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Computer simulation of the binding of amonafide and azonafide to DNA

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Summary

Intercalative binding of the antitumor drugs amonafide and azonafide to the oligonucleotide duplex d(GGCCGGCCGG)•d(CCGGCCGGCC) was compared using molecular dynamics in vacuum with the AMBER force field. A number of reasonable possible binding conformations were obtained, with the azonafide complexes favored over the amonafide complexes in net binding enthalpy. In comparison with amonafide, the larger chromophore of azonafide permits greater DNA distortion and wider side-chain swings, without falling out of the intercalation site. The best model obtained was used for further dynamics on amonafide and azonafide with solvent and counterions present, and again the azonafide complex had a more favorable enthalpy. Furthermore, the enthalpy change on going from solvent into the intercalation site was less unfavorable for azonafide. These results are consistent with the stronger DNA binding of azonafide compared to amonafide, as observed in relative melting transition temperature increases and tumor inhibition in cell cultures.

Introduction

Amonafide (**1**) and its nitro analogue mitonafide (**2**) are new antineoplastic agents [1,2], which have received clinical trials [3–6]. They act by inhibiting macromolecular synthesis, especially of DNA [2]. This effect appears to be based on DNA intercalation [7], which results in protein-associated single-strand breaks mediated by inhibition of the DNA nicking–closing enzyme topoisomerase II [8]. As part of our program on the design and synthesis of new antitumor agents, we became interested in analogues of amonafide that might possess increased DNA binding strength, in the expectation that this effect would result in increased antitumor potency. One such analogue is azonafide (**3**), which has the amino group of amonafide replaced by a new aromatic ring, extending the chromophore from naphthalene to anthracene [9]. Preliminary molecular modeling (W.A. Remers, unpublished results), using only molecular mechanics, indicated significant overlap of the anthracene chromophore with DNA base pairs and a stronger net binding energy for azonafide compared to amonafide for the DNA segment d(GGCCGGCCGG)•d(CCGGCCGGCC). Azonafide (**3**) was synthesized and found to be considerably more potent than amonafide (**1**) against a variety of tumors in cell culture. Azonafide also raised the melting transition temperature (ΔT_m) of calf thymus DNA by a greater amount than did amonafide (15.2 versus 3.5 °C) [9]. Although the intercalation of azonafide has not been proven rigorously, the supporting evidence is strong. It includes the ΔT_m value, which is consistent with strong intercalators such as doxorubicin, a shift in visible absorption maximum from 443 to 457 mm in the presence of DNA [8], and the mode of action, which comprises protein-associated single-strand breaks involving inhibition of topoisomerase II [10].

Based on these results, we thought it desirable to develop a more sophisticated model for the binding of amonafide and azonafide to an appropriate DNA segment. Such a model could help in explaining the apparently stronger binding of azonafide and it might aid in the design of more potent azonafide and amonafide analogues. Our finding that in a series of amino-substituted azonafides there was a high correlation of antitumor potency with ΔT_m values indicates that DNA binding strength contributes more to activity than other factors, such as partition coefficient [11]. Consequently, there is a reasonable possi-

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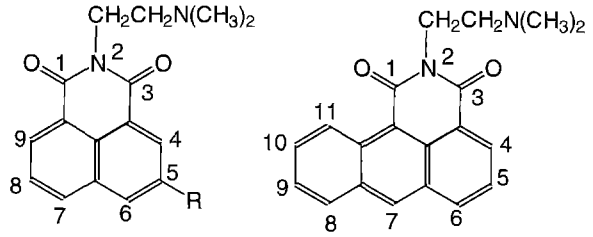

1 R = NH_2 (amonafide)
2 R = NO_2 (mitonafide)

3 (azonafide)

Fig. 1. Structures of amonafide, mitonafide and azonafide.

bility that the design of stronger DNA-binding molecules may be translated into greater antitumor potency.

In order to develop a DNA binding model suitable for the optimization of intercalating structures, we took into account the following considerations.

(1) A DNA sequence should have GC pairs at the ends to prevent fraying during molecular dynamics at elevated temperatures, it should have GC pairs at its center because many intercalating agents, including mitonafide, are GC specific [12,13], and it should be sufficiently long to approximate a region of DNA. The decanucleotide duplex d(GGCCGGCCGG)•d(CCGGCCGGCC) was chosen both for these reasons and because the coordinates of this duplex, unwound to permit intercalation between the central base pairs, were available (kindly provided by Dr. Michael Corey, Burroughs Wellcome Laboratories, Research Triangle Park, NC).

(2) The drug molecules should be protonated on the side-chain nitrogen. Amonafide and azonafide have pK_a values of 7.3, which means that they are approximately 67% protonated at the pH of 7.0 used in the ΔT_m measurements and in the antitumor assays. It is expected that protonated molecules would compete favorably with unprotonated ones for DNA binding sites.

(3) A minimum of four possible binding modes can be envisaged for amonafide or azonafide with the chromophore intercalated: with the side chain in the major groove or the minor groove, and the chromophore inserted one way (closer to dG5 and dG6, see Fig. 3) or flipped over (closer to dC15 and dC16) for each of major and minor groove selectivity. The drugs should be modeled in all four of these modes, with the side chain near the center of the groove. If the side chain moves to one side during dynamics, it should be redocked with the side chain as far as possible to the opposite side to make certain that conformational space was searched completely. Temperatures should be raised until the helix almost becomes unstable, notably by bending and loss of interstrand hydrogen bonding, in order to overcome local minima in the calculations.

(4) The initial molecular dynamics simulations should be performed in vacuum. Charges on the phosphate groups should be reduced to approximate the effects of counterions [14] and to remove the otherwise dominating

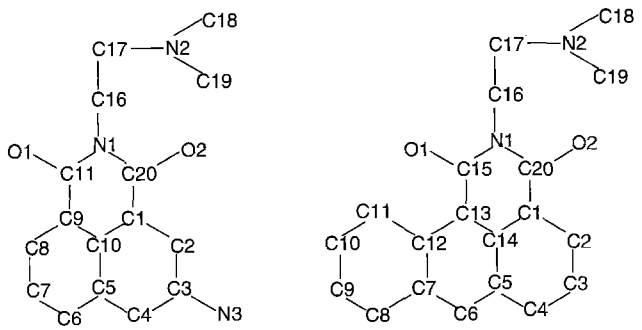

Fig. 2. Numbering of amonafide (left) and azonafide (right) for AMBER files. Hydrogen atoms bear the numbers of heavier atoms to which they bind (e.g., H2, HN3A).

