Theor Chem Acc (1998) 99:312–319
DOI 10.1007/s002149800m17

Theoretical
Chemistry Accounts

Regular article

An ab initio study of the conformational preferences of Hoechst 33258 in gas-phase and aqueous solution environments

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Received: 3 March 1998 / Accepted: 29 May 1998 / Published online 19 August 1998

Abstract A quantum mechanical study of the conformational preferences of Hoechst 33258, a synthetic minor groove-binding drug, has been performed in both gas-phase and aqueous solution. Gas-phase calculations were performed at the HF/6-31G(*d*) and MP2/6-31G(*d*) levels of theory, whereas calculations in the aqueous solution phase were performed using the PCM model with the 6-31G(*d*) basis set. The molecule was divided into three fragments, which were submitted to a systematic and detailed conformational study. The results clearly indicate that Hoechst 33258 does not adopt a planar conformation in either the gas-phase or aqueous solution. Thus, a folded conformation is not induced by binding of the molecule to DNA, but is an intrinsic property of the compound.

Key words: Hoechst 33258 – ab initio calculations – Solution calculation – Minor groove binding drugs – Conformational properties

Introduction

The comparison between the structures of the complexes formed by drugs and DNA oligonucleotides has allowed us to visualize the fine details concerning the manner in which a minor groove-binding drug interacts with a B-DNA helix [1]. On the other hand, the study of the mechanism of the binding interaction between the drug and DNA has been of great interest in recent years, since knowledge of this mechanism would be very useful for the design of new synthetic drugs [2–5]. Such studies have suggested that the approach of the drug to DNA occurs through interactions involving charged groups. Contrary to common belief, in the approach step the hydrogen bonds would be rather passive [5].

The study of the conformation of the uncomplexed drug would also provide important information for both the molecular recognition and approximation processes. This point has been investigated by several research groups [6–8] for Hoechst 33258, i.e. 2-(4-hydroxyphenyl)-5-[5-(4-methylpiperazine-1-yl)benzimidazo-2-yl]-benzimidazole (see Fig. 1). Hoechst 33258 is a chromosome-standing agent with antihelmintic activity [9, 10] which binds strongly to double-stranded B-DNA with specificity for AT-rich sequences [11–14]. The interaction of Hoechst 33258 with DNA has been extensively studied using X-ray crystallography [15–20] and NMR spectroscopy [21–26].

The molecular structure of Hoechst 33258 is shown in Fig. 1. It is an N-methyl piperazine derivative with two benzimidazole groups and one phenol group. The conformational preferences of uncomplexed Hoechst 33258 in both gas-phase and aqueous solution were investigated in recent work using semiempirical quantum mechanical calculation [6]. The results indicated that Hoechst 33258 does not adopt a planar conformation, but is similar to those observed in DNA complexes by X-ray crystallography. Furthermore, in aqueous solution the conformation deviated from planarity more than in the gas phase. In a more recent work, Sapse et al [7] reinvestigated the conformation of Hoechst 33258 in the gas phase at the ab initio Hartree-Fock (HF) level using the STO-3G and 3-21G minimal basis sets. These levels of theory predicted a fully planar conformation for the torsional angles between the three aromatic rings of the drug. More recently, Sapse et al. [8] studied the effect of the solvent on the conformation of Hoechst 33258 using the SM1a mode [27]. The authors found that in solution the two benzimidazole ring are not coplanar, whereas the phenol and the nearest benzimidazole ring remain in the plane, the latter trend being probably due to the use of HF/STO-3G energies to estimate the gas-phase contribution to the conformational free energy in solution.

Crystal and solution structures of Hoechst 33258-DNA complexes [15–26] indicate that van der Waals interactions with the DNA minor groove constrain the piperazine ring to a stiff chair conformation. However, most of the X-ray structures have been solved at moderate atomic resolution and therefore the accuracy for the conformational details is somewhat poor. On the other hand, NMR constraints are usually compatible with more than one conformation of the ligand and

