Computational analysis of drug resistance of taxanes bound to human β-tubulin mutant (D26E)

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Abstract

The single-point mutation D26E in human β-tubulin is associated with drug resistance seen with two anti-mitotic taxanes (paclitaxel and docetaxel) when used to treat cancers. The molecular mechanism of this resistance remains elusive. However, docetaxel and a third-generation taxane, cabazitaxel, are thought to overcome this resistance. Here, structural models of both the wildtype (WT) and D26E mutant (MT) human β-tubulin were constructed based on the crystal structure of pig β-tubulin in complex with docetaxel (PDB ID: 1TUB). The three taxanes were docked into the WT and MT β-tubulin, and the resulting complexes were submitted to three independent runs of 200 ns molecular dynamic simulations, which were then averaged. MM/GBSA calculations revealed the binding energy of paclitaxel with WT and MT β-Tubulin to be −101.5 ± 8.4 and −90.4 ± 8.9 kcal/mol, respectively. The binding energy of docetaxel was estimated to be −104.7 ± 7.0 kcal/mol with the WT and −103.8 ± 5.5 kcal/mol with the MT β-tubulin. Interestingly, cabazitaxel was found to have a binding energy of −122.8 ± 10.8 kcal/mol against the WT and −106.2 ± 7.0 kcal/mol against the MT β-tubulin. These results show that paclitaxel and docetaxel bound to the MT less strongly than the WT, suggesting possible drug resistance. Similarly, cabazitaxel displayed a greater binding propensity against WT and MT β-tubulin than the other two taxanes. Furthermore, the dynamic cross-correlation matrices (DCCM) analysis suggests that the single-point mutation D26E induces a subtle dynamical difference in the ligand-binding domain. Overall, the present study revealed how the single-point mutation D26E may reduce the binding affinity of the taxanes, however, the effect of the mutation does not significantly affect the binding of cabazitaxel.

Keywords: Taxane; Point-mutation; Drug resistance; Binding affinity; Docking; Molecular dynamics simulations; Dynamic cross-correlation matrix.

Introduction

Microtubule is primarily composed of α- and β-tubulin heterodimers and is an important component of the cytoskeleton involved in many essential cellular functions, including maintenance of cellular shape, intracellular transport, and chromosome segregation. Interference with the tubulin-microtubule dynamics can cause mitotic arrest and eventually trigger the signals for apoptosis. Thus, tubulin is a very attractive target for developing anticancer drugs [1].

Taxanes are an important class of chemotherapeutic agents used for the treatment of cancer. Taxanes tamper with the spindle microtubule dynamics thereby preventing eukaryotic cancer cell division from passing the spindle checkpoint due to the inability to achieve proper metaphase spindle fiber configuration in mitosis, leading to cell cycle arrest and apoptosis [2]. Categorically, taxanes are classified as microtubule-stabilizing agents [3, 4]. There are currently three generations of taxanes on the market: paclitaxel (tradename Taxol®), docetaxel (tradename Taxotere®), and cabazitaxel (tradename Jevtana®) [5]. Structurally, they all only differ in two R groups attached to the aromatic rings (**Figure 1**). The tubulin subunits, each of which possesses a paclitaxel-binding site, have a characteristic GTP binding site **(**Figure S1 and Table S1&2). One of which undergoes GTP-hydrolysis located, which is near the Taxol binding site and at the dimer-dimer interface. In high concentrations, taxanes cause the microtubules to create bundles which inhibit the disassembly of the dimers and strengthen lateral interactions between proto-filaments [6]. It thwarts the crucial step of the disassembly of the microtubule proteins [7]. At low concentrations, taxanes suppress the microtubule dynamics without affecting the polymer mass. In both cases, the

taxanes impair the microtubules' ability to properly attach to and move chromosomes during the G2/M phase of the cell cycle [2]. Lastly, the cell fails to pass the spindle checkpoint before entering cell division and re-entering interphase and instead goes into cell-cycle arrest and subsequent cell death [2]. To remedy the side effects of Taxol (such as alopecia, myelosuppression, and neuropathy) associated with the use of Taxol [8], docetaxel was approved to be in combination with doxorubicin and cyclophosphamide [9]. Docetaxel, a 2nd generation taxane, is a group of diterpenes derived from the European yew tree *Taxus baccata* [3]. These two generations of taxanes differ in only two R-groups and hence have slight dissimilarities in their chirality (**Figure 1**).