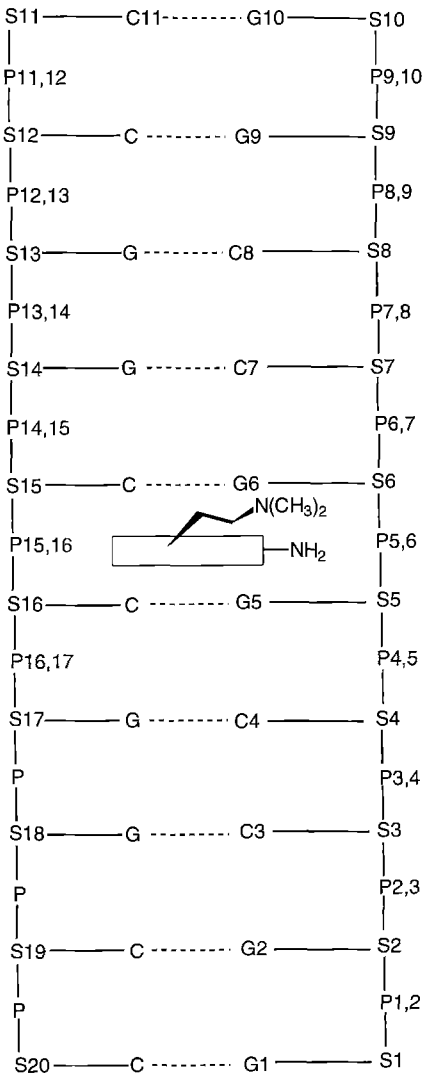

Fig. 3. Schematic representation of d(GGCCGGCCGG)•d(CCGGCCGGCC). S stands for sugar. In this illustration, the amino group of intercalated amonafide (N3) points toward a guanine (dG6) and the side chain is up and to the right of the major groove. Thus, its orientation is maj GUR.

TABLE 1
ADDITIONAL BOND, BOND ANGLE AND TORSIONAL PARAMETERS NOT GIVEN IN REF. 19.

Bond	k_b (kcal/mol Å^2)	R_b (Å)	Angle	k_a (kcal/mol rad^2)	a (deg)	Dihedral	k_d (kcal mol)	a (deg)	n
C-C	469.0	1.394	C-C-C	85.0	120.0	X-C-C-X	4.0	180.0	2
C-HC	340.0	1.084	C-C-N	70.0	118.9				
			C-C-HC	35.0	120.0				
			C-C-O	80.0	119.25				

attraction between the negatively charged phosphate oxygens and protonated nitrogen on the drugs. A dielectric constant significantly higher than 1.0 should be used to approximate the effect of solvent.

(5) On completion of the simulations in vacuum, the best models should be subjected to dynamics in the presence of water to see how much they might change. Modeling the drugs alone in water and comparing the results with those obtained on the solvated drug–DNA complexes provides an opportunity to determine the overall driving force for DNA intercalation as measured by net DNA binding enthalpy and desolvation enthalpy [15].

Methods

Following the considerations just outlined, a number of models for the intercalative binding of amonafide and azonafide with d(GGCCGGCCGG)•d(CCGGCCGGCC) were prepared. Structures for the drugs were built using CHEMLAB II [16] and the coordinates were transferred into AMBER 3.0 [17]. Preliminary minimization was conducted using the all-atom parameters contained in this program [18]. Some additional parameters not included in AMBER, listed in Table 1, had to be defined. A distance-dependent dielectric constant was used and the structure was refined until the rms gradient was less than 0.1 kcal/mol Å. The cutoff distance was 99 Å and the nonbonded pair list was updated every 100 cycles. The refined coordinates were used to calculate partial atomic charges (ESP) with QUEST 1.0 [16]. These charges are listed in Table 2. The structures were again refined by molecular mechanics. The oligonucleotide duplex was also generated in the NUCGEN module of AMBER, using modified phosphate groups with the charge on each phosphate oxygen not attached to sugars reduced by 0.25 (to –0.597) in the AMBER force field. These charges were developed by Hingerty et al. from values provided in AMBER, and were based on an earlier development by Taylor and

TABLE 2
ESP ATOMIC CHARGES FOR AMONAFIDE AND AZONAFIDE CATIONS

Amonafide				Azonafide			
Atom[a]	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C2	–0.047	H16B	0.108	C2	0.088	O1	–0.431
H2	0.094	C17	–0.421	H2	0.054	N1	–0.413
C3	0.431	H17A	0.187	C3	–0.124	C16	0.044
N3	–0.903	H17B	0.166	H3	0.076	H16A	0.112
HN3A	0.329	N2	0.535	C4	–0.082	H16B	0.108
HN3B	0.336	C18	–0.403	H4	0.078	C17	–0.421
C4	–0.280	H18A	0.158	C5	0.091	H17A	0.187
H4	0.120	H18B	0.178	C6	–0.199	H17B	0.116
C5	0.091	H18C	0.151	H6	0.116	N2	0.535
C6	–0.113	C19	–0.522	C7	0.108	C18	–0.403
H6	0.089	H19A	0.198	C8	–0.159	H18A	0.158
C7	–0.101	H19B	0.166	H8	0.082	H18B	0.178
H7	0.048	H19C	0.220	C9	–0.047	H18C	0.151
C8	0.090	HN2	0.215	H9	0.063	C19	–0.522
H8	0.073	C20	0.743	C10	–0.076	H19A	0.198
C9	–0.355	O2	–0.422	H10	0.071	H19B	0.166
C10	0.188	C1	–0.374	C11	–0.132	H19C	0.220
C11	0.685			H11	0.086	HN2	0.215
O1	–0.413			C12	0.221	C20	0.711
N1	–0.398			C13	–0.483	O2	–0.381
C16	0.440			C14	0.233	C1	–0.341
H16A	0.112			C15	0.698		

[a] The numbering of atoms for the two compounds is indicated in Fig. 2.