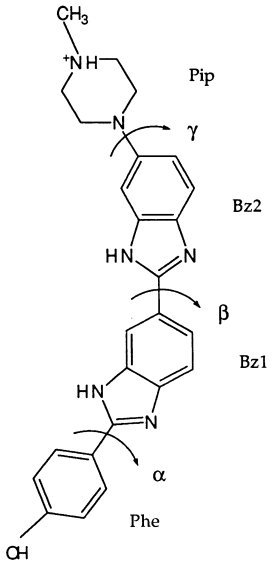

Fig. 1. Atomic scheme of Hoechst 33258. This drug has four structural units: a phenol ring (*Ph*), two benzimidazole rings (*Bz1* and *Bz2*), and a piperazine ring (*Pip*). The torsional angles between the four stuructural units are indicated by α, β and γ

therefore it is not possible to define the conformation. In addition, the accurate determination of the chair conformation for the piperazine ring presents an additional problem in that the number of chair conformers for the piperzine ring depends not only on the position of the methyl group attached to the protonated nitrogen atom, but also on the orientation of the lone pair of the unprotonated nitrogen atom. It is well known that the inversion of a lone pair which belongs to an aminic nitrogen atom requires a low activation energy. Detailed knowledge of the conformational preferences of the piperazine ring is required in order to ascertain the role of this charged group in the interaction with DNA.

In this work we have investigated the conformational preferences of Hoechst 33258 in both gas-phase and aqueous solution using ab initio computational methods at the HF/6-31G(*d*) and MP2/6-31G(*d*) levels.

2 Methods

2.1 Gas-phase calculation

The conformational preferences of the aromatic groups for Hoechst 33258 were determined by measuring the values of the torsion angles α and β between adjacent rings (Fig. 1). Since the large size of the Hoechst 33258 molecule makes it difficult to handle from a computational point of view, the use of appropriate fragments is required. The conformational preferences of α and β were investigated in fragments 1 and 2, which are shown in Fig. 2. In order to have a better understanding of the conformational differences between compounds with two aromatic rings like 1 and 2, and those with only one aromatic ring, we have extended the study to 3 (Fig. 2). This is a fragment of netropsin, another minor groove-

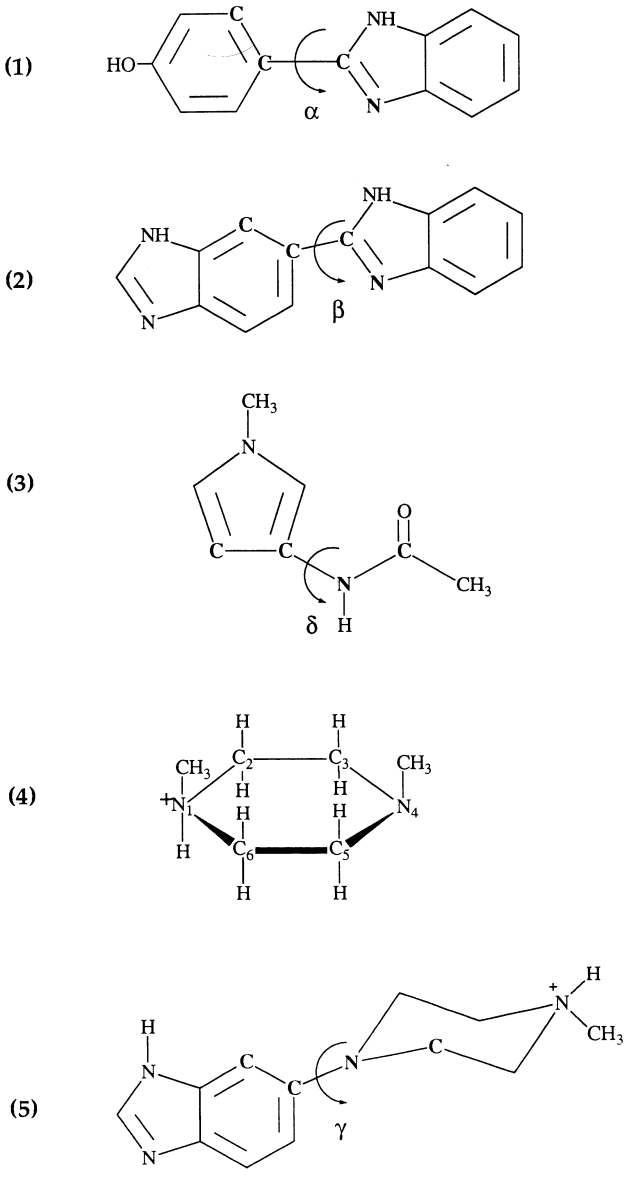

Fig. 2. Atomic scheme of the molecular systems investigated in the present work: **1** fragment of Hoechst 33258 consisting of a phenol ring attached to a benzimidazole ring; **2** fragment corresponding to the central part of Hoechst 33258 in which two benzimidazole rings are directly attached; **3** netropsin consisting of a *N*-methylpyrrole ring bonded to an acetamide group; **4** piperazine ring analogue; and **5** fragment of hoechst 33258 containing a benzimidazole ring bonded to a piperazine ring. Atoms indicated with *bold letters* are used to define the dihedral angles investigated

binding drug, that consists of an N-methyl-pyrrole ring bonded to an acetamide group.

