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Joint Experimental and Theoretical Investigation of the Comparative DNA Binding Affinities of Intercalating Anthracycline Derivatives

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SUMMARY

The comparative binding affinities for poly(dA-dT) and poly(dG-dC) of novel antitumor anthracyclines are reported. The data concern, besides the parent compound adriamycin (ADM), 4-demethoxy 6-deoxy 6-aminodaunomycin (**II**), 9-deoxy-ADM (**III**), 4-demethyl-6-*O*-methyl-ADM (**IV**), and 3′-deamino-3′-hydroxy-4′-epi-ADM (**IV**). Theoretical computations are performed in parallel for their comparative binding affinities to model double-stranded hexanucleotides, $d(GCGCGC)_2$, $d(TATATA)_2$, and $d(CGTACG)_2$, using the SIBFA (sum of interactions between fragments computed *ab inito*) procedure. The computations reproduce in a very satisfactory manner the most salient features of the experimental comparative binding affinities. These encompass, in particular, a higher affinity for the $d(TATATA)_2$ oligomer of **II** than that of ADM, despite the absence of the 14-OH substituent in **II**, a marked reversal of the CG versus TA sequence selectivity of the neutral compound **V**, favoring the $d(CGCGCG)_2$ oligomer over the $d(TATATA)_2$ one; and the deleterious effect incurred on the binding affinities by the presence of an *O*-methyl substituent at position 6 of the chromophore.

It is well established experimentally today that anthracyclines showing antitumor properties have structural features that allow their intercalation into double helical DNA (1–4). A number of studies have been performed aiming at the exploration of this phenomenon at a molecular level, both by experimental and theoretical methods (5–9). An important result was achieved when the three-dimensional structure of the d(CGTACG)-DNM crystal complex was determined by X-ray diffraction studies (10).

Outstanding compounds among the anthracyclines are DNM and ADM which rank among the most powerful antitumor agents currently in use in chemotherapy (11–12). Because DNA constitutes a major target for DNM and ADM complexation, the elucidation of the inherent base sequence preferences was and is considered one of the major tasks in studies of the mechanism of action of these drugs.

Whereas several contradictory experimental results were published in the past (13–18), a very recent study, involving a combination of several experimental techniques (19), demonstrated the existence of a well defined pattern of sequence preferences for DNM and ADM in terms of triplets of base pairs. Such a pattern, as well as the prefered receptor triplets d(ACG) and d(TCG), were in full conformity with the predictions published by us in 1985, on the basis of theoretical computations (7) indicating preferential intercalation of the chromophore between the two C-G base pairs and interaction of the cationic side chain with the A-T base pair upstream.

As a continuation of our work on anthracycline binding to oligonucleotides (7–9), we report here results of a joint experimental and theoretical investigation of the binding to a representative DNA sequence of a series of anthracycline analogs, differing by their substitution patterns on the chromophore and the side chain on ring A.

The results of the theoretical computations are compared with the experimental binding affinities. The compounds considered in this study are those shown in Fig. 1. The three-dimensional model of DNA employed is the same as that used in the X-ray determination of the $d(CGATCG)_2$-DNM complex (10) in which the subscript 2, used in this paper, indicates a double-stranded hexanucleotide with the same composition of bases. In this paper, we compare the specificity, as determined experimentally for poly d(G-C) and poly d(A-T), with the theoretical results computed, in the framework of the SIBFA method (20, 21), with DNA models represented by the hexanucleotides $d(CGCGCG)_2$ and $d(TATATA)_2$, similarly to the procedure used for the theoretical investigation of the base sequence selectivity of DNM and ADM. The intercalation site

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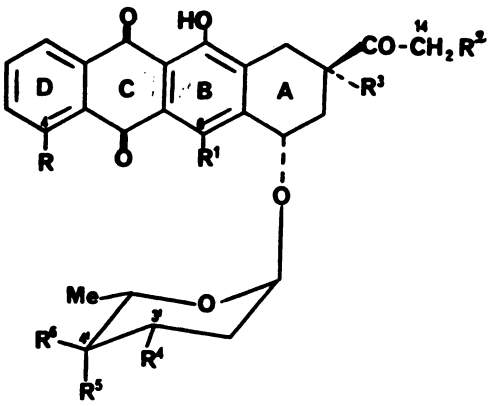
ABBREVIATIONS: DNM, daunomycin; ADM, adriamycin; SIBFA, sum of interactions between fragments computed *ab initio*.



