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Model Building and Molecular Mechanics Calculations of Mitoxantrone-Deoxytetranucleotide Complexes: Molecular Foundations of DNA Intercalation as Cytostatic Active Principle[#]

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Summary. Several intercalation complexes of the antitumor-active drug mitoxantrone with base paired tetranucleotides were constructed by molecular modeling using computer graphics and molecular mechanics calculations. The mitoxantrone molecule favours DNA binding into CG intercalation site. The two side chains of the drug are orientated into the major groove and fixed by hydrogen bonds with the nucleotide bases. This molecular study can be helpful for understanding the mode of action of cytostatically active compounds and to design new structurally related compounds of the anthraquinone drug type.

Keywords. DNA intercalation model; Mitoxantrone-deoxytetranucleotide complexes; Molecular mechanics.

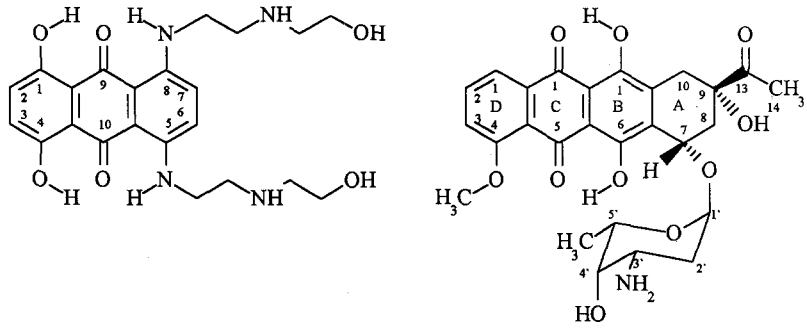
Modellentwicklung und molekülmechanische Berechnung von Mitoxantron-Deoxytetranukleotid-Komplexen: Molekulare Aspekte der DNA-Interkalation als Grundlage einer zytostatischen Wirkung

Zusammenfassung. Verschiedene Interkalationskomplexe aus dem antitumor-aktiven Arzneistoff Mitoxantron und basengepaarten Tetranukleotiden wurden mit Hilfe der Methoden des Molecular Modelings unter Einsatz von Computergraphik und molekülmechanischen Rechnungen konstruiert. Mitoxantron bevorzugt die Einlagerung in eine CG-Basensequenz. Beide Seitenketten des Wirkstoffs orientieren sich nach der großen Rinne und sind über Wasserstoffbrücken mit den DNA-Basen verbunden. Diese molekulare Studie soll dazu beitragen, den Wirkmechanismus zytostatisch aktiver Substanzen zu verstehen und neue strukturell verwandte Verbindungen vom Anthrachinon-Typ zu entwerfen.

Introduction

Mitoxantrone (**1**, Fig. 1) is a cytostatic chemotherapeutic drug for the therapy of several human tumors [1]. The naturally occuring antitumor active drug

[#] Dedicated to Prof. Dr. Dr. h.c. mult. *H. Oelschläger* on the occasion of his 75th birthday



1 2

Fig. 1. Mitoxantrone (**1**); Daunorubicin (**2**)

daunorubicin (**2**, Fig. 1) was the lead compound for the development of this synthetic drug. The decisive role for the development of mitoxantrone was the knowledge about the mode of action of daunorubicin and several other anthracycline antibiotics; they form stable intercalation complexes with the DNA [2–8]. Several physical and biophysical methods have proven that one of the main principles of cytostatic action of mitoxantrone consists of complexation with DNA followed by subsequent inhibition of several DNA enzymes [9, 10]. Whereas intercalation with DNA has been experimentally documented on the molecular level in the anthracycline series by several X-ray structures [2–8], no X-ray structure of an oligonucleotide mitoxantrone complex is available so far.

For a rational development and design of drugs, first of all the threedimensional structure of the biologically relevant drug-receptor complexes has to be known. In this paper, we focus on the structural aspects of potential mitoxantrone-DNA interactions by model building and molecular mechanics calculations. There are several important considerations to be directly addressed in a structural survey of the various intercalation complexes regarding the specific molecular behaviour of mitoxantrone: the determination of favourable DNA intercalation geometries with mitoxantrone and the detailed mode(s) of interaction of mitoxantrone with these geometries to produce the intercalated drug-DNA complexes. From the results we try to analyse the conformational characteristics of the complexes for optimal drug binding and the geometrical (conformation, size) requirements for intercalation with optimal binding and prediction of base sequence selectivity. It has already been shown that molecular mechanics techniques yield models – although not as accurate as X-ray or NMR structures – which are precise enough and suitable to study intercalation processes and help to understand the mechanism of action via DNA-intercalation. *In vivo*, DNA-intercalation of anthraquinones results in subsequent topoisomerase inhibition [11–17].

We have therefore built, energy-minimized, and studied intercalation complexes formed by mitoxantrone and several deoxytetranucleotide duplexes.