Rotational profiles were computed for 1, 2 and 3 spanning the torsional angles in the range 0–180° in steps of 30°. The structure at each point of the rotational profile was obtained at the ab initio HF/6-31G(*d*) level [28] from geometry optimization but by fixing the torsional angle value. The Møller-Plesset (MP) perturbation treatment [29] was used to compute the electron correlation corrections to the energy. Thus, MP2/6-

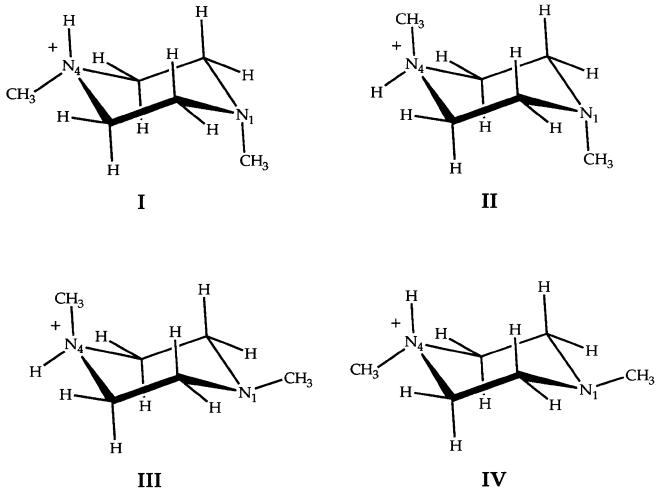

Fig. 3. Chair conformations of the model compound **4**

31G(*d*) calculations were performed considering the structures optimized at the HF/6-31G(*d*) level. Furthermore, the minimum energy conformations were characterized at the HF/6-31G(*d*) level by full geometry optimizations, the nature of the minimum state being verified by frequency analyses.

The conformational preferences of the piperazine ring were investigated using the analogue 4 (Fig. 2). For this purpose four chair conformations, which are shown in Fig. 3, were considered. The conformational study of 4 has been carried out at higher levels of theory than those of 1, 2 and 3 due to its small size. Thus, geometry optimizations were performed at both the ab initio HF/6-31G(*d*) [25] and HF/6-31+G(*d*, *p*) [28, 30] levels. Force constant analyses were carried out to verify the minimum nature of the conformations. MP2 corrections were computed from the structures optimized at the HF/6-31+G(*d*, *p*) level. The small correction up to the MP4 level was computed with the 6-31G basis set and added to the MP2/6-31 + G(*d*, *p*) value [31] this level of theory being denoted MP2/6-31 + G(*d*, *p*)//HF/6-31G + G(*d*, *p*) + MP4 #. This approximation has been used previously and gives reasonable estimations for the correlation effects [32–34].

Finally, the spatial arrangement of the piperazine ring in Hoechst 33258 was investigated in fragment 5 (see Fig. 2). The relative orientation between the benzimidazole and the piperazine rings is described by the dihedral angle γ. A previous study[6] showed that the rotational profile for this angle corresponds to that typically found in rotations around C(sp^2)-C(sp^3) bonds, which display three "theoretical" minima located at $\gamma \approx 60°$, 180° and −60°. Therefore, the conformational preferences of 5 were investigated by locating and characterizing all the minima at the HF/6-31G(*d*) level using, as starting points in the geometry optimizations, the "theoretical" minima. Subsequently, the effect of the electron correlation on their relative stability was analyzed by performing single point energy calculations at the MP2/6-31G(*d*) level.

2.2 Aqueous-phase calculations

In the description of the solvent we have used the polarizable continuum model (PCM) developed by Tomasi and coworkers [35, 36] as implemented in the Gaussian-94 computer program [37]. Calculation with this self-consistent reaction field (SCRF) method have been performed in the framework of the ab initio HF level using a 6-31G(*d*) basis set [PCM/6-31G(*d*)].

