Molecular Modeling of the Antitumor Agents Azinomycins A and B: Force-Field Parametrization and DNA Cross-Linking-Based Filtering

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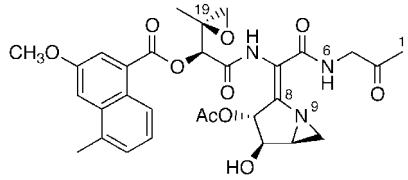
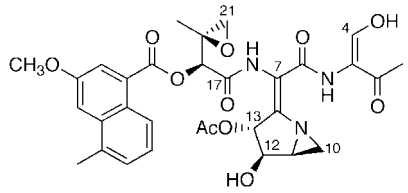
We describe a molecular modeling study on the DNA cross-linking agents azinomycins A and B. Analysis of five force fields and development of a new set of parameters were performed using ab initio calculations and experimental data to evaluate properly the potential energy profile of this structurally unique class of natural products. Critical fragments were identified on the basis of their potential role in determining the conformational shape of the agents relative to the site of action on the DNA duplex. A new conformational analysis protocol was developed on the basis of a hypothetical cross-linking mechanism where the distance and topology of the reactive sites on DNA were transformed into geometrical descriptors that were used to filter minimized conformations.

Introduction

Azinomycins A (**1a**) and B (**1b**) are antitumor agents that were isolated from cultures of *Streptomyces griseofuscus*.[1] The azinomycins possess an intricately functionalized structure that contains the unprecedented aziridino[1,2-*a*]pyrrolidine ring system. The azinomycins exhibit potent in vitro cytotoxic activity and significant in vivo antitumor activity against P388 leukemia in mice.[2] Biological evaluation of these agents has been hampered by chemical instability and poor availability from natural sources.[3] The presence of electrophilic epoxide and aziridine rings suggests that the azinomycins act by covalent alkylation and cross-linking of DNA, in a manner similar to mitomycin C.[4] Lown and Majumdar[5] demonstrated that carzinophilin covalently cross-links native DNA without prior activation. These workers demonstrated that alkylation occurs within the minor groove at G residues. More recent studies on carzinophilin/oligonucleotide interactions by Armstrong and coworkers[6] were interpreted to show cross-link formation between the agent and N7 of G/N7 of A within the major groove of DNA.



Azinomycin A (**1a**)



Azinomycin B (**1b**)

To date, the site and mechanism of action of these agents remains unclear, and this fact provided the impetus for the studies described herein. For any type of developmental studies on these agents to proceed in a logical manner, an understanding of the shape and reactivity of the agents is essential. The goal of our studies was the exploration of the conformational space of the azinomycins by molecular modeling as part of a program to elucidate the mechanism of cross-linking to the DNA double-helix.[7] No modeling work or high-field NMR data on these potentially useful antitumor agents has been reported.

We compared standard molecular mechanics force fields and their classification of low-quality parameters.[8]

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(1) Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *39*, 1527. Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554.

(2) Ishizeki, S.; Ohtsuka, M.; Irinoda, K.; Kukita, K.-I.; Nagaka, K.; Nakashima, T. *J. Antibiot.* **1987**, *40*, 60. In vitro cytotoxicity: IC_{50} = 0.07 μg/mL (**1a**) and 0.11 μg/mL (**1b**) against L5178Y cells. In vivo antitumor activity: 193% ILS at 16 μg/kg **1b** (3/7 survivors) against P388 leukemia; 161% ILS at 32 μg/kg **1b** (5/8 survivors) against Erlich carcinoma. In the same system, mitomycin C exhibited a 204% ILS at 1 mg/kg against P388 leukemia.

(3) Azinomycin B is apparently identical to carzinophilin A, an antitumor agent isolated in 1954 from *Streptomyces sahachiroi*: Hata, T.; Koga, F.; Sano, Y.; Kanamori, K.; Matsumae, A.; Sugawara, R.; Hoshi, T.; Shimi, T.; Ito, S.; Tomizawa, S. *J. Antibiot. Ser. A.* **1954**, *7*, 107.