COMPOUND		R	R^1	R^2	R^3	R^4	R^5	R^6
I	ADM	OCH_3	OH	OH	OH	NH_2	OH	H
II	4-DEMETHOXY-6--DEOXY-6-AMINO--DNM	H	NH_2	H	OH	NH_2	OH	H
III	9-DEOXY-ADM	OCH_3	OH	OH	H	NH_2	OH	H
IV	4-DEMETHYL-6-O--METHYL-ADM	OH	OCH_3	OH	OH	NH_2	OH	H
V	3'-DEAMINO-3'--HYDROXY-4'-EPI--ADM	OCH_3	OH	OH	OH	OH	H	OH

Fig. 1. Structures of the anthracyclines investigated.

for the compounds considered is thus maintained between the base pair Pu6-Pyl′ and Py5-Pu2′ (see Fig. 2).

Procedures

Experimental methods. Poly d(A-T) and poly d(G-C) were purchased from Boehringer Mannheim and titrated spectrophotometrically at 260 and 254 nm (ϵ = 6600 and 8400 M^{-1} cm^{-1}, respectively). The drug-DNA complexes were formed directly in 1-cm optical path length quarz cuvettes upon mixing of appropriate volumes of the DNA and drug stock solutions. The complex solutions were then titrated by the addition of aliquots of a solution of the drug. The measurements were performed on a Perkin Elmer MPF 44AP spectrofluorometer at room temperature. DNA nucleotide concentrations 44AP spectrofluorometer at room temperature. DNA nucleotide concentrations ranged from 5×10^{-8} to 3×10^{-4} M. The different drug concentrations employed were in the range 2×10^{-7} to 1×10^{-5} M. A nonlinear fitting procedure (22) was applied in order to evaluate the binding parameters, as reported in Ref. 23, and the simple model of a single class of independent binding sites (24) was used for the determination of the K_n. The calculations were performed using the program of Fletcher and Shrager (25).

Theoretical methods. As in our preceding studies devoted to anthracycline intercalator-oligonucleotide complexes (5–9), we assume a rigid model for the oligonucleotides fixed in the appropriate conformation but allow a large flexibility of the drugs along their dihedral angles. The conformational energy of the drug and the intermolecular oligonucleotide-drug interactions are computed and energy minimized, using the SIBFA procedure (20, 21).

Results and Discussion

The results of the computations are reported in Table 1, in which are listed, in addition to the experimentally determined affinities for the d(A-T) and d(G-C) polynucleotides, the theoretically computed overall binding energies, δE, and their differences, $\delta\delta E$, with respect to the most favorable value of δE taken as energy zero. Also reported in Table 1 are the differences of A-T versus G-C binding affinities of the drugs, computed as the difference between δE values for $d(TATATA)_2$ and $d(CGCGCG)_2$. Let us recall that δE is, itself, the sum of the oligonucleotide-ligand intermolecular interaction energy, E, the conformational energy of the ligand, E_c, spent upon binding to the oligonucleotide, and the unstacking energy of the oligonucleotide, $E_{unstack}$, necessary for the generation of the intercalation site.

In Table 2, a further analysis is performed of the comparative contributions of the chromophores of II (4-demethoxy-6 deoxy-6 amino-DNM) and I (ADM) to the overall stabilization of their respective $d(CGCGCG)_2$ and $d(TATATA)_2$ complexes. Finally, we report in Table 3 an analysis of the binding energetics of the neutral compound V to the oligonucleotides $d(TATATA)_2$ and $d(CGCGCG)_2$, in which, in addition to the terms E, E_c, and $E_{unstack}$, the separate contributions to E of the electrostatic (E_{MTP}), repulsion (E_{rep}), polarization (E_{pol}), and charge-transfer (E_{ct}) components are reported.

Representations of the complexes of II with $d(CGTACG)_2$

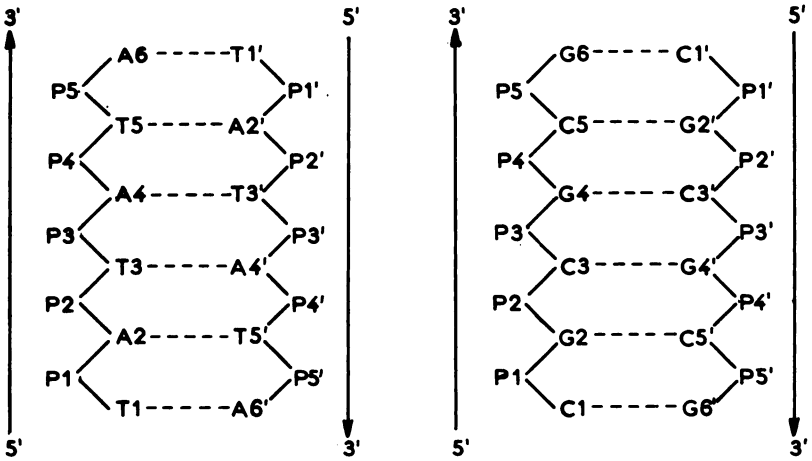


Fig. 2. Base numbering adopted for the self-complementary double-stranded hexanucleotides.