The PCM considers the solvent as a continuous dielectric with a cavity accurately modeled on the solute. The solvent reacts against the solute charge distribution, generating a reaction field (V_R). The electrostatic interaction between the solute and the solvent is introduced as a perturbation operator in the solute Hamiltonian (Eq. 1)

$$(H^\circ + V_R)\psi = E\psi \tag{1}$$

The reaction field operator was described in terms of an apparent set of point charges spread over the solute/solvent interface, i.e. the solute cavity (Eq. 2). The apparent charge density was determined by solving the Laplace equation (Eq. 3) at the cavity surface by imposing the suitable boundary conditions. In Eq. 3 both the solute V_ρ, and the solvent V_σ, contributions to the electrostatic potential are taken into account. It should also be noted that the solute electrostatic potential, V_ρ, was rigorously computed from HF/6-31G(*d*) wavefunctions.

$$V_R = \sum_i \sigma(s_i)S_i/|r - r_o| = \sum_i q_i/|r - r_o| \tag{2}$$

$$\sigma(s_i) = -\{\varepsilon - 1/4\pi\varepsilon[\partial(V_\rho(r) + V_\sigma(r))/\partial n]\} \tag{3}$$

The conformational free energy (ΔG_{conf}) in solution was estimated by adding $\Delta\Delta G_{sol}$ to the gas-phase energy computed at the highest theoretical level (Eq. 4).

$$\Delta G_{conf} = \Delta E + \Delta\Delta G_{sol} \tag{4}$$

3 Results and discussion

3.1 Conformational preferences of the aromatic fragments for Hoechst 33258

The rotational profiles computed for 1 and 2 in the gas phase at both HF/66-31G(*d*) and MP2/6-31G(*d*) levels are displayed in Fig. 4a and b respectively. The relative energies and torsional angles of the minima and saddle points for 1 and 2 are complied in Table 1. It is worth noting that the minima do not correspond to planar conformations. Thus, the two minima predicted for 1, *anti-gauche* and *syn-gauche*, are almost isoenergetic and occur at a torsional angle of $\alpha = 162.6°$ and $\alpha = 19.1°$, respectively. The two minima are separated by a barrier that corresponds to the *gauche-gauche* ($\alpha = 90.0°$) conformation. This is not favored with respect to the *anti-gauche* minimum by 3.6 kcal/mol at the MP2/6-31G(*d*) level. The fully planar *anti* ($\alpha = 180.0°$) and *syn* ($\alpha = 0.0°$) conformations are 0.3 and 0.7 kcal/mol,

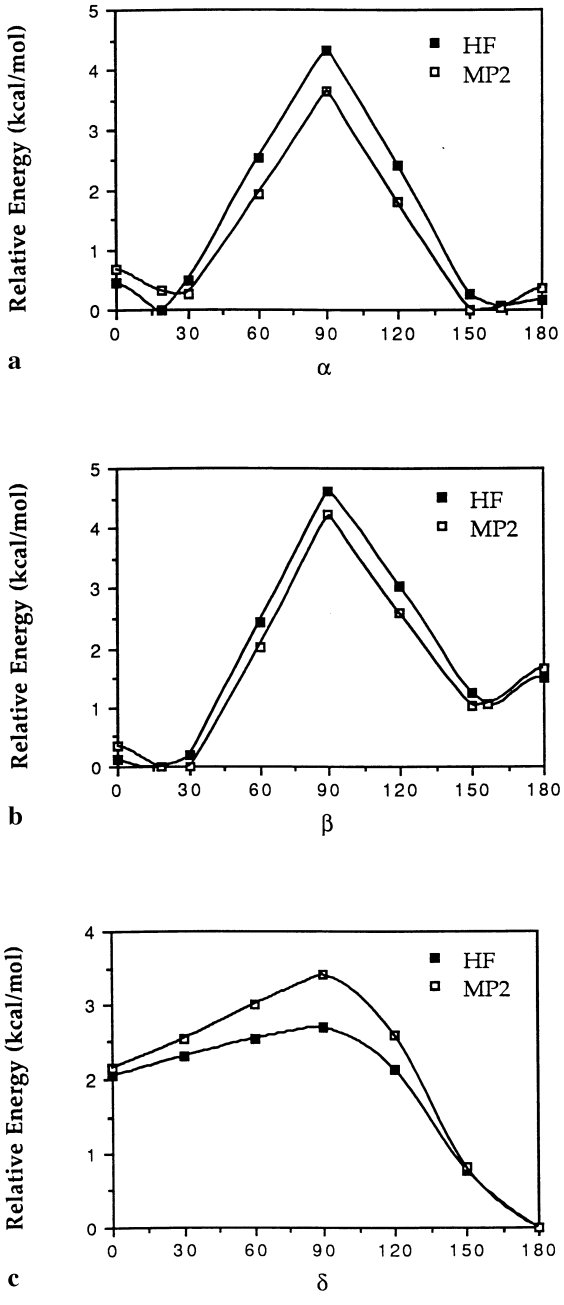

Fig. 4. Evolution of the gas-phase conformational energy as a function of the dihedral angles α, β and δ for the fragments 1 **(a)**, 2 **(b)** and 3 **(c)** respectively. Rotational profiles were computed at both HF/6-31G(*d*) and MP2/ 6-31G(*d*) levels

respectively, less favored than the *anti-gauche* minimum at the MP2/6-31G(*d*) level.