TABLE 3
INTERACTION ENTHALPIES (kcal/mol) FOR AMONAFIDE INTERCALATED WITH d(GGCCGGCCGG)•d(CCGGCCGGCC)

Docking orientation[a]			MD time[b]	Intramolecular[c]			Distortion[d]			Intermolecular[e]				Net binding[f]	HN2 H-bonds[g]
Groove	Start	End		Total	DNA	Drug	DNA	Drug	Total	Chrom	Amine	Chain	Total		
maj	CUM	CUR	80	−286.3	−231.8	12.6	7.8	0.2	8.0	−36.3	−2.7	−18.1	−54.4	−46.4	dG6 N7, O6
maj	CUL	CUR	20	−273.4	−222.3[h]	12.8	17.3	0.4	17.7	−33.2	−1.0	−15.9	−51.1	−33.4	dG6 N7, O6
maj	CDR	CDR	80	−288.0	−234.8	12.9	4.8	0.5	5.3	−35.9	−2.6	−17.5	−53.4	−48.1	dG5 N7, O6
maj	GUR	GUR	60	−283.8	−228.7	12.9	10.9	0.5	11.6	−35.2	−0.9	−18.1	−53.3	−41.7	dG6 O6
maj	GDM	GDR	60	−294.2	−239.6	13.0	0	0.6	0.6	−36.4	−0.2	−18.2	−54.6	−54.0	dG5 N7, O6
maj	GDL	GDR	40	−282.6	−228.3	12.9	11.3	0.5	11.8	−36.1	−0.3	−18.2	−54.3	−42.5	dG5 N7, O6
min	GUM	GMR	80	−284.5	−229.2	13.3	10.4	0.9	11.3	−34.1	−1.5	−17.5	−51.6	−40.3	none[i]
min	GUL	GMR	100	−286.6	−228.6	13.2	11.0	0.8	11.8	−38.1	−1.6	−17.7	−55.8	−44.0	none
min	GDR	GUM	60	−291.8	−238.1	12.9	1.5	0.5	2.0	−36.1	−2.0	−17.5	−53.6	−51.6	P7, 8
min	GDR	GDR	120	−282.6	−226.9	13.6	12.7	1.2	14.9	−36.7	−1.4	−19.0	−55.7	−40.8	P16, 17
min	CDM[j]	C,−,L	40	−281.7	−232.9	13.0	6.7	0.6	7.3	−34.7	−1.4	−17.6	−52.3	−45.0	none
min	CUL	CUR	20	−291.5	−235.6	13.1	4.0	0.7	4.7	−35.2	−0.3	−20.6	−55.9	−51.2	dC15 O2

[a] Maj indicates a side chain in the major groove and min indicates a side chain in the minor groove. For the starting orientation, C indicates that the amino group on the chromophore was placed on the side closer to cytosine residues above and below the gap and G indicates that this amino group was closer to the guanines; U means that the side chain was up (toward dG6 and dC15) and D means that it was down; L indicates that the side chain was placed at the left side of the groove, M means that it was in the middle, and R indicates that it was at the right side of the groove. The orientation of this side chain after dynamics (End) follows the same designations as at the start, with the addition of a dash to indicate that the chain was neither up nor down.

[b] The total time for molecular dynamics in picoseconds.

[c] The enthalpy of the oligonucleotide minimized in the absence of amonafide is −239.6 kcal/mol and the enthalpy of amonafide in the absence of oligonucleotide is 12.4 kcal/mol.

[d] Distortion enthalpies are obtained by subtracting enthalpies of uncomplexed oligonucleotide and amonafide from their values in the complexes.

[e] Intermolecular enthalpies are divided into interactions of the chromophore and protonated side chain with the polynucleotide. The contribution of the NH_2 group to chromophore binding is listed separately.

[f] Net binding enthalpies are obtained by adding the total intermolecular and total distortion enthalpies.

[g] Hydrogen bonds formed between the proton on the side-chain nitrogen (N2) and various atoms on the oligonucleotide.

[h] This enthalpy was decreased by a broken base pair (dG1-dC20). The drug binding pattern was nearly identical to that of the preceding example.

[i] An H-bond was made to P6,7 and then broken during dynamics.

[j] This orientation is the only one possible for the C,D orientation. Significant changes are not possible due to steric hindrance.

Olson derived from Manning's original treatment [14]. No other changes were made in the parameters. The dielectric constant was varied until a standard setting of 3.0 was established. It should be noted that this oligonucleotide duplex does not have a gap for intercalation. Subsequent calculations of DNA distortion resulting from drug intercalation will include the enthalpy required to unwind the DNA to generate the intercalation gap. An attempt to conduct dynamics on the opened but unbound decanucleotide duplex resulted in collapse of the gap and considerable distortion of the structure.

For the drugs intercalating DNA, coordinates of the d(GGCCGGCCGG)•d(CCGGCCGGCC) duplex with an intercalation gap between the central base pairs [13] were read into the EDIT module of AMBER, then the drugs were docked in the intercalation gap using the interactive program MIDAS [19] and the approach described above. Amonafide and azonafide were docked in one orientation (amino group or third ring closer to G or C, see Fig. 3) as well as flipped over to give the opposite orientation. This was done for the side chain both in the major groove and in the minor groove. For each of these four orientations, the side chain was placed in the groove in the up (toward the 3' end of the first DNA strand) or down orientation. The following code is used for the docking orientation: NH_2 or third ring of chromophore toward cytosines, C; toward guanines, G; side chain up, U; down, D; or in the middle, M; side chain to the right, R; left, L; or middle, M. Thus, the orientation of the drug in Fig. 3 is CMM. If the side chain moved to one side of the groove during molecular dynamics, it was docked again with the side chain at the opposite side of the groove to ensure that conformational space was thoroughly searched. In a few cases, a third docking was made. Coordinates of the drug docked into the DNA helix were transferred into AMBER for energy refinement with molecular mechanics under the conditions described above.