Materials and Methods

All operations were performed with the aid of the molecular modeling program packet SYBYL 5.5 [18], installed on a Micro VAX 3000. The molecular mechanics calculations were realized with AMBER [19]

and TAFF [20] (TRIPOS associated force field) in SYBYL using the *Powell* minimizer. For base paired B-DNA, oligonucleotide specific parameters from *P. A. Kollman* were used [19], whereas the calculations of the intercalation complexes including the non nucleic structure of the drug were performed with TRIPOS force field parameters as implemented in SYBYL. Validation and success of calculations of drug-receptor complexes using the TRIPOS parameters in comparison to using the AMBER parameters is well documented [20]. For the electrostatic term in force field calculations for refinement of oligonucleotides, the nucleinic charge from the AMBER program was used [19]; the atomic charges of the drug-DNA complexes were calculated by the method of *Gasteiger* and *Hückel* [21]. The molecular mechanics calculations revealed no significant differences for the DNA complex geometries by using alternatively MOPAC-AM1 charges [22] for the drug; thus, *Gasteiger* and *Hückel* charges were used. A distance dependent dielectric constant of the form $\varepsilon = cR_{ij}$ ($c = 4$) was employed throughout in the electrostatic term [18]. The complex was further energy minimized with TAFF until the energy gradient was less than 0.01 kcal/mol. The graphical visualizations were performed with an Evans & Sutherland PS 390 system.

The interaction energy *E-inter* of the various complexes is calculated by

$$E\text{-}inter = E\text{-}tot - E\text{-}oligo - E\text{-}drug. \tag{1}$$

The potential energy term for the whole complex is *E-tot*; for its tetranucleotide, it is *E-oligo*, and for mitoxantrone or other intercalators, it is *E-drug*. *Van der Waals* and electrostatic contributions to the interaction energy *E-inter* between DNA and the drug are given as *E-int-vdW* and *E-int-elec*, respectively. All changes in the conformational energy are summed up in *E-int-conf*. It should be noted that

$$E\text{-}inter = E\text{-}int\text{-}elec + E\text{-}int\text{-}vdW + E\text{-}int\text{-}conf \tag{2}$$

In this paper, only the energy values of Eq. 2 are given, because only these values are relevant to the discussion of the complexes. It has also to be noted that the calculated values have no physical meaning and thus should only be considered for comparing the different drug-DNA complexes to find optimal binding geometries.

Results and Discussion

Model building, geometric aspects, and energy minimization

No X-ray structure of mitoxantrone (**1**) is known. Therefore, the X-ray structure of 1-(2-(diethylamino)-ethylamino)-anthracen-9,10-dione [23] was used as starting geometry to build up the drug molecule. This structure was modified with the aid of the module BUILDING in SYBYL. The more basic secondary amines in the two side chains of mitoxantrone were constructed in the protonated form. To get a conformation with an energy near the global minimum of mitoxantrone, a conformational search in SYBYL using the SEARCH option was performed.

To build up the tetranucleotide–mitoxantrone complexes, it was necessary to construct a tetranucleotide-duplex with an intercalation site between the second and third base pair. As starting structure for the tetranucleotides $d(TCGA)_2$ and $d(TTAA)_2$, the canonical B-DNA of the X-ray structures of a daunorubicin-$d(CGATCG)_2$ [3] and of an idarubicin-$d(TGATCA)_2$ intercalation complex [6] were used. In these complexes, two drug molecules are intercalated between the first and second and between the fifth and sixth base pairs in the 5′ and 3′ chain direction. In these complexes, the space between the stacked base pairs at the intercalation site is enlarged from genuine 3.4 to 6.7 Å, respectively.

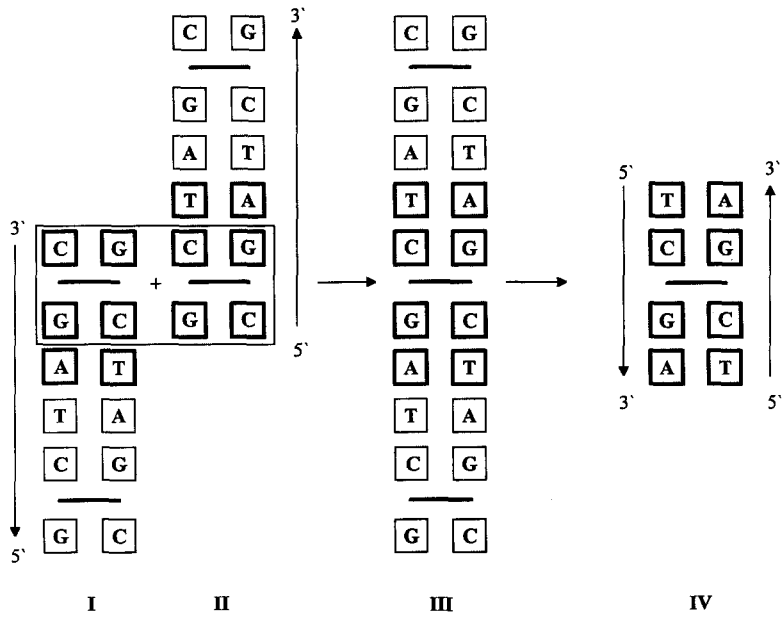


Fig. 2. Schematic construction of $d(TCGA)_2$ (**IV**) with intercalation site between the second and third base pair starting from the nucleinic part of an X-ray crystal structure of the $d(CGATCG)_2$-daunorubicin complex [3] by the superposition and deletion strategy described in the text

