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Molecular Modeling of the Interaction of Some Phenoxazone–Antitumoral Drugs with DNA

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Abstract

Motivation. The study of the interactions between double stranded deoxyribonucleic acid (DNA) and different binding agents is of major importance in the understanding of biochemical processes. Many aspects concerning the structural factors determining one type or another of the drug–DNA interaction mechanism (intercalation or minor groove binding) are not yet elucidated, especially in aqueous solutions. The objective of the present paper is to evaluate the contribution of the different structural factors that determine the main binding mechanism, intercalation or minor grove binding, for several iminoquinone (phenoxazinone) drugs, using molecular modeling and quantum–chemical calculations.

Method. The models of the drug–nucleic acid complexes were built by manual docking followed by molecular mechanics optimization with implicit solvent effect using OPLS force field. In order to identify and analyze intermolecular interactions for the drug–DNA complexes, the SHB_interactions program, based on EH–calculated Mulliken overlap populations as a quantitative quantum chemical criterion, was used.

Results. Structural modeling of the solution drug–DNA complexes and energetic analysis outlines that the substituents in 1,9 positions are essential for the intercalative binding mode. The calculated binding energies vary in the same order as the biological activity questiomycin < 1,9–diacetyl– 2–aminophenoxazine–3–one < protonated 5H–pyrido[3,2–a]phenoxazine–3–one < actinomycin D. Mulliken overlap populations (OP) analysis allows to identify the classical N–H...O bonds, as well as C–H...O(N) bonds, which represent 97% from the OP due to H–bonds. The selectivity and biological activity is probably due to the H–bonds O5: 2H2 G4 and O3: 2H2 G12 present in all minor groove complexes.

Conclusions. Our results show that, in spite of the drastic approximations implied, the combined use of molecular mechanics modeling with Mulliken overlap populations (SHB_interactions program), may provide useful information about the structural factors controlling the binding mechanism of the phenoxazinone drugs to dsDNA and outline the specific H–bonds and other atom–atom interactions which contribute to the stabilization of drug–DNA complexes.

Availability. The source code for SHB_interactions program, written in C, instructions and some examples are available at http://gw–chimie.math.unibuc.ro/staff/cbendic/shb/SHB_interactions.html.

Keywords. SHB_interactions; overlap population; iminoquinone; drug–DNA complexes; intercalation; minor groove.

Dedicated on the occasion of the 75th birthday to Professor Lemont B. Kier.

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Abbreviations and notations

OPLS, Optimized Potentials for Liquid Simulations	QPy, 5H–pyrido[3,2–a]phenoxazine–3–one
OP, overlap population	$QPyH^+$, protonated 5H–pyrido[3,2–a]phenoxazine–3–one
MB, methylene blue	Cin, Cinnabaric acid
ActD, actinomycin D	Cet, 1,9–diacetyl– 2–aminophenoxazine–3–one
Que, questiomycin	Eth^+, ethidium bromide

1 INTRODUCTION

The study of the interactions between double stranded deoxyribonucleic acid (DNA) and different binding agents is of major importance in the understanding of biochemical processes like replication, repair, recombination and expression of genes. The pharmacological activity of the antitumoral drugs is generally attributed to their tight interaction with DNA, which causes inhibition of transcription elongation by the blockage of RNA–polymerase [1,2].

The iminoquinone moiety is characteristic for a large number of antitumoral drugs and plays an important role in the antineoplastic action of actinomycin D, one of the most employed drugs in the treatment of highly malignant tumors [3–6]. Although other drugs with better therapeutic action and less cardiotoxicity have been used in the last years, actinomycin D containing a 2–amino phenoxazine–3–one ring substituted with two cyclic pentapeptides in 1,9 positions, is still considered as a model compound in the study of DNA–drug interactions [7–10].

The structural features to which the antineoplastic activity of these drugs is usually attributed are: the presence of a planar aromatic moiety, consisting of three or four aromatic rings, which ensures the intercalation of the drug between specific base–pairs, and the presence of a sugar, amine or peptide substituent, which may ensure the minor or major grove binding.