Tumor cell types developed resistance to paclitaxel and docetaxel after multiple cycles of therapy during clinical trials, thus confirming the drug resistance tendency of cancer cells in vivo. In the cell line KB-15-PTX/099, a single-point mutation, D26E, was identified in the NH_2 terminus of class 1 β-tubulin. Consequently, the MT drug binding site becomes less favorable to the cytotoxic effect of toxoids [10]. This mutation decreases paclitaxel's binding affinity as well as destabilizes microtubule assembly. The understanding of drug resistance to both generations of taxanes prompted the development of more potent and efficacious taxanes (new generation) to circumvent the drug resistance of cancer cells. In 2010, a new generation taxane, cabazitaxel (trade name: Jevtana) (**Figure 1**), was approved by the FDA for the treatment of metastatic hormone-resistant prostate cancer after unsuccessful treatment with paclitaxel and docetaxel [11, 12]. The relative binding affinities to the D26E MT in the KB-15-PTX/099 cell line are in the order of paclitaxel < docetaxel ≤ cabazitaxel.

This research is aimed at examining the binding affinity of these three generations of taxanes against WT β-tubulin and the known D26E MT and to evaluate possible changes in the

drug interactions with β-tubulin due to the point mutation. Therefore, MM/GBSA binding energy calculation coupled with dynamic cross-correlation matrix (DCCM) analysis was performed to assess the mutational impact on protein-ligand dynamics and interactions. The MM/GBSA results provided a statistically significant correlation between in vitro ligand binding energies and in silico ligand binding energies. DCCM analysis provided insights into the overall dynamic change of the protein-ligand complexes.

Computational Methods and Materials

System Setup and Docking

The human FASTA sequence of β-tubulin was retrieved from UniProt (UniProtKB – entry Q9H4B7 "TBB1_HUMAN") (Figure S2). A homology model for human β-tubulin was built using the crystal structure of pig β-tubulin complexed with docetaxel as a template (PDB ID: 1TUB)[13]. The prime toolkit of Maestro (Schrödinger, Inc.) was used for the homology modeling. A point mutation (D26E) was then introduced to the structure at residue in the full-length sequences on the β-tubulin monomer of WT to introduce the MT form. Both the WT and MT human β-tubulin models were processed using Maestro's Protein Preparation Wizard [14]. During the preparation, the protein was preprocessed to assign correct bond orders, add hydrogen atoms and missing side chain atoms, create disulfide bonds, and delete water beyond 5 Å. The protein charge state was optimized using PROPKA at a pH of 7.4. A restrained minimization was done to relax the protein using an OPLS3 force field [15].

The 3D structures of paclitaxel, docetaxel, and cabazitaxel were obtained from the Zinc database and processed in Maestro's Epik module [16], in which each ligand geometry was optimized, the ionization and tautomeric states were generated using pKa calculations at a pH of

7. The lowest tautomeric state for each ligand structure was minimized to a best-fit structure, with respective R-groups highlighted (Figure S3).

Protein Docking

Maestro extra precision (XP) docking [17] was performed for each ligand (paclitaxel, docetaxel, and cabazitaxel) into the β-tubulin WT and the MT. First, the fully prepared, merged, protein-ligand (doc) complex was used to generate the receptor grid file. The center of the ligand was used to define the active site of the receptor. The grid file was generated using a van der Waals scaling factor of 1 and a partial charge cutoff of 0.25 [18, 19]. The prepared ligands, paclitaxel, docetaxel, and cabazitaxel, were docked into the generated grid of the human WT and MT β-tubulin (**Figure 2**) with no constraints using an OPLS3 force field and their docking scores were calculated using an XP scoring function [15, 18-20].