As shown in Fig. 2, fitting of two identical oligonucleotide duplexes **I** and **II** derived from the X-ray structure of the $d(CGATCG)_2$-daunorubicin intercalation complex [3] over base pairs 1/2 and base pairs 5/6 was performed to give rise to a decanucleotide duplex **III**. By this procedure, an intercalation site is constructed between the base pairs 5 and 6. Then, the first and last three base pairs were deleted from the decanucleotide duplex to produce $d(TCGA)_2$ with an enlarged intercalation site between the second and third base pairs (type **IV** in Fig. 2). For the construction of $d(TTAA)_2$ starting from the $d(TGATCA)_2$-idarubicin complex, the base pairs two and five were substituted to become a $d(TAATTA)_2$-hexanucleotide, no X-ray analysis of an ApT or TpA intercalation site being available so far. Then, the same procedure as demonstrated in Fig. 2 was realized. The thus constructed tetranucleotide duplex $d(TCGA)_2$ and $d(TTAA)_2$ were refined using the AMBER molecular mechanics method [19]. Starting from these base paired tetranucleotides, eight complementary base paired sequences were built up by manual exchange of the terminal base pairs. These sequences all posses a central $d(\text{pyrimidine-3'-5'-purine})_2$ sequence. For validation of the generated geometries we compared these with some relevant X-ray crystal structures. The constructed tetranucleotide duplexes with a prepared intercalation site are very similar to the backbone structure of the B-DNA-hexanucleotides from the X-ray complexes [3, 5, 6] and to a pure B-DNA dodecamer [24] (Table 1). The increased base pair distance at the intercalation site induces first of all a change in the angle ζ [25] (for definition see footnote in Table 1). Further geometric changes of the DNA by intercalation is the buckling of the base pairs of the intercalation site and a slight unwinding, demonstrated in the unwinding angle [25]. Both values keep unchanged on the basis of the construction mode of the tetranucleotides. Roll, slide, twist angle, propeller twist, and the furanose

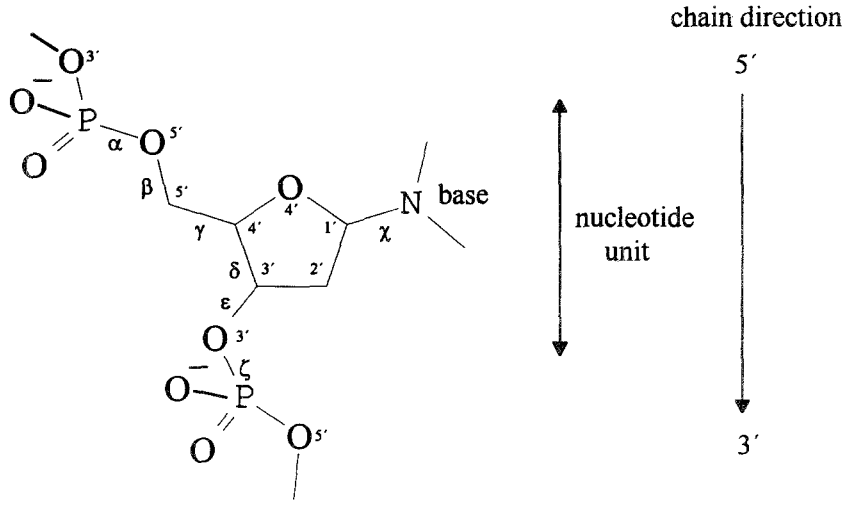


Fig. 3. Atomic numbering scheme and definition of torsional angles for a polyribonucleotide chain, counting the nucleotides is from top to bottom, *i.e.*, in the direction O5′–O3′; the chain direction is from 5′ to 3′ end. For details see Ref. [25].

Table 1. Average backbone torsion angles and their standard deviations (in parentheses) of the constructed tetranucleotides $d(TCGA)_2$ and $d(TTAA)_2$ in comparison with the corresponding values of the anthracycline-hexanucleotide complexes and of a dodecanucleotide

Oligonucleotide	Sugar-phosphate backbone and glycosyl torsion angles[a]						
	α	β	γ	δ	ε	ζ	χ
$d(TCGA)_2$	−67.7	156.1	55.5	130.9	−139.5	−140.6	109.8
	(8.3)	(19.1)	(4.3)	(11.7)	(34.9)	(46.6)	(11.3)
$d(TTAA)_2$	−71.5	154.5	55.3	138.3	−142.2	−127.6	111.6
	(7.7)	(20.8)	(2.1)	(10.9)	(23.5)	(50.4)	(19.9)
$d(CGATCG)_2$-	−61.9	158.7	46.0	128.3	−148.0	−99.7	−111.5
daunorubicin [3]	(33.8)	(27.5)	(28.5)	(8.9)	(32.1)	(64.4)	(25.7)
$d(TGATCA)_2$-	−65.5	164.8	52.6	126.5	−140.4	−128.0	−105.0
idarubicin [6]	(12.2)	(20.2)	(14.9)	(11.9)	(26.4)	(58.1)	(34.8)
$d(TGTACA)_2$-	−67.8	169.6	50.4	130.6	−145.2	−122.1	−108.8
4′-epiadriamycin [5]	(8.2)	(21.1)	(2.8)	(20.8)	(27.9)	(52.4)	(30.0)
$d(CGAGAATTCGCG)_2$	−57.1	171.5	48.3	131.8	−172.7	−101.9	−109.3
[24]	(25.6)	(24.8)	(28.9)	(23.9)	(25.0)	(22.5)	(19.9)

[a] Backbone torsion angles are defined as P–α–O5′–β–C5′–γ–C4′–δ–C3′–ε–O3′–ζ–P and the glycosyl angle as O4′–C1′–χ–N1–C2 for pyrimidines and O4′–C1′–χ–N9–C4 for purines

conformation [25] were not significantly modified by an intercalator; therefore these values are not given in Table 1.

