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QUARTERLY

The geometry of intercalation complex of antitumor mitoxantrone and ametantrone with DNA: Molecular dynamics simulations*°

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Intercalative binding of the antitumor drugs ametantrone and mitoxantrone to the dodecamer duplex d(CGCGAGCTCGCG)$_2$ was studied by applying molecular dynamics in water with the GROMOS 87 force field. A number of reasonable binding orientations were tested by short pre-simulations. It was shown that in energetically favourable orientation the anthraquinone chromophore is perpendicular to the direction of inter-base hydrogen bonds. Helically shaped side-chains of the drugs fit to the minor groove. The best orientation obtained in pre-simulations was applied in the main simulations. Small but significant differences were found between structures of intercalation complexes of the two drugs with the dodecamer duplex, the mitoxantrone complex possessing more favourable energy. The molecular nature of interactions responsible for those differences has been discussed.

The discovery of the antitumor activity of 1,4-bis[(aminoalkyl)amino] anthracene-9,10-diones [1-6] such as ametantrone (**AMT 1**) and mitoxantrone (**MIT 2**) has led to numerous physicochemical and pharmacological studies on the mechanism of their antitumor action [7]. Despite the efforts of intensive chemical and biological research, the mechanism of action of the anthracenediones has not been fully clarified. The overall picture of the data available strongly suggests that the ability to bind DNA is a necessary though not a sufficient condition for the drug activity [8].

The interaction of MIT and other anthracenediones with DNA has been studied by a variety of biochemical as well as physico-

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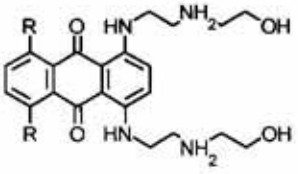
Abbreviations: AMT, ametantrone; MIT, mitoxantrone; MD, molecular dynamics.

chemical techniques. Results of those studies have led to the first suggestion concerning the structure of the DNA-anthracenedione intercalation complex [7]. In this model the long axis of the anthraquinone chromophore lies perpendicularly to the axis of the base pair hydrogen bonds, with both side-chains occupying the major groove.



1 AMT; R = H

2 MIT; R = OH

Scheme 1. Chemical structures of anthracenediones studied: 1 ametantrone AMT and 2 mitoxantrone MIT.

Experimental evidences of molecular structure of the anthracenedione-DNA complex on atomic level have not been reported yet, and computer modelling studies reported up till now have been done only for very simple systems. Therefore, application of more sophisticated computer-modelling techniques, such as molecular dynamics (MD), seems to be reasonable. Molecular dynamics simulations have been increasingly used to study DNA itself as well as to study interactions of DNA with various agents [9–19].

In this study we present the results of extensive computer simulations on a dodecamer duplex d(CGCGAG|CTCGCG)$_2$ with an MIT or AMT molecule intercalated between G-C base-pairs. The finding of the most probable orientation of the anthracenedione molecule and its conformation in the intercalation complex were the main goals of this study.

Because of the lack of experimental data concerning the orientation of the intercalated anthracenedione, the main simulations were preceded by four short pre-simulations with different relative orientations of the intercalator.

The main simulations of the complexes were performed with the intercalators in such an orientation which corresponds to the maximal interaction energy obtained in pre-simulations.

METHODOLOGY

The dynamics simulations reported in this work were performed with GROMOS 87 [20], a set of molecular dynamics programs from the University of Groningen, implemented on the VAX 6310 computer at the Computer Center of the Camerino University. The periodic boundary condition was applied, all bond lengths were constrained to their equivalence values using SHAKE algorithm [21], and distances related to Watson-Crick hydrogen bonds were restrained to 0.21 nm during all simulations [18]. A dielectric constant of 1 and a cut-off of 0.8 nm were used for non bonded interactions, whereas a cut-off of 1.2 nm was used for long term electrostatic interactions. These cut-off values were proved in previous simulations of this kind to be acceptable and represent a compromise between accuracy and computational efficiency [9, 22]. An appropriate number of Na^+ counterions and water molecules were added to the dodecamer-drug system. In order to save computation time the intercalation phenomena were analysed with a DNA fragment in which the intercalation cavity was pre-formed.