However, many aspects concerning the structural factors determining one type or another of the drug–DNA interaction mechanism are not yet elucidated, *i.e*., the structure of the intercalator–DNA complexes in aqueous solutions [11,12]. Molecular modeling is a powerful tool to suggest possible structures for these complexes and to identify the structural features and specific interactions that determine their stability [13].

Recently, the binding of a dye molecule, methylene blue (MB) was reanalyzed and the minor groove binding was found to be predominant for the DNA– MB complex, especially at increased salt concentrations [13]. The energetic analysis performed has shown that the non–electrostatic (van der Waals) energy favors minor groove binding of MB over both intercalative and major groove binding.

Our previous studies [10,15] have shown that the use of molecular mechanics simulations corroborated with a method using as quantum–chemical criterion the Mulliken overlap populations, (SHB_interactions program [16]), allows an insight in the nature of the non–electrostatic interactions which contribute to the stability of the actinomycin D–DNA intercalation complexes,

for which both experimental and theoretical data indicated a major contribution of the non–electrostatic interactions to their stability. The results have indicated that a contribution of about 70% to the stability of the drug–ssDNA complex is due to classical H–bond interactions, and to other atom–atom interactions for the drug–dsDNA complex.

The objective of the present paper is to evaluate the contribution of the different structural factors that control the main binding mechanism, intercalation or minor groove binding, for several iminoquinone (phenoxazinone) drugs, using the same theoretical approach based on the coupling of molecular mechanics simulations with SHB_interactions analysis. To this purpose the following compounds were investigated: 2–aminophenoxazine–3–one (questiomycin–**Que**) and 5H–pyrido[3,2–a]phenoxazine–3–one (**QPy**), which contain only unsubstituted three and respectively four aromatic cyclic moieties; 2–aminophenoxazine–3–one 1,9–substituted with smaller COCH3 and COOH groups (**Cet** and Cinnabaric acid – **Cin**) comparatively with actinomycin D (**ActD**); and positively charged species protonated 5H–pyrido[3,2–a]phenoxazine–3–one (**QPyH**$^+$), studied comparatively with the typical intercalator ethidium bromide (**Eth**$^+$), in order to outline the role of electrostatic contribution to the binding mechanism. The protonated **QPyH**$^+$ was considered because of its high antitumoral activity that could be due, according to literature data [14] to the positive charge of the nitrogen pyridine atom (pK_a = 5.9) at physiological pH.

To facilitate comparison with the model drug previously studied [10–13], ActD–dsDNA complex was also calculated using the present approximations and methods as for the other drugs. Differing from our previous study, where only in vacuum optimizations were employed, molecular mechanics optimization with implicit solvent effect based on OPLS force field was used. The structural modeling of the drug–dsDNA complexes was performed using an oligonucleotide 5'–d(GpApApGpCpTpTpC)–3' octamer. Because of the lack of X–ray or NMR–determined structures (pdb–files), structural modeling of the solution drug–DNA complexes and energetic analysis, coupled with a quantum–chemical analysis based on Mulliken overlap populations (SHB_interactions program) were used to obtain information about the binding modes of the investigated drugs to DNA. The results are compared with literature experimental data regarding the antitumoral activity [14].

2 COMPUTATIONAL DETAILS

The structures of the drugs were built using the HyperChem Release 6.01 program and geometries were optimized by the semiempirical AM1 method (EF optimization algorithm with RMS gradient of 0.01 kcal/mol Å). The model structures of the drug–nucleic acids complexes were built by manual docking started with step by step intercalation of the drug between the GpC pairs, followed by molecular mechanics optimization with implicit solvent effect using OPLS force field with the scale factor D = 10. An extensive energy minimization was obtained by Polak–Ribiere

algorithm with RMS gradient of 0.01 kcal/mol Å). The different binding modes as intercalation of the drug between adjacent base pairs, as well as the insertion into the minor or major groove of the DNA helix, were considered.