Results for 2 are similar to those for 1, the minima being located in twisted conformations rather than in planar ones. Thus, the global minimum appears in the *syn-gauche* conformation ($\alpha = 18.3°$), whereas the local minimum corresponds to the *anti-gauche* conformation ($\alpha = 156.3°$). The second minimum lies 1.1 kcal/mol above the *syn-gauche* conformation at the MP2/6-31G(*d*) level. The energy barrier between the two minima is 4.2 kcal/mol, being 0.6 kcal/mol higher than that predicted for 1 at the same theoretical level. The planar *syn* and *anti* conformations are 0.4 and 1.7 kcal/mol less favored than the global minimum at the MP2/6-31G(*d*) level.

The rotational profiles indicate that both 1and 2 do not adopt a planar conformation in the gas-phase. This conformational behavior is similar to that found for other aromatic compounds constituted by two rings attached by a single bond [38, 39]. The conformational preferences predicted for 1 and 2 at the ab initio HF/6-31G(*d*) and MP2/6-31G(*d*) levels are in good agreement with those previously reported at the AM1 level [6]. However, this semiempirical method overestimated the deviation of the inter-ring dihedral angles α and β with respect the planarity by about 10°. On the other hand, the present results are in poor agreement with those reported at the HF/3-21G and HF/STO-3G levels [7, 8], which tend to overestimate the stability of the planar conformations.

In order to improve our understanding of the conformational preferences of compounds constituted by two bonded aromatic rings, we have extended our study to compound 3 (see Fig. 2). The rotational profiles computed for 3 at the HF/6-31G(*d*) and MP2/6-31G(*d*) levels are displayed in Fig. 4c, while the minimum energy conformations of 3 correspond to the planar *syn* ($\delta = 1.4°$) and *anti* ($\delta = 179.3°$) structures, the latter being favored by 2.1 kcal/mol with respect to the former. The low stability of the *syn* conformation is due to the repulsive interactions between the hydrogen atom of the amide group and a hydrogen atom of the N-methylpyrrole ring. The two minima are separated by a rotational barrier of 3.4 kcal/mol. These results together with those provided for 1 and 2 indicate that compounds constituted by one aromatic ring attached to an amide group, which are frequently found in minor groove-binding drugs, adopt a fully planar conformation, whereas those with two adjacent aromatic rings are not

Table 1. Gas phase relative energies (kcal/mol) and torsional angles (degrees) of the minimum energy conformations and saddle points predicted for fragments 1, 2 and 3

Fragment	Level[a]	syn	syn-gauche	gauche-gauche	anti-gauche	anti
1	HF/6-31G(*d*)//HF/6-31G(*d*)	–	0.0 ($\alpha = 19.1°$)	4.3 ($\alpha = 90.0°$)	0.1 ($\alpha = 162.6°$)	–
	MP2/6-31G(*d*)//HF/6-31G(*d*)	–	0.3	3.6	0.0	–
2	HF/6-31G(*d*)//HF/6-31G(*d*)	–	0.0 ($\beta = 18.3°$)	4.6 ($\beta = 90.0°$)	1.2 ($\beta = 156.3°$)	–
	MP2/6-31G(*d*)//HF/6-31G(*d*)	–	0.0	4.2	1.1	–
3	HF/6-31G(*d*)//HF/6-31G(*d*)	2.0 ($\delta = 1.4°$)	–	2.7 ($\delta = 90.0°$)	–	0.0 ($\delta = 179.3°$)
	MP2/6-31G(*d*)//HF/6-31G(*d*)	2.1	–	3.4	–	0.0

[a] Level of geometry optimization//level of energy calculation

able to retain such an arrangement. Thus, the degree of π–π conjugation between the adjacent units seems to be lower in the latter case than in the fully planar conformation.

The free energy of solvation profiles computed for 1, 2 and 3 using the PCM/6-31G(*d*) model are displayed in Fig. 5a. Table 2 shows the free energies of solvation of the minima and saddle points for the three compounds. In all cases water tends to destabilize the fully planar conformations *syn* and *anti*. Further more, the most favoured solvent-solute interactions correspond to the *gauche-gauche* conformations, which correspond to the maximum of energy in the gas-phase rotational profiles.