(4) Tomasz, M.; Lipman, R.; McGuinness, B. F.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 5892 and references therein. For a recent review, see: Tomasz, M. *Chem. Biol.* **1995**, *2*, 575.

(5) Lown, J. W.; Majumdar, K. C. *Can. J. Biochem.* **1977**, *55*, 630.

(6) Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144.

(7) For synthetic studies on the azinomycins, see: Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1992**, *57*, 5813. Coleman, R. S.; Carpenter, A. J. *Tetrahedron* **1997**, *53*, 16313. Coleman, R. S.; Kong, J.-S. *J. Am. Chem. Soc.* **1998**, *120*, 3538.

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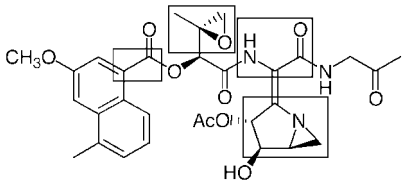

Figure 1. Segments of the azinomycins examined for reparametrization in AMBER*.

Using geometrical descriptors based on least-squares deviations, we compared structures generated with various force fields with ab initio and semiempirical generated structures or with experimental data derived from the Cambridge Structural Database. The AMBER* force field parameters were modified so as to maximize agreement between calculated/experimental geometries and those generated using original AMBER*. The next step explored the conformational space using the Monte Carlo technique applied to the 11 rotatable bonds of the azinomycins. The energies were calculated using our modified AMBER* force field and the implicit model of solvation GB/SA. We developed a conformational analysis protocol based on energetic and geometrical criteria, taking into account structural information relative to the most probable sites of interaction with B-DNA. This computational protocol was used to select azinomycin A and B conformers potentially able to cross-link DNA. To our satisfaction, the global minimum of azinomycin A determined by Monte Carlo simulation was in the final set of conformers obtained using our mechanism-based filter.

Results and Discussion

Force Field Analysis and Parametrization. Our molecular modeling study on azinomycins started with the evaluation[9] of the five force fields AMBER*, OPLS*, MM2*, MM3*, and MMFF as implemented in MacroModel.[10] The regions of the azinomycins that we felt required specific attention with respect to parametrization are identified in Figure 1 and include the naphthalene−carboxyl bond, the spiroepoxide, the dehydroamino acid, and the azabicyclic ring system. The rationale for inclusion of these particular segments of the agents is largely due to the hypothesis of their potentially critical role in the biological mechanism of action. Specifically, the epoxide and aziridine rings are the most likely sites of electrophilicity in determining the cross-linking ability of the natural products. Furthermore, given the potential role of the naphthalene as an intercalative binding group, the conformation about the indicated bond will play a major role in the recognition of duplex DNA, the putative site of action. Finally, the two indicated bonds of the dehydroamino acid will determine the overall conformation of the backbone and, hence, the spatial orientation of the electrophilic epoxide and aziridine rings.

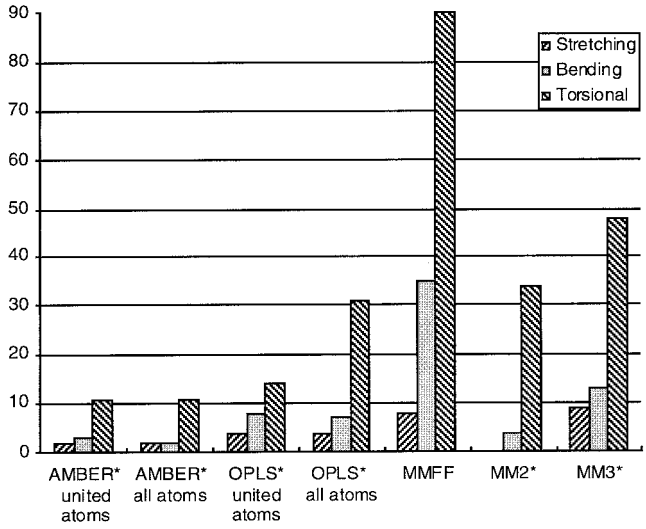

Figure 2. Number of low-quality parameters present in the five force fields implemented in MacroModel.