The initial co-ordinates of ametantrone and mitoxantrone with side chains expanded in a mean plane of the ring system were obtained from molecular modelling of the molecules by program INSIGHT II (BioSym Inc.).

Comparison of different orientations of ametantrone

The ametantrone molecule was inserted to the pre-formed intercalation cavity of the dodecamer duplex d(GCCGAG|CTCGCG)$_2$ in four different orientations (Fig. 1).

In all orientations the center of the quinone ring was laid on the helix axis and the plane of anthracenedione ring system was equidis-

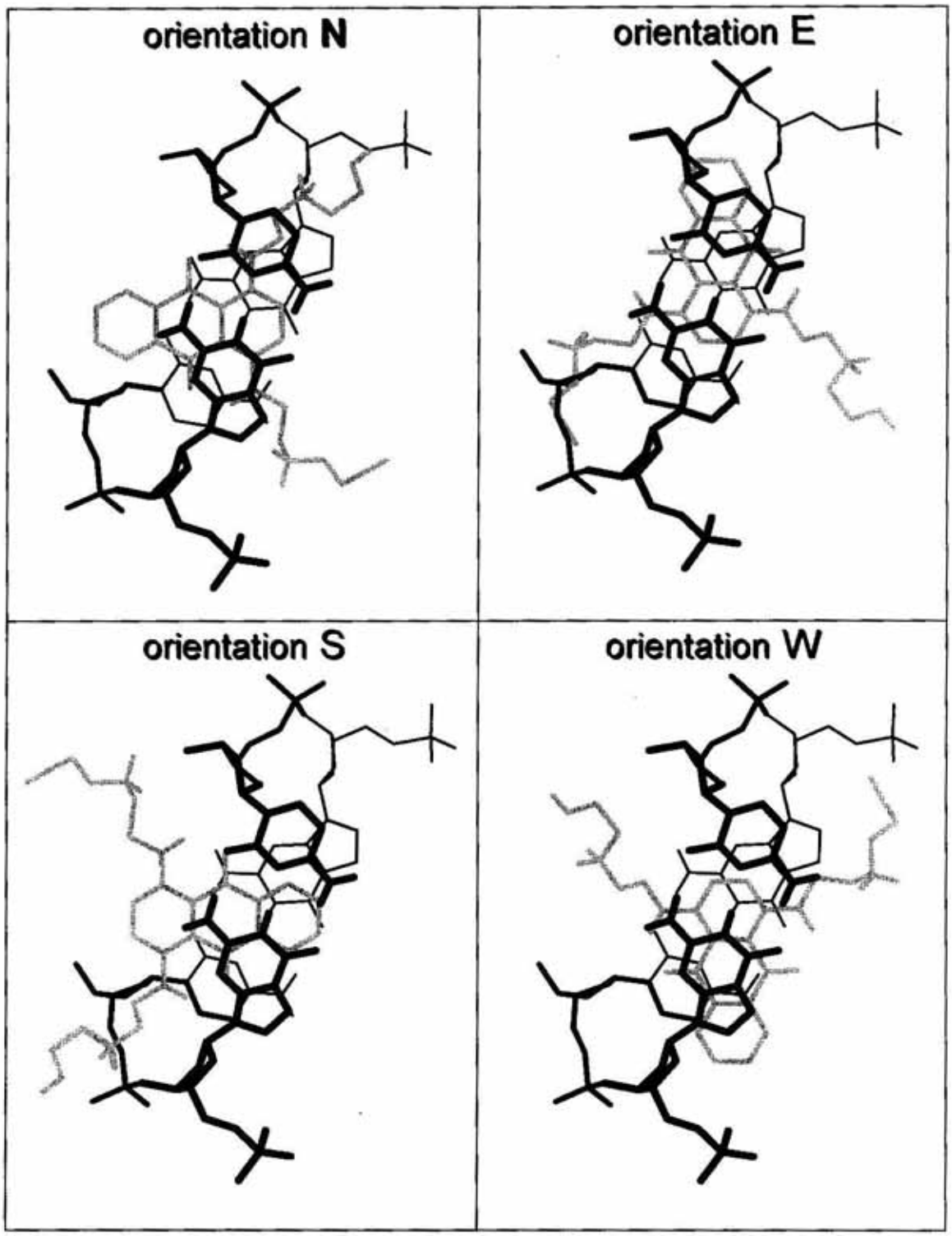


Figure 1. Four starting geometries of the AMT-dodecamer intercalation complex considered in pre-simulation step.

