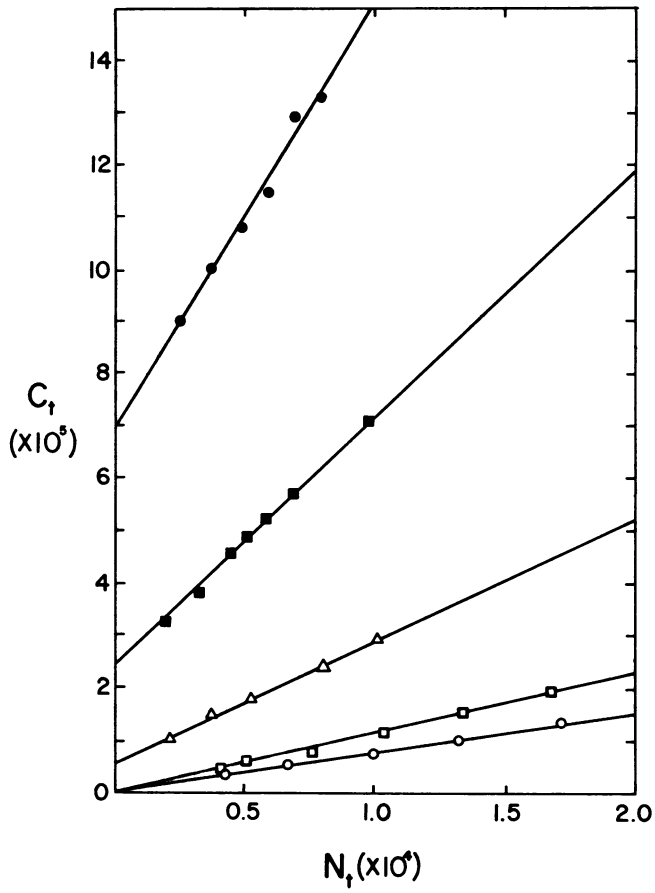
where C_T' = total drug concentration, $\nu' = C_B/N_T$, C_B' = bound drug concentration, N_T' is the total DNA concentration in base pairs, C_F' = free drug concentration, and all quantities are those determined at the principal maximum in a viscometric titration (5,10). Using experimentally determined ν' values from equation (1), unwinding angles ϕ_u can be calculated from equation (2):

$$\phi_u = \frac{\phi_s \cdot \nu_s'}{\nu_u'} \qquad (2)$$

where ϕ_s is the known unwinding angle for a standard compound such as ethidium bromide, and ν_u' and ν_s' are determined from a plot according to equation (1) for the unknown and standard respectively (5,10). The ν_s' and ν_u' values can also be determined independently through a single viscometric titration and appropriate thermodynamic characterization of the ligand-DNA interaction. The Vinograd method does not require assumptions about binding models for fitting binding data and allows a direct linear plot for minimization of the error in determining ν' values. For these reasons we feel this is the preferable method for quantitation of unwinding.