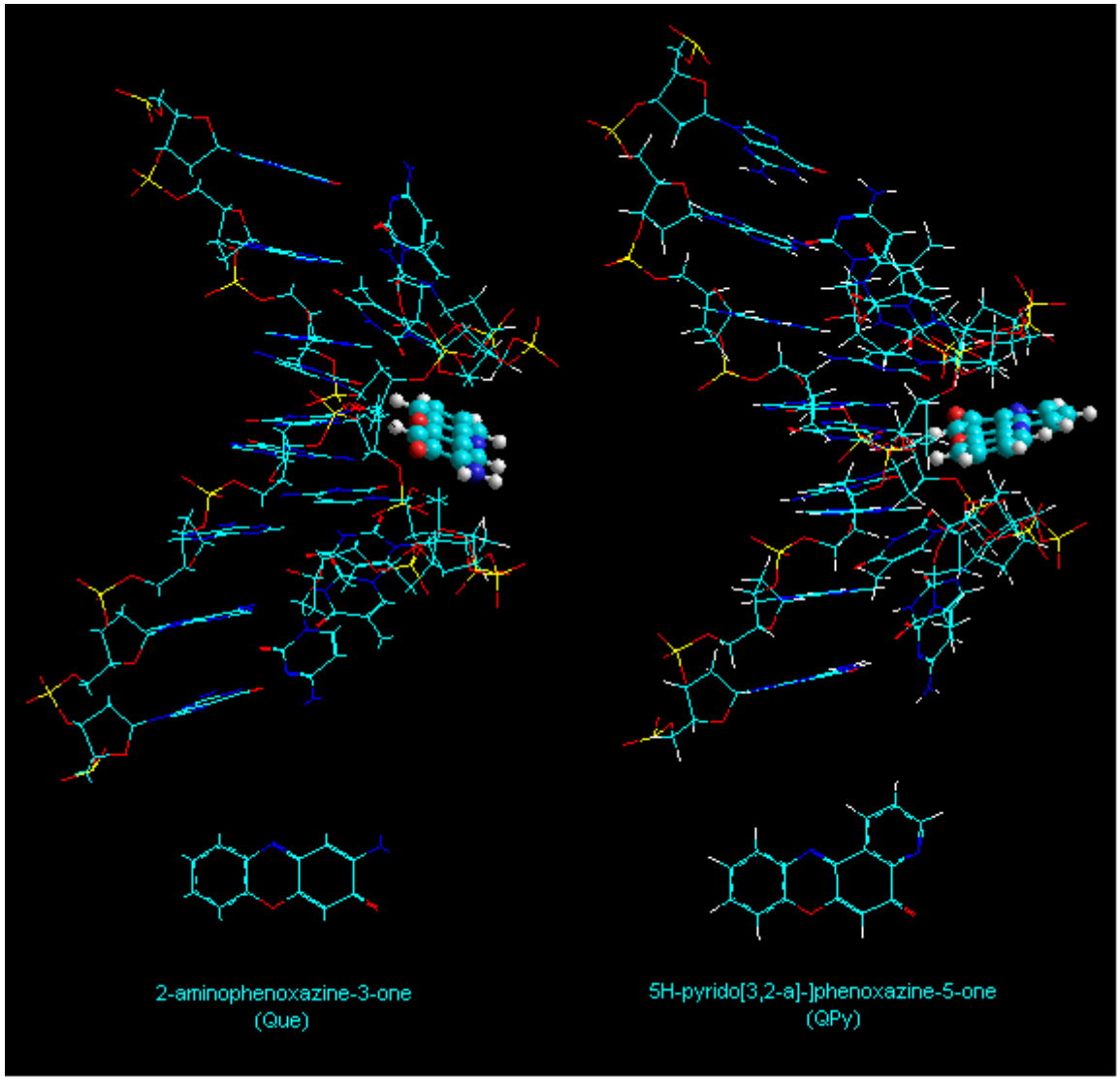

Figure 1. The OPLS optimized drug–5'–d(GpApApGpCpTpTpC)–3' complexes: 2–aminophenoxazine–3–one and 5H–pyrido[3,2–a]phenoxazine–3–one.