For building up the intercalation complexes by docking of mitoxantrone into the enlarged intercalation cavity of the base paired tetranucleotide, favoured orientations of the drug were taken from the structure elements of the X-ray structures of the anthracycline–hexanucleotide complexes [3, 6]. Former molecular modeling

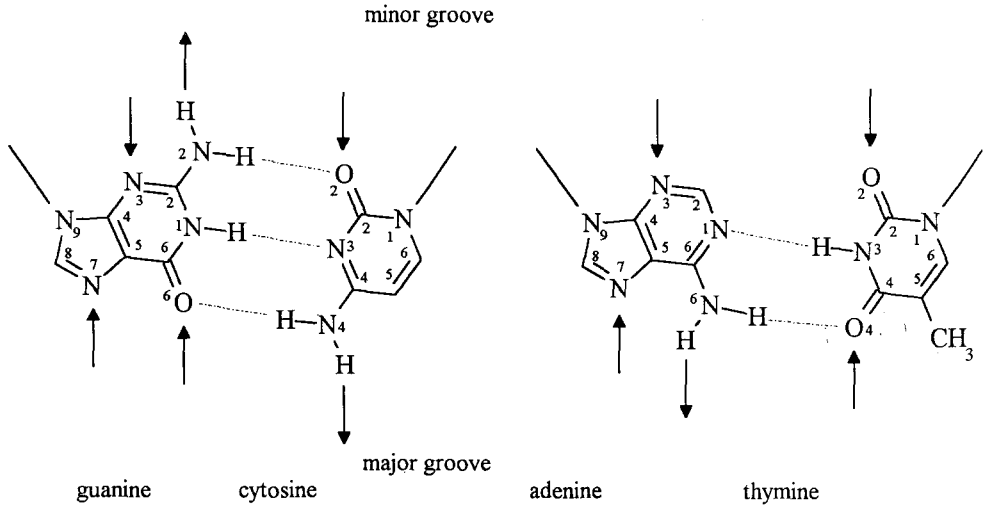


Fig. 4. The hydrogen-bond donor and acceptor sites given by arrows for GC and AT base pairs in the major and in the minor groove of DNA

studies with mitoxantrone did not take into account anthracycline-DNA complex geometries for drug preorientation [17, 26]. Alternative complex geometries to our results are discussed. The generated mitoxantrone–tetranucleotide complexes were then minimized by molecular mechanics. From these calculations, the anthraquinone chromophore is best orientated nearly perpendicular to the long axis of the two stacked base pairs (N1-pyr-N9-pur, Fig. 4). An edge-on alignment orientation of the chromophore, *e.g.* nearly parallel to base the pair axis, and a "straddle binding" resulting thereof with one side chain in the major and one side chain in the minor groove as discussed in earlier literature [27, 28] was ruled out on the basis of the anthracycline–hexanucleotide X-ray analysis [2–8].

According to several docking experiments and energy minimizations, we constructed a complex with orientation of the two side chains of the drug both either in the minor or both in the major groove. These chains form hydrogen bonds with the heteroatoms and the heteroatom hydrogens of the base pairs. The base pair heteroatoms show a pattern of hydrogen bond acceptors and donors dependent on the nucleotide sequence (Fig. 4). In the case of the anthracycline intercalators, the C9–OH and the protonated amino group of the sugar moiety are involved in hydrogen bonds with DNA. The necessity of the C9–OH group for the antitumor activity has been proven [29]. X-ray structures of pure DNA-minor groove binders, for example a distamycin DNA complex [30], are bound to oligonucleotides by several hydrogen bonds. Thus, hydrogen bonds play a decisive role for the stabilization of DNA-drug complexes, and the stability of the DNA-drug complexes is a condition for significant biological activity. X-ray structures of anthracycline oligonucleotide complexes [2–8] rule out any (principially possible) ionic interaction of the ammonium group with the phosphate anion as already discussed before [31]. Therefore, an electrostatic interaction of the mitoxantrone side chains with the phosphate group was not taken into consideration in our model studies.

Energy refinements of some base paired nucleotide segments of X-rayed anthracycline–oligonucleotide complexes [3, 6] with TAFF conform that not only two

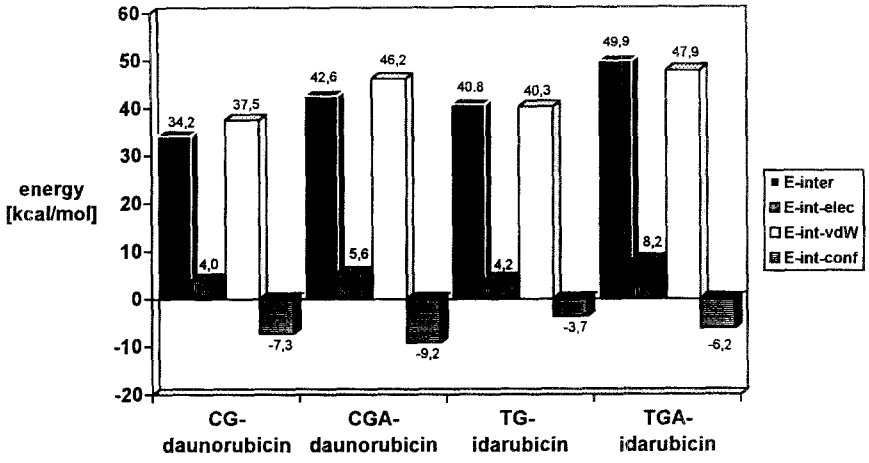


Fig. 5. Potential energy values (kcal/mol), deduced from TAFF calculations of oligonucleotide fragments of anthracycline-hexanucleotide complexes

