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MOLECULAR DYNAMICS SIMULATION OF MICROTUBULE AND ITS INHIBITOR COMPLEXES: IN SILICO SEARCH FOR ANTICANCER DRUGS

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ABSTRACT

In this paper molecular dynamics simulation were performed to search for the better antitubilin drugs which would be the substitute of microtubule binding agents. The new antitubulin drugs are taken from the large field of botanical extracts and marine organisms which have the capability to acquire a comfort zone with the microtubule. The set of thirteen different molecules were selected and interacted with the microtubule. The GLIDE modules SP and XP were used for the molecular docking simulation. Docking simulations have been performed at the taxol binding site of the microtubule. The docking energy and interactions suggest that the molecule 7, 10 and 11 have a better tendency of binding with the microtubule in addition to the taxol molecule. The results of better-docked complexes have been subjected to molecular dynamics simulations for 15ns using DESMOND. The validations of binding capabilities were checked by studying average RMSD variations and dynamical pathway observations during the course of molecular dynamics simulation. It has been observed that molecules 7 and 10 transits quickly to a comfort zone and remains stable throughout the dynamics. Consequently, the reported compounds 7 and 10 might be developed as a better substitute for taxol.

KEYWORDS: Microtubule, Tubulin binding agents, Docking, Molecular dynamics simulation, Schrodinger

INTRODUCTION

Microtubules are highly dynamic, cylindrical, cytoskeletal protein filaments that play a relevant role in the regulation of various cellular functions, such as intracellular migration and transport, cellular architecture maintenance, cell signaling and mitosis. Microtubules are built by the polymerization of α- and β- tubulin subunits that combine to form a αβ-heterodimer, which then assembles in a

filamentous tube-shaped structure[1]. This arrangement results in the formation of long protein fibers called protofilaments. Approximately, 13 protofilaments arrange in parallel to form a C-shaped protein sheet, which then curls around into a pipe-like structure called microtubule (Figure-1). In cells, microtubules exist in a continuously dynamic state of growing and shortening through the reversible association and disassociation of αβ-tubulin heterodimers. This dynamic behavior is controlled by the exchange of one GTP molecule for GDP in the β-subunit of a microtubule end, while other GDP unit is tightly bound to a α-tubulin and is non-exchangeable. A microtubule with a GTP molecule at the β-end will be stable and continue to grow, whereas a microtubule with the GDP molecule at the β-end will be unstable and rapidly depolymerize. This process and its control of microtubules length are vital to the proper functioning of the mitotic spindle in cell division[1].

Due to their crucial role in mitotic events, microtubules serve as important drug targets for anticancer compounds. Ligands targeting tubulin are broadly known as Tubulin Binding Agents (TBAs) and comprise a wide class of chemically diverse compounds that disrupt microtubule dynamics and inhibit mitosis, ultimately leading to cell death. TBAs can be classified into four main general types with respect to their binding site on the αβ-tubulin dimer: (a) The first group includes compounds that bind to the vinca alkaloids site which is located at the interface between two longitudinally aligned αβ-tubulin heterodimers; (b) The second group comprises compounds that bind to the colchicines site at the interface between the α- and β- subunits of the same tubulin heterodimer; (c) The third group is formed by the compounds that bind to the paclitaxel (Taxol) site on the luminal surface of the β-tubulin subunit, and (d) The fourth group comprises of compounds that bind to the laulimalide site on the exterior of β-tubulin. These four binding sites are targeted by a number of structurally unrelated compounds, which limits the identification of consistent pharmacophores for TBAs binding at the specific interaction sites. Moreover, the very recent discovery of new tubulin binding domains has evidenced the versatility and still unexplored features of this protein as a receptor for anticancer compounds and has broadened the possibilities for the discovery of novel TBAs[2].