Following the molecular mechanics, the resulting structures were subjected to molecular dynamics using a 3.0 dielectric constant and a temperature increase from 10 to 400 K. Other investigators have used $\varepsilon = 4.0$ to obtain faster decline of electrostatic forces with distance, but in our model this made the helix unstable. Watson–Crick hydrogen bonds were broken at the ends of the helix. A maximum temperature of 400 K was satisfactory when belly dynamics were used, but a temperature of 500 K caused helix instability. Initial belly dynamics with the

TABLE 4
INTERACTION ENTHALPIES (kcal/mol) FOR AZONAFIDE INTERCALATED WITH d(GGCCGGCCGG)•d(CCGGCCGGCC)

Docking orientation[a]			MD time[b]	Intramolecular[c]			Distortion[d]			Intermolecular[e]				Net binding[f]	HN2 H-bonds[g]
Groove	Start	End		Total	DNA	Drug	DNA	Drug	Total	Chrom	Ring 3	Chain	Total		
maj	GUM	GUR	80	−292.4	−235.9	24.6	3.7	1.0	4.7	−38.4	−7.5	−17.8	−56.4	−51.7	dG6 N7, O6
maj	GUL	GDR	60	−290.9	−233.5	25.6	6.1	2.0	8.1	−38.7	−7.3	−18.7	−57.4	−49.3	dG5 N7, O6[h]
maj	CDM	CUM	40[i]	−280.5	−231.6	25.6	8.0	2.0	10.0	−38.2	−7.5	−10.6	−48.8	−38.8	none
maj	CDR	CDR	100	−297.7	−237.2	25.3	2.4	1.7	4.1	−40.8	−7.4	−19.7	−60.5	−56.4	P4,5
min	CUM	CDR	100	−281.2	−226.7	25.0	12.9	1.4	14.3	−36.7	−6.4	−17.8	−54.5	−40.2	P15,16
min	CUL	CUM	120	−289.8	−223.9	25.1	15.7	1.5	17.2	−41.1	−7.1	−24.8	−65.9	−48.7	P15,16
min	CUM	CDL	60	−295.7	−234.4	25.7	5.2	2.1	17.3	−40.0	−6.5	−21.2	−61.2	−53.9	P5,6
min	GMM	GDL	40[j]	−283.9	−226.4	24.5	13.2	0.9	14.1	−37.6	−5.4	−19.9	−57.5	−43.4	P5,6
min	GMR	GDL	60	−296.5	−236.6	24.7	3.0	1.1	4.1	−38.9	−6.3	−21.0	−59.9	−55.8	P5,6
min	GUR	GUR	20	−294.0	−232.5	25.6	7.1	1.0	8.1	−38.7	−4.3	−22.9	−61.6	−53.5	P15,16

[a] Maj indicates a side chain in the major groove and min indicates a side chain in the minor groove. For the starting orientation, C indicates that the amino group on the chromophore was placed on the side closer to cytosine residues above and below the gap and G indicates that this amino group was closer to the guanines; U means that the side chain was up (toward dG6 and dC15) and D means that it was down; L indicates that the side chain was placed at the left side of the groove, M means that it was in the middle, and R indicates that it was at the right side of the groove. The orientation of this side chain after dynamics (End) follows the same designations as at the start.

[b] The total time for molecular dynamics in picoseconds.

[c] The enthalpy of the oligonucleotide minimized in the absence of azonafide is −239.6 kcal/mol and the enthalpy of azonafide in the absence of oligonucleotide is 23.6 kcal/mol.

[d] Distortion enthalpies are obtained by subtracting enthalpies of uncomplexed oligonucleotide and azonafide from their values in the complexes.

[e] Intermolecular enthalpies are divided into interactions of the part of the chromophore corresponding to that of the amonafide chromophore, ring 3 of the chromophore, and protonated side chain with the oligonucleotide. The contribution of ring 3 to the chromophore binding is listed separately.

[f] Net binding enthalpies are obtained by adding the total intermolecular and total distortion enthalpies.

[g] Hydrogen bonds formed between the proton on the side-chain nitrogen (N2) and various atoms on the oligonucleotide.

[h] The H-bond switches from N7 to O6 during dynamics.

[i] Upon further dynamics the DNA bends progressively worse until at least 100 ps.

[j] Dynamics were continued until 80 ps. The drug–DNA interaction remained the same, but distortions in the total DNA increased slightly.

[k] Dynamics were continued until 60 ps. The drug–DNA interaction remained the same, but distortions in the total DNA increased slightly.

drug and one base pair on both sides free to move were run for 5 ps, then a second run of 15 ps was made with the drug and two base pairs on both sides free to move. Finally, full dynamics were run in 20 ps increments until no significant further change in conformation occurred, according to visual inspection and comparison of enthalpies. When the enthalpy of the helix became progressively worse because of distortions, the simulation was terminated. In one example (Table 4, conformation 3) the result is given at the end of 40 ps because it is a unique conformation, despite its relative instability. In all dynamics simulations, the time step was 0.001 ps, the nonbonded cutoff was 8 Å, and the nonbonded pair list was updated every 25 cycles. SHAKE was applied with all bonds constrained and the end point was rms < 0.1 kcal/mol Å. The simulations were conducted with velocity scaling (coupling to a bath). Results of the molecular dynamics simulations are given in Tables 3 and 4.

For simulations that included the presence of water and counterions, the complexes with the lowest net binding enthalpy for amonafide and azonafide after molecular dynamics in vacuum (Tables 3 and 4) were chosen as starting structures; however, small distortions induced in these dynamics became magnified in the calculations in solvent and this approach was thus abandoned. Instead, both drugs were newly docked, both with approximately the same starting conformation, which was based on the best resulting conformation for azonafide (CMM). Thus, the NH_2 group or third ring of the chromophore pointed toward cytosine and the side chain was in the major groove and centered. These complexes were placed in a 3×3 cube of TIP3P water [20] and the solvent shell thickness was reduced to 8 Å using the EDIT module of AMBER. The oligonucleotide alone was solvated to the same shell thickness. These operations resulted in 2236 waters for DNA alone, 2421 waters for the amonafide complex, and 2457 waters for the azonafide complex. Counterions were placed on the bisector of the phosphates at an initial distance of 4.0 Å between the phosphorus atom and the counterion. The charges on phosphate oxygens were returned to their values in AMBER and a distance-independent dielectric constant of 1.0 was used. The free drugs also were solvated using a cutoff of 10 Å for the water shell.