but three base pairs are involved in complexation with the drug on the basis of the potential energy values. In Fig. 5, various potential energy terms from molecular mechanics calculations of some fragments of anthracycline–hexanucleotide complexes are given. The *E-drug* value for daunorubicin was deduced from an optimal energy minimum structure resulting from a daunorubicin X-ray structure [32]. The *E-drug* value for idarubicin was derived from the same X-ray analysis [32] after transformation of daunorubicin with the SYBYL Build option.

The protonated amino group of the anthracyclines interacts with a third base pair. This can be demonstrated by analysis of the X-ray geometry and also by calculation of *E-inter*. There is a significant decrease of the *E-inter* values if a trinucleotide instead of a dinucleotide complex is calculated (Fig. 5); so the interaction between the amino group and the third base pair is demonstrated. The anthracycline drugs have only one "side chain" in the form of the amino sugar, thus a minimum of three nucleotides in the DNA is necessary for optimal DNA binding, whereas mitoxantrone with two side arms favours binding with a tetranucleotide sequence of DNA. This conclusion was supported by an X-ray analysis of a d(GAATCTTC)$_2$-actinomycin complex [33]. The two depsipeptide rings of actinomycin D interact with a tetranucleotide sequence in the solid state.

Both side chains in mitoxantrone are connected by two hydrogen bonds of type **I** and **II** (Table 2, Fig. 6) with the tetranucleotide. These hydrogen bonds contribute significantly to the stabilization of the mitoxantrone-tetranucleotide complexes, as it is demonstrated by force field calculations. The hydrogen bonds of type **I** are formed between the protonated amino group of each side chain and a hydrogen acceptor of the second or third base pair. The hydrogen bonds of type **II** are formed between the hydroxy group of each side chain and the first or fourth base pair (for details see Table 2).

All mitoxantrone-tetranucleotide complexes have a pseudo two fold axis in the long axis of the mitoxantrone chromophore similar to a recently reported X-ray structure of the actinomycin D-hexanucleotide complex [33] which has an approximately two fold symmetry around the short axis of the chromophore.

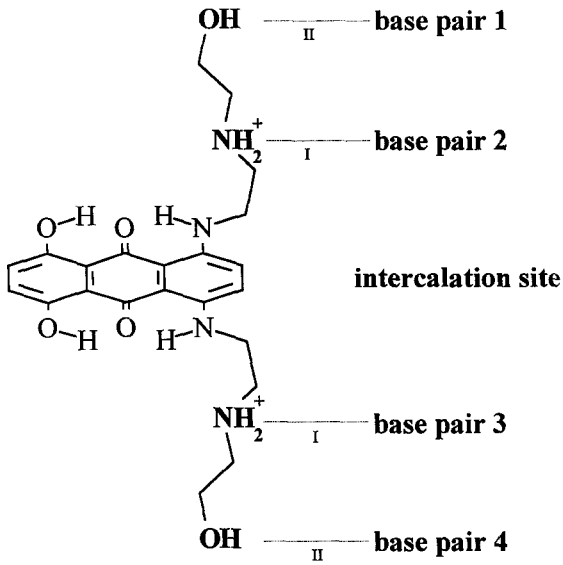


Fig. 6. Schematic illustration of a tetranucleotide-mitoxantrone intercalation complex with two different types (**I** and **II**) of hydrogen bonds for each side chain

Table 2. Geometries of the hydrogen bonds between mitoxantrone and base paired tetranucleotides of the intercalation complexes after energy refinement using TAFF

Base sequence of the tetranucleotides	Complexes (abbreviation see[a,b])	Hydrogen bonds **I** with specification of the participated base pair heteroatoms and of angles[c] (°) and distances[d] (Å)		Hydrogen bonds **II** with specification of the participated base pair heteroatoms and of angles[c] (°) distances[d] (Å)	
d(ACGT)$_2$	acgt-ma[a]	G–N7	G–O6	T–O4	
		131.3/112.3	127.3/152.6	121.0/126.0	
		2.33/2.72	1.75/1.58	2.20/2.60	
d(CCGG)$_2$	ccgg-ma	G–N7	G–O6	G–O6	
		134.2/103.2	119.9/136.2	144.0/122.0	
		2.40/2.63	1.83/1.73	2.78/2.65	
d(GCGC)$_2$	gcgc-ma1	G–N7	G–O6	C–N4	
		131.8/125.4	118.9/121.8	122.0/127.3	
		2.03/2.21	2.22/1.99	2.25/2.13	
d(GCGC)$_2$	gcgc-ma2	G–N7	G–O6	G–O6	
		124.6/135.1	130.4/128.9	121.2/129.0	
		2.53/2.50	1.72/1.79	2.76/2.54	
d(TCGA)$_2$	tcga-ma	G-N7	G–O6	A–N6	T–O4
		124.0/132.5	130.9/131.6	126.6/122.2	152.2/154.6
		2.55/2.53	1.71/1.75	2.49/2.47	2.39/1.98
d(ACGT)$_2$	acgt-mi[b]	C–O2		T–O2	
		135.9/137.5		139.9/154.1	
		2.68/2.18		2.68/2.69	
d(CCGG)$_2$	ccgg-mi	C–O2		G–N2	
		163.7/147.9		135.3/147.8	
		1.78/2.03		2.18/1.80	