From a mechanistic point of view, TBAs can be classified as either inhibitor of tubulin polymerization (microtubule destabilizing agents) or promoters of microtubule assembly and stabilization (microtubule stabilizing agents)**.** Polymerization inhibitors typically bind to the vinca or colchicines sites, and decrease microtubule polymer mass by blocking the addition of tubulin dimmers (e.g. colchicine) or by promoting a conformational change or microtubule ends that impedes further tubulin self-association (e.g. vinblastine). On the other

hand, polymerization promoters bind to the paclitaxel (e.g., paclitaxel and epothilones) or laulimalide (e.g. laulimalide and peloruside A) sites and increase microtubule polymer mass by promoting tubulin self-association and impeding microtubule depolymerization[1]. Although the molecular basis for the action of TBAs is not yet fully understood, the conformational changes induced by TBA binding in the protein are recognized as key factors for the biological activity of stabilizing and destabilizing agents[1,3].

Over the years, TBAs such as paclitaxel, docetaxel, vinblastine, and vincritine have been widely employed in the treatment of different types of cancer[4]. However, severe problems such as acquired and intrinsic resistance, negative side effects, neurotoxicity, low solubility, and poor bioavailability have limited their clinical success and encourage the search for novel, more potent active species with improved therapeutic properties[4a-4c,5]. Currently, a number of TBAs are in various stages of preclinical and clinical development have entered clinical use, and more active compounds are still being discovered[6]. Most of these species are synthetic or semisynthetic analogs of parent TBAs or are natural compounds with structures similar to known tubulin-targeting lignads[7]. Nevertheless, a major challenge regarding this issue involves the discovery of new chemical scaffolds capable of efficiently and adequately interacting with tubulin to overcome the current limitations of TBAs.

To address this need, computer-aided drug design methods have emerged as valuable tools for improving the efficacy and reducing the costs associated with the synthesis and discovery of new TBAs as well as for enhancing the current understanding about the molecular basis underlying the biological activity of these compounds[8]. To date, a number of ligand-based (e.g. QSAR, COMFA, and pharmacophore modeling)[9] and structure based (e.g. Molecular docking and molecular dynamics)[9a,10] computational studies have been aimed at the elucidating the binding modes of TBAs and the molecular features responsible for their efficacious interaction with tubulin. Nevertheless, the structural diversity among TBAs and the insufficient knowledge about the three-dimensional details of the TBA-tubulin association have traditionally hindered computational approaches from leading to significant advances in the development of novel active ligands. However in the past two years a number of high resolution three-dimensional structures of tubulin complexes with a series of TBAs have been released[2b,3a,6d,11] providing a valuable resource for the discovery of novel ligands targeting tubulin. Surprisingly, despite that the number high- quality crystalline structures for TBA-tubulin complexes has increased rapidly, there are very few reports dealing with the rational design of novel TBAs using structure-based computational methods[12] which constitute a unique research opportunity and the main goal of the present study.

METHODOLOGY

Glide Docking

The three-dimensional crystal structures of refined tubulin alpha-beta dimer (PDB ID: 1JFF), determined by X-ray crystallography, were retrieved from the Protein Data Bank (http://www.rcsb.org/). Coordinates of the ligand molecules were taken from the reported entries[13] while the coordinates of taxol were extracted from the protein complex (1JFF) (Figure 2). The problem of missing residues is corrected using the Prime application while added water, more than one molecule, chain breaks, alternate locations etc. are corrected using protein preparation wizard of the Schrödinger suite[14]. 1JFF contains two chains, 'A' and 'B', one of which, containing 'taxol', was retained while the other chain was deleted for the purpose of docking. GDP, GTP, Zn, and Mg of 1JFF were removed from the structure because they played no critical role in taxol binding. Before docking, the preparations of proteins and ligands were carried out using Protein Preparation and Ligprep Wizards, respectively, of the Suite. Docking studies have been carried out using Glide (Grid-based Ligand Docking with Energetics)[15] in Standard Precision(SP) and Extra-Precision(XP) modes. Glide carries out an exhaustive conformational search, improved by a heuristic screen that rapidly eliminates conformations deemed not to be suitable for binding to a receptor, such as conformations that have long-range internal hydrogen bonds. During the docking simulation, the ligands were treated as flexible and the receptor was kept rigid, which facilitates the generation of multiple configurations of the receptor–ligand complexes. The docking complexes were ranked by Glide scores (G-scores). The G-score takes into account a number of parameters like hydrogen bonds, hydrophobic contacts, Coulombic, van der Waals, polar interactions in the binding site, metal binding terms and energy penalties for freezing rotatable bonds and buried polar groups. The Extra-Precision (XP) docking protocol of Glide includes protein-ligand structural motifs leading to enhanced binding affinity, in addition to unique water desolvation energy terms[16]. Protein–ligand complexes, retrieved from the RCSB website, were prepared using the Protein-Preparation Wizard of the Schrödinger Suite, where hydrogens were added and subsequent refinement of the structure was carried out. It was observed that no water molecules were involved in the hydrogen bonding of ligand with protein. Therefore, all the co-crystallized water molecules were removed and bond orders were reassigned. Further, whole systems were minimized to an RMSD of 0.30 Å. Other parameters were taken as reported in our earlier paper[16]. Missing residues were corrected using the Prime module and the Build Panel of the Schrödinger Suite.