Our evaluation started with the analysis of parameter quality, and the results are displayed in Figure 2. The number of low quality parameters, as classified in MacroModel, were found to vary markedly with respect to force field. On the basis of this classification of stretching, bending, and torsional parameters for each bonded component, AMBER* appeared to be the best parametrized force field for this class of molecule. On the other hand, MMFF seemed to be poorly parametrized. The most important consideration is that of the parameters that play a significant role in determining the three-dimensional shape of the agents. This classification, in any case, is simply the starting point for the improvement of parameters for modeling the azinomycins.

Upon careful examination of the poorly parametrized bond terms in AMBER*, the following fragments (**2**−**5**) were identified as models for the development of new parameters (Figure 3). The basis for comparison included two criteria: (1) structures derived from high-level ab initio calculations and (2) experimental data derived from X-ray crystallography.

The aziridino[1,2-*a*]pyrrolidine substructure **2** is the most extraordinary structural feature of the azinomycins. The 1-azabicyclo[3.1.0]hexane ring is unprecedented in natural products chemistry,[11] but similar systems have been synthesized.[12] Thus, an accurate model of the natural agents required the development of high-quality parameters. Semiempirical and ab initio calculations on this system were used to generate these parameters. We used the structure minimized with RHF theory and 6-31G* basis set as the reference for comparison with

(8) All calculations were preformed on Silicon Graphics Indigo Impact R10000 computers using the programs MacroModel version 5.5 (Columbia University, New York, 1996) and Spartan version 4.0 (Wavefunction, Inc., Irvine, CA, 1995).

(9) Gundertofte, K.; Liljefors, T.; Norrby, P.-O.; Pettersson, I. *J. Comput. Chem.* **1996**, *17*, 429.

(10) MacroModel: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440. MM2: Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. MM3: Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551. AMBER: Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Nguyen, D. T. *J. Comput. Chem.* **1986**, *7*, 230. OPLS: Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657. AMBER*: McDonald, D. Q.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7743. MMFF: Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.

(11) For the structurally related natural product ficellomycin, see: Kuo, M.-S.; Yurek, D. A.; Mizsak, S. A. *J. Antibiot.* **1989**, *42*, 357.

(12) Brockman, R. W.; Shaddix, S. C.; Williams, M.; Struck, R. F. *Cancer Treatment Rep.* **1976**, *60*, 1317. Schnabel, F. M., Jr.; Trader, M. W.; Laster, W. R., Jr.; Shaddix, S. C.; Brockman, R. W. *Cancer Treatment Rep.* **1976**, *60*, 1325. Hammer, C. F.; Heller, S. R.; Craig, J. H. *Tetrahedron* **1972**, *28*, 239. Gassman, P. G.; Fentiman, A. F., Jr. *J. Org. Chem.* **1967**, *32*, 2388. For a review of 1-azabicyclo[3.1.0]hexanes, see: Black, D. S. C.; Doyle, J. E. *Adv. Heterocycl. Chem.* **1980**, *27*, 1.

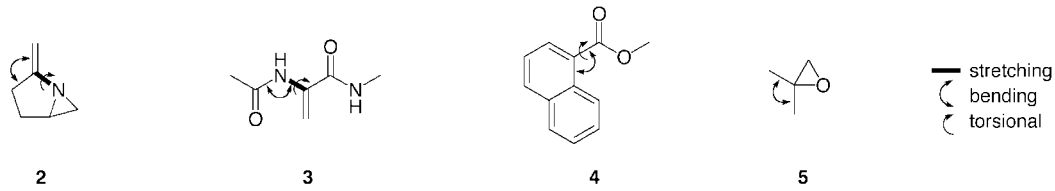

Figure 3. AMBER* low-quality parameters.