MD Simulation System Setup

All six molecular dynamics simulation systems were built using the prepared and refined receptor-ligand complexes from the Glide XP docking as input files. Like successfully applied on other protein systems previously [21-23], an SPC water solvent model [24] using an orthorhombic solvent box with 10Å water buffer was used to model each system. Each system was neutralized with Na+ ions and then Na+ and Cl- ions were added to the solvent to give a physiological ionic concentration of 0.15 M. Finally, an OPLS3 force field [15] was used to model the system. Ligand charges were assigned using a combination of the Cramer and Truhlar CM1A charge model [25], and a set of bond charge correction terms (BCC) [26].

Relaxation and Simulation Protocols

Desmond simulation package [27] was used to run all simulations. First, all systems were relaxed using the default relaxation protocol consisting of multiple stages: 1) A low-temperature (10 K) Brownian dynamics simulation under the NVT ensemble for 100 ps. 2) Simulation under the NVT ensemble, small time steps, and restraints on solute heavy atoms for 12 ps. 3) Simulation under the NVT ensemble and restraints on solute heavy atoms for 12 ps. 4) Simulation under the NPT ensemble and restraints on solute heavy atoms for 12 ps. 5) Simulation under NPT with temperature 300 K, pressure 1 bar and no restraints for 1.5 ns. After the relaxation, three 200 ns-long production runs were conducted for each of the three systems under the NPT ensemble (300 K and 1 bar) using the default protocol. In detail, temperature was controlled using the Nosé-Hoover chain coupling scheme [28] with a coupling constant of 1.0 ps. The pressure was controlled using the Martyna-Tuckerman-Klein chain coupling scheme [28] with a coupling constant of 2.0 ps. M-SHAKE [29] was applied to constrain all bonds connecting hydrogen atoms, enabling a 2.0 fs time step in the simulations. The k-space Gaussian split Ewald method [30] was used to treat long-range electrostatic interactions under periodic boundary conditions (charge grid spacing of ~1.0 Å, and direct sum tolerance of 10^{-9}). The cutoff distance for short-range non-bonded interactions was 9 Å, with the long-range van der Waals (VDW) interactions based on a uniform density approximation. To reduce the computation, non-bonded forces were calculated using an r-RESPA integrator [31] where the short-range forces were updated every step and the long-range forces were updated every three steps. The trajectories were saved at 40.0 ps intervals for further analysis in the future.

Simulation Interaction Diagram (SID) Analysis

The simulation interaction diagram (SID) tool implemented in Desmond was used to analyze dynamic behaviors and interactions of proteins with ligands during MD simulations. The parameters computed include root mean square deviation (RMSD); 2D protein-ligand contacts, including hydrogen bonds, hydrophobic, ionic, and water-bridge contacts; root mean square fluctuation (RMSF); secondary structure changes, and ligand torsional profile.

Trajectory Clustering Analysis

Desmond trajectory clustering tool [32] was used to group structures obtained from the simulations. The backbone RMSD matrix was used as a structural similarity metric and hierarchical clustering with average linkage [32] was selected as the clustering method. The merging distance cutoff was set to be 2Å. The centroid structure (the structure having the largest number of neighbors in the structural family) was used to represent the structural family.

MM/GBSA Binding Energy Calculations

MM/GBSA binding energy was calculated using frames from the last 100 ns since the RMSDs of most of the systems simulated showed the tendency to converge beyond this time. OPLS3 force field, VSGB 2.0 solvation model [33], and the default Prime procedure were used for MM/GBSA calculation. The performance of this method had been evaluated by previous studies done on diverse protein-ligand complexes. A significant correlation coefficient of 0.63 was calculated between the experimental and predicted binding affinity [34]. Moreover, compared to Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA), MM/GBSA displayed a better correlation factor and more flexibility in terms of applications to multi-target comparisons [35]. The default procedure consists of three steps: receptor alone (minimization), ligand alone

(minimization), and receptor-ligand complex (minimization). The total binding free energy equation is computed as follows:

$$\Delta G_{(bind)} = [E_{complex(minimized)}] - [E_{ligand(minimized)} + E_{receptor\ (minimized)}].$$

To gain a more detailed understanding of binding nature, the individual interaction terms (Coulombic + H-bond + GB solvation+ VDW + π- π stacking + self-contact + lipophilic) were grouped into three categories: (1) $E_{electrostatics,}$ (2) $E_{VDW,}$ and(3) $E_{lipophilic,}$ where $E_{electrostatics}=(H_{bond} + E_{coulomb} + E_{GB\_solvation})$, $E_{VDW} = (E_{VDW}+E_{\pi-\pi} + E_{self\text{-}contact})$ and $E_{lipophilic.}$

Because solute conformational entropy is not included in MM/GBSA analysis, the binding energies by MM/GBSA may overestimate the true binding free energy [36].