The AMT molecule is shown in grey. The major groove is on the right. The intercalator and two neighbouring base-pairs are presented only.

tanced from the mean planes of the neighbouring base-pairs. The following protocol of calculation was applied for each orientation:

◆i) the dodecamer/AMT complex has been placed in the center of a rectangular 3.017 × 3.127 × 4.996 nm. The minimum dis-

tance between any atom in the complex and the walls of the box was equal to 0.4 nm; 1235 water molecules and 20 Na^+ counterions were inserted into the box.

- ii) 100 steps of steepest descent minimisation with restrained hydrogen bonds distances were applied to the entire system. The list of non bonded interactions was updated after each step. An additional potential which restrained distances related to Watson-Crick hydrogen bonds to 0.21 nm, was used in this and all further calculations.
- iii) the energetically minimised system was termalized at 300 K during 10 runs of 100 MD steps of 1 fs. The atomic velocities were reassigned according to a Maxwell-Boltzmann distribution at the beginning of the first run and between runs if the mean temperature during the run deviated from 300 K by more than 5 K. The list of non bonded interactions was updated every 10 steps.
- iv) the system obtained in step iii) was next relaxed by 10 ps MD simulation (5000 steps of 2 fs). During relaxation the system was coupled to an external thermal and pressure bath of 300 K and 1 barr with time constants equal to 10 fs (for temperature) and 50 fs (for pressure). The list of non bonded interactions was updated every 20 steps. During the last 2 ps of the relaxation step the co-ordinates of the system were saved every 0.1 ps.

The mean values of the dodecamer-AMT interaction energy as well as of intramolecular topological tensions from the last 2 ps of the relaxation were taken into account in choosing the best orientation of the intercalator.

The MIT molecule was inserted to the preformed cavity in the orientation selected during pre-simulation of AMT and the above protocol was performed only for this single orientation.

Molecular dynamic simulations of mitoxantrone and ametantrone intercalated to the dodecamer

The main molecular dynamics simulations of the two anthracenedione drugs intercalated to the dodecamer duplex were performed starting from the relative orientation of the intercalator chosen during pre-simulation. The protocol described above was followed by 40 ps molecular dynamics simulation for each drug.

The systems were coupled to thermal and constant pressure bath with the same constants as for the pre-simulations. The distance restraints were also applied. The co-ordinates of the simulated systems were saved every 0.2 ps and formed trajectories of 200 geometries. These trajectories have been then carefully analysed.

RESULTS

The intercalator orientation

Short molecular dynamics simulations of the dodecamer duplex d(CGCGAGCTCGCG)$_2$ together with AMT in water was used to select the energetically plausible geometry of the intercalated anthracenedione molecule for the main simulations. Four different starting orientations (Fig. 1) of the intercalator in relation to the axis of inter-base (G6-C19) hydrogen bonds were examined as described in Methodology. The sum of energy of the AMT–dodecamer non bonded interactions and energy of intramolecular topological tensions was used as a criterion for the selection. It must be emphasised that this approach is not designed to provide exact quantities of the intercalation energy but only to provide a guide to relative differences and ranking orders of different intercalation patterns.

Table 1. Energetic terms, in kJ/mol, of intermolecular non-bonded interactions and intramolecular tensions* for ametantrone–DNA geometries obtained during the last 2 ps of the relaxation step for different starting geometries

Energy term	Starting orientation			
(kJ/mol)	N	E	S	W
Intramolecular tensions*	2,390 ± 60	2,320 ± 50	2,290 ± 50	2,280 ± 50
Ametantrone–DNA electrostatic interactions	−1,460 ± 40	−2,370 ± 60	−2,580 ± 30	−2,090 ± 70
Ametantrone–DNA Lenard-Jones interactions	−250 ± 10	−220 ± 10	−230 ± 10	−240 ± 10
Sum	680 ± 70	−270 ± 80	**−520 ± 60**	−50 ± 80

*This term is calculated as the sum of energies resulted from the deviation of bond angles, dihedral angles, and improper dihedral angles, and distance constrains from their optimal values.