Table 1. Azabicyclohexane Molecular Mechanics and Semiempirical Force Field Comparison

force field	rms (Å) vs RHF/6-31G* reference
AMBER*	0.166
OPLS*	0.109
MMFF	0.074
MM2*	0.681
MM3*	0.683
AM1	0.327
PM3	0.349
MNDO	0.361

semiempirical and molecular mechanics simulations. Rauk et al.,[13] found that RHF theory and the 6-31G* basis set was as accurate as higher level ab initio methods in the geometry optimization of 1-azabicyclo-[3.1.0]hexane systems. Because of the rigidity of this ring system, we used the initial geometry provided by molecular mechanics as the starting point for further optimizations using the appropriate computational protocols. The results in terms of atomic coordinate deviation are reported in Table 1.

The results show that the MMFF minimum energy conformation was most similar to the ab initio reference structure. OPLS* and AMBER* also provided acceptable results. Other molecular mechanics and semiempirical force fields were unable to effectively reproduce the RHF/6-31G* geometry. Despite the better agreement of MMFF conformations with calculated ab initio geometries, we elected to modify the AMBER* force field because of its proven utility in modeling oligonucleotide systems and because of its ability to be used in the more flexible all-atom and united-atom notations. In addition, on the basis of our analysis of parameter quality, AMBER* was considered as the most appropriate force field among the five implemented in MacroModel to describe the azinomycins.

Parametrization of the AMBER* force field for the azabicyclic fragment **2** of the azinomycins was performed by introducing a new substructure into the original force field that contained the appropriate stretching, bending, and torsional parameters. The root mean square (rms) deviation between the ab initio and modified AMBER* structures was reduced to 0.074 Å and was judged acceptable.

The internal degree of freedom that most dramatically affected the conformational space of the azinomycins was the dehydroamino acid[14] dihedral angle CO−NH−C═C of **3**, principally because of its location in the middle of the backbone of the agents. As a result, this bond was judged critical for effective parametrization, which was conducted using a different philosophy compared to the azabicyclic ring. In the original torsional energy profile, two degenerate minima were located at 0° and 180° (Figure 4a) and were in extremely poor agreement with an experimentally derived population. Because this substructure of the azinomycins is commonly occurring, we were able to identify 49 X-ray crystal structures that contained this fragment.[15] The values of the CO−NH−C═C torsional parameter obtained from the Cambridge Structural Database (CSD) are plotted in Figure 5. Our modified set of AMBER* parameters (Figure 4b) was able to reproduce the expected population.

The CSD torsional distribution revealed two preferred angles centered at ±120°, with only two structures exhibiting a torsional value near 0°. The original AMBER* potential energy surface was unable to reproduce this experimental distribution, so a new set of parameters was added using the substructure tool of MacroModel to reproduce the experimentally determined torsional distribution.

The naphthoate (**4**) Ar−CO AMBER* low-quality torsion was monitored using ab initio and semiempirical calculations in order to compare the potential energy profile with the original molecular mechanics force field. The potential energy surface was essentially identical between the RHF/6-31G*, AM-1, and AMBER* dihedral simulations.

The epoxide fragment **5** is another important fragment of the natural agents, and an X-ray crystal structure has been published for a synthetically prepared system.[16] The agreement between the (original) AMBER*-minimized structure and the X-ray structure[15] was acceptable (rms = 0.064 Å), and the (original) AMBER* structure similarly agreed well with the ab initio structure (rms = 0.061 Å).

Conformational Searching and Energy Evaluation. The parametrization of AMBER* force field allowed us to perform an energetic evaluation more accurately than with the original set of parameters. To explore the conformational space of azinomycin A, we adopted a molecular mechanics protocol based on the Monte Carlo algorithm.[17] In this simulation, we considered 11 internal torsional degrees of freedom (i.e., rotatable bonds, Figure 6) generating 7000 conformations (i.e., Monte Carlo iterations). Each of these was minimized using 1000 iterations with the Truncated Newton Conjugate Gradient algorithm[18] with a gradient convergence threshold of 0.01 kcal/Å·mol. Similar conformations were

(13) Rauk, A.; Yang, D.; Tsankov, D.; Wieser, H.; Koltypin, Yu.; Gedanken, A.; Shustov, G. V. *J. Am. Chem. Soc.* **1995**, *117*, 4160.