In order to identify and analyze intermolecular interactions for the drug–nucleic acids complexes obtained by molecular mechanics optimization, the SHB_interactions program [16] was used. This program cuts off from the PDB structure of the drug–nucleic acids complex the residue–residue or drug–residue pairs that possess atoms placed at a distance less than 3.5 Å., adds hydrogen atoms to satisfy the oxygen and phosphorus valence according to their hybridization state, and performs EH calculation of the overlap population [17]. The use of the EH method is justified because it is the only semiempirical method where the ZDO approximation is not used and the direct calculation of Mulliken overlap population is done. The simplicity of this method and the approximations that are

made are compensated by the use of the overlap population as a relative criterion in the interpretation of the results. Its simplicity, offers the possibility to perform such calculations for a large set of biopolymer structures in a relative short time.

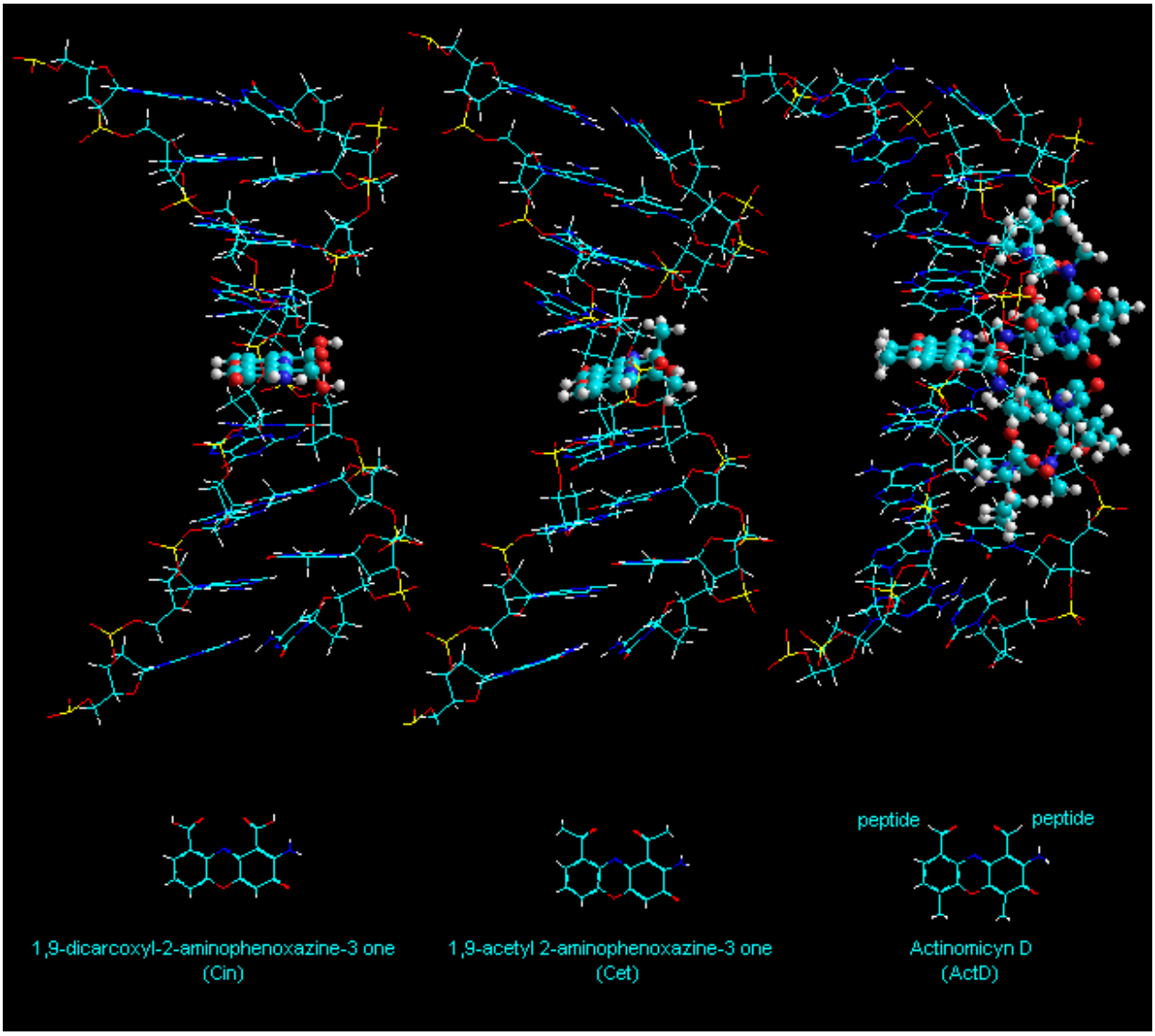

Figure 2. The OPLS optimized drug–5'–d(GpApApGpCpTpTpC)–3' complexes: 1,9–dicarboxyl–2–aminophenoxazine–3–one, 1,9–acetyl–2–aminophenoxazine–3–one and actinomycin D.

The capability of the overlap population to measure the strength of atom–atom intermolecular interactions, is justified qualitatively: the more positive the electronic population of atomic overlap distribution, the greater the overlap distribution contributes to the atom–atom interaction, chemical bond being a classical example. Our previously results reflect this capacity of the overlap population (OP) to make distinction between different H–bond types [16–18]. The classical H–bonds appear to be responsible for the selectivity and biological activity of the drug. In addition, the use of the OP as a quantum selection criterion presents the advantage to detect not only the weaker

H–bonds like C–H…O(N), but also any other atom–atom intermolecular interactions responsible for the stability of the drug – DNA complexes.