Table 1 details energetic terms which have been obtained. The Lenard-Jones interactions between the intercalated AMT molecule and dodecamer as well as intramolecular tensions are practically the same in all orientations studied. The main differences between the orientations occur in electrostatic interactions.

High electrostatic attraction observed in orientation **S** (−2,580 ± 30 kJ/mol), where both side chains occupy the minor groove, results from adjustment of the side chains to the shape of the minor groove and from distribution of its polar and ionic constituents. It is noteworthy that the side chains being in this orientation do not lay in the plane of the anthraquinone ring system but reproduce the helical shape of the groove.

In orientation **N**, in which both side chains are located in the major groove, the electrostatic attractive forces are the lowest (−1,460 ± 40 kJ/mol). In this orientation the side chains seem to be surrounded by the water molecules and electrostatic interactions may be only of indirect character.

In the case of orientations **E** and **W** we deal with a medial situation. The chain placed in the minor groove interacts strongly, but that located in the major groove and surrounded by water interacts rather weakly. This is reflected in values of electrostatic interactions (Table 1) which are intermediate between those characteristic for orientations **S** and **N**.

Characteristic differences are also observed between hydrogen bond patterns formed by side chains of AMT in different orientations. When both side chains are placed in the major groove (orientation **N**) they both will be able to form hydrogen bonds, but only with phosphate groups. It is also noteworthy that only terminal OH groups are able to form hydrogen bonds with the dodecamer in these orientations. In orientation **S** both chains form hydrogen bonds and, in contrast to other orientations, the bonds are formed also by hydrogens from charged, aliphatic amino groups.

As a result of the above presented data the orientation **S**, in which both side-chains are placed in the minor groove, has been chosen for the main dynamics simulation.

Drug–dodecamer intercalation complexes

During the main 40 ps simulations the atomic co-ordinates saved every 0.2 ps formed two trajectories of 200 geometries: one for AMT and one for MIT. The molecules of either drug were intercalated to the dodecamer in orientation **S** chosen during the pre-simulation of AMT.

A comparison of mean values of dihedral angles in the two dodecamer duplexes has shown that in both complexes conformations of the DNA molecule are practically the same. The mean distances between central base-pairs

Table 2. The list of hydrogen bonds between drug molecule and DNA observed during main simulations of the drug–DNA complexes

Donor	Acceptor	Bond participants	Drug	
			AMT	MIT
Drug side chain — carbohydrate-phosphate backbone				
Drug	7Cyt	N3+-HN31+→O4*	85.5%	
	7Cyt	N3+-HN31+→O5*	5.0%	
	7Cyt	N3+-HN32+→O4*	4.5%	
Drug	6Gua	O6+-HO6+→O3*	19.0%	
Drug	7Cyt	O6+-HO6+→O2P		28.5%
	7Cyt	O6+-HO6+→O5*	5.5%	
Drug	22Gua	O6+-HO6+→O2P		5.0%
Drug	9Cyt	O6*-HO6*→O4*		9.5%
Drug side chain — base				
Drug	6Gua	N3+-HN31+→N3		12.0%
		N3+-HN31+→N2		11.0%
		O6+-HO6+→N3	38.0%	
		O6+-HO6+→N2	6.0%	
Drug	8Thy	O6*-HO6*→O2		40.0%
6Gua	Drug	N2-HN22→O6+	35.5%	
18Gua	Drug	N2-HN22→O6*		48.5%
Drug ring system — base				
6Gua	Drug	N2-HN22→NC1		50.0%
6Gua	Drug	N2-HN22→OC9	4.0%	
18Gua	Drug	N2-HN22→NC4	28.5%	10.0%
18Gua	Drug	N1-HN1→OC9		5.0%
Drug	6Gua	NC1-HNC1→N3		26.5%
Drug	7Cyt	NC1-HNC1→O2	67.5%	9.5%
Drug	18Gua	NC4-HNC4→N3	5.0%	

Numbers in the table relate to per cent of relaxation time when particular hydrogen bond has been observed. The hydrogen-acceptor distance shorter then 0.25 nm and donor-hydrogen-acceptor angle larger than 120° were used as the criteria of the hydrogen bond existence.

are equal to 0.742 ± 0.025 nm for AMT and 0.727 ± 0.016 nm for MIT and do not differ significantly.