(*Continued*)

Table 2. (*Continued*)

d(GCGC)$_2$	gcgc-mi	C–O2	G–N2
		119.7/146.8	143.9/158.6
		2.49/1.95	1.79/1.94
d(TCGA)$_2$	tcga-mi	C–O2	A–N3
		152.7/145.8	149.5/155.6
		1.89/2.68	2.59/2.63
d(ATAT)$_2$	atat-ma	A–N7	T–O4
		124.8/139.8	140.5/157.0
		2.39/1.79	2.50/2.53
d(CTAG)$_2$	ctag-ma	A–N7	G–O6
		139.4/124.9	139.2/154.8
		1.99/2.64	2.80/2.80
d(GTAC)$_2$	gtac-ma	A–N7	C–N4
		133.9/123.4	123.9/127.9
		1.78/2.44	2.40/2.52
d(TTAA)$_2$	ttaa-ma	A–N7	A–N6
		122.5/133.2	126.4/123.8
		2.44/1.86	2.72/2.62
d(ATAT)$_2$	atat-mi	T–O2	A–N3
		168.6/129.9	146.1/132.2
		1.68/1.74	2.15/2.33
d(CTAG)$_2$	ctag-mi	T–O2	G–N2
		136.7/141.4	124.6/128.1
		1.75/1.73	2.23/2.61
d(GTAC)$_2$	gtac-mi	T–O2	G–N2
		158.8/126.3	128.5/122.5
		1.69/1.80	2.07/1.91
d(TTAA)$_2$	ttaa-mi	T–O2	T–O2
		166.4/134.4	123.0/159.1
		1.57/1.69	2.56/1.86

[a] ma: implementation of both side chains of mitoxantrone into the MAjor groove; [b] mi: implementation of both side chains of mitoxantrone into the MInor groove; [c] angle heteroatom-hydrogen heteroatom; [d] distance between hydrogen and acceptor-heteroatom

On the basis of all considerations mentioned above, from the eight constructed tetranucleotide duplexes seventeen different mitoxantrone complexes were formed with intercalation of the drug between the second and third base pair. The side chains were orientated both in the major or alternatively both in the minor groove. During the TAFF force field calculations using the Tripos parameters, the tetranucleotide duplexes were defined as aggregates and therefore they remained geometrically unchanged. This procedure seems to be allowed because the tetranucleotide duplexes used are geometrically very similar to the X-ray structures of the hexanucleotide–anthracycline complexes [3, 5, 6].

Comparing the force field energy terms of seventeen calculated mitoxantrone–tetranucleotide intercalation complexes (Table 3), *E-int-vdW* and *E-int-elec* con-

Table 3. Potential energy values (kcal/mol), deduced from TAFF calculations of the constructed mitoxantrone-tetranucleotide complexes[a]

Tetranucleotide complexes	*E-inter*	*E-int-elec*	*E-int-vdW*	*E-int-conf*
acgt-ma	43.0	12.5	39.6	−9.1
ccgg-ma	43.7	12.1	38.4	−6.8
gcgc-mal	44.0	12.5	38.4	−6.9
gcgc-ma2	45.3	11.7	39.3	−5.7
tcga-ma	45.7	12.6	39.5	−6.4
acgt-mi	28.6	8.8	31.5	−11.7
ccgg-mi	32.0	12.0	31.3	−11.3
gcgc-mi	26.9	11.5	32.5	−17.1
tcga-mi	28.9	9.4	32.8	−13.3
atat-ma	26.8	8.2	37.3	−18.7
ctag-ma	30.7	8.3	39.3	−16.9
gtac-ma	30.4	7.8	41.7	−19.1
ttaa-ma	28.9	6.9	40.9	−18.9
atat-mi	22.6	17.0	28.3	−22.7
ctag-mi	26.4	15.3	31.5	−20.4
gtac-mi	19.0	14.9	26.7	−22.6
ttaa-mi	25.8	16.4	24.1	−14.7

[a] Abbreviation: see Table 2

tribute mostly to the complex stabilization energy. The *van der Waals* energy (*E-int-vdW*) is induced first of all by the enlargement of the contact area of the interacting chromophore of the drug with the groups of the base paired oligonucleotide. In TAFF, the hydrogen bonds are taken into consideration during the calculation of the *van der Waals* and the electrostatic energy. The value for *E-drug* of mitoxantrone, necessary to calculate the energy values shown in Table 3, is deduced from an appropriate energy minimum structure resulting from a conformational search of mitoxantrone.

