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Docking experiments showing similar recognition patterns of paclitaxel when interacting with different macromolecular targets

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Abstract

Using the Protein Data Bank crystallographic model of paclitaxel with tubulin as reference, a comparative interaction study of the antitumor drug with known macromolecular targets such as β-cyclodextrin and Dickerson's DNA dodecamer was carried out by molecular modeling techniques. AMBER* united atoms was found to be the most appropriate force field for our study. Conformational search of paclitaxel was performed using a water environment. A large set of conformers was selected for automatic "quasi-flexible" docking calculations performed by the "in-house" software MOLINE. A proper docking protocol was based on a crystallographic model and validated by a remarkable low atomic coordinate deviation. Using this method, a similar pattern via benzamide interaction was established for molecular recognition of paclitaxel cyclodextrin and DNA. The results are supported by our previous observations and other author's experimental data.

Keywords: Paclitaxel; Cyclodextrin; DNA; Tubulin; Docking

1. Introduction

Paclitaxel (Fig. 1) is one of the most promising anticancer drugs recently approved for therapy in treating ovarian cancer [1].

Many research groups have extensively studied conformational properties of paclitaxel to obtain information about the most populated conformers in solution [2–4] and to find reasonable pharmacophore models that are useful for the drug design of new analogs [5]. Some authors have reported three different macromolecules that can bind taxanes with high affinity: β-cyclodextrin [6], Dickerson's dodecamer [7], and the subunit β of tubulin [8]. These macromolecules display an increasing level of complexity and chemical diversity but are able to recognize the antitumor agent. Crystallographic data are only available for the interaction with tubulin, which represents the main target macromolecule responsible for the known mechanism of action of taxanes. β-Cyclodextrin is widely used as carrier [9] to overcome solubility problems and poor pharmacokinetic profile of paclitaxel. DNA is another potential target for explaining paclitaxel biological effects such as apoptosis [10].

As a follow up of a previous study [11], a molecular modeling study is here reported with the aim to compare the binding mode of paclitaxel towards different macromolecules. In the said paper, the interactions of paclitaxel with three differently methylated β-cyclodextrins were investigated using molecular modeling and spectroscopic methods. Thus, our "quasi-flexible" automatic docking methodology MOLINE [12] was successfully applied as a host–guest inclusion molecular system. Recently, the same computational procedure was tested for characterizing the binding modes of small ligands with acetylcholinesterase [13] and for novel inhibitors with cyclooxygenase 1 and 2 [14].

2. Experimental

All calculations were carried out using a Linux cluster with 12 Intel Pentium IV 1.5 MHz processors equipped with MACROMODEL [15] version 7.2 and MOLINE version

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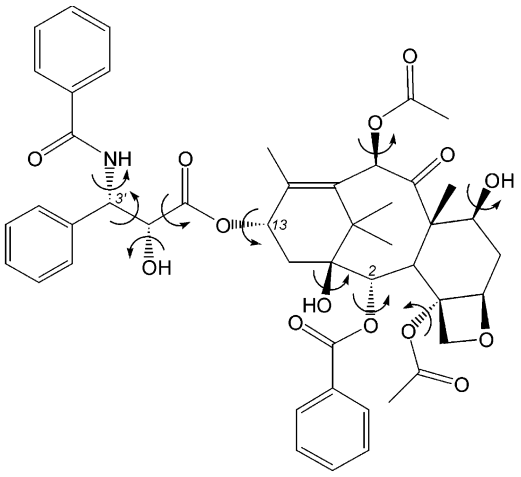

Fig. 1. Structure, rotatable bonds and main numbering scheme of paclitaxel.

6.5. According to our computational approach [12], a complete conformational search of the isolated drug was performed followed by a selection of conformers of the three macromolecules to be used in the ''quasi-flexible'' docking with paclitaxel.

2.1. Conformational search of paclitaxel

The conformational search of paclitaxel was performed by starting with the crystallographic conformation extracted from the complex with tubulin deposited in the Protein Data Bank (PDB) with the code 1JFF [8]. This structure was used to assess the quality of the force fields implemented in MACROMODEL as well as in our MOLINE package. Quality evaluation was performed by carrying out energy minimization of the X-ray coordinates both in vacuo and in GB/SA solvent models [16]. This was followed by computation of the root mean square (RMS) deviation with respect to the X-ray conformer. Since the molecule contains a very flexible side-chain on C13 and the rigid macrocyclic scaffold, the RMS deviation was separately measured for the entire molecule and taxane ring (Table 1).