All the ligands were prepared using the Ligprep module of the Schrödinger Suite, which produces structures with various ionization states, tautomers, stereochemistries, and ring conformations. The bond orders were

modified according to their data and different conformers were generated. Each generated conformer was subjected to a full minimization in the gas phase with the Optimized Potential for Liquid Simulations (OPLS2005) all-atom force field[17] to eliminate the bond length and bond angles biased from the crystal structure. Prepared ligands and receptors were used as the initial coordinates for docking purposes. The first stage of ligand docking is the receptor grid generation. The receptor grid was generated using a 1.0 van der Waals radius scaling factor, 0.25 partial charge cut-off without any constraint[18]. Partial charges for the ligand atoms were obtained from the OPLS-AA force field[19], which do not reflect the influence of the environment on the atomic charges. The location of taxol was taken as a binding site for docking of all the ligands. In the minimization of ligands, the distance-dependent dielectric constant with a value of 2.0 and a conjugate gradient algorithm with 1,000 steps have been used. After docking, to improve the geometry of the configurations, post-docking minimizations were performed. Post-docking minimization specifies a full force-field minimization of those configurations which are considered for the final scoring. The docking results were used for binding energy calculations and docking scores.

Molecular dynamics simulation

The coordinates of the best docking configurations were subjected to molecular dynamics simulation using DESMOND. A suitable number of Na+ counter-ions were used to neutralize the complexes. The whole system was immersed in a Monte-Carlo-equilibrated, periodic TIP3P water bath, which extended approximately 10.0 A in each direction. Model systems were generated using the OPLS2005 force field. The Verlet-leap-frog algorithm was used in numerical integration with a 1.0-fs time step for minimization and 2.0 fs for dynamics. A cut-off of 9.0 A was applied to the Lennard-Jones interactions and constant pressure was maintained with isotropic molecule- based scaling. Water molecules and counter-ions were the first energy minimized by steepest descent followed by conjugate-gradient energy minimization with the satisfactory convergence of RMS gradient of 0.1 kcal / mole-Å. The whole system was subjected to 10,000 steps of steepest-descent minimizations followed by conjugate-gradient minimization. The velocities were assigned at 100K to all atoms according to Boltzmann's distribution. The temperature of the sample was raised from 100K to 298K over 2ps by scaling velocities according to the Berendsen algorithm followed by a constant pressure dynamics run over 25ps at 298K. After the initial equilibration, a molecular dynamics production run was carried out for 15.0 ns. [20]

RESULTS AND DISCUSSION

The results of the glide docking of taxol and other twelve compounds are depicted in Table-1. It has been found that the minimum docked glide energy for taxol is -70.890 while glide score is -8.956 kcal/mol respectively in XP docking while that of SP docking the values comes out to be -73.126 and -8.559 kcal/mol.

The glide energy values of the best fit compounds as shown in table-1 indicates that the minimum docked energy for compound 10 attains the values -55.381 and -8.416 in XP docking and for SP docking the values comes out to be -51.646 and -6.955 and these values are in close proximity to that of taxol. Similar trends are followed by compounds 11 and compound 7 also. As we proceeds for these compounds their energy values comes to be -47.359 and -7.363 for XP docking and -49.058 and -6.308 for SP docking for compound 11. If we observe the values for compound 7, we see that the values for XP docking is -43.097 and -7.100 while that for SP docking is -53.558 and -7.168 kcal/mol.