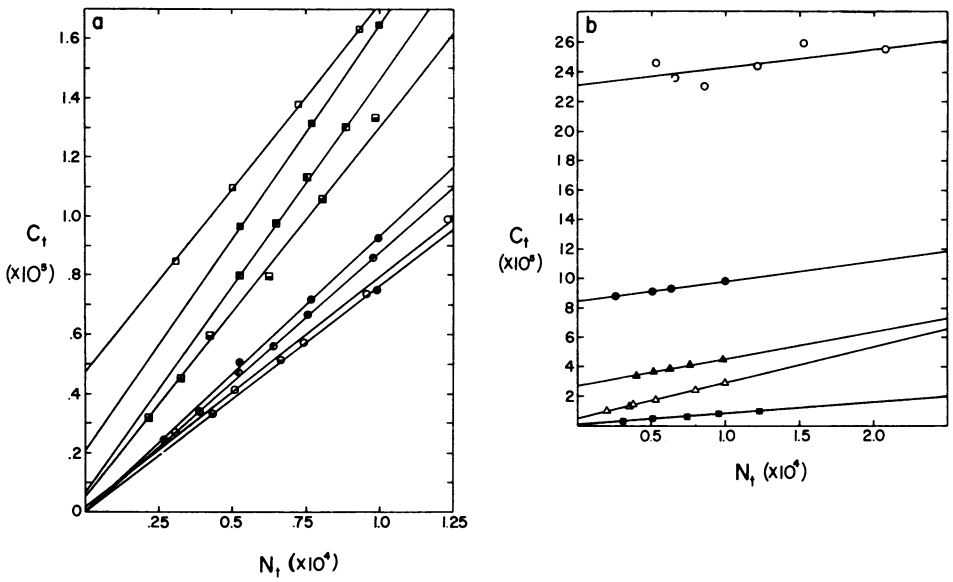
Plots for the compounds shown in Figure 1 are collected in Figure 3 at a single ionic strength with the total DNA concentration varied as illustrated. The effect of changing salt concentration is indicated with similar plots in Figure 4 for ethidium, 9AA, and chloroquine. Quinacrine is omitted from this Figure to prevent the confusion of many overlapping lines. Because of the weak binding of 9ATHA and 4AQ, they could not be quantitatively analyzed at all higher ionic strengths. Results from Figures 3, 4 and other similar experiments are collected in Table 1. 9ATHA was analyzed at the two lowest ionic strengths while 4AQ could be quantitatively examined only at the lowest ionic strength. The problem with compounds which bind weakly is that large amounts of compound are required to unwind the DNA and the resulting titration curves have only broad flat maxima. This gives large errors in evaluating C_T' for equation (1).

We have also encountered some difficulty in analyzing the quinacrine unwinding angle at the lowest ionic strength. In contrast to chloroquine, the



3. A plot of N_T vs C_T according to equation (1) for ethidium (○), 9AA (□), chloroquine (△), 4AQ (●), TH9AA (■), in PIPES buffer at 25°C. The solid lines in the figure were calculated using a linear regression computer program. Slopes and standard deviations were determined from the computer program. C_T is the total drug concentration and N_T is the DNA concentration in base pairs.

quinacrine unwinding angle apparently increased at low ionic strength. We now feel that this might have been due to partial precipitation of the DNA by quinacrine creating an apparently low C_T' value for unwinding. Titrating with more dilute drug solutions, with slow addition, and with continuous mixing during addition eliminated these problems. The quinacrine unwinding angle of approximately 17° apparently is essentially ionic strength independent.



4a. Viscometric titrations for ethidium (◒) .007 M Na^+, (◐) .017 M Na^+, (●) .037 M Na^+, (○) .107 M Na^+, and 9-aminoacridine (◒) .007 M Na^+, (◧) .017 M Na^+, (■) .037 M Na^+, (□) .107 M Na^+, as a function of ionic strength in PIPES buffer with added NaCl to obtain the desired ionic strength.

4b. Viscometric titrations for ethidium (■) (all plots from Figure 4a overlap) and chloroquine, (△) .007 M Na^+, (▲) .017 M Na^+, (●) .037 M Na^+, (○) .107 M Na^+, plotted as in Figure 4a according to equation (1). All titrations were conducted at 25°C in PIPES buffer with NaCl added to achieve the desired ionic strengths. The scales are changed in Figures 4a and 4b to show all data. DNA concentrations (N_T) are in base pairs.

Literature values for the quinacrine unwinding angle vary from 26° (15) to 17° (16) based on 26° for ethidium. This may be due to experimental problems similar to those encountered by us.