Solvated DNA and DNA–drug complexes were refined according to the Ross method (W. Ross, University of California, San Francisco, CA, personal communication) of shaking and gradual warming. The initial step was

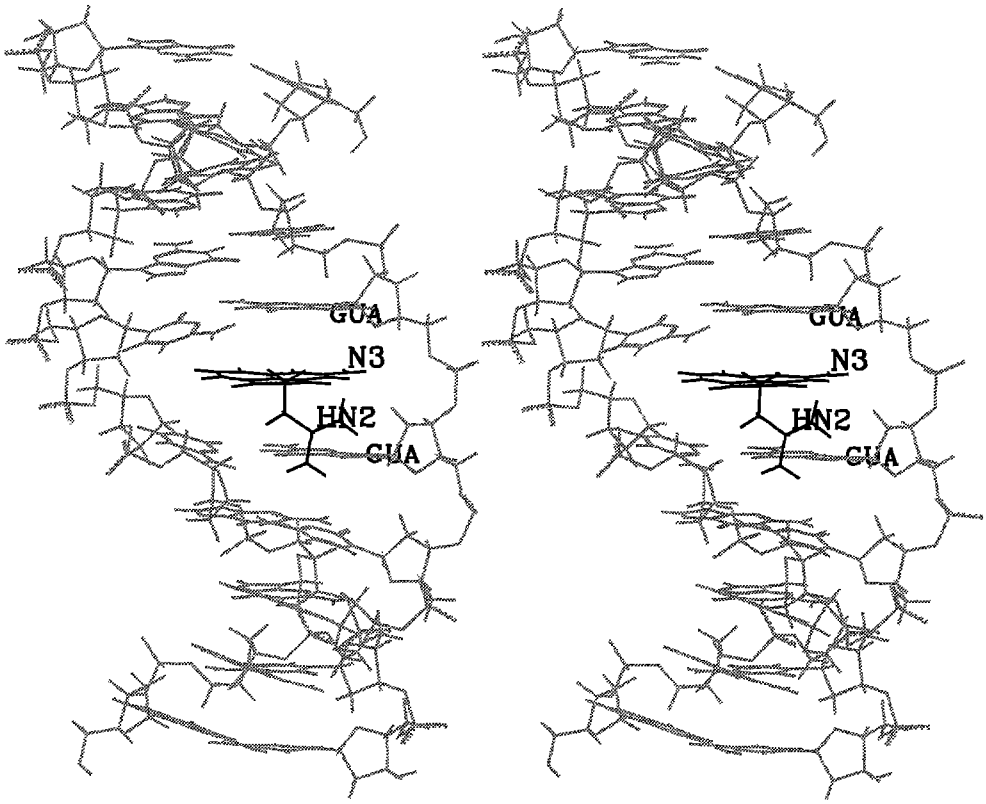

Fig. 4. Stereoview of the lowest enthalpy conformation for the amonafide–DNA complex.

minimization to a gradient of less than 0.5 kcal/mol Å using molecular mechanics in AMBER 4.0 [21] and allowing the drug, solvent, and the two central base pairs to move. A 9 Å cutoff distance was applied. Following this refinement, molecular dynamics were performed with the SANDER module of AMBER 4.0. Initial dynamics permitted only water to move, with the temperature at 1500 K for 3 ps and ramped down to 300 K for 2 ps, and with a 0.02 time constant for the solvent. Another phase of molecular mechanics, with water, the two central base pairs and the drug free to move, was conducted until an rms of 0.5 kcal/mol Å was obtained. Next, dynamics were run with all atoms free to move, except that the counterions were constrained to distances of 2 to 4 Å from the phosphate oxygens. The temperature was ramped up from 10 to 300 K during 5 ps and held constant for 10 ps. A final dynamics run was conducted. Starting at 300 K and without constraints, the run was performed at 300 K for 10 ps. This was followed by molecular mechanics refinement until the rms was 0.2 kcal/mol Å. Analysis of the final conformations utilized the ANAL module of AMBER 4.0 and was confined to the intercalation area of the drug (if any) and two base pairs above and below, because the ends of the DNA had lost some Watson–Crick hydrogen bonds, as often occurs in dynamics.

Uncomplexed amonafide and azonafide were placed in 3×3 cubes of TIP3P water and the solvent shell thickness was reduced to 10 Å. The resulting complexes were refined by molecular mechanics until the rms was 0.2 kcal/mol Å. Dynamics were run for 10 ps at 300 K with all atoms free to move, no ramping, and a cutoff distance of 8 Å. Following another molecular mechanics refinement, dynamics were repeated under the same conditions and then molecular mechanics were conducted a final time. The results of the first and second dynamics runs displayed very little difference.

Results and Discussion

In the initial molecular dynamics simulations in vacuum, phosphate groups were assigned a full negative charge and a dielectric constant of $\varepsilon = 1.0$ was used. As a result of using these parameters, the amonafide and azonafide molecules moved and changed side-chain conformation to give strong binding with phosphate groups on the oligonucleotide. This problem has been observed by others and a variety of methods have been devised to overcome it and to provide complexes that more closely resemble the situation where solvent and counterions are present. These methods are based on increasing the dielectric constant and reducing charges on the phosphate group. We found that reducing the phosphate charges to one half [18] and increasing the dielectric constant to 3.0 produced a variety of drug nucleus and side-chain orientations at the intercalation site, without permitting the oligonucleotide to unravel when the temperature for the dynamics simulation was increased. Under these conditions, and using belly dynamics for initial simulations,

temperatures of 400 K could be used. The standard protocol that evolved included docking the drug into the oligonucleotide in a variety of orientations (Tables 3 and 4), minimizing the enthalpy by molecular mechanics until the rms was 0.1 kcal/mol Å, conducting 5 ps of belly dynamics with only the drug and one base pair on each side unconstrained and then 15 ps of belly dynamics with the drug and two base pairs on each side unconstrained, and finally fully unconstrained dynamics in 20 ps segments until no further change was observed in the conformation of the complex.

The relatively large number of dockings was used to ensure that conformational space was searched completely. As expected, some of these dockings led to nearly identical conformations after molecular dynamics. Nevertheless, the number of resulting unique models was greater than the theoretical minimum of four per drug. As shown in Table 3, the 12 initial dockings for amonafide give final orientations that could be reduced to 8 unique ones by combining orientations that are nearly the same (e.g., three for CDR). The resulting unique orientations are the following: CUR, CDR, and GUR for the major groove and GMR, GUM, GDR, CML and CUR for the minor groove. It is difficult to select a single preferred mode of binding from this group of models, because the enthalpy differences among the best ones are small. Thus, only 2.7 kcal/mol separates GDR in the major groove, GUM in the minor groove, and CUR in the minor groove; however, the GDR conformation was chosen for comparison with the best azonafide model in calculations on solvent effects.