The docking of molecules within the taxol binding site exhibits several interactions. The interaction diagrams of the better complexes of the better docking scores as obtained through XP docking are shown in Figure 3. On the basis of XP docking, the best docked complex of the taxol exhibit hydrogen bonding interaction with residues Asp26 and Thr276. It has also been observed through SP docking that Taxol binds with Thr276. Residue His229 divulge aromatic π-π interactions with taxol. Hydrogen bonding interaction with Thr276, Gly370, Asp26 and Asp226 has been observed in the docking complex of molecule 7 with 1JFF. Through SP docking, the interaction of molecule 7 is also observed with Arg278 residue. The best XP docking complex of molecule 10 demonstrate hydrogen bonding interactions with Ser236, Gly370 and Arg278. Residue Thr276 also interact with molecule 10 which is observed through SP docking. The SP and XP dockings of compound 11, demonstrate hydrogen bonding interactions with residue Thr276 and Pro274, Ser236 respectively. Residues Phe272, Leu275, Pro274, Leu371, Phe83, Val23, Ala233, Pro360, Cys213, Leu230, Leu217, Leu219 and Leu227 form a hydrophobic enclosure to the binding site. Polar interactions with the ligands have been observed through residues Gln282, Ser277, Thr276, His229, Ser236. Salt bridge interactions with ligands are also possible through Arg369, Arg320 and Arg278 residues which are present within the active site of protein.

On the basis of docking results, the better complexes have been extracted to study their stability with time. The molecular dynamics simulation of the better docked complexes of molecules TXL, molecule 7 and molecule 10, as obtained through XP docking, have been run for 15 ns using DESMOND. To see whether structures were fully converged, the root mean square deviations of the averaged simulated structures were observed. Small values of rmsd [Figure 4] indicate that the structures were fully converged. It seems that after few nanosecond of simulation the structures of compound 10 and 7 transits quickly to an equilibrium position, attains a small oscillations and remains stable throughout the dynamics.