Since it has been shown that the unwinding angle of ethidium is essentially independent of ionic strength (7,8,14), unwinding angles for other compounds can be calculated, using equation (2), relative to ethidium at any ionic strength desired. Unwinding angles at several different ionic strengths were calculated from ν' values in Table I and are also included in Table I. It is clear from these results that the apparent unwinding angles for chloroquine, 9ATHA and probably 4AQ are ionic strength dependent. A plot of unwinding angles from Table I as a function of the square root of the sodium

TABLE I

INTERCALATION UNWINDING ANGLES AS A FUNCTION OF Na^+ CONCENTRATION

$[Na^+]$	Ethidium	Quinacrine	9-AA	Chloroquine	9ATHA	4AQ
0.007 M	26°[a]	17.3° ± 0.6°	16.0° ± 0.6°	8.6° ± 0.4°	3.9° ± 0.2°	2.5° ± 0.2°
	.077[b] ± .004	.117 ± .004	.125 ± .005	.234 ± .010	.510 ± .022	.81 ± .074
0.017 M	26°	18.0° ± 1.5°	16.1° ± 0.3°	13.0° ± 1.7°	13.8° ± 0.9°	
	.087 ± .003	.127 ± .011	.140 ± .003	.174 ± .025	.164 ± .012	
0.037 M	26°	18.3° ± 0.4°	16.8° ± 0.3°	17.3° ± 1.0°		
	.093 ± .005	.132 ± .003	.144 ± .003	.140 ± .008		
0.107 M	26°	15.9° ± 0.6°	16.0° ± 0.2°	16.8° ± 7.2°		
	.077 ± .004	.126 ± .030	.125 ± .002	.119 ± .090		
0.207 M	26°	16.7° ± 0.7°				
	.079 ± .003	.123 ± .005				

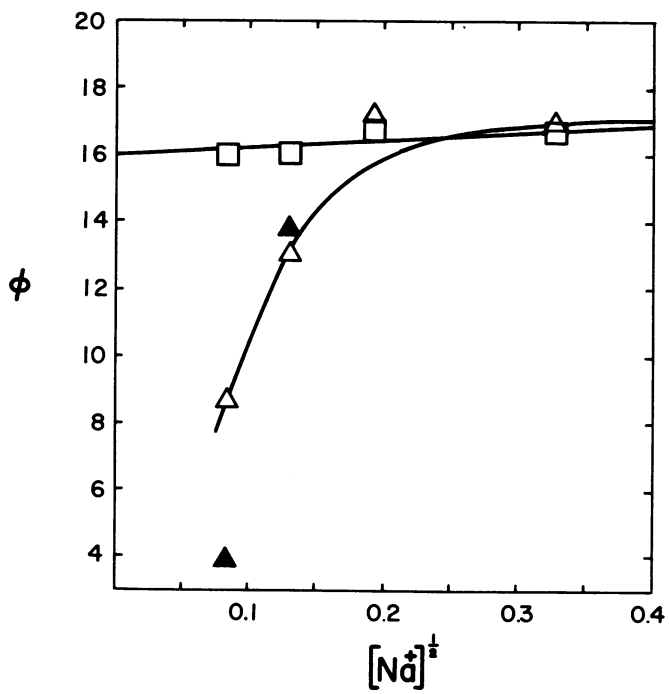
a) Standard value; b) ν values are below the unwinding angles and were determined from the slope of C_T versus N_T plot by a linear regression computer program. Errors were taken from the 95 percent confidence limits.

The unwinding angles were determined from Equation (24) using ν values determined as described; errors are relative to the deviations in ν.

ion molarity is shown in Figure 5. The chloroquine results indicate that the unwinding angle approaches a limiting unwinding angle of approximately 17° at high ionic strength. The 9ATHA results could not be analyzed at high enough ionic strength to establish a limit, but the behavior as a function of ionic strength is obviously similar to chloroquine. An empirical plot of Φ versus the reciprocal of the sodium ion molarity (not shown) gave the following limiting unwinding angles ($1/[Na^+] = 0$): quinacrine, 17°; 9AA, 17°; and chloroquine, 20°.