(14) Alagona, G.; Ghio, C.; Pratesi, C. *J. Comput. Chem.* **1991**, *12*, 934. Alemán, C.; Perez, J. J. *Biopolymers* **1993**, *33*, 1811. Palmer, D. E.; Pattaroni, C.; Nunami, K.; Chadha, R. K.; Goodman, M.; Wakamiya, T.; Fukase, K.; Horimoto, S.; Kitazawa, M.; Fujita, H.; Kubo, A.; Shiba, T. *J. Am. Chem. Soc.* **1992**, *114*, 5634.

(15) References to these structures are available in the Supporting Information.

(16) Konda, Y.; Machida, T.; Sasaki, T.; Takeda, K.; Takayanagi, H.; Harigaya, Y. *Chem. Pharm. Bull.* **1994**, *42*, 285.

(17) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

(18) Ponder, J. W.; Richards, F. M. *J. Comput. Chem.* **1987**, *8*, 1016.

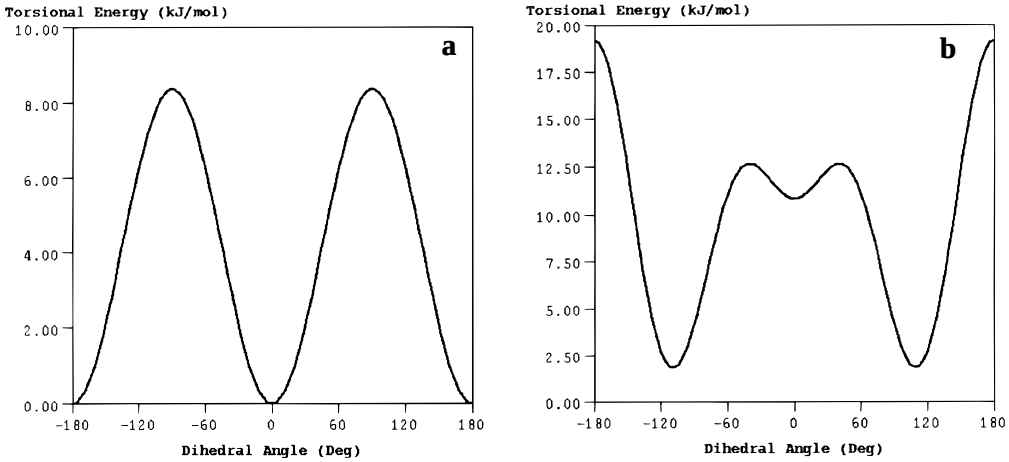

Figure 4. AMBER* CO−NH−C═C torsional energy profiles.

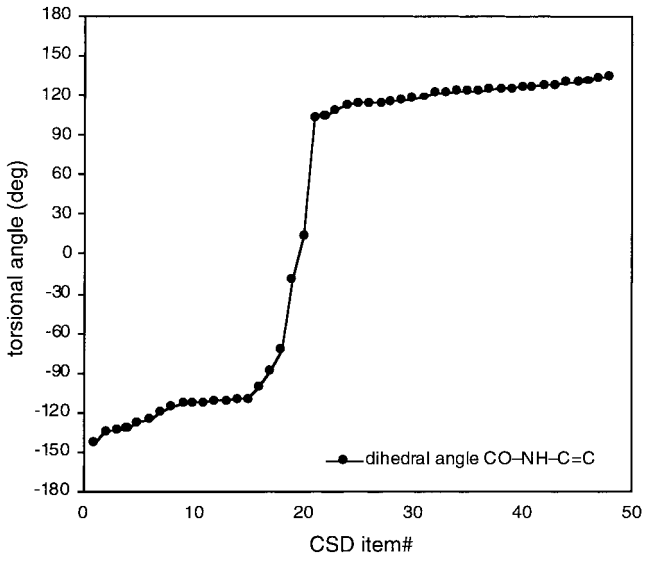

Figure 5. Torsional distribution for CO−NH−C═C obtained from Cambridge Structural Database (CSD).