The energy requirement for conformational changes of the intercalator to construct the optimal binding geometries has to be compensated by the energy gain of *E-int-vdW* and *E-int-elec* (Table 3, abbreviations see Table 2). The calculations demonstrate that the intercalation of mitoxantrone between a $d(CG)_2$ base pair in a pyr-3′-5′-pur sequence with orientation of both side chains into the major groove is energetically mostly favoured (Table 3). In Fig. 7a, one of these complexes is shown. This result is compatible with experimental findings for mitoxantrone by analysis of sequence selectivity [34]. According to our calculations and geometrical analyses, this binding geometry is therefore favoured, because only in this case a three-centered hydrogen bond **I**, connecting the guanine N7 and the guanine O6 with one ammonium hydrogen, is formed (Fig. 7b).

This fact was also supported by comparison of energy values of some structural subunits of the complexes (Fig. 8). The calculated complexes of the pure chromo-

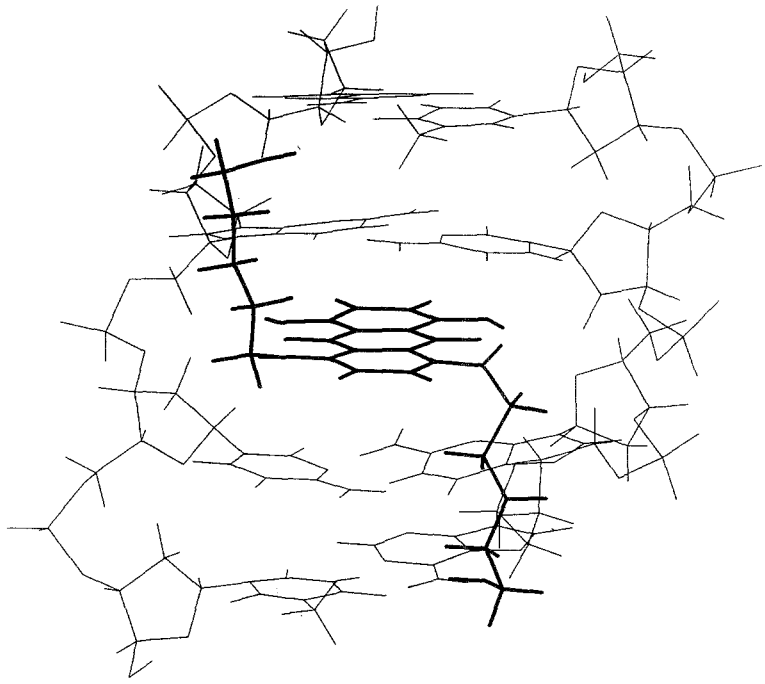


Fig. 7a. Intercalation of mitoxantrone into $d(TCGA)_2$; view approximately perpendicular to helix axis; the drug is viewed from the major groove; the energetically mostly favoured intercalation complex structure (tcga-ma) is presented; the two side chains are orientated into the major groove

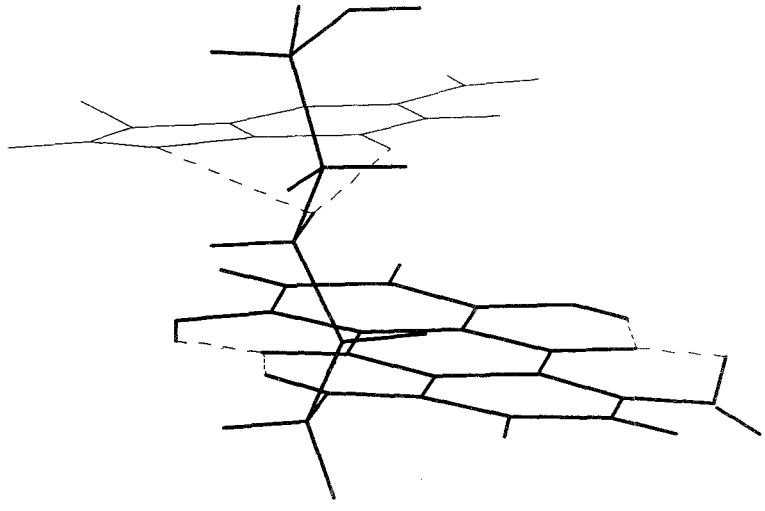


Fig. 7b. Enlarged part of Fig. 7a, showing explicitly the three-centered hydrogen bond between one mitoxantrone side chain and guanine (hydrogen bond of type **I**)

phore structure of mitoxantrone ((1,4-dihydroxy-5,8-diamino)-anthracen-9,10-dione) with the tetranucleotide duplexes $(TCGA)_2$ and $(TTAA)_2$ revealed no significant site selectivity on comparing the interaction energies *E-inter*. From calculations of complexes constructed with a mitoxantrone derivative without the terminal

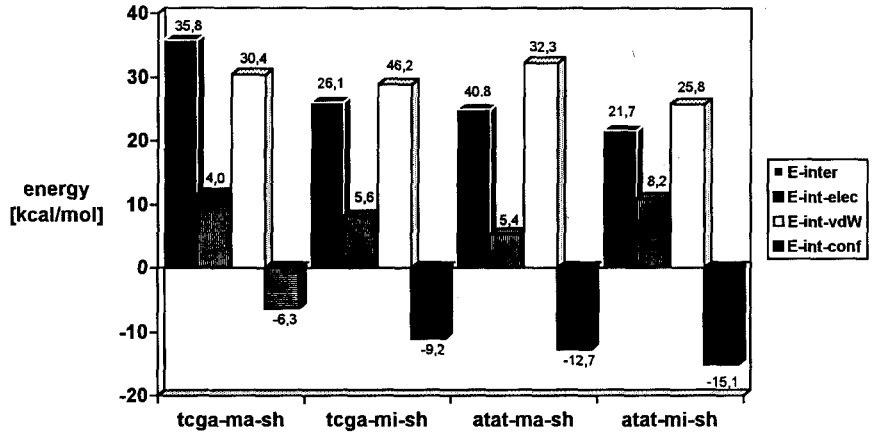


Fig. 8. Potential energy values (kcal/mol), deduced from TAFF calculations of complexes of mitoxantrone subunits with base paired tetranucleotides