Dynamic Cross-Correlation Matrix Analysis

To gain a further understanding of the overall stability of the protein-ligand complexes, a dynamic cross-correlation matrix (DCCM) was generated for all six systems using Carma [37]. The program Carma calculates covariance and pairwise correlations (Cij):

$$C_{ij} = \frac{\langle \Delta\vec{r}_i(t) \cdot \Delta\vec{r}_j(t) \rangle}{(\langle \Delta\vec{r}_i(t)^2 \rangle \langle\ \Delta\vec{r}_j(t)^2 \rangle)^{1/2}}$$

between pairs of atoms across and the MD trajectory. The extracted MD trajectory included 428 atoms, where atoms 1-427 correspond to the protein Cα atoms and atom 428 corresponds to the center atom of the ligand. This trajectory of each system was used to calculate the covariance matrix which was then converted to the DCCM.

Results

MMGBSA binding energy suggests that the D26E mutation weakens ligand binding

The efficacy of the three ligands was estimated using MM/GBSA calculations of binding energies (ΔE) on WT and MT β-tubulin (**Table 1**). Although paclitaxel, docetaxel, and cabazitaxel are all taxane derivatives, their interaction patterns with the WT and D26E MT strain were different. Paclitaxel was found to have a binding energy of −101.5 ± 8.4 and −90.4 ± 8.9 kcal/mol against the WT and MT β-Tubulin, respectively. The calculated binding energy difference $\Delta\Delta G^2$ between the two systems is 11.1 kcal/mol. Therefore, the bonding energy was more favorable in the WT, with stronger attractive forces.

The IC_{50} values of paclitaxel are 2.4 ± 0.4 nmol/L and 71.2 ± 17.6 nmol/L for the WT and MT β-Tubulin. The expected resistance fold change is 29 times larger in the MT form than in the WT β-Tubulin based upon the ratio of IC_{50} values. The binding energy of docetaxel was estimated to be −104.7 ± 7.0 kcal/mol with the WT and −103.8 ± 5.5 kcal/mol with the MT β-tubulin. The resulting ΔΔG for docetaxel was smaller (0.9 kcal/mol) than that of paclitaxel. The expected IC_{50} values are 0.84 ± 0.46 nmol/L for the wildtype and 8.1 ± 1.9 nmol/L for the MT, with an expected resistance fold change 9.7 times higher in the MT form. Consequently, there is a large discrepancy in resistance change between both paclitaxel's and docetaxel's response to a mutation in β-tubulin. Since a higher-fold resistance factor indicates the drug treatment is not so effective, the higher expected resistance folds change values for paclitaxel than that of docetaxel underlies the higher efficacy of docetaxel. Interestingly, cabazitaxel was found to have a binding energy of −122.8 ± 10.8 kcal/mol against the WT and −106.2 ± 7.0 kcal/mol against the MT β-tubulin. These data show that paclitaxel and docetaxel bound to the MT less strongly than the WT, suggesting possible drug resistance. The difference between the two systems is 16.6 kcal/mol. Further experimental/computational analysis is yet to be done to determine the expected resistance fold change for cabazitaxel bound to the MT and WT β-tubulin. These

results suggest that the mutation reduced the binding affinity of all three generations of taxanes with the largest binding energy change observed in the case of cabazitaxel bound to the WT and MT β-tubulin.