A clearer illustration of the effect of aqueous solution on the conformational preferences of 1, 2 and 3 can be obtained from the conformational free energies. These are estimated by adding the gas-phase relative energies computed at the MP2/6-31G(*d*) level to the relative free energies of solvation. The variations of the relative conformational free energies ($\Delta\Delta G_{conf}$) with the interring dihedral angle for 1, 2 and 3 are shown in Fig. 5b, whereas the values for gas-phase minima and saddle points are included in Table 2. Water exerts two different effects on fragments 1 and 2, which illustrate the tendency of the solvent to break the planarity between the two aromatic rings. First, the planar *syn* and *anti* conformations are less stable in aqueous solution than in the gas phase. Second, there is a large stabilization of the *gauche-gauche* conformation which introduces a considerable reduction (about 15–25%) in the energy barrier.

On the other hand, there is a change in the relative stability between the *syn-gauche* and *anti-gauche* conformations. This trend can be easily explained by considering the magnitude of the dipole moments. For 1 and 2 the dipole moment of the *anti-gauche* minimum energy conformation (2.36 and 1.61 Debye respectively) is lower than that of the *syn-gauche* one (4.60 and 6.20 Debye respectively). For fragment 3, the *trans* conformation is the most favored in aqueous solution. Furthermore, there is a strong stabilization of the *gauche-gauche* conformation (about 65%), being of similar energy to the *syn* one.

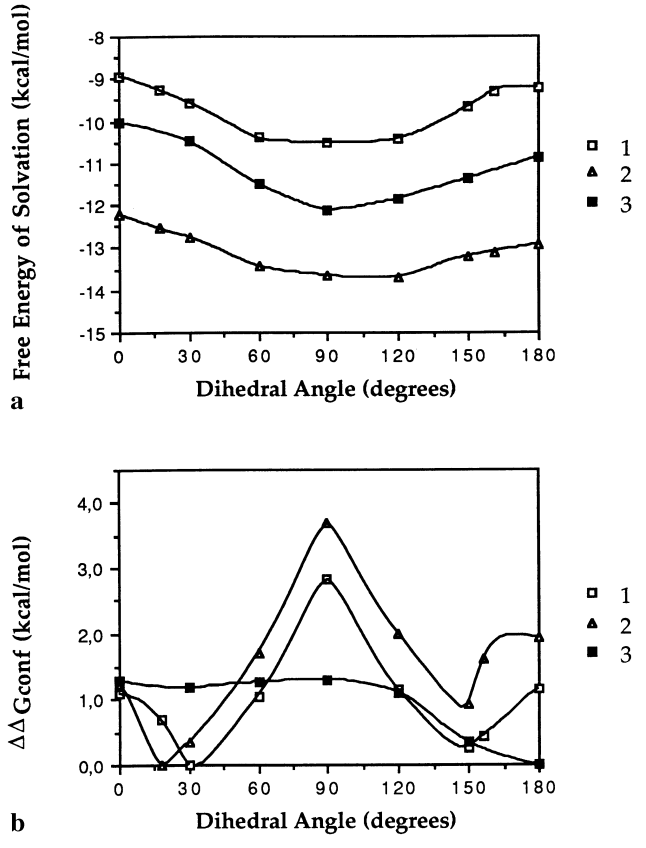

Fig. 5. Evolution of **a** the free energy of solvation (ΔG_{sol}) and **b** the relative conformational free energy ($\Delta\Delta G_{conf}$) as a function of the dihedral angles α, β and δ for the fragments **1**, **2** and **3** respectively

3.2 Conformational preferences of the piperazine ring in Hoechst 33258

The relative energies of the four conformers of the piperazine ring (see Fig. 3 for nomenclature) are listed in Table 3. Note that the introduction of correlation effects does not modify the results when the conformational preferences of 4 are investigated. It can be noted that IV is the lowest energy conformation. This conformer has the two bulky methyl groups in the equatorial orientation, whereas the hydrogen atom bound to N4 and the lone pair of N1 are in the axial orientation. Therefore, it seems to be stabilized mainly by the absence of repulsive steric interactions between the methyl groups and the axial hydrogens. Furthermore, it should be noted that a weak interaction of an electrostatic nature between the lone pair of N1 and the axial hydrogen atoms attached to the nearest endocyclic carbon atoms is also possible.