In both simulated complexes side-chains of the drugs fit to the minor groove of the dodecamer. It is important to note that the shape of this groove is significantly deformed near the intercalation place. This fitting results in formation of hydrogen bonds between drugs side-chains and the dodecamer (Table 2). It should be emphasised that hydrogen bonds are formed not only between side-chains of the drugs and oxygen atoms of phosphates and sugars but also between the side-chains and the base moieties. The presence of such bonds confirms deep penetration of the chains into the minor groove. On the other hand, the formation of hydrogen bonds occurs not only with neighbouring nucleotides but also with more distant ones. This indicates that side-chains are stretched along the groove.

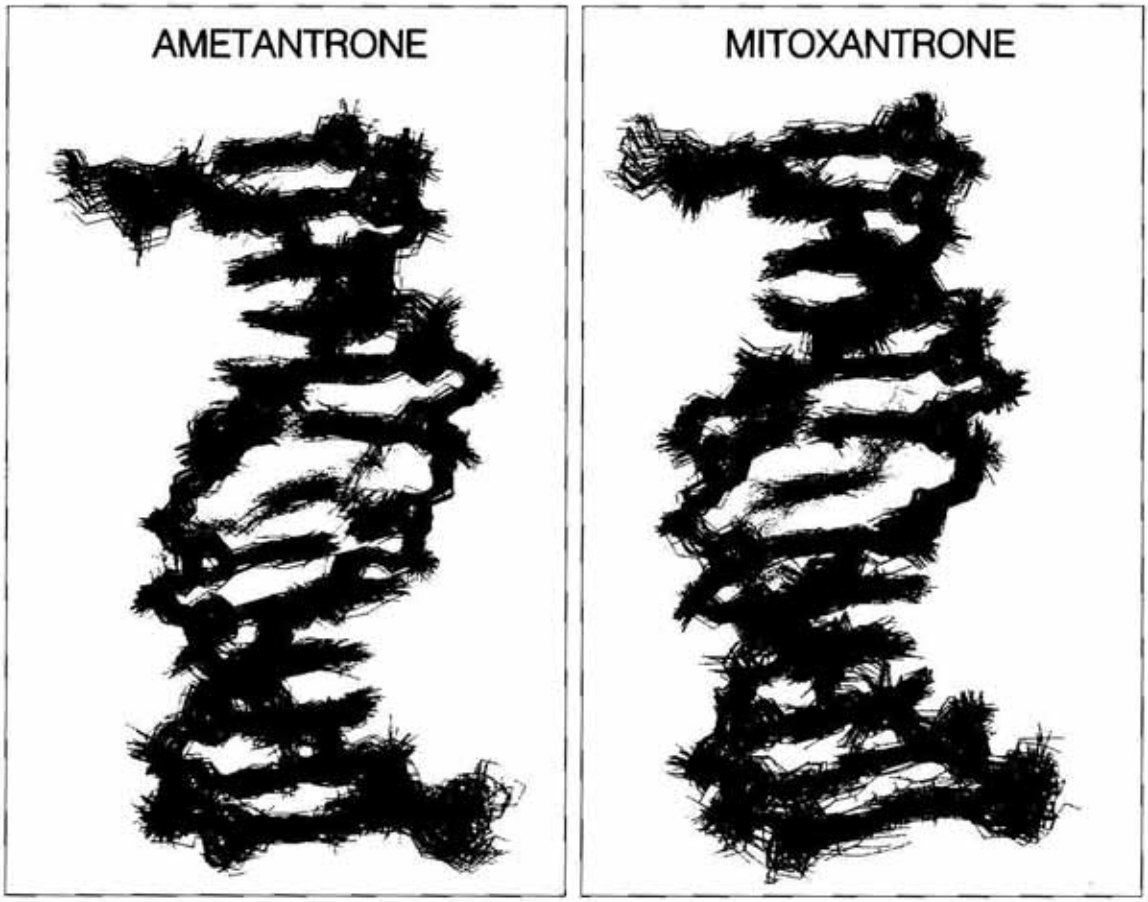


Figure 2. Composite of 40 snapshot geometries of the anthracenedione–dodecamer complexes obtained during main simulations.

The phosphorus atoms in each snapshot have been fitted to their positions in the first snapshot. The sodium cations and water molecules are omitted for clarity.