Table 3 gives a breakdown of binding and distortion enthalpies into their components for amonafide. Total intermolecular binding energies do not differ greatly among the various orientations, falling in the range of −51.1 to −55.9 kcal/mol, and their component binding enthalpies for chromophore, amino substituent on the nucleus (N3), and side chain are also very close. The main difference in net binding enthalpies (total of intermolecular binding and distortions) lies in the distortion of the oligonucleotide (DNA) and amonafide (drug) that occurs when the enthalpy of the system is minimized. There is almost no distortion in the drug or decanucleotide in the GDR final orientation in the major groove, but the CUR orientation has 17.3 kcal/mol distortion in the decanucleotide. Hydrogen bonding by the protonated tertiary amine in the side chain (N2) contributes to the stability of the complexes, although some of the resulting minor groove orientations do not have such bonds. The major groove binding models all have H-bonds to O6 of either dG5 or dG6 and all models but one have additional H-bonding to N7 of these guanines. For the minor groove orientations, H-bonds are formed with a phosphate oxygen or with O2 of dC15, or no H-bond to the

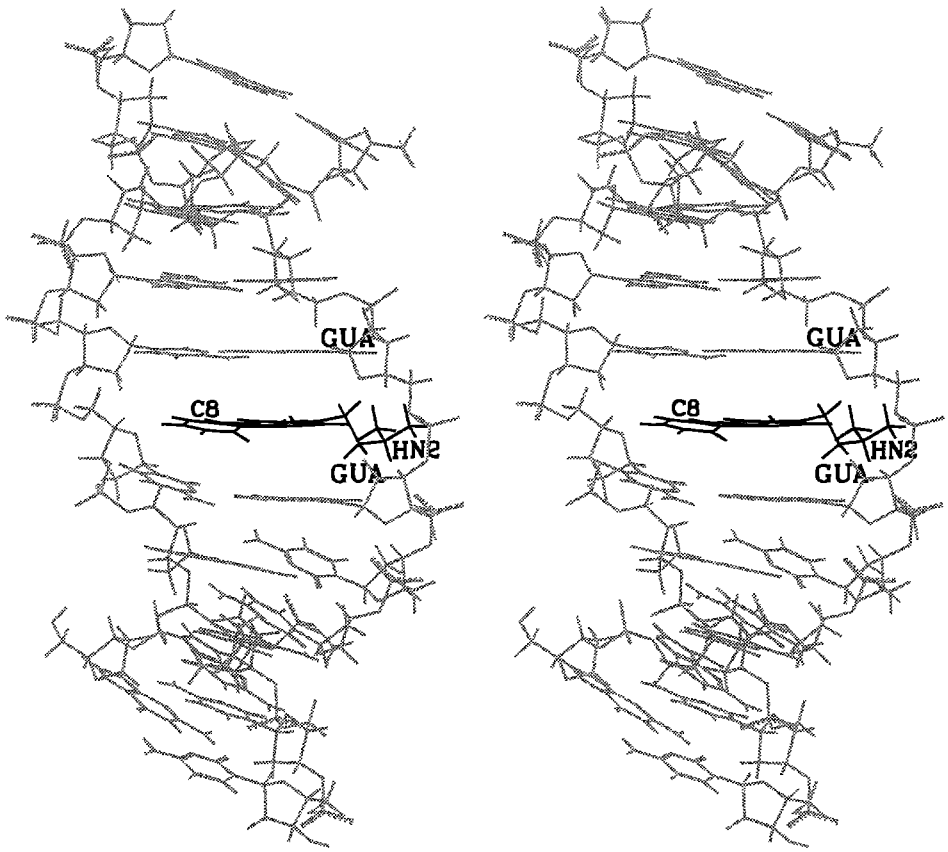

Fig. 5. Stereoview of the lowest enthalpy conformation for the azonafide–DNA complex.

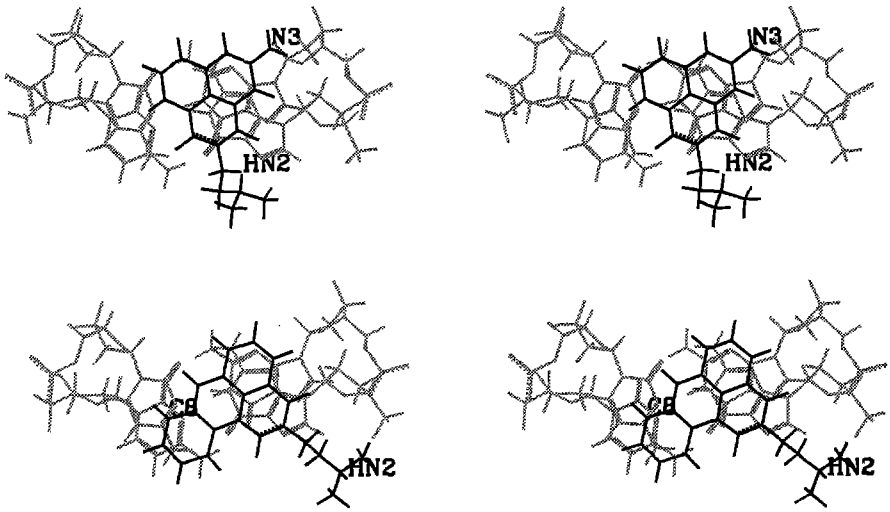

Fig. 6. Stereoviews of the central portions of the amonafide (top) and azonafide (bottom) complexes, showing overlaps of the chromophores and DNA base pairs.

DNA is formed. The drug side chain is more likely to bind with a phosphate in the minor groove than in the major groove, because the former is narrower and the phosphates are closer to the center.

The results of molecular dynamics simulations on azonafide intercalation complexes with the decanucleotide are given in Table 4. There are four and five distinct conformations for major and minor groove binding, respectively. It is again difficult to select a single preferred binding mode, because the CDR conformation in the major groove and the GMR conformation in the minor groove have an enthalpy difference of only 0.5 kcal/mol. The former conformation was used for a comparison with the best amonafide model in calculations on solvent effects. Total intermolecular binding enthalpies vary among the azonafide models from −48.9 to −61.6 kcal/mol, which is a wider spread than that observed for the amonafide models. The distortion in the decanucleotide duplex varies from 2.4 to 14.5 kcal/mol (1–7% of the total DNA enthalpy), but there is little distortion of azonafide in any model. In comparison with amonafide, the larger chromophore of azonafide permits a greater reach and wider side-chain swings, without falling out of the intercalation site. The side chain of azonafide bound to DNA displays less tendency to rotate than that of amonafide, because in most cases interactions with phosphate oxygens are strongly preferred to those with atoms on the bases. There are, however, interactions with N7 and O6 of guanines in the major groove and with O2 of dC15 in the minor groove.