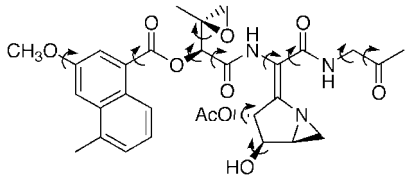

Figure 6. Rotatable bonds used in Monte Carlo simulation.

deduplicated with a cutoff of 1 kcal/mol and an rms threshold of 0.25 Å.

Aqueous solvation effects were taken in account using the implicit water model GB/SA.[19] Our protocol for conformational searching in vacuo included a large energy window (ca. 12 kcal/mol) to include all potentially relevant conformers for evaluation with the GB/SA water model. We included a large enough sampling of conformational space (7000 conformations) to ensure that low-energy conformations were not missed, and this was confirmed by the Monte Carlo frequency factors of the lowest energy geometries. The adopted protocol consisted of 1000 iterations of the Truncated Newton Conjugate

(19) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

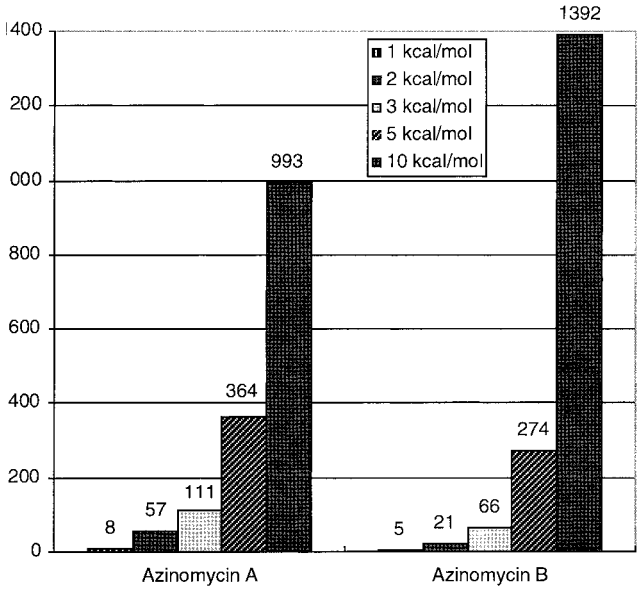

Figure 7. Conformational distribution displayed in energy windows.

Gradients with a convergence criterion in the energy minimization of 0.0001 kcal/mol. Similar conformations were deduplicated with a cutoff of 1 kcal/mol and an rms threshold of 0.5 Å. In Figure 7, the number of minimum-energy conformers obtained in the conformational search with AMBER* united-atoms and all-atoms reported into five energy windows starting from the global minimum.

Conformational Analysis and Filtering Strategy. The high conformational mobility of the azinomycins, as evidenced by the number of conformations obtained, indicated that we needed to develop an effective filtering protocol to simplify the results. To identify bioactive conformations, we used both energetic and mechanistic-based geometrical criteria. An energy threshold of 3 kcal/mol from the global minimum was invoked as an initial filter.

The geometrical criterion that we adopted was based on the hypothesis that azinomycins would interact covalently in an interstrand fashion with two nucleoside bases disposed 5′ to each other.[6] Our mechanism-based filter does not assume a binding mode, but simply uses an experimentally determined binding site to confirm that a significant population of azinomycin A and B