TABLE 1

Experimental [poly d(G-C), poly d(A-T)] and computed (hexanucleotide) binding affinities of the investigated compounds

Compound	Poly d(G-C), k_n	$d(CGCGCG)_2$		Poly d(A-T), k_n	$d(TATATA)_2$		$d(CGTACG)_2$		$\delta(E_{TA} - E_{CG})$
		δE	δδE		δE	δδE	δE	δδδE	
	M^{-1}	kcal/mol		M^{-1}	kcal/mol				
I	1.07×10^6	−407.1	14.6	1.18×10^6	−418.7	3.0	−421.1	0.6	11.6
II	2.62×10^5	−408.2	13.5	3.18×10^6	−419.6	2.1	−421.7	0.0	11.4
III	1.8×10^5	−401.4	20.3	3.8×10^5	−415.6	6.1	−415.5	5.2	14.2
IV	1.46×10^4	−345.7	76.0	2.4×10^4	−351.1	70.6	−347.5	74.2	5.4
V	1.35×10^5	−70.7		3×10^4	−62.5				−8.2

TABLE 2

Values of the separate chromophore-backbone and chromophore-base interaction energies for binding of I and II to $d(CGCGCG)_2$ and $d(TATATA)_2$.

	$d(CGCGCG)_2$	$d(TATATA)_2$
	kcal/mol	
Chromophore-backbone		
II	−25.6	−26.3
	−8.5	−8.5
I	−15.7	−16.8
	0.7	0.4
Chromophore-base		
II	−13.7	−12.7
	1.5	0.1
I	−17.5	−15.7
	−1.1	−0.4

TABLE 3

Binding energetics of V with $d(CGCGCG)_2$ and $d(TATATA)_2$

Energies in kcal/mol (see text for definitions).

	$d(CGCGCG)_2$	$d(TATATA)_2$
	kcal/mol	
ΔE_{inter}	−81.2	−73.0
E_{MTP}	−26.1	−18.1
$E_{mono\text{-}mono}$	−21.3	−17.1
E_{rep}	+66.9	53.8
E_{pol}	−6.1	−7.2
E_{disp}	−91.4	−84.9
E_{ct}	−24.5	−16.6
E_c	584.1	583.7
E_{tor}	2.6	4.1
$E_{unstack}$	10.5	9.5
$\delta_{c\,rel} = \delta E_c + \delta E_{tor}$	0.0	1.0
$\delta E = \Delta E + \delta E_c + E_{unstack}$	−70.7	−62.5

and of V with $d(CGCGCG)_2$ are given in Figs. 3 and 4, respectively.

The results of Table 1 indicate that, in the interaction with the regularly alternating oligomers, all the cationic derivatives display a marked preference for $d(TATATA)_2$ over $d(CGCGCGC)_2$. For binding to the preferred $d(TATATA)_2$ sequence, they rank in the following order: 4-demethoxy-6-deoxy-6-amino-DNM (II) > ADM (I) > 9-deoxy-ADM (III) ≫ 4-demethyl-6-*O*-methyl-ADM (IV). This ordering is the same as the one determined in the experimental study with poly d(A-T), although the difference in relative affinity of 4-demethoxy-6-amino-DNM (II) as compared with ADM (I) seems underestimated by the present computations.