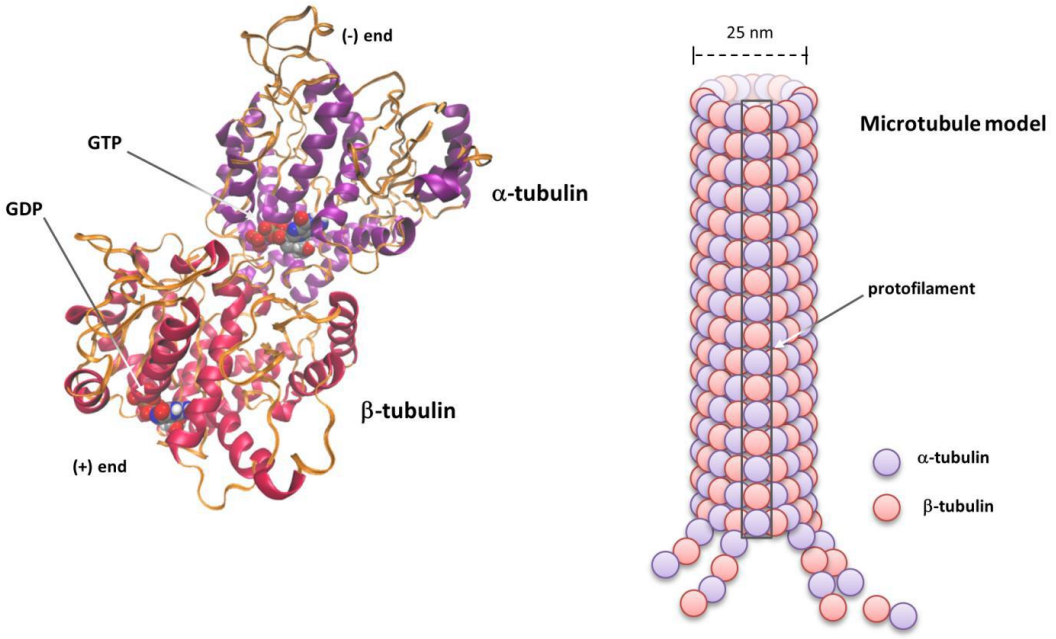

Figure 1: Structure of the αβ-tubulin heterodimer and microtubule model

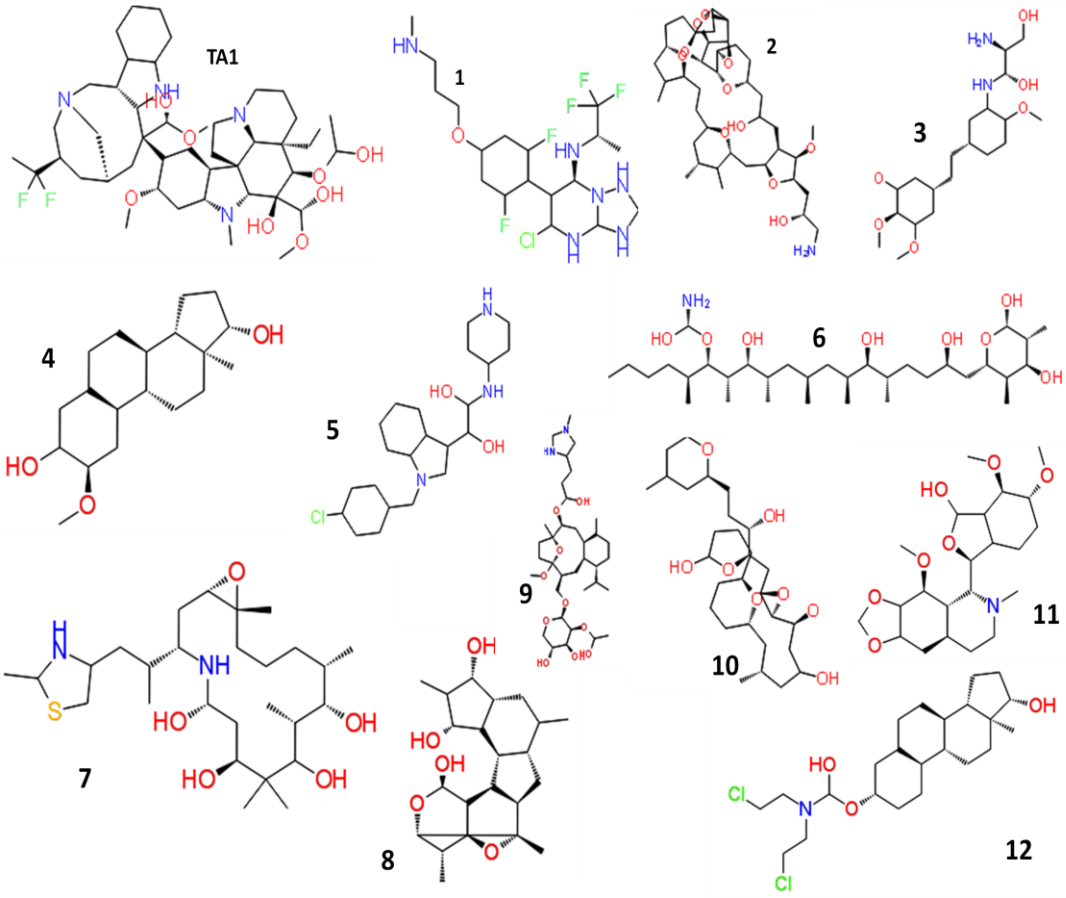

Figure 2: Chemical structure of the molecules chosen for the docking studies