Generally, the RMS deviation relative to the taxane ring is lower than 0.35 Å with all force fields. AMBER* united atoms is definitively the superior method with values lower than 0.2 Å. This force field works well for the entire molecule including GB/SA $CHCl_3$ while in water the all atom notation gave the lowest RMS deviation. In vacuo MM3* was found to be the optimum method.

In order to obtain the most reasonable set of molecular mechanic parameters to adopt for the rest of the molecular modeling study, a Monte Carlo (MC) conformational search of paclitaxel using all force fields cited above was performed.

The MC search was conducted by randomly exploring the 10 rotatable bonds depicted in Fig. 1. This procedure generated 5000 conformations. The drug and energy minimization was conducted using a maximum 2000 truncate Newton conjugate gradient (TNCG) iteration with a gradient convergence threshold of 0.01 kcal/Å mol in vacuo. In order to avoid convergence problems the same simulation in GB/SA water and chloroform was carried out using a threshold of 0.1 kcal/Å mol. All conformers within 11.95 kcal/mol, corresponding to the standard 50 kJ/mol MACROMODEL energy window, were compared to the PDB X-ray conformation of the drug as a reference. The relative energy corresponding to the closest MC generated structure of paclitaxel was considered in this second force field analysis. The optimal RMS deviation and relative energies found in the MC search of the drug are reported in Table 2.

Clearly only two force fields, AMBER* united atoms and MM3*, are able to reproduce the reference conformer in all conditions within the energy range equal to 11.95 kcal/mol. Remarkably, in vacuo, MM3* provided the closest conformer relative to the X-ray model with a relative energy equal to 0.7 kcal/mol and a low RMS deviation of 0.867 Å. In GB/SA solvents, AMBER* united atoms achieves the lowest RMS deviations (0.760 and 0.931 Å) and relative energies of 2.59 and 2.28 kcal/mol. The convergence in all MC searches was considered satisfactory as revealed by the average number of duplicates larger than 2.

Table 1
Total and macrocycle RMS deviations after energy minimization of the bioactive paclitaxel conformer with different molecular mechanic force fields and solvating models

Force field	Total RMS (Å)			Macrocycle RMS (Å)		
	Vacuo	GB/SA H_2O	GB/SA $CHCl_3$	Vacuo	GB/SA H_2O	GB/SA $CHCl_3$
AMBER* united atoms	1.102	1.039	0.774	0.179	0.186	0.145
AMBER* all atoms	1.098	1.033	1.029	0.292	0.304	0.297
OPLS* united atoms	1.592	1.497	1.583	0.226	0.227	0.221
OPLS* all atoms	1.535	1.392	1.532	0.310	0.302	0.312
MM3*	1.048	1.039	1.001	0.305	0.300	0.309
MM2*	1.108	1.106	1.064	0.328	0.345	0.319

Table 2
Relative energies and RMS deviations of closest MC generated paclitaxel conformers to the PDB structure with different molecular mechanic force fields and solvating models

Force field	Vacuo		GB/SA H_2O		GB/SA $CHCl_3$	
	E relative (kcal/mol)	RMS (Å)	E relative (kcal/mol)	RMS (Å)	E relative (kcal/mol)	RMS (Å)
AMBER* united atoms	4.96	0.990	2.59	0.760	2.28	0.931
AMBER* all atoms	11.69	1.322	> 11.95	–	> 11.95	–
OPLS* united atoms	3.99	1.157	> 11.95	–	0.29	1.208
OPLS* all atoms	8.3	2.571	> 11.95	–	11.28	3.021
MM3*	0.7	0.867	4.87	0.874	4.42	0.798
MM2*	3.06	1.213	9.89	2.076	> 11.95	–

Conformers within 11.95 kcal/mol above the global minimum were considered.

The docking experiment incorporates the first 56 conformers within 3 kcal/mol above the global minimum obtained in GB/SA water with AMBER* united atoms force field and corresponding to a Boltzmann population at 300 K greater than 96%.