The relative energy ordering of the remaining conformers was the following: IV < III < I < II. This energy

Table 2. Free energies of solvation (ΔG_{sol}; kcal/mol) and relative conformation free energies ($\Delta\Delta G_{conf}$; kcal/mol) of the minimum energy conformations and saddle points predicated for fragments 1, 2 and 3

Fragment	#	syn	syn-gauche	gauche-gauche	anti-gauche	anti
1	ΔG_{sol}	–	−9.8	−10.5	−9.5	–
	$\Delta\Delta G_{conf}$	–	0.0	2.8	0.3	–
2	ΔG_{sol}	–	−12.7	−13.6	−13.2	–
	$\Delta\Delta G_{conf}$	–	0.3	3.7	0.0	–
3	ΔG_{sol}	−10.8	–	−12.1	–	−10.1
	$\Delta\Delta G_{conf}$	1.3	–	1.3	–	0.0

Table 3. Energy differences (kcal/mol) between the four conformers of 4 (see Fig. 3) calculated at different levels of theory

Level[a]	I	II	III	IV
HF/6-31G(*d*)//HF/6-31G(*d*)	4.4	7.2	2.7	0.0
HF/6-31 + G(*d,p*)//HF/6-31G(*d,p*)	4.6	7.6	2.8	0.0
MP2/6-31G(*d,p*)//HF/6-31 + G(*d,p*)	4.0	7.1	2.4	0.0
MP2/6-31G(*d,p*)//HF/6-31 + G(*d,p*) + MP4[b]	4.3	7.2	2.6	0.0

[a] Level of energy calculation/level of geometry optimization
[b] MP4 correction computed with the 6-31G basis set

order is that traditionally expected for a 1,4-disubstituted cyclohexane. Conformer III has the methyl group attached to N4 in an axial orientation, which is not favored by repulsive steric interactions with the axial hydrogens. The energy difference with respect to IV is 2.6 kcal/mol. Thus, the energy difference between III and IV must be associated with the repulsive steric interaction generated by one axial methyl group.

Conformer I loses the weak electrostatic interaction of the lone pair since it has an equatorial orientation. This conformer is 4.3 kcal/mol less favored than IV at the highest level of theory. The fact that both III and I have one methyl group in an axial position provides an estimate of the energy associated with this electrostatic contribution. Thus, the difference between the relative energies of III and I can be basically associated with the interaction between the axial lone pair and the neighboring hydrogen atoms. This value is −1.7 kcal/mol. Finally, conformer II has the two methyl groups in axial positions, and is 7.2 kcal/mol destabilized with respect to IV. This relative energy indicates that our estimation of the steric contribution for each methyl group is reasonable, differing by only 0.8 kcal/mol from the expected value.

A polar solvent like water may exercise a large influence on the conformational preferences of charged compounds. Computed results for 4 are shown in Table 4. According to the results, conformer II is the better-hydrated conformation. More specifically, the ΔG_{sol} values indicate that I, III and IV are disfavored with respect to II by approximately 0.5–1.5 kcal/mol. The stabilization of II can be easily explained by the large interaction with the solvent generated by the equatorial orientation of both the lone pair of N1 and the polar hydrogen attached to N4 in the ammonium cation. Thus, conformer II, which is the least stable in the gas phase (7.2 kcal/mol at the highest level of theory), is only about 5.8 kcal/mol less favored than IV in water.

Single-crystal X-ray studies of Hoechst 33258 complexed with different DNA sequences found either a chair or a twisted-chair conformation for the piperazine ring [13, 14, 16–18]. A comparison of the conformational parameters of IV with those determined for the piperazine ring in Hoechst 33258-DNA complexes indicates a good agreement (data not shown). Thus, in four of the seven structures the ring adopts a chair conformation [13, 16–18] like that obtained as the lowest energy minimum in the gas phase and aqueous solution. In two structures the piperazine adopts a twisted-chair conformation [14, 16] which is probably stabilized by van der Waals interactions with the DNA minor groove. Finally, in one structure a boat conformation [16] was found. This is an unexpected conformation due to the strong repulsive interactions between the sybstituents. On the other hand, NMR studies of Hoechst 33258-DNA complexes found that the piperazine ring usually adopts a chair conformation [19–24].