2-hydroxyethyl-groups (1,4-dihydroxy-5,8-*bis*-(2-amino-ethylamino)-anthracen-9,10-dione) and the tetranucleotide duplex – for example tcga-ma-sh (**sh**ortened chain) – very different values for the interaction energy were obtained (Fig. 8). Thus, we assume that the complex stabilization and site selectivity are controlled first of all by the interaction of the side chain amino group with the DNA on the basis of hydrogen bonds. The complex with the highest value for *E-inter* is as expected tcga-ma-sh with the three-centered hydrogen bond.

The hydrogen bonds formed by the 2-hydroxyethyl group in mitoxantrone (type **II** hydrogen bonds) seem to yield a significant energetical contribution for complex stability, whereas the oligonucleotide sequence specificity was less influenced by this type of hydrogen bond (compare the energy values in Table 3). However, the hydroxyethyl group can interact with the oligonucleotide as hydrogen donor and also as hydrogen acceptor. Thus, for hydrogen bond type **II** several interaction modes are possible. In the case of the complexation of mitoxantrone with *d*(GCGC)$_2$ with insertion of both side chains into the major groove, two geometrical orientations (gcgc-mal and gcgc-ma2) were studied. In gcgc-mal, a hydrogen bond is built between the oxygen of the hydroxyl group and a hydrogen of the cytosine-N4 of the base pair 1 or 4, respectively. In gcgc-ma2, the hydrogen bond is built between the hydrogen of the hydroxyl group and the guanine-O6. The calculated difference of the energy values of these complexes amounts to only 1.3 kcal/mol. These results are compatible with the structural characteristics of the X-ray structure of the *d*(GAAGCTTC)$_2$-actinomycin D complex which revealed several binding modes for the two cyclic substituents of the phenoxazone chromophore with the base paired oligonucleotide [33].

The other calculated complexes – intercalation of drug between base pairs TA or CG, both with side chain orientation into the minor groove – are slightly less energetically favoured than the intercalation of the drug between the CG base pair with the side chains in the major groove (Table 3). These results are supported by experimental findings: the interaction of the side chains of mitoxantrone in the minor

groove of a CG base pair takes place only when any interaction with the major groove is sterically hindered [9]. However, on the basis of our calculations intercalation of mitoxantrone between TA base pairs with orientation of both side chains into the minor groove seems also possible (Table 3).

Structure antileukemic activity relationship studies of *bis*-(substituted) aminoalkylamino-anthraquinones [35] demonstrated that a removal of the terminal group of the side chain gives rise to compounds with lower antileukemic activity. Thus, the nitrogen atom in the center of the side chain playes an important role in the magnitude of cytostatic effects. Replacement of the nitrogen atom by a methylene group or by a sulfur atom leads to inactive compounds. The results of these structure activity relationship therefore support our DNA-interaction models.

Conclusion and some remarks to base-drug stacking

According to the presented molecular modeling studies, mitoxantrone intercalates DNA first of all from the major groove, and the hydroxyl or amino groups of the side chains are involved in hydrogen bonds with pyrimidine and purine base heteroatoms of the base paired oligonucleotides. Different binding modes are discussed. An interstrand cross-linking of the DNA helices is established (Fig. 7a and 7b), and a CG intercalation site selectivity (pyr-3′-5′-pur sequence) is obvious, fully compatible with anthracycline-DNA intercalation complexes. Our model is also consistent with physicochemical and electron microscopic studies of the CG-selective interaction of mitoxantrone with DNA [9, 10] as well as with structure/antileukemic activity relationship studies [35].

The given mitoxantrone–oligonucleotide intercalation model is constructed *in vacuo*, and an extrapolation of our results to the real conformative interaction process of the drug with DNA in a biological system has to be examined carefully and critically [17]. The calculated potential energy from molecular mechanics method cannot normally provide data on free energy values that have direct relevance to thermodynamic measurements of free energy and entropy, although relative binding energies calculated for a closely related series of drugs that do not displace different numbers of water molecules should principially correlate with experimental enthalpies and even changes in free energies [17]. Our model calculations are quite successful in predicting correct complex geometries; they do not reflect the balance of driving forces for intercalation. The intercalative driving force to base-drug stacking is indeed a phenomenon of liquid water structure in reality [25]. Therefore, the calculated (absolute) interaction energies based first of all upon hydrogen bonding and *van der Waals* forces have to be relatived carefully with the real interaction state of DNA-drug complexes in condensed phase or *in vivo*. Probably hydrophobic effects not considered in the present calculations play also an important role in the drug-base stacking interaction [25].

However, the presented results predict undoubtely intercalation of the drug on the basis of a theoretical screening of several binding modes to base paired tetranucleotides of B-DNA type. Moreover, such an approach can be helpful to understand the mode of actions of cytostatically active intercalators and to design structurally related compounds of the anthraquinone drug type.

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