2.2. β-Cyclodextrin conformations

β-Cyclodextrin is characterized by a number of rotatable bonds much higher than paclitaxel. An MC conformational search is practically impossible. Therefore, a different computational strategy was used to represent conformations able to include ligands into the cleft. ''Open'' conformations were generated considering the symmetric structure of the macrocycle ring when the glycosidic bond backbone was fixed as $\psi = 120^\circ$ and $\varphi = 116^\circ$. The torsional bonds pertinent to the secondary hydroxyl groups were fixed equally for all residues ($\chi_2 = -37^\circ$ and $\chi_3 = -43^\circ$). Three conformations have been generated: *gauche*+ (100°), *gauche*− (100°), and *trans* (170°) rotamers of the χ_5 equal for all the residues [11]. In Fig. 2 the superimposition of the three ''open'' structures is reported.

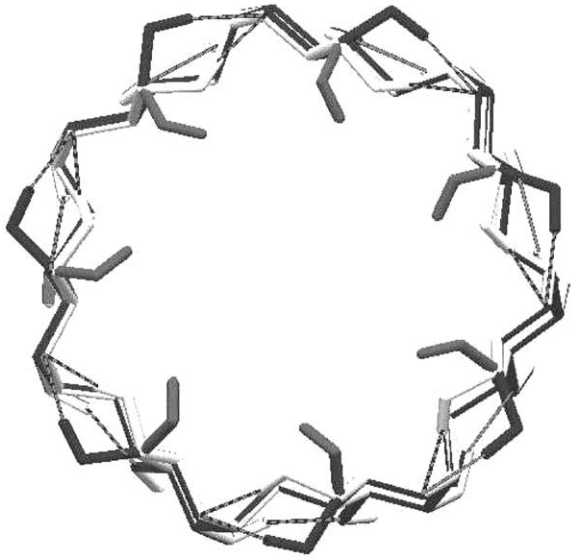

Fig. 2. Superimposition of ''open'' β-cyclodextrin symmetric conformations with χ_5 *gauche*+ (gray), *gauche*− (black) and *trans* (white) domains.

2.3. Dickerson's conformer

The evidence of DNA interaction with paclitaxel is reported in the literature for Dickerson's dodecamer [10] which is defined as the palindrome duplex sequence $d(CGCGAATTCGCG)_2$ with two terminal ''GC-rich'' areas and one internal ''AT-rich'' area. The B-form is generally considered as the most stable conformation of the DNA duplex [17]. The dodecamer conformation was generated with the MACROMODEL builder. As previously reported for other DNA molecular modeling experiments [18,19], the effects of the electrostatic overestimation due to the absolute negative charge of the duplex were prevented by the inclusion of Na^+ counterions located 2.5 Å from each anionic oxygen of the phosphate (Fig. 3). The sodium ion energy is not evaluated in the standard MM3* force field calculation and prompted us to adopt the AMBER* for the charge assignment and docking simulation.

2.4. Tubulin model

The co-crystallographic structure of tubulin interacting with paclitaxel [8] was the main model for our computational study. It contains both subunits of the protein, the guanosine-5′-diphosphate, the guanosine-5′-triphosphate and the drug bound to the β chain. This subunit containing 445 amino acids was computationally isolated from the other components. The scope was to define the binding pocket of the ligand with the 69 residues surrounding the drug within 15 Å from the paclitaxel C13 position. The binding cleft was fixed by filling the residues partially selected in the last operation and then subjecting the structure to an AMBER* united atoms energy calculation and charge assignment.