To understand the energetic basis of drug resistance at the residue level, the key residues involved in ligand binding based on cutoff values (−1 kcal/mol to +1 kcal/mol for ΔΔE) were identified (**Table 2** and **Figure 3)**. Our MM/GBSA analysis identified R318 and Q276 as the key residues essential for binding to WT β-tubulin by the three taxanes. On the other hand, none of the taxanes have direct interactions with these residues in the MT protein. Eleven residues in paclitaxel-bound β-tubulin collectively contributed 3.3 kcal/mol to the total binding energy. In the case of docetaxel-bound β-tubulin, 13 residues collectively contributed 0.5 kcal/mol to total binding energy. For cabazitaxel bound β-tubulin, 12 residues collectively contributed 11.2 kcal/mol to the total binding energy (Table S3). Furthermore, the key residues that weaken the binding of drugs relative to the MT and thus contribute to anti-drug resistance were identified.

Convergence of molecular dynamic simulations

For each docked complex, the stability was examined through three 200 ns MD simulations averaged over time. To check the convergence of the MD simulation trajectories towards the end of the simulation time, we investigated the protein Cα and ligand RMSD plots with predefined atom selections (backbone, side chains, heavy atoms, Cα), which were pre-computed for each trajectory against simulation time **(Figure 4**). The protein-ligand RMSDs from the three individual trajectories of the paclitaxel, docetaxel, and cabazitaxel WT and MT β-tubulin systems are available in the supporting document (Figure S3-S5**)**. The relatively flat plots within the last 200 ns indicate that the complex systems reached a steady state, particularly after

150 ns. Thus, both the average protein Cα RMSD and average ligand RMSD values were calculated using the last 50 ns of the MD simulation (Table S4). Convergence of the MD simulation trajectories is further indicated by the relatively low standard deviations of both the protein and ligand RMSD values as seen in Table S4. Monitoring the RMSD of the protein Cα terminus can help gain insights into its structural conformation throughout the simulation trajectory, thereby providing an indication of the conformational stability of the protein receptor and whether the simulation equilibrated properly. No significant protein Cα RMSD differences between the wildtype and MT β-Tubulin were observed in both the paclitaxel and docetaxel systems. However, there was a significantly higher protein Cα RMSD value in the cabazitaxel WT system, suggesting that the structural conformation was more stable in the D26E mutant system. Among the mutant systems, cabazitaxel had the lowest protein Cα RMSD value, indicating that it has higher conformational stability and showed lower drug resistance. The ligand RMSD can indicate how stable the ligand is with respect to the protein, as well as the evolution of its internal conformation. Paclitaxel and docetaxel showed more table ligand RMSD trends when bound to the MT than when bound to the WT β-Tubulin. On the other hand, cabazitaxel showed lower fluctuations when bound to the WT system in comparison to the MT system. This agrees with the MM-GBSA analysis which showed that the cabazitaxel WT and MT systems had binding energies of −122.8 ± 10.8 kcal/mol and −106.2 ± 7.0 kcal/mol, respectively.

Different protein-ligand interaction between the wildtype and mutant β-Tubulin

The differential effects of the D26E point mutation on the ligand-receptor systems averaged over the three individual trajectories were seen in the 2D schematics of the protein

residues and their ligand interaction sites (**Figure 5**). The timeline and types of different interactions in the averaged WT and MT complexes are also shown in **Figure 6.** The protein-ligand interaction 2D diagram and histogram for the three individual trajectories of the paclitaxel, docetaxel, and cabazitaxel WT and MT β-Tubulin systems were provided in the supporting document (Figures S6-S8). Using the protein-ligand interaction histograms of the three averaged trajectories, twelve key residues involved in binding the WT and MT β-Tubulin systems were identified (**Table S3**). Overall, the four distinct protein-ligand interactions observed were hydrogen bonds, ionic interaction, water bridges, and hydrophobic contact. Paclitaxel interacted with the WT and MT residues via hydrogen bonding and water bridges. Additionally, paclitaxel exhibited hydrophobic interactions as well as positively charged interactions, with a polar interaction with both WT and MT forms. Docetaxel formed comparable hydrogen bonds and hydrophobic interaction with both the WT and MT forms. Interestingly, cabazitaxel formed more persistent hydrophobic interactions, positively charged, and hydrogen bonds with the WT than the MT.