One of the main drawbacks of these crystal structures is their relatively low atomic resolution. Furthermore, the electron density in the minor groove corresponding to the drug is in general poorly defined. This introduces two undesirable effects. First, the final geometries of the

Table 4. Free energies of solvation (ΔG_{sol}; kcal/mol) in aqueous solution and relative conformational free energies ($\Delta\Delta G_{conf}$; kcal/mol) for the four conformers of 4 obtained from PCM/6-31G(*d*) calculations

Method	I	II	III	IV
ΔG_{sol}	−53.0	−53.4	−52.4	−52.0
$\Delta\Delta G_{sol}$	0.4	0.0	1.0	1.4
$\Delta\Delta G_{conf}$	3.4	5.8	2.2	0.0

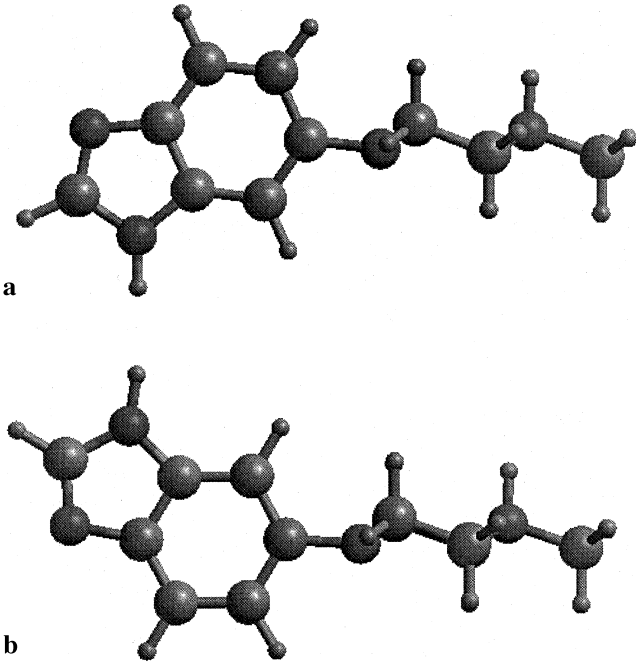

Fig. 6. Minimum energy conformations for fragment 5 computed at the HF/6-31G(*d*) level. Minima 5a and 5b are displayed at the top and bottom, respectively

crystallographic model can be rather poor providing large thermal parameters. Second, the interpretation of the electronic density maps at the early stages of the refinement can be very ambiguous. Such interpretation is more difficult in the flexible parts of the drug, like the piperazine ring and its exocyclic sybstituents in Hoechst 33258. The results described in this work would provide a useful framework with which future structural determinations of Hoechst 33258 complexed with DNA can be compared.

3.3 Conformational preferences of the bond between the benzimidazole and piperazine rings

Geometry optimizations at the HF/6-31G(*d*) (see level sect. 2) predict two minimum energy conformations for fragment 5 (Fig. 2). Such minima are displayed in Fig. 6. The values of the dihedral angle γ in the two minima are 65.9° and −114.3°, which will be denoted 5a and 5b, the former being 0.7 kcal/mol less stable than the latter. In all cases the piperazine ring retains a chair conformation almost identical to IV (see sect. 3.2), which was used as a starting conformation in the geometry optimizations. A detailed inspection of Fig. 6 reveals that the benzimidazole ring is perpendicular to the piperazine ring in the two minimum energy conformations of 5. The difference between the two minima results from a rotation of 180° of the benzimidazole ring with respect to the chair conformation of the piperazine ring.

PCM/6-31G(*d*) calculations provide ΔG_{sol} values of −56.0 and −55.1 kcal/mol for 5a and 5b respectively. Thus, interactions with the solvent are 0.9 kcal/mol more favored for 5a than for 5b. Calculation of $\Delta\Delta G_{conf}$ reveals that both conformers are almost isoenergetic, 5a being only 0.2 kcal/mol less favored than 5b. These results are in good agreement with those reported by Fede et al. [20] using NMR spectroscopy, according to which several orientations of the piperazine ring with respect to the close benzimidazole ring have been detected.

4 Conclusions

The results presented in this work reveal that DNA minor groove-binding drugs constituted by aromatic groups like Hoechst 33258 do not adopt a planar conformation in either the gas-phase or in aqueous solution. Comparison of our results with those previously reported indicate a qualitative agreement with those obtained at the AM1 semiempirical level [6] whereas no agreement is found with those computed at the HF/3-21G and HF/STO-3G levels [7, 8].

X-ray crystallography and NMR studies of Hoechst 33258-DNA complexes have attributed the deviation with respect to the planarity of the drug to the curve of the minor groove. However, the present results indicate that the distortion of the drug is intrinsic to the whole molecule. Thus, the torsion between planar groups is a property of the compound, although, in drug-DNA complexes the intermolecular interactions could provide a modulation of the drug conformation. These results agree with a thermodynamic and spectroscopic study of the DNA-binding behaviour of Hoechst 33258 [14] according to which the formation of the drug-DNA complex induces a negligible conformational arrangement of either the host DNA or the drug.

Acknowledgements. The author is indebted to the Centre de Supercomputació de Catalunya (CESCA) for computational facilities.

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