Stereopairs for the lowest enthalpy conformations of amonafide and azonafide with the decanucleotide duplex

TABLE 5
INTERACTION ENTHALPIES (kcal/mol) FOR SOLVATED AMONAFIDE AND AZONAFIDE AND THEIR SOLVATED COMPLEXES WITH d(GGCCGGCCGG)•d(CCGGCCGGCC)[a]

Compound	Drug	Drug–DNA complex							Net enthalpy changes			
	Drug–wat	Drug–wat	DNA	DNA dist[b]	Drug	Drug dist[c]	Drug–DNA	Group–DNA[d]	Hydration[e]	DNA binding	Process[f]	Difference[g]
Amonafide	−54.6	−25.8	−47.9	15.6	12.8	0.4	−47.9	−1.8	28.8	−31.9	−3.1	−4.7
Azonafide	−49.7	−24.2	−39.5	24.0	22.9	0.0	−57.3	−10.3	25.5	−33.3	−7.8	

[a] The side chain was centered in the major groove and the NH_2 group or third ring was directed toward cytosine (CMM).
[b] The enthalpy of uncomplexed DNA is −63.5 kcal/mol and distortions are calculated by subtracting this number from the enthalpy of DNA in the complex. The value for uncomplexed DNA is that obtained for the oligonucleotide not open for intercalation. Thus, the distortion includes unwinding the strands to form the intercalation site.
[c] Enthalpies for the uncomplexed drugs are 12.4 and 22.9 kcal/mol for amonafide and azonafide, respectively. Drug distortion enthalpies are calculated by subtracting these numbers from their enthalpies in the complexes.
[d] The group is the 5-NH_2 of amonafide or the four new CHs of the third ring of azonafide.
[e] The enthalpy change on hydration is the result of loss of binding enthalpy between drug and water that occurs on intercalation. It is obtained by subtracting the value for the drug–water interaction from the corresponding value of the drug–water interaction in the complex.
[f] The net enthalpy change of the process results from the drug going from water into the solvated DNA intercalation complex. It is obtained by adding the net hydration enthalpy change to the net DNA binding enthalpy change.
[g] The difference in enthalpy for the intercalation processes for amonafide and azonafide partially explains the greater binding ability of azonafide.

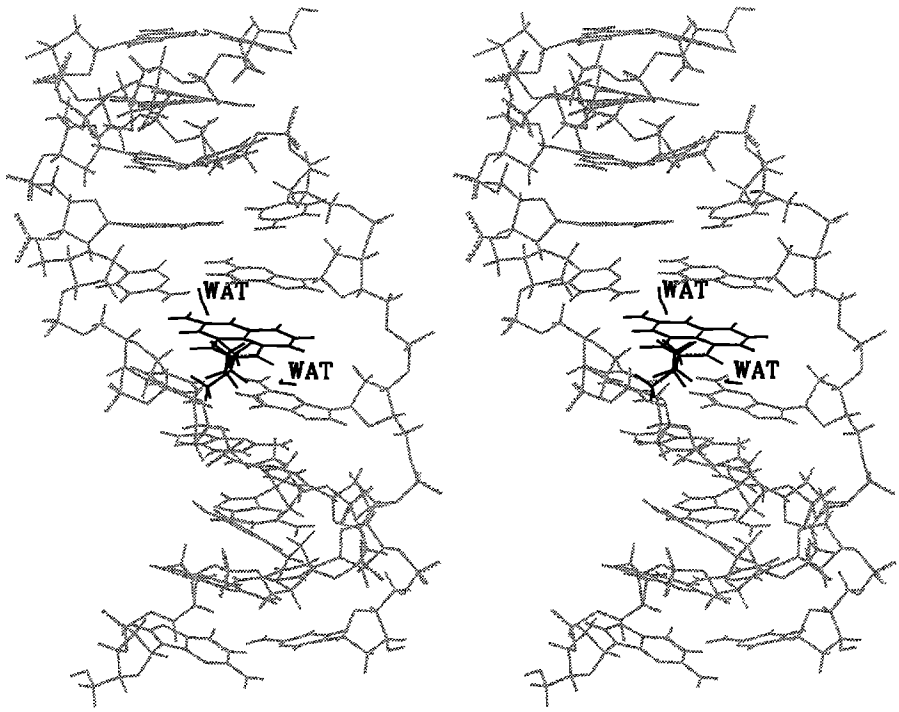

Fig. 7. Stereoview of the conformation of the solvated amonafide–DNA complex. Only those water molecules that form H-bonds to amonafide are shown.

are shown in Figs. 4 and 5, and the central portions of these complexes are shown in Fig. 6. These figures illustrate the substantial overlap of the chromophores with base pairs in both cases, with the third ring of azonafide providing greater overlap. This factor is probably related to the preference of azonafide ring 3 for the cytosine side

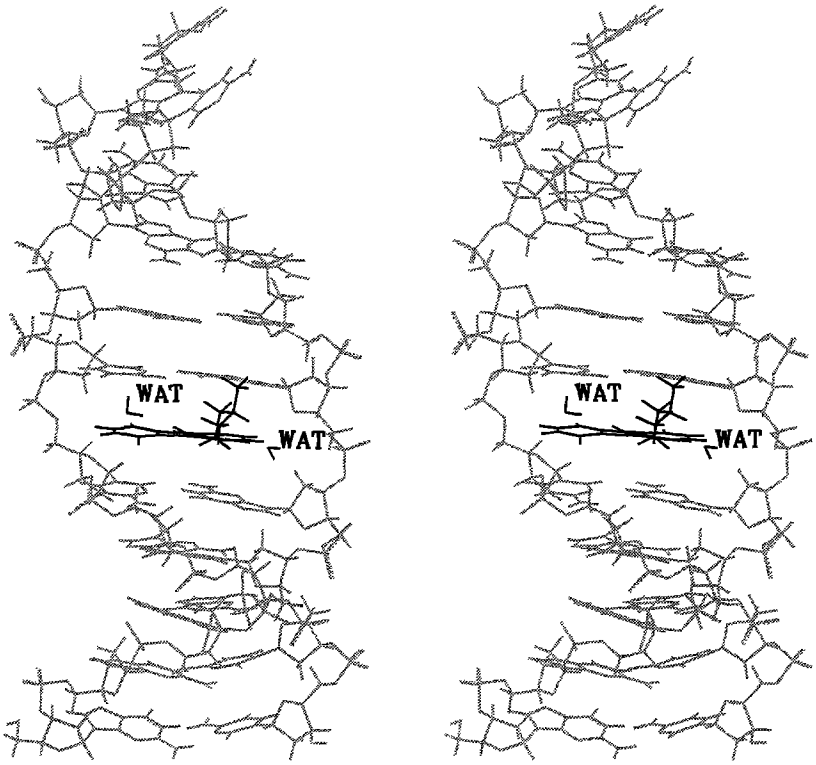

Fig. 8. Stereoview of the conformation of the solvated azonafide–DNA complex. Only those water molecules that form H-bonds to azonafide are shown.