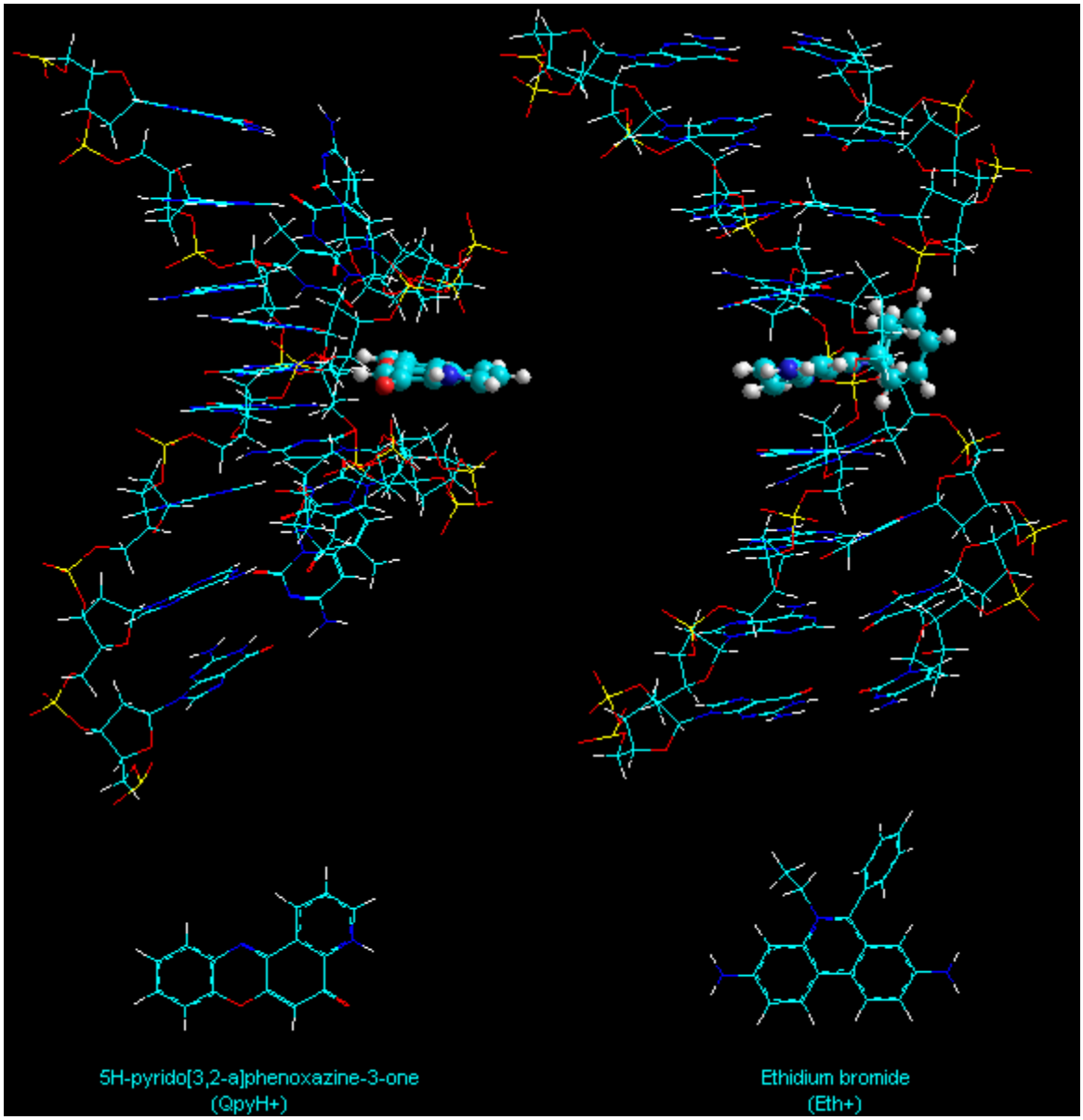

Figure 3. The OPLS optimized drug–5'–d(GpApApGpCpTpTpC)–3' complexes: 5H–pyrido[3,2–a]phenoxazine–3–one and ethidium bromide.

3 RESULTS AND DISCUSSION

The fully optimized drug – DNA complexes obtained by the procedure described in the preceding section, are presented in Figures 1–3. The unsubstituted phenoxazinone derivatives **Que** and **QPy** (Figure 1) are placed in the minor groove, even if manual docking initially started with intercalation of the drug between the GC base pairs of DNA. The 1,9 substituted compounds **Cin** and **Cet**, as well as **ActD** (Figure 2), remain intercalated, with the 1,9 substituents oriented towards the minor groove.

The charged drug, protonated **QPyH**$^+$, compared with the typical intercalator ethidium bromide (**Eth**) are presented in Figure 3. Although both drugs are positively charged, the **QPyH**$^+$ is disposed in the minor groove, whereas ethidium bromide remains intercalated, with ethyl and phenyl groups almost perpendicular to the planar condensed rings and placed in the minor groove. The different behaviors outlined in Figures 1–3 are reflected in the binding energy ($E_{bind.}$ defined as the difference between the OPLS energy of the complex and the sum of the energies of the molecules with the geometry frozen as in the complex), and the relative contribution of van der Waals (vdW) vs. electrostatic terms, presented in Table 1.