It is noteworthy that, despite the absence of the 14-OH substituent, (see Fig. 1) analogue II is endowed with a binding affinity comparable to, and indeed larger than, that of ADM. Let us recall in this connection that the additional presence of such a substituent in ADM resulted in an enhancement of the binding affinity to oligonucleotides of ≈ 6–7 kcal/mol (7, 8),

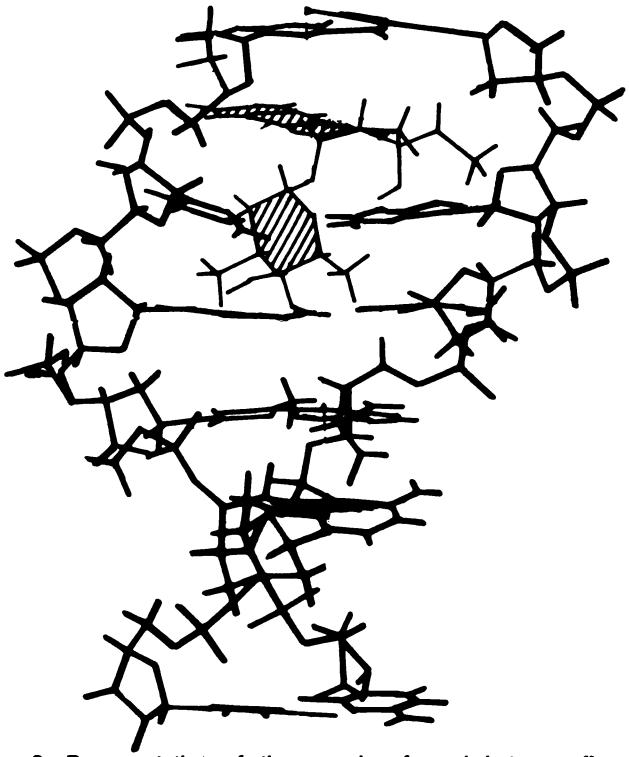


Fig. 3. Representation of the complex formed between II and $d(CGTACG)_2$.

with respect to DNM. The enhanced affinity of 4-demethoxy-6-deoxy-6-amino-DNM, with respect to ADM, is due principally to a more favorable (by about 15 kcal/mol) chromophore-backbone interaction energy for the former than for the latter. This preference is due essentially to the electrostatic energy contribution, E_{MTP}, which has negative values in the complexes of 4-demethoxy-6-deoxy-6-amino-DNM (−8.5 kcal/mol) and a positive, destabilizing one in the complexes of ADM (≈0.5 kcal/mole). The more favorable values of the chromophore-backbone interaction energies of the former are compensated to some extent by chromophore-base interaction energies that are ≈4 kcal/mol less favorable for it than for ADM (≈−13 kcal/mol for the former as opposed to ≈−16.5 kcal/mole for the latter, as averaged for the two sequences). This reversal of the order of preferences is, again, due predominantly to E_{MTP}, which now attains positive values in the complexes of 4-demethoxy-6-deoxy-6-amino-DNM. Such a discriminatory role of E_{MTP}, even though in neither the chromophore-backbone nor in the chromophore-base interactions is it the dominant contribution numerically, is a most persistent and characteristic feature of the present computations.

Intercalation of the chromophore of 4-demethoxy-6-deoxy-

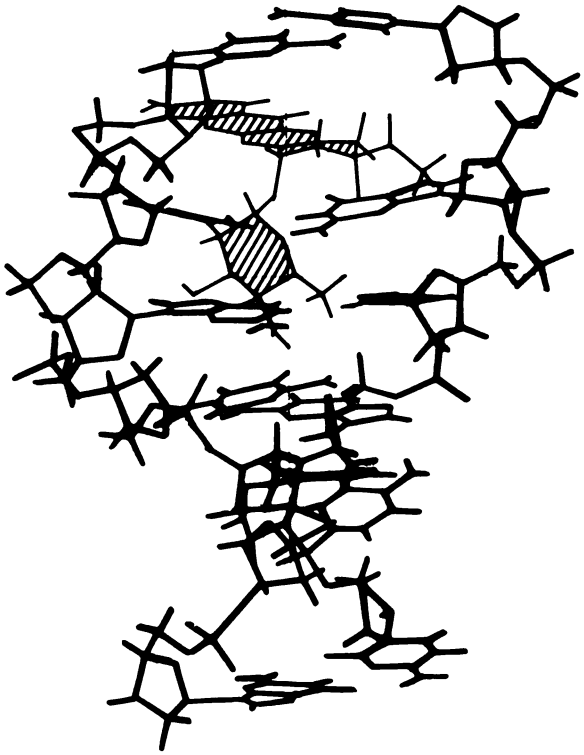


Fig. 4. Representation of the complex formed between V and d(CGCGCG)$_2$.

6-amino-DNM is stabilized, with respect to that of ADM, by a somewhat elongated hydrogen bond between one H of the 6-amino substituent and O_3, the esteric oxygen of the intercalation site ($d_{O3'-H}$ = 2.58 Å whereas the corresponding distance involving the 6-hydroxy hydrogen in ADM is 3.0 Å).