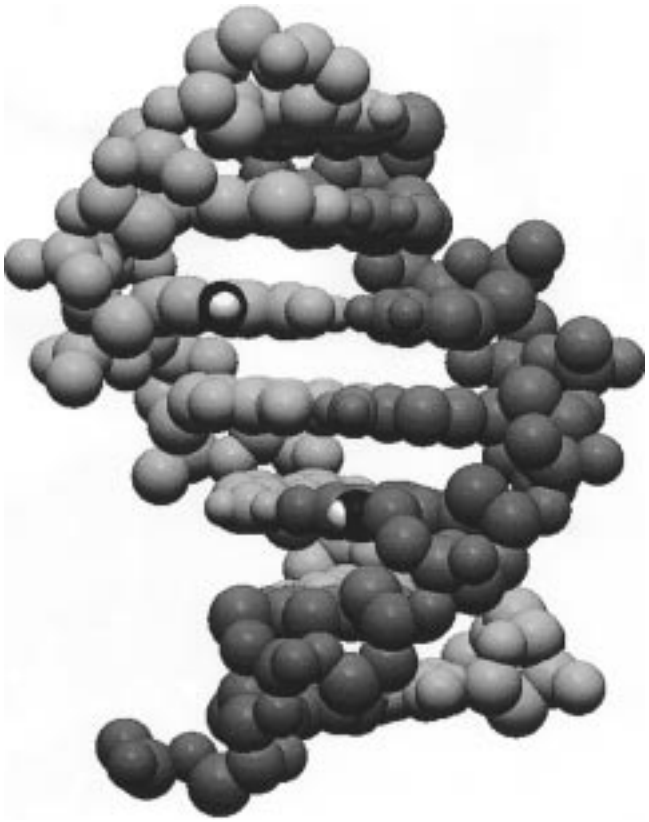

Figure 8. CPK model of putative DNA binding/cross-linking site containing the sequence d(GCC): dG N7 atoms shown in black; nitrogen lone pairs shown in white.

conformers are competent to cross-link at this known site. To measure geometrical descriptors to use as filtering parameters, we considered a DNA fragment in B-conformation containing the triplet 5′-GCC-3′, a sequence reported to undergo effective covalent cross-linking. The mechanistic model we formulated for DNA cross-linking assumes that the C21 of the epoxide and C10 of the azabicyclic system are the relevant electrophilic atoms that alkylate DNA. We assumed a standard backside attack model, which requires that the trajectory of the nucleophile is collinear with the carbon−heteroatom bond being broken. On the DNA model, this was translated into two geometrical descriptors: the linear distance between N-7 atoms of each dG base and the dihedral angle between the nucleophilic N7−lone pair vectors (Figure 8). The values of the relevant geometrical descriptors in canonical B-DNA are approximately 8.5 Å and 62°, respectively.[20]

This filter was applied to azinomycin distances between the C-21 of the epoxide and C-10 of the azabicyclic system with a tolerance of ±0.5 Å. The dihedral angle was related to the vectors collinear with the O20−C21 and C10−N9 bonds. The filtering tolerance was set to ±30°. Another filtering constraint was applied as a result of the ^{1}H NMR evidence for hydrogen bonding between the N5−H and the aziridine N9 position.[1] The filtering value for the H bond was set to 2.5 ± 0.5 Å.

(20) These values are based on the standard model of B-DNA as proposed by Arnott and implemented in MacroModel. See: Arnott, S.; Campbell-Smith, P.; Chandresekharan, P. *CRC Handbook of Biochemistry*; CRC Press: Boca Raton, FL, 1976; Vol. 2.