Table 1. The binding energies and the vdW and electrostatic contributions for the investigated drug–DNA complexes.

Drug	Localization	Contribution (Kcal/mol)		$E_{bind.}$ (Kcal/mol)
		vdW	Electrostatic	
Que	Minor Groove	–40.81	0.46	–40.35
QPy	Minor Groove	–43.90	0.65	–43.25
Cet	Intercalation	–51.01	0.50	–50.51
Cin	Intercalation	–48.67	0.74	–47.93
$QPyH^+$	Minor Groove	–43.32	–44.59	–87.91
Eth^+	Intercalation	–54.84	–44.14	–98.98
ActD	Intercalation	–126.97	–1.58	–128.55

Analysis of data in Table 1 shows that for **Que**, which contains the same planar phenoxazone moiety as **ActD**, with no substituents in sites 1,9 and 4,6, the smallest binding energy is obtained, and is practically due to vdW interactions. The drug is ejected in the minor groove, even if initially the manual docking started with intercalation between the GC base pairs of DNA. For **QPy**, with a planar moiety consisting of four condensed rings, the situation is similar and the binding energy is only 3 kcal/mol higher. The same situation was encountered for methylene blue (MB) in a recent study [13]. The energetic analysis for the solution–DNA complexes has shown that the considerable deformation of DNA in the intercalation complex requires a high energy and therefore the minor groove binding is favored over intercalation.