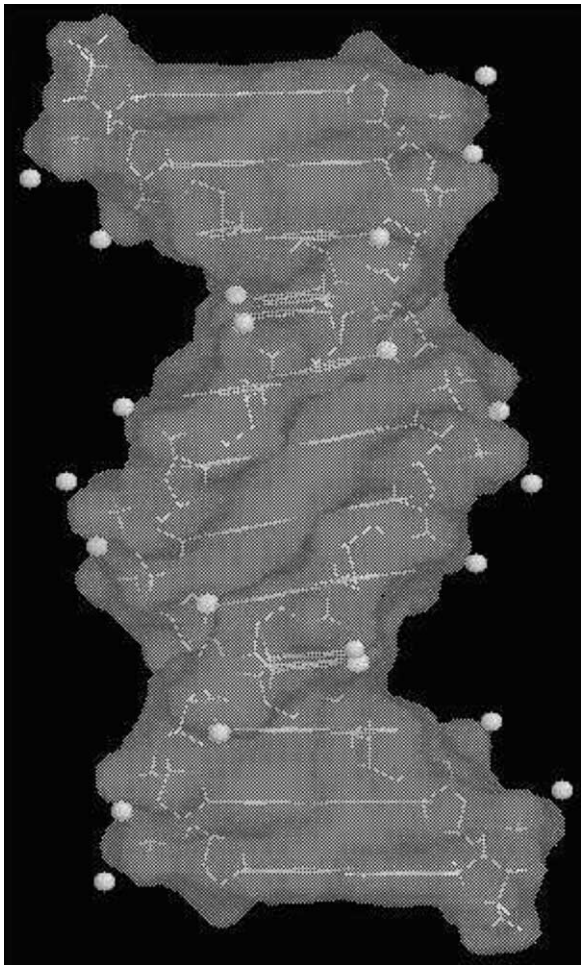

Fig. 3. Minor groove viewing of Dickerson's dodecamer in B-form with Na^+ counterions (white spheres).

2.5. Docking experiments

Several docking protocols have been tested in order to find the most appropriate one by using the tubulin complex as a reference model. Adequate setting of the protocol parameters, binding cleft of the protein, and the crystallographic conformer of paclitaxel were considered. According to the MOLINE methodology [12], both the grid resolution G_R and the van der Waals compression factor χ were systematically varied by selecting 6 and 0.8, respectively, which corresponds to the best compromise between speed simulation and adequate reproduction of the crystallographic data. The adopted resolution generated exactly 65.712 configurations for the tubulin–paclitaxel complex. The lowest RMS deviation equal to 0.0664 Å was found with the global energy minimum (−50.35 kcal/mol) and considered remarkably low for validating the computational protocol (Fig. 4). Other details about the docking protocol are the activation of the selection module SEL-5FD and the number of cycles of the rigid optimization process equal to three each with 100 simplex iterations [12]. The force field used for the protocol validation was AMBER* united atoms having a dielectric constant of 80.

Docking experiments with the same protocol were carried out considering the 56 paclitaxel conformers against the other two macromolecules. After the rigid docking, the most stable complexes within 3 kcal/mol above the global minimum were subjected to GB/SA water energy minimizations with MACROMODEL. The duplicated structures were identified and removed by considering conformers within an energy window of 1 kcal/mol and RMS lower than 0.25 Å computed onto the paclitaxel atomic coordinates.

With β-cyclodextrin, the three "open" symmetric structures (Fig. 2) were subjected to the docking calculations generating exactly 65.712 per couple of host/guest conformers. The most stable complexes within 10 kcal/mol above the global minimum were used for rigid docking optimizations. Finally, 3216 conformations corresponding to a large ensemble within 3 kcal/mol were fully minimized with AMBER* united atoms 2000 TNCG iterations in GB/SA water. After conformational reduplication, 1219 bimolecular complexes were considered for the analysis of results. The Boltzmann distribution of the complexes computed at 300 K revealed nine most stable conformers representing more than 99%.

Similarly, a docking experiment was carried out using Dickerson's dodecamer with counterions in B-form and the 56 low energy paclitaxel conformers. In order to prevent distortion of the DNA duplex, the 814 most stable complexes obtained above within 3 kcal/mol were used in a constrained energy minimization applying a 24 kcal/mol constant force on the nucleic acid and atomic sodium coordinates [17,18]. The same force field and number of TNCG iterations in GB/SA water were used. Finally, 129 paclitaxel–DNA complexes were considered for the analysis of results. The Boltzmann distribution of the complexes computed at 300 K revealed only three very stable conformers representing almost 100%.

The analysis of the docking experiments for complexes with β-cyclodextrin and Dickerson's dodecamer were performed adopting a simple distance descriptor coupled with the Boltzmann probability computed at 300 K. As previously reported in another communication [11], the analysis was carried out considering two dummy atoms averaging the fourteen β-cyclodextrin oxygen atoms in C2/C3 positions (center of the large entrance) and the seven methylene C6 carbons (center of the small entrance) and measured the distances with respect to the C13 atom of the paclitaxel. An arbitrary threshold of 2.5 Å was considered for deriving the Boltzmann probability of the pattern. These descriptors can easily give an idea of the inclusion direction (large or small entrance). Another set of dummy atoms was created for the three aromatic rings of paclitaxel and