The same ordering of the four compounds is also observed for binding to the d(CGCGCG)$_2$ sequence, with, however, substantially smaller values of δE. The preferred (by 1 kcal/mol) binding to this sequence of 4-demethoxy-6-deoxy-6-amino-DNM as compared with ADM, is at variance with the experimental result obtained with poly(dG-dC), which indicates the reverse ordering. This is the only discrepancy with respect to experimental results in the present investigation. Further elaboration, including a relaxation of the oligonucleotides upon binding, will be attempted to clarify this point.

As compared with ADM, removal of the (9-OH substituent (see Fig. 2) to yield 9-deoxy-ADM (III) results in a decrease in the binding affinity and an increase, from 11.6 kcal/mol to 14.2 kcal/mol, of the AT versus GC preference. This situation is a reflection of the loss of the stabilizing interaction involving this substituent with N3 and the 2-amino group of a guanine of the intercalation site (7, 8). The other features of the binding stereochemistry of the compound are, on the other hand, closely similar to those of ADM (2).

Introduction of a methyl group in position 6, as in 4-demethyl-6-*O*-methyl-ADM (IV), has a deleterious effect on the binding affinity, owing to a severe steric repulsion involving this substituent and the DNA backbone. As a result, the preferred binding position has the chromophore significantly unstacked from the base pairs of the intercalation site and the side chains shifted away from the electron-rich atoms of the minor groove.

Concerning the interactions with the mixed d(CGTACG)$_2$ sequence, the three derivatives behave in a variable way; whereas 4-demethoxy-6-deoxy-6-amino-DNM shows a preference for the mixed oligonucleotide, as does ADM, 9-deoxy-ADM manifests an equal attraction for the mixed and pure AT sequences, whereas 4-demethyl-6-*O*-methyl-ADM displays an overall preference for this last sequence.

From that point of view, the binding of the neutral compound (V) to the sequences d(CGCGCG)$_2$ and d(TATATA)$_2$ introduces an interesting new aspect because, in agreement with experimental results, it now occurs with a significant preference for the GC sequence over the AT sequence. Again, the value of the energy difference, ≈8.2 kcal/mol, stems from the corresponding difference of the electrostatic contribution, which amounts to −26.1 and −18.1 kcal/mol in the GC and AT oligomers, respectively. (Let us also note that, out of these, only 4 kcal/mol are contributed by the sole charge-charge component of E_{MTP}, reemphasizing the necessity of a complete refined representation of this contribution.)

This compound is one of the very few intercalative compounds reported to date that elicits a GC selectivity while having its side chain located in the minor groove. Other examples of such a situation are actinomycin D (26) and some derivatives in the series of acridine alkylamides synthesized by Roques, Le Pecq, and co-workers (27, 28).

The preferential binding of V to the d(CGCGCG)$_2$ oligomer is assisted at the level of the side chain by one H-bond involving the hydrogen of the hydroxyl group R^4 on the sugar ring (see Fig. 1) and $O_{1'}$, of the deoxyribose residue linked to base C5 of the intercalation site, and another H-bond between the H of hydroxyl substituent R^6 and O_1, of the deoxyribose linked to base G4′ on the other strand (and two base-pairs below the intercalation site, d=2.15 Å in the two cases). Additional interactions occur between the two oxygen atoms of these two substituents and the two amino hydrogens of the N2 group of base G4 below the intercalation site, although the angles O · · · NH are ≈100°, below the optimal values encountered for H-bonding interactions.

Its binding to the d(TATATA)$_2$ oligomer is only assisted, at the level of the side chain of V, by two hydrogen bonds involving the hydrogen atoms of hydroxyls R^4 and R^6 and O_1, of deoxyribose atoms linked, respectively, to bases T5 and A4′, with O · · · H distances of 2.15 and 2.23 Å, respectively.

The summed interaction energies of the two hydroxyl substituents with the two bases G4 and C5 of d(GCGCGC)$_2$ and with the two corresponding bases A4 and T5 of d(TATATA)$_2$ amount to −7.8 kcal/mol and −3.6 kcal/mol, respectively. This difference accounts for a substantial fraction of the overall stabilization of 8.2 kcal/mol in favor of the former. An additional contribution to this stabilization is brought about by the more favorable chromophore-base interaction in the GC oligomer than in the AT oligomer one, as outlined above.