Substitution in the 1,9 positions (**Cet** and **Cin**), even with smaller, less bulky substituents in comparison with **ActD**, brings about an increase of $E_{bind.}$ and of the vdW contribution (entries 3 and 4 in Table 1). Therefore the presence of substituents in sites 1,9 disposed in the minor groove seems to be essential for the intercalative binding mode. A possible explanation for this could be a partial compensation of the high deformation energy of DNA in the intercalation process, by the interactions of the substituent groups placed in the minor groove. Support for this assertion is given by the comparison with Act D, for which a much higher binding energy is obtained, arising mainly from vdW interactions. In this case the intercalated planar moiety is the same as for **Cet** and **Cin**, and consequently the deformation energy would be the same, but the cyclic pentapeptides in sites 1,9, disposed in the minor groove compensate the deformation energy required by intercalation.

Examination of the results obtained for the positively charged drugs, protonated **QPyH**$^+$ and ethidium bromide, shows a binding energy in the range of –85 up to –100 kcal/mol, arising, as

expected, from the important electrostatic contribution of the charged drug in the minor groove with the backbone phosphate groups, but also from a high vdW contribution. The different binding mode observed for the two drugs and the higher binding energy found for the typical intercalator ethidium bromide [19] may be due to the phenyl and ethyl substituents, almost perpendicular to the planar condensed rings and oriented towards the minor groove, which ensures additional stabilization of the complex by a higher vdW contribution.

Table 2. Overlap population for H–bond and other atom–atom interaction for the investigated drug/dsDNA complexes

Drug	Overlap population		Specific H – bonds				
	H–bonds	Other atom–atom interactions	Type	DNA	Drug	r H–A	O P
Que	0.0213	0.1926	N–H…O	2H2 G 4	O5	2.54	0.0076
				2H2 G 12	O3	2.61	0.0041
			C–H…O(N)	N3 G 4	H6	2.75	0.0072
				O2 C 13	H4	2.52	0.0018
QPy	0.0322	0.1498	N–H…O	2H2 G 12	O5	2.54	0.0061
				2H2 G 4	O3	2.71	0.0048
			C–H…O(N)	2H5* T 15	N2	2.68	0.0092
				N3 G 12	H6	2.69	0.0065
Cet	0.0062	0.1157	N–H…O	O4* C 13	1HN2	2.99	0.0021
			C–H…O(N)	N9 G 4	H7	3.18	0.0007
				O2 C 5	1H18	2.65	0.0007
Cin	0.0161	0.1536	N–H…O	O4* C 5	1HN2	3.01	0.0028
				1H4 C 5	O5	3.54	0.0020
				1H4 C 13	O5	3.41	0.0015
			C–H…O(N)	H5 C 5	O3	3.82	0.0015
				H6 C 5	O3	3.88	0.0015
$QPyH^+$	0.0190	0.1446	N–H…O	2H2 G 12	O5	2.65	0.0052
				2H2 G 4	O3	2.66	0.0027
			C–H…O(N)	N3 G 12	H6	2.78	0.0072
				1H5* T 6	N2	3.39	0.0017
				O2 C 5	H4	2.58	0.0011
Eth	0.0177	0.1392	N–H…O	O4* C 13	2HN3	2.64	0.0045
				O4* C 5	2HN8	2.56	0.0040
			C–H…O(N)	O4* C 13	H4	2.71	0.0026
				N3 G 4	H16	2.92	0.0010
ActD	0.04930	0.72705	N–H…O(N)	O4* C 13 [a]	2HN2 PCZ 17 [a]	2.50	0.0064
				2H2 G 12 [a]	O THR 18 [a]	2.58	0.0041
				2H2 G 4 [a]	O THR 23 [a]	2.51	0.0040
				N3 A 3	2HN MVA 27	2.75	0.0029
				N3 G 4 [a]	H THR 18 [a]	2.87	0.0024
				2H2 G 12	OG1 THR 18	2.86	0.0022
				N3 G 12 [a]	H THR 18 [a]	2.69	0.0021
				O4* T 7	1HN SAR 21	3.21	0.0011
			C–H…O(N)	O3* A 3	2HG1 MVA 27	2.88	0.0063
				O4* C 5	H8 PXZ 17	2.61	0.0019
				N3 G 12	HA MVA 22	2.77	0.0018
				O4* T 7	1HA SAR 21	3.16	0.0015
				O4* G 12	3HG2 MVA 22	2.70	0.0012
				H4* G 4	OG1 THR 23	3.21	0.0011
				O6 G 12	2H15 PXZ 17	2.73	0.0011
				O4* G 4	HA MVA 27	3.08	0.0011