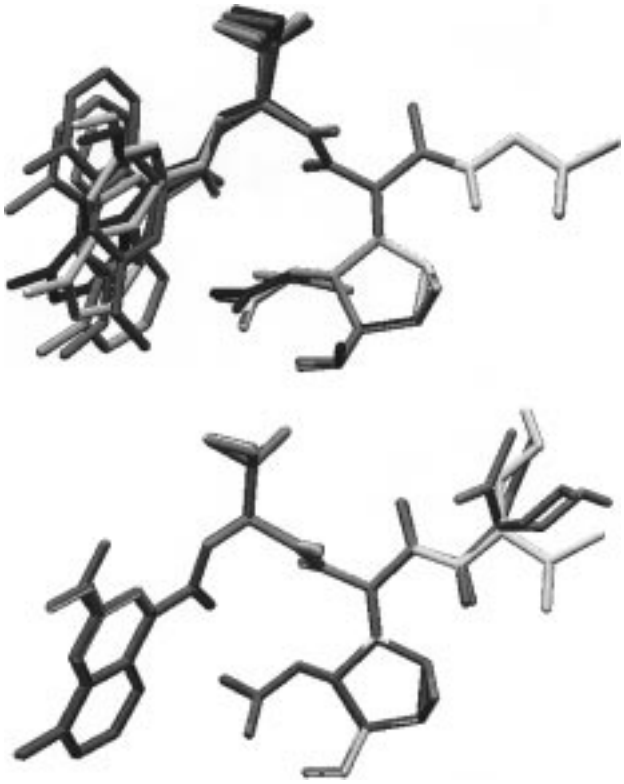

Figure 9. Polytube models of superimposed minimum energy conformations obtained by modified AMBER* (united-atoms), using mechanism-based filtering protocol. Top: azinomycin A (**1a**). Bottom: azinomycin B (**1b**).

Table 2. Data for Modified AMBER* (United-Atoms) Conformers Resulting from Mechanism-Based Filtering

conformation no. (frequency[a])	rel energy (kcal/mol)	population (%) at 300 K[b]	weighted population[c] (%)
	azinomycin A		
1 (64)	0.00	19.8	36.5
20 (11)	1.48	1.7	0.5
25 (11)	1.62	1.3	0.4
40 (13)	2.39	0.4	0.1
46 (13)	2.51	0.3	0.1
	azinomycin B		
8 (38)	1.70	2.5	7.1
15 (8)	2.50	0.7	0.4
20 (2)	2.64	0.5	0.1

[a] Number of occurrences in Monte Carlo simulation after deduplication. [b] Boltzmann distribution. [c] Boltzmann distribution weighted with frequency factor.

The results of application of this mechanistic-based filtering protocol provided a consistent family of conformers for both azinomycin A and B (Figure 9). Two main differences can be observed between **1a** and **1b**. Azinomycin A is characterized by extended conformation in the terminal ketone portion, azinomycin B by lower variability in the spatial position of the naphthoate ring system.

Both modified AMBER* notations give similar conformational results. In the case of AMBER* united-atoms, the presence of the global minimum structure obtained in the conformational search of azinomycin A indicated an agreement between the computational approach adopted and the filter protocol based on the mechanistic hypothesis. Table 2 presents data related to filtered minimum energy conformations shown in Figure 9. These data show that conformers capable of binding to

the putative cross-linking site on duplex DNA are among the low-energy conformers obtained by the computational protocol. This protocol included Monte Carlo simulation, minimization using the GB/SA water model, and filtering by energy and mechanistic considerations, as described above. In the case of azinomycin A, the overall weighted Boltzmann population generated by filtering consisted of almost 40% of the total. Weighted populations were obtained from a frequency factor that represents the number of times an identical conformer is found, which is used as a statistical coefficient in the calculation of the Boltzmann populations. The results with azinomycin B were less satisfactory, with the weighted Boltzmann population consisting of slightly less than 8% of the total.

Conclusions

In this paper, we present a molecular modeling study on the antitumor agents azinomycin A and B. Starting with an evaluation of standard molecular mechanics force fields as implemented in the MacroModel package, AMBER* was found to be the most appropriate force field in terms of number of low-quality parameters. The remaining AMBER* low-quality parameters have been analyzed and improved. We used ab initio and semiempirical methods and experimental data where available to obtain more effective parametrization.

The new AMBER* force field was used for energy evaluations in a conformational search. We selected a molecular mechanics protocol based on the standard Monte Carlo algorithm. This search was repeated with both notations for AMBER*. Refinements using the implicit solvation model GB/SA in water were performed starting from the energy minimum conformations obtained in vacuo. The analysis of the conformer set so obtained was based on a multicriteria, sequential filtering technique. This approach was developed using energetic considerations and an explicitly defined mechanism-based geometrical protocol that provided one self-consistent cluster of structures for each agent. We feel that our study will be useful in defining the mechanism of action of this class of antitumor agents using theoretical recognition approaches.

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Supporting Information Available: Lists of references to dehydroalanine crystal structures with CSD access codes and modified AMBER* parameters (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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