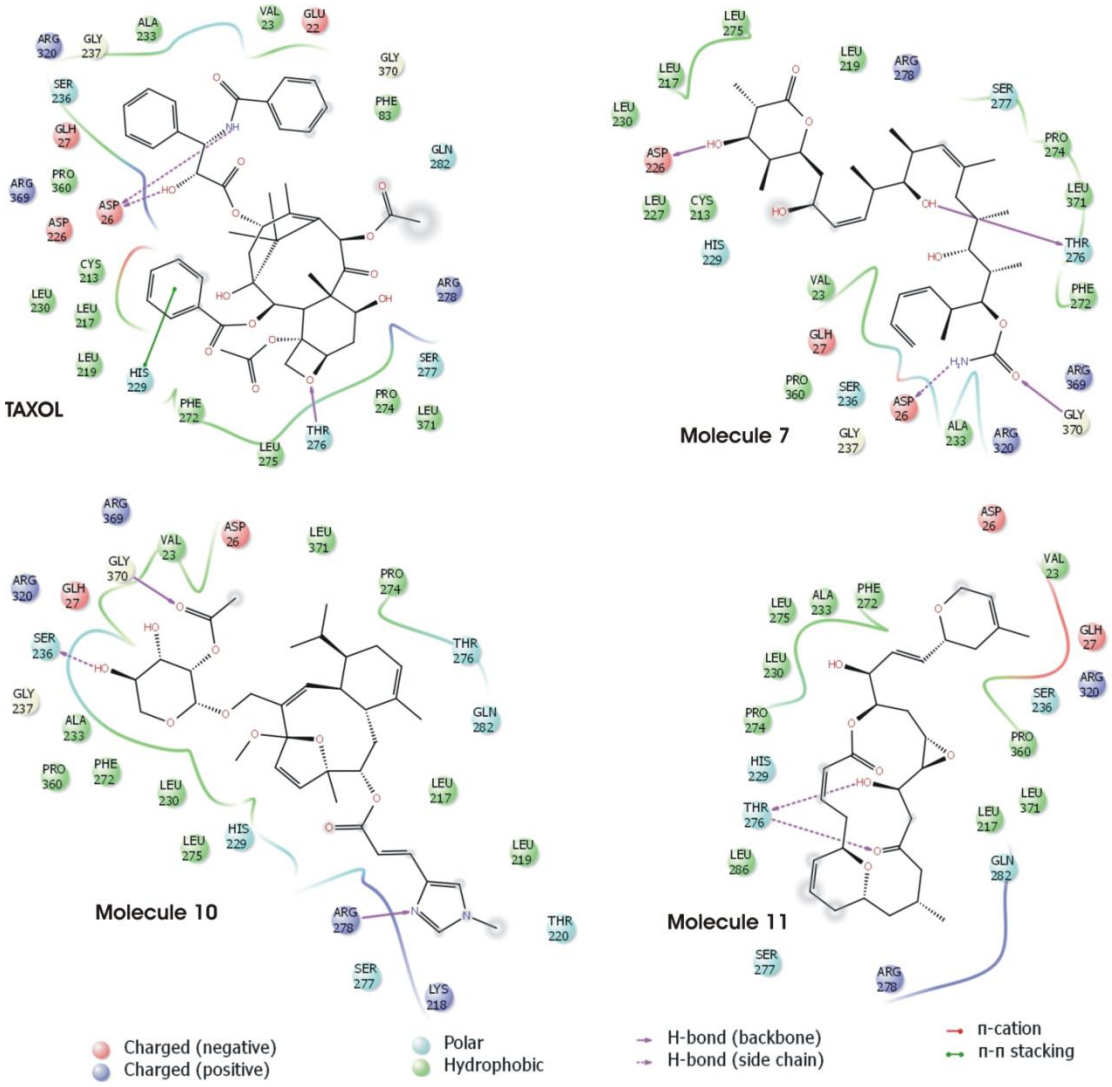

Figure 3: Interactions of the molecules (TXL, Molecules 7, 10 and 11) within the conserved active sites of the microtubule as obtained through Glide-XP docking