Residual fluctuations and secondary structure change

The protein Cα RMSF plots for the three individual trajectories of the six systems are shown in Figures S9-S11. The residue fluctuation values were plotted against the protein residue indices over the 200 ns simulation time. Protein Cα RMSF plots, averaged over the three trajectories, illustrate the differential conformational dynamics response to the binding of the three drugs (**Figure 7**). Comparing the WT systems, the cabazitaxel bound system was found to show the lowest fluctuation with the docetaxel bound system having the second lowest fluctuation and third the paclitaxel bound system. It is worth noting that binding site residues

showed smaller fluctuations. However, it is not clear whether the WT system has lower fluctuations than the MT system. Nonetheless, a higher fluctuation was seen in the αβ dimer interface and the rest of the secondary structure regions. On the other hand, the non-secondary structure region between helix 1 (α1) and sheet 2 (β2) is associated with higher fluctuation. To further examine the allosteric effects of the D26E single-point mutation, the average protein RMSF values on specific regions of the protein were examined (**Table S5**), including the N-terminal (residues 1-121), the middle region (residues 122-161), the C-terminal (residues 262-421), the overall protein, and the residues of the β chain in contact with the α chain (**Table S5, column 5**). The middle region exhibited a lower fluctuation in comparison to both the end terminal regions, indicating that it is tightly packed in the protein core while the end terminals are exposed to the solvent. Interestingly, the most fluctuation is seen in the protein-protein heterodimer interface, in which the β-tubulin (Chain B) is in contact with the α-tubulin (Chain A). Taxanes, such as paclitaxel, docetaxel, and cabazitaxel, are mitotic inhibitors that stabilize the GDP-bound tubulin in the microtubule, thus inhibiting the wildtype microtubule deformation during cellular mitosis, yet the D26E mutation in β-tubulin reduces the stabilizing effects of these drugs. Thus, it makes sense that the D26E mutation induced a higher structural fluctuation of the interfacing residues on β-tubulin, which might weaken the protein-protein interaction between the β-tubulin and α-tubulin, leading to an easier disassembling of the spindle (**Figure S1**).

Upon examining the ligand RMSF diagrams for the averaged trajectories of each system, the ligand structures did not exhibit high fluctuation. For each of the six systems, the ligand RMSF diagrams for the individual trajectories can be found in Figures S12-S14. Upon observation, all three ligands, paclitaxel, docetaxel, and cabazitaxel contained eight rotatable

bonds. The torsion angles and timelines from the individual trajectories of the six systems were provided in the supporting document (Figures S15-S17). Interestingly, the secondary structures- alpha helices and beta sheets- are maintained over time for both the WT and MT forms (**Figure 8)**. Provided in the supporting document are the illustrations of the secondary structures for the individual trajectories of the six systems (Figures S18-S21).

Protein and ligand conformational changes

To explore the mutational impact on three generations of taxanes binding poses and ligand-receptor interaction with microtubules, trajectory clustering was performed to extract the most abundant structure of each complex (Figures S22-S24). The respective representative structures were superimposed (**Figure 9**). Except for unstructured regions, there were no significant conformational differences between the WT and MT β-tubulin structures for all the 3 systems. Unstructured regions of proteins are known to show an increased movement due to high beta factor [38]. Slight changes in the rotations and angles influenced most of the chiral centers for the three ligands and thus affecting their binding positions, apparently due to variation in their R-groups (**Figure 9**). Nonetheless, their binding mode and orientations are the same in both the WT and MT β-tubulin.

Dynamical changes in the protein and ligand

To explore the overall stability of the protein-ligand complexes, the DCCM of the six systems was calculated by the Carma program, as outlined in the methods section. Provided in the supporting document is a pairwise comparison of the DCCMs from all six mutant systems (**Figures 10-12**). As shown in the DCCMs, the single-point mutation D26E induces a very

different dynamic response in the ligand-binding region. The DCCMs of both paclitaxel and docetaxel are comparable, but it can be shown that the ligand-binding domain region of both mutant systems appears to have a stronger negative (blue) correlation in comparison to their respective wildtype systems which display more positive (yellow) correlations (**Figures 10-11**). Amongst the WT systems, cabazitaxel appears to display the strongest positive correlation, demonstrating the least dynamical change in the system over time. In the cabazitaxel DCCM pairwise comparison, the ligand-binding domain region also appears to be more yellow (+0.4) in the wildtype system in comparison to the D26E mutant system, which appeared bluer (-0.4 to -0.6) (**Figure 12**). Overall, DCCM analysis demonstrates a reduction in correlation for the D26E mutant systems of paclitaxel, docetaxel, and cabazitaxel, providing further indication that this D26E single-point mutation can induce dynamical changes in the system over time. These dynamic changes can contribute to the reduction of binding affinity.