It should be realized, on the other hand, that the theoretical binding affinities of compound V, which show an outstanding correlation with the experimental results in terms of selectivity, diverge considerably from those of compounds I–IV. The much lower value of the stabilization energy can be related to the absence of a cationic charge on V. In fact, at physiological pH, the daunosamine residue of I–IV is largely in the protonated form (and was considered as such in the calculations). Contributions due to its strong electrostatic interactions with strong

negative molecular potential of the minor groove of the DNA are absent in the stabilization energies for compound V.

Let us recall, in this connection, that upon studying the binding of DNM to hexanucleotides [7], we observed that the binding energies dropped greatly (from ≅−420.4 to ≅134.3 kcal/mol) when the anionic charges of the phosphates were neutralized by countercation binding. A further drop in the absolute values of the overall energy balances is to be expected, when taking into account the dehydration energies of the ammonium group. Whereas it is justified to neglect these factors in a *comparative* approach within the homogeneous series represented by cationic compounds I–IV, they should be considered when comparing anthracycline derivatives with neutral and charged sugar moieties. Experimental values of dehydration enthalpies for NH_4^+ and $CH_3NH_3^+$ of, respectively, −83.8 and −75.7 kcal/mol have been reported (29). Therefore, if we substract the value of the dehydration enthalpy of methyl-ammonium ion, namely −75.7 kcal/mol, from the value of −134.3 kcal/mol, calculated for DNM when the phosphate groups are neutralized, we obtain a value of −58.6 kcal/mol, which is of the same order of magnitude as the value obtained for compound V.

Conclusions

In comparing the theoretical and experimental data previously reported, two important points have to be mentioned. The theoretical calculations were obtained using double-stranded hexanucleotides that are simple models for the true DNA molecules on which the experimental affinities were determined; these calculations do not consider solvation effects.

Nevertheless, the present computations have enabled us to account for some of the most salient features of the experimental comparative binding affinities of anthracycline analogs. Thus, we compute a higher binding affinity for $d(TATATA)_2$ of compound II (4-demethoxy-6-deoxy-6-amino-DM) than of compound I (ADM) despite the absence of the 14-OH substituent in the former. Analysis of the factors involved in this preference has demonstrated the more favorable chromophore-backbone interactions occurring with II, as compared with I, which more than compensate for the loss of the stabilizing interactions involving the 14-OH substituent of ADM.

A remarkable reversal of CG versus TA sequence preference is found for compound V (3′-deamino-3′-hydroxy-4′-epi-ADM). The origin of the CG sequence preference of V may be interpreted, in the framework of our theoretical computations, in terms of more favorable interactions of the two hydroxyl substituents on the sugar ring with the base C5 of the intercalation site and the base G4 immediately upstream of it than with the corresponding base T5 and A4 in the $d(TATATA)_2$ sequence, on one hand, and of more favorable chromophore-base interactions in the $d(CG)_2$ intercalation site on the other hand.

The increase (from 11.6 to 14.2 kcal/mol) of the TA versus CG sequence preference of III (9-deoxy-ADM), as compared with I (ADM) can be described quantitatively in terms of the loss of the hydrogen bond involving the 9-hydroxyl oxygen and the 2-amino group of guanine G2′ at the intercalation site. Actually, compound III manifests equal affinities for the regular alternating sequence $d(TATATA)_2$ and the mixed sequence $d(GCTACG)_2$. This contrasts with ADM, for which a distinct preference (of 2.6 kcal/mol) is computed for the mixed sequence, containing the interacting triplet $d(ACG)_2$, over the regular sequence $d(TATATA)_2$. It is worth noting, in this respect, that III has reduced antitumor activity, as compared with ADM.

The dramatic loss of DNA affinities of IV, as compared with the other compounds, can be attributed to the protruding effect of its 6-methyl substituent.

Binding of anthracyclines to DNA is a necessary but not sufficient condition for the exhibition of antitumor properties (30-31). In fact, compound III is still a good intercalator but is devoid of pharmacological activity whereas IV is a poor intercalator but shows noticeable activities against mouse tumours (32). Intercalated drugs interact with enzymes involved in the replication and transcription of DNA and, particularly, with DNA topoisomerase II (33). It appears that the C-9 hydroxy group is an important structural component of the interaction and that its absence in III determines the loss of bioactivity of this compound. The geometry determined for compound IV after theoretical energy refinement indicates a somewhat more external position of the intercalated chromophore with respect to the intercalation geometries of I–III and V. As a consequence, the daunosamine residue protrudes more markedly toward the exterior within the minor groove and could be in a more suitable orientation for the interaction with the DNA enzymes.

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