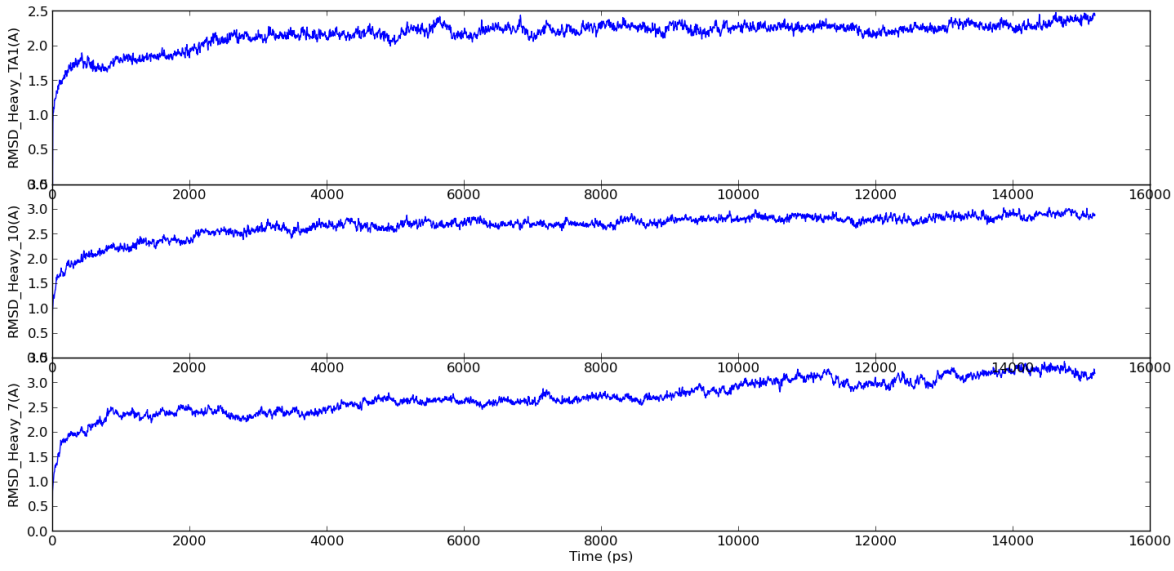

Figure 4: Average RMSD of heavy atoms of the complexes during MD simulation

Table 1: Docking score and energy of taxol and other compounds 1-12.

Compound	XP docking		SP Docking	
	Glide score(kcal/mole)	Glide Energy (kcal/mole)	Glide Score (kcal/mole)	Glide Energy (kcal/mole)
TA1	-8.956	-70.890	-8.559	-73.126
10	-8.416	-55.381	-6.955	-51.646
11	-7.363	-47.359	-6.308	-49.058
07	-7.100	-43.097	-7.168	-53.558
08	-6.411	-43.678	-5.894	-41.711
06	-6.363	-38.861	-5.869	-42.993
03	-6.271	-43.800	-6.260	-45.440
02	-6.169	-45.574	-5.832	-40.658
01	-5.683	-56.838	-5.719	-53.526
05	-5.634	-16.532	-5.886	-29.022
04	-5.611	-46.299	-5.710	-42.678
14	-5.426	-42.121	-5.097	-33.961
09	-5.195	-37.271	-6.070	-36.961
12	-4.722	-37.312	-5.537	-37.675

CONCLUSION

Here, we have used computational methods for evaluating the effect of compounds binding to tubulin and examined the stability and strength by monitoring the conformational variations. A series of botanical extracts which are capable of acquiring comfort zone with microtubule were selected and massive docking was carried out. The screening of a range of botanical species and marine organisms has provided satisfactory new antitubulin agents. With the help of molecular dynamics simulation, we attempted here to give some antitubulin drugs which can be substitute for microtubule binding agents. A set of twelve different molecules along with taxol were selected and interacted with microtubule within the taxol binding site. Molecular docking simulation has been performed through SP and XP both modules of GLIDE. Better docked complexes have been subjected to molecular dynamics simulation and the results have been analyzed. On the basis of docking energy and interactions, apart from taxol molecule, molecules 7 and 10 were found to have better tendency of binding with microtubule. In order to further validate binding capability, molecular dynamic simulations of the best docked complexes were carried out for 15 ns using DESMOND. The average RMSD variations and dynamical pathway observations indicate that the molecules 7 and 10 transits quickly to a comfort zone and remain stable throughout the dynamics. Molecule 10 shows better stability than molecules 7 and the reference drug Taxol. With the help of above observed outcomes we conclude that these compounds are less toxic than taxol and perhaps they can be considered for preclinical tests in order to be picked as a better substitute for taxol due to their indication of low toxicity.

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