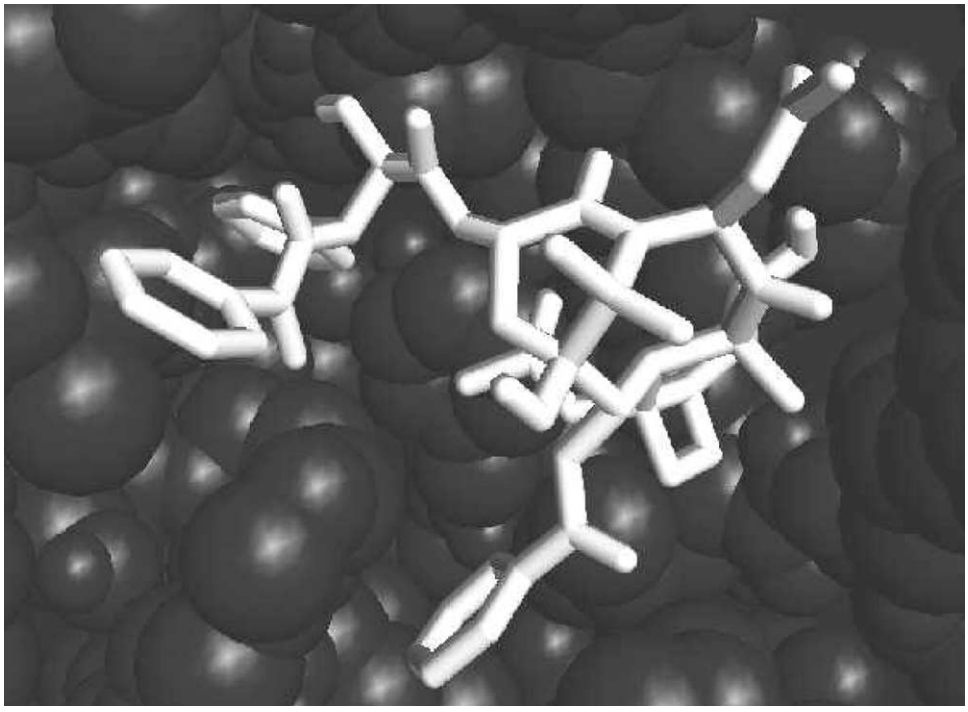

Fig. 4. Polytube comparison (RMS = 0.0664 Å) between the paclitaxel crystallographic position (white model) and the MOLINE docking (gray model) within the β-tubulin cleft (spacefill black representation).

was used to evaluate the individual recognition of such moieties.

Since only three useful complexes were obtained, they were visually compared in order to identify similar geometrical descriptors for each DNA groove/paclitaxel ring recognition site. Interestingly, all three complexes revealed interactions with the "AT-rich" section of Dickerson's dodecamer. Table 3 summarizes the results of the analysis.

3. Results and discussion

The choice of a suitable force field is always a crucial step when a molecular modeling study is carried out. Despite several published papers reporting molecular modeling experiments on paclitaxel, this study was carried out with the evaluation and the selection of the most suitable set of molecular mechanic parameters reproducing the conformation of the drug co-crystallized within the tubulin binding pocket and recently reported into the PDB. Using this information, as reference for a comparative conformational search of the antitumor agent, only two of the six force fields tested resulted reliable for describing the conformational properties. The limit of the reference from the PDB model should be considered, firstly because the resolution factor was 3.5 Å and secondly because the crystallographic conformation is determined in the solid state. Regarding the resolution of the model, the same authors earlier reported the first co-crystallographic tubulin model [20] with similar resolution but with docetaxel instead of paclitaxel. Comparison of the two models revealed similar recognition and conformation of the two related antitumor agents showing that in the solid state the "bioactive" structure is conserved.

Looking at the results reported in Table 1 it is not surprising that the closest RMS values were found with AMBER* united atoms and MM3* using GB/SA $CHCl_3$ rather than H_2O. The binding pocket of the tubulin protein can actually be considered closer in terms of electrostatic behavior to chloroform rather than in water. Unfortunately, no better implicit model of solvation was available in MACROMODEL for describing the environment where non-covalent interaction occurs between the drug and the tubulin-binding pocket.