To further elucidate the dynamical changes of the protein-ligand complexes due to the D26E single-point mutation, the correlation coefficients between the ligand center atom position and the protein residue Cα position (1-427) were extracted from the DCCMs and plotted in **Figures S25-S27.** The differences between the D26E mutant and wildtype system are shown in **Figure 13**, in which the twelve key residues and location of the D26E single-point mutation are indicated by red and black arrows, respectively. Overall, the cabazitaxel system showed stronger correlations throughout the correlation plot in comparison to both the paclitaxel and docetaxel systems (**Figure 12**). To this, the docetaxel system notably showed a strong negative correlation in comparison to paclitaxel and cabazitaxel around residues 90 to 190. Ultimately, the correlation coefficient plots further demonstrate that the D26E single-point mutation induces varying dynamical changes in the systems of paclitaxel, docetaxel, and cabazitaxel over time.

Discussion and Conclusion

This study sought to get insights into the binding of cabazitaxel to WT and MT β-tubulin in comparison to two older taxanes, paclitaxel and docetaxel. The change in free energy can be better understood in the fold change of the binding for each drug to the WT and MT proteins. Paclitaxel was found to have the largest fold change between the two forms of β-tubulin. Docetaxel showed a comparable binding affinity to both the WT and MT β-tubulin, suggesting that the drug might overcome the effect of mutation seen with paclitaxel. These trends of binding affinity among the 3 generations of taxanes, to some extent, agree with the existing data. Cabazitaxel was found to be 10 times more effective than second-generation taxanes such as docetaxel [11]. Docetaxel is known to have a stronger binding affinity to the WT β-tubulin than paclitaxel [39] and has wider cell cycle activity than paclitaxel [40], although cabazitaxel is stronger than paclitaxel [41]. The rotations in the poses of the drugs and the changes in interactions can explain the differences in binding affinity for these taxanes [42]. Moreover, the MM/GBSA calculations identified R318 and Q276 as the key residues essential for binding of the taxanes to WT β-tubulin. A hydrogen bond between Q276 and Taxol's oxetane ring is critical for Taxol's stabilizing activity [42].

Furthermore, no significant protein Cα-RMSD differences between the WT and MT β-Tubulin were observed in all the 3 systems. Paclitaxel and cabazitaxel showed relatively more stable RMSD trends when bound to the WT than when bound to the MT. On the other hand, docetaxel did not show a significant change in RMSD when bound to the WT and MT, suggesting that the drug binding was not affected by the β-Tubulin mutation. This is consistent with the residual fluctuation data which suggested that residues in the region of the secondary

structure of the protein did not fluctuate significantly. Also, the ligand RMSD indicates how stable the ligand is with respect to the protein, as well as the evolution of its internal conformation [43]. Due to variations in the R-groups, the rotations and angles influenced most of the chiral centers for the three ligands and thus affecting their binding positions, apparently due to variation in their R-groups (**Figure 11**). Nonetheless, their binding mode and orientations remained the same in both the WT and MT β-tubulin. According to the DCCM analyses, the D26E single-point mutation induces dynamical changes in the systems over time.

Taken together, our data suggest that paclitaxel and cabazitaxel demonstrated an increased binding affinity for the WT compared to the MT version. On the other hand, the binding affinity of docetaxel for the WT was not so different from that of the MT form. Interestingly, cabazitaxel was found to have the highest binding affinity for both the WT and MT β-Tubulin even though it is not clear as to whether the drug could overcome the effects of the mutation. This study examined the binding of taxanes to both the WT and MT β-Tubulin, providing insights into the mutation effects on the overall dynamics and interaction of the protein with the ligands.

Supporting Information

Additional images, figures, diagrams, and tables are included in the supporting materials.

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