DISCUSSION

We have for some time been interested in the effects of drug molecular structure on intercalation complexes of quinoline and acridine derivatives (cf. 3-5). We had hoped to quantitatively compare unwinding angles for a closely related series of compounds, 4AQ, 9ATHA, and 9AA, which bridge the



5. Results from Table I are plotted as Φ versus the square root of the Na^+ concentration. Quinacrine (▲), chloroquine (Δ), and 9-aminoacridine (□) all have approximately the same ϕ at $[Na^+]$ of 0.327 as seen from Table I.

quinoline and acridine ring systems. Our previous results suggested that these ring systems might behave differently even when similarly substituted (5). Results with 4AQ and 9ATHA at low ionic strength, shown in Figure 3 and Table I, however, present a problem in that their apparent ν values are greater than 0.5, which is the maximum allowed by the intercalation exclusion principle (11,12). These results could be explained by a nonintercalative binding mode which also contributes to ν. Since this "outside" binding usually is primarily an electrostatic interaction, it should be eliminated at lower salt concentrations than intercalative binding. For example, the apparent ν' could be expressed as the sum of two terms:

$$\nu' = \nu'_o + \nu'_i \qquad (3)$$

where ν'_o and ν'_i are the values for outside bound and intercalated ligand respectively, determined from the ν' value at the maximum in a viscometric titration. It is generally assumed that ν'_i, corrected for superhelix density, is independent of salt. If the above assumptions are correct, a plot of Φ, determined from ν', versus $[Na^+]$ should increase and approach the intrinsic intercalation unwinding angle. We have analyzed chloroquine up to a sodium ion concentration of 0.107 M and the results are as predicted above and suggest that the apparently high ν values obtained for chloroquine at low ionic strength are due to nonintercalative binding. It has long been recognized that outside binding is more sensitive to ionic strength changes than intercalation (17). The application of the ion condensation theory for polyelectrolytes to DNA-ligand interactions provides a framework for understanding these effects. A plot of log K_{obs} versus -log $[Na^+]$ (where K_{obs} is the observed equilibrium constant for the ligand DNA interaction) will have a slope equal to the number of ion pairs formed between the ligand and DNA times a constant. The intercalated complexes of dicationic ligands like chloroquine and quinacrine form two ion pairs (18) while monocations like 9AA and ethidium form only one pair. With outside binding, however, the ligands stack along the outside of the polyanionic double helix (17) and the resulting number of ion pairs could be quite large. The slope of the log K_{obs} versus -log $[Na^+]$ plot will thus be much larger for outside binding than for intercalation. For compounds such as the quinolines which stack well along DNA, but are weak intercalators, more of the compound may be bound outside than by intercalation at low ionic strength. The unwinding angles for 9AA and quinacrine change only slightly over the sodium ion concentration that can be analyzed suggesting that for the low ν' values obtained experimentally for these compounds, intercalation is the only signifi-

cant binding mechanism.

An important finding from these experiments is that quinacrine, 9AA, and chloroquine all approach a limiting unwinding angle of approximately 17° at high salt. It has also been shown that proflavine has an unwinding angle of approximately 17° at ionic strengths of 0.02 (19) and 0.2 (20). These results suggest that aminoacridines and perhaps aminoquinolines, substituted as in the above compounds, have a common intercalation unwinding angle even though they have significant differences in structure and binding constant. This value of 17° is significantly below the unwinding angle of 26° for ethidium and similar diaminophenyl phenanthridines which vary in quaternizing group (propidium and dimidium) (21). Waring has suggested that an unwinding angle below 26° could be caused by nonintercalative binding (9). Although outside binding affects the apparent unwinding angle for chloroquine dramatically (Figure 5), the limit approached is near 17° not 26°. Since 9AA, quinacrine (Figure 5) and proflavine (16,17) also give 17° unwinding angles at ionic strengths above 0.05, it would seem that some molecular feature of the intercalated acridine complex accounts for this lower unwinding angle relative to ethidium. The anthracycline drugs have apparent unwinding angles of 12° (9) and it will be of interest to test these and other ring systems to determine if their unwinding angles are characteristic of the intercalated complex or of a competing nonintercalative binding mode. From all of these results it seems likely that there is, in general, a free energy minimum for unwinding the double helix in an intercalation complex of around 17°. This value could then be increased as with phenanthridines (21) or decreased as with anthracyclines (9) due to other stabilizing features (such as hydrogen bonds) which are predicted to exist in the phenanthridine (6,22) and anthracycline (23) complexes with DNA.

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