Table 3
Geometrical descriptors expressed as average Boltzmann probability percentages at 300 K computed onto the complex ensembles with AMBER* united atoms and GB/SA water

Paclitaxel aromatic ring	β-Cyclodextrin		DNA dodecamer	
	Small	Large	Minor	Major
Phenyl in 3′ side-chain	0.00	0.00	0.08	0.00
Benzamide in 3′ side-chain	100.00	0.00	99.77	0.15
Benzoate in 2 macrocycle	0.00	0.00	0.00	0.00

The small and large recognition patterns refer to the cavity entrance direction of the inclusion, minor and major to the DNA grooves.

Only the MC search analysis (Table 2) was able to demonstrate the limit of the other four force fields because the crystallographic paclitaxel conformation was never reproduced in some solvating environments.

The applicability of both AMBER* united atoms and MM3* was finally determined by the DNA dodecamer built as a neutral structure with sodium counterions. MM3* had no parameters for the Na^+ atom type. Moreover AMBER* united atoms is widely accepted as the best parameterized force field for biopolymers. In our previous communication [11], only β-cyclodextrins interacting with paclitaxel were considered and the adopted force field was MM3*. In this case AMBER* united atoms was selected to provide a better comparison of the recognition processes with the other macromolecules.

There are a few differences in the results when carrying out the docking experiments with the various force fields. In GB/SA water with MM3* the results indicated most of the recognition process (84.2%) due to the inclusion of the C3′ phenyl ring and partially (13.6%) to the inclusion of the benzamide moiety into the small cavity of β-cyclodextrin [11]. With AMBER* united atoms, using the same solvation parameters, the ring inclusion pattern is inverted (Table 3) with a higher benzamide probability complexing into the small cavity. In Fig. 5, the global minimum complex geometry responsible for more than 50% of the Boltzmann probability at 300 K is reported. Interestingly, the stabilization in the small cavity of the conformer is due to five hydrogen bonds between the drug side-chain and the primary hydroxyl groups of the cyclodextrin.

The three most stable conformers obtained by docking paclitaxel and Dickerson's dodecamer were analyzed graphically and illustrated in Fig. 6. The first observation of these results was attributed to the preferential recognition of the paclitaxel with the "AT-rich" area of the dodecamer. The global and the second energy minima that represent more than 99.9% of the Boltzmann population at 300 K interact with the DNA in this region. The third one, with a very poor probability of 0.08%, interacts with the dodecamer at the interface region between the "AT-rich" and "GC-rich" areas. This strong preference for the "AT-rich" area is in perfect agreement with the recent observations made by Bischoff et al. [21] that arrived at the same conclusion spectroscopically. The second observation was ascribed to the DNA groove recognition analysis. Table 3 and Fig. 6 show a preference for the minor groove where the most stable complexes were detected. This result is in agreement with Gopala Krishna et al. that found a comparable paclitaxel affinity constant for the other minor groove binders [6]. The last observation was attributed to the ring preference analysis. In Table 3 and Fig. 6, the main role of the benzamide moiety was pointed out with respect to the other two-phenyl rings. Only, the third conformation with a modest population of 0.08% was able to interact with the DNA minor groove and the phenyl in the 3′ position of the side-chain. Unlike previous citations, no statistically important interaction of the paclitaxel macrocycle was observed in the docking ensemble after GB/SA water energy minimization.

The comparison of the binding modes of paclitaxel with chemically different and complex macromolecules was carried out by a well validated docking methodology revealing similar patterns. The side-chain of the drug plays a crucial role not only in the mechanism of action by interacting with the tubulin β subunit (Fig. 4), but also the macrocycle and the phenyl groups fit closely into the binding pocket. The interaction energy computed for the crystallographic binding mode was estimated lower than −50 kcal/mol. The docking experiments carried out with cyclodextrin and DNA showed negative global minimum interaction energies lower than −33 and −35 kcal/mol, respectively. In both cases, as summarized in Table 3 and shown in Figs. 5 and 6, the benzamide is the main interacting moiety of