A visual inspection of trajectory geometries has shown (right panel on Fig. 2B) that the ring system of MIT is practically coplanar with the neighbouring base pairs (mean value of an angle between the planes is equal to 5.7° ± 2.3). Small deviations from the coplanarity are random and do not favour any particular orientation. On the contrary, the ring system of the AMT (left panel on Fig. 2) has a tendency to a small but significant inclination according to the minor groove run (mean value 13.1° ± 3.0). This difference in the ring system orientation reflects in relations observed for the two drugs between the ring system plane and side-chains (Fig. 3). For MIT the fitting of the chains to the DNA minor groove is a result of helicoidal twisting of the side-chains, whereas in the case of AMT it is in a significant part a result of the ring system inclination. Thus, side-chains of AMT exhibit much less twisting.

It is noteworthy that two aromatic hydroxyl groups present in the MIT molecule do not form hydrogen bonds with neighbouring base pairs. As one can see on Fig. 3 these groups may exist in two conformations. In one of them they form hydrogen bonds with quinone oxygen atoms whereas in the other they interact with water molecules placed in the major groove.

An analysis of energetic terms stored during the main simulations also confirms differences between complexes formed by the two drugs (Table 3). The intramolecular topological tensions are practically the same in both complexes. However, intermolecular nonbonded interactions are significantly higher in the case of the MIT complex. The highest

difference is observed for electrostatic drug-dodecamer interactions.

DISCUSSION

The results presented in this paper strongly suggest that anthracenediones in an intercalation complex prefer orientations in which both side-chains occupy the minor groove of the DNA. Our simulations indicate that superiority of the drug orientation with side-chains in the minor groove results from their fitting well to the groove shape. The observed network of hydrogen bonds shows that side-chains which are fitted to the groove are elongated to such extent that they can interact with polar atoms from at least four nucleo-

Table 3. Energetic terms, in kJ/mol, of intermolecular non-bonded interactions and intramolecular topological tensions for drug-DNA complexes obtained during the last 5 ps of the main simulations.

Energy term	Complex with	
(kJ/mol)	AMT	MIT
Intramolecular tensions*	2,350 ± 60	2,370 ± 50
Drug–DNA electrostatic interactions	-2,350 ± 20	-2,630 ± 20
Drug–DNA Lenard-Jones interactions	-230 ± 10	-300 ± 10
Sum	-230 ± 65	**-560 ± 65**

*This term is calculated as the sum of energies resulted from the deviation of bond angles, dihedral angles, and improper dihedral angles, and distance constrains from their optimal values.