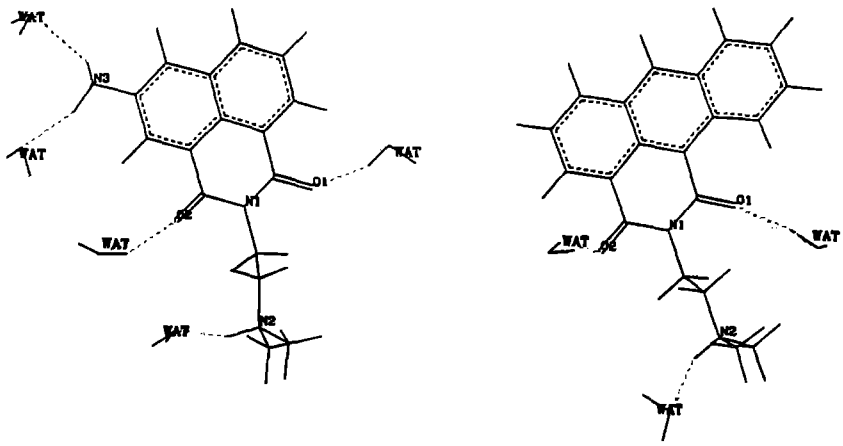

Fig. 9. Conformations of solvated amonafide (left) and azonafide (right). Only those waters that form H-bonds to the drugs are shown.

of the base pairs. Rotation of the amonafide side chain to make a bifurcated H-bond with N7 and O6 of dG5 is clearly apparent. The same H-bond could be made by the azonafide side chain, but this might lessen the chromophore overlap, which is probably why an H-bond to P4,5 is preferred.

Table 5 gives the results of molecular dynamics in water with counterions present at the phosphate groups of the DNA. It is evident that azonafide binds more strongly than amonafide (–57.3 and –47.9 kcal/mol, respectively); however, azonafide binding induces more distortion than amonafide in the DNA (24.0 and 15.6 kcal/mol, respectively). These distortion enthalpies include unwinding of the DNA to form the intercalation site. They are obtained by subtracting the enthalpies of solvated, uncomplexed DNA (–63.5 kcal/mol) from the enthalpies of DNA in the complexes. Distortions induced in both drug molecules are nil or small. The net enthalpy change for DNA binding is given by the sums of these values; it favors azonafide over amonafide by –1.4 kcal/mol. When the drug intercalates, it goes from an all-water environment into an environment consisting of both water and DNA. Solvation of the drug side chain is largely retained in the complex. Thus, Figs. 7 and 8 show the side chain surrounded by water and making two H-bonds to water and none to DNA. The NH_2 group of azonafide protrudes out of the complex sufficiently far to make two H-bonds to water. (The number of H-bonds varies from one to two, depending on the cycle of dynamics). Nevertheless, there is a net loss of enthalpy for drug–water binding attending intercalation. Table 5 shows that a net enthalpy loss of 28.8 kcal/mol occurs when amonafide moves from solvent into intercalation. The corresponding change for azonafide requires 25.5 kcal/mol. Combining the net drug–DNA binding enthalpy and the partial loss of solvation enthalpy affords the net enthalpy change for the overall process, which is –3.1 kcal/mol for amonafide and –7.8 kcal/mol for azonafide. Thus, azonafide intercalation is favored over amonafide intercalation by –4.7 kcal/mol. This result is consistent with the greater ΔT_m value for azonafide.

The analysis presented above is for enthalpy changes and not free energy changes, because it neglects entropy effects. We do not have quantitative estimates of the entropy changes resulting from intercalation, but it is evident from Fig. 9 that amonafide is H-bonded to four water molecules and azonafide is H-bonded to two water molecules before intercalation, whereas Figs. 7 and 8 show the same number of H-bonds to water after intercalation. This result indicates no difference in entropy change. The larger size of azonafide suggests that it might require more ordered waters than amonafide and, consequently, have a higher entropy effect when these water molecules are released upon intercalation. This factor would also favor the DNA binding of azonafide.

Conclusions

The comparative DNA binding of the related antitumor drugs amonafide and azonafide was studied by molecular dynamics in vacuum. These simulations indicated that a significant number of reasonable conformations exists for the intercalation of the compounds. In each conformation there is an induced fit with small distortions occurring in the DNA and drug in order to provide maximum drug–DNA interaction. The protonated amino side chains of the drugs acquired a variety of orientations, directed by interactions with H-bond acceptors on the DNA. A small advantage was observed for the binding of azonafide over amonafide, based on either the single best conformation of each or the average of all conformations (–49.2 kcal/mol versus –44.9 kcal/mol).

When the dynamics simulations were repeated with water and counterions present, the side chains changed their binding interactions from the DNA to water molecules. Based on the single complex of each drug used for the comparison in solvent, there was a small advantage in enthalpy for azonafide binding compared with amonafide

binding. A further advantage for azonafide was found in the enthalpy change occurring when the drugs move from water into the intercalation complex that includes both DNA and water interactions. Qualitative entropy considerations suggested that entropy changes were approximately the same for azonafide and amonafide binding, or possibly favoring azonafide binding. The more favorable changes on azonafide binding are consistent with the greater stability of complexes formed between azonafide and calf thymus DNA as measured by melting transition temperature increases.

It is theoretically possible to use the methods developed in this study to predict the comparative DNA binding ability of unknown molecules; however, in the present case and many others it is simpler to synthesize the compounds and measure their binding strengths. On the other hand, if the approximate geometry of the complex can be assigned without an extensive search of possible binding modes, this method may be useful. For example, the groove binding sites and conformations of compounds such as netropsin are known and an estimation of the comparative binding strength of analogues could be made rather quickly.

Acknowledgements

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