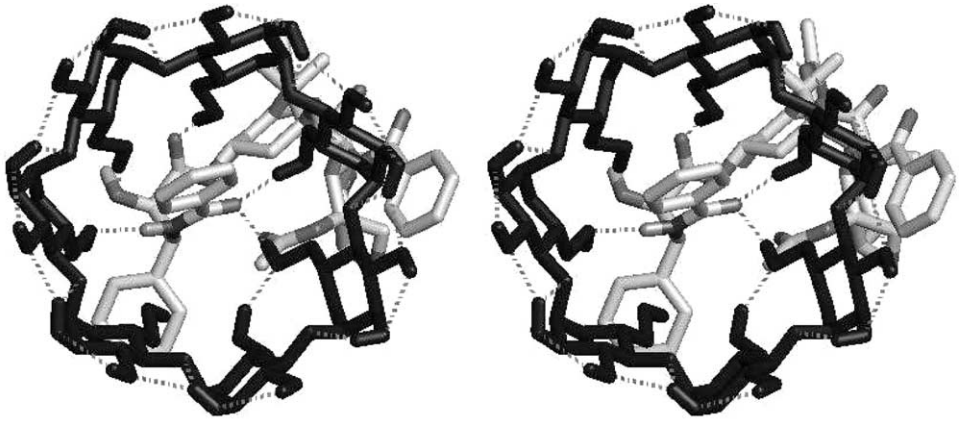

Fig. 5. Stereoview of the polytube model of the global minimum complex between paclitaxel (atom type color) and β-cyclodextrin (black). Hydrogen bonds are represented as dotted lines.

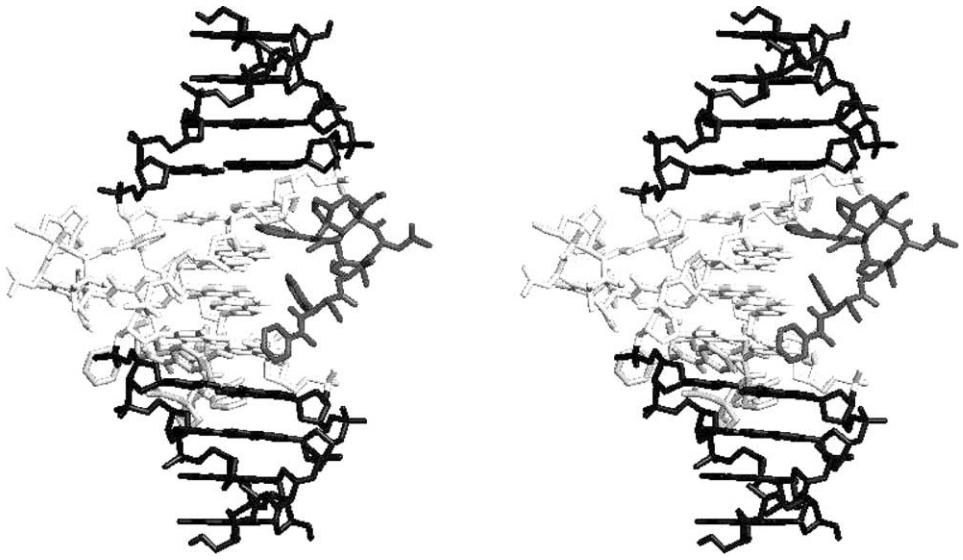

Fig. 6. Stereoview of the polytube model of the three main complexes between paclitaxel and DNA. The light gray (a), dark gray (b), and gray (c) models represent the global, the second, and the third energy minimum orientations of the drug, respectively. The A–T and G–T nucleotides are displayed in white and black respectively. Sodium ions are not shown for clarity.

the drug proving the importance of the side-chain in the molecular recognition of paclitaxel with the above macromolecules. Finally, the superimposition with respect to the PDB drug structure of the paclitaxel conformers of the most stable complexes surprisingly revealed very little differences, especially in the side-chain conformation.

4. Conclusions

A comparative interaction study between paclitaxel and known macromolecular targets was performed. The use of a detailed conformational search analysis with appropriate force fields and a validated docking approach using the crystallographic PDB model as the experimental reference was achieved. Based on the relative energy and RMS quality, AMBER* united atoms was selected as the method of choice for the entire computational work. A large set of paclitaxel conformers including the closest X-ray structure was generated by MC simulations and subjected to the ''quasi-flexible'' docking study. Remarkably, low RMS deviation with respect to the crystallographic reference was found with a MOLINE protocol adopted for the rest of the simulations. Few and very stable complex conformers indicated a strong preference of the drug to interact with the different macromolecules maintaining similar recognition patterns via the benzamide moiety. The results are in good agreement with our previous observations and with experimental data reported by other authors.

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