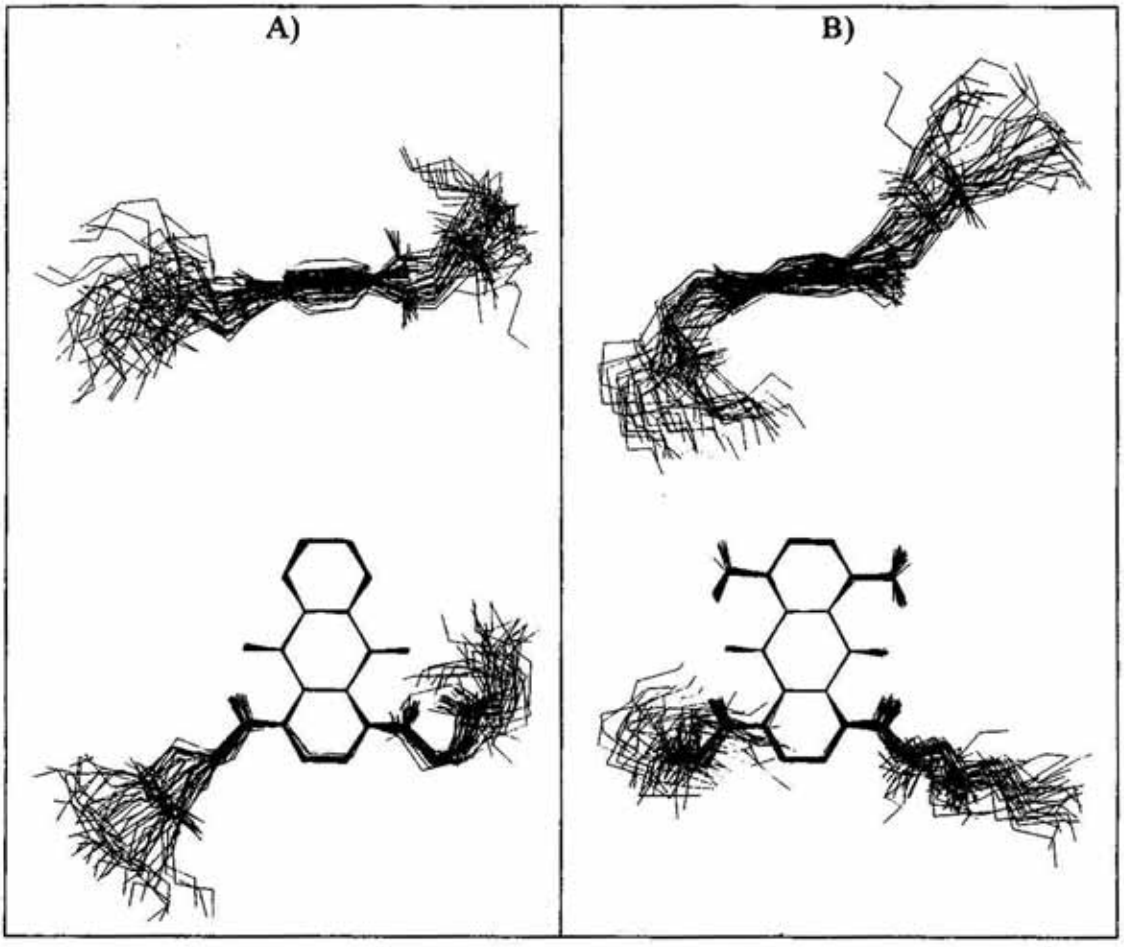


Figure 3. Orthogonal views of composite of 40 snapshot geometries of: A) AMT, and B) MIT stored during the mains simulations.

The quinone ring of the drug molecule in each snapshot has been fitted to its position in the first snapshot. The dodecamer duplex and water molecules are omitted for clarity.

tides (Table 2). Such kind of interactions cannot be detected when a model with a short part of the DNA is used. It is noteworthy that the minor groove is not as small as one could expect. Results of X-ray analysis of intercalation complex monocrystals revealed that even substituents of relatively large size fit very well to this groove. Such a situation has been observed for the sugar moiety of tetracycline antibiotic adriamycine [23] and even for its N-morpholyl derivative [24].

The comparison of features of the complexes formed by MIT and AMT leads to notable conclusions. Our simulations indicate that the complex formed by MIT is energetically more favourable. This is in agreement with well known experimental results [25]. The explanation of this superiority was one of the purposes of our study. As the two drugs differ only in the presence of two phenolic groups in MIT molecule, our attention was focused on these groups. We expected that the hydroxy groups could form hydrogen bonds with the neighbouring base-pairs. However, such interactions have not been observed during the main simulation. The most important difference between energetic terms of the complexes results from electrostatic interactions (Table 3). It may have two sources. First, the presence of two aromatic hydroxyl groups changes the electrostatic field of the MIT ring system and such modified field may better interact with electrostatic field of the intercalation cave. Secondly, the side-chains of MIT are able to interact stronger with the minor groove walls in spite of the fact that side-chains of the two drugs are identical. This is because the fitting of the side-chains depends not only on their chemical structure but also on their conformation and space orientation. In the case of MIT, the chains fit to the groove due to their characteristic helical shape, whereas in the case of AMT mainly due to the inclination of the ring system inside the intercalation cave (Fig. 2). This inclination of about 13° may induce weaker electrostatic interactions between the AMT ring system and the neighbouring base pairs. However, it is not clear how the presence of the phenolic groups affects the orientation of the ring system inside the cave. One of the explanations is based on the observation that two phenolic groups in *para* position increase the width of the MIT ring system and decrease its inclination.

Thus, the results of theoretical simulations presented in this paper suggest a molecular explanation of higher affinity of MIT to DNA. The molecule of MIT is wider than the molecule of AMT. As a result MIT in the intercalation cave is in a more coplanar orientation. Such orientation results in better electrostatic interactions between the drug and base pairs and, in consequence, the MIT-DNA complex is energetically more favourable than the AMT one.

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