[a] Specific H–bonds evidenced also in [10] for the crystallographic structure (1dsc) of the ActD–dsDNA complex.

It is interesting to note that the binding energies in Table 1 vary in the same order as the biological activity Que < **Cet** < **QPyH$^+$** < **ActD** [14], *i.e.*, $E_{bind.}$ may be considered as a possible criterion for the antitumoral activity of these drugs.

The results of the Mulliken overlap populations analysis using the SHB_interactions program are presented in Table 2. These results are used to identify the specific H–bonds and other atom–atom interactions which contribute to the stabilization of the drug–DNA complexes and determine the favored binding mechanism. It may be observed that for the unsubstituted **Que** and **QPy**, for which the minor groove binding type is favored, the contribution of the H–bonds represents 10–20% from the total overlap population. The specific H–bonds imply classical N–H...O bonds, as well as C–H…O(N) bonds, and represent 97% from the OP due to H–bonds. The selectivity and biological activity of these drugs is probably due to the H–bonds O5: 2H2 G 4 and O3: 2H2 G 12 present in all minor groove complexes.

For the 1,9–substituted drugs, where the intercalative binding mode appears to be predominant, the H–bonds contribution decreases to 5–10% and that of specific H–bonds to 50–60% from the overlap population due to H–bonds. For the charged species **QPyH$^+$** and ethidium bromide the H–bonds contribution is about 12%, almost entirely due (70–93%) to specific H–bonds (N–H...O(N) or C–H...O(N)).

For **ActD** the H–bond contribution is comparable to the other intercalative not charged drugs (6%), the remaining part being due to other atom–atom interactions; about 85% from the OP due to H–bonds is due to specific H–bonds (N–H...O(N) or C–H...O(N)) of the pentapeptidic chains in the minor groove, as outlined by the data in Table 2. Comparison with the previous calculations [10] where the crystal X–ray geometry of the complex was used without further optimization, in this case smaller overlap populations for the specific H–bonds are observed, corresponding to greater distances between the interacting groups.

4 CONCLUSIONS

Structural modeling of the solution drug–DNA complexes and energetic analysis performed outlines the important role of the substituents in sites 1,9 disposed in the minor groove for the intercalative binding mode. A possible explanation for this could be a partial compensation of the high deformation energy of DNA in the intercalation process, by the interactions of the substituent groups placed in the minor groove.

For the positively charged drugs, a high binding energy was obtained, arising, as expected, from the important electrostatic contribution of the charged drug in the minor groove with the backbone

phosphate groups, but also from a high vdW contribution that ensures additional stabilization of the complex.

Mulliken overlap populations analysis allows identifying the specific H–bonds and other atom–atom interactions that contribute to the stabilization of drug–DNA complexes and determine the favored binding mechanism. The selectivity and biological activity of these drugs is probably due to the H–bonds O5: 2H2 G 4 and O3: 2H2 G 12 present in all minor groove complexes. The binding energies vary in the same order as the biological activity **Que** < **Cet** < **QPyH$^+$ < ActD**, *i.e.*, $E_{bind.}$ may be considered as a possible criterion for the antitumoral activity of these drugs. The binding energies and overlap populations analysis allow explaining both the stabilization of drug–DNA complexes and the favored binding mechanism.

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