CHAPTER 3

MOLECULAR INTERACTION OF NOSCAPINOIDS AT THE INTERFACE OF γ-TUBULIN DIMER
Chapter 3

Abstract

γ-tubulin plays crucial role in the nucleation and organization of microtubules during cell division. Recent studies have also indicated their role in the regulation of microtubule dynamics at the plus end of the microtubules. Moreover, γ-tubulin has been found to be over-expressed in many cancer types like carcinomas of the breast and glioblastoma multiforme etc. These studies have led to immense interest in the identification of chemical leads that might interact with γ-tubulin and disrupt its function in order to explore γ-tubulin as potential chemotherapeutic target. Recently a colchicine interacting cavity was identified on γ tubulin that might also interact with other similar compounds. In the same direction we found a class of compounds, noscapinoids (noscapine and its derivatives) that fits well in a cavity close to the interface of γ-tubulin dimer. Molecular interaction of Noscapine and two of its derivatives- amino-noscapine and bromo-noscapine was investigated by molecular docking, molecular dynamics simulation and binding free energy calculation. All noscapinoids displayed stable interaction throughout simulation of 10 ns. The predictive binding free energy (Δ$G_{\text{bind}}$) indicates that noscapinoids bind strongly with γ-tubulin dimer. However, bromo-noscapine showed the best binding affinity (Δ$G_{\text{bind}}$ -37.6 kcal/mol) followed by noscapine (Δ$G_{\text{bind}}$ -29.85 kcal/mol) and amino noscapine (Δ$G_{\text{bind}}$ -23.99 kcal/mol) using the MM-PBSA method. Similarly using the MM-GBSA method also, bromo-noscapine showed highest binding affinity with Δ$G_{\text{bind}}$ value of -43.64 kcal/mol followed by amino-noscapine with Δ$G_{\text{bind}}$ value of -37.56 kcal/mol and noscapine with Δ$G_{\text{bind}}$ value of -34.57 kcal/mol. The results thus generate compelling evidence that these noscapinoids indicate a great potential for preclinical and clinical evaluation.
3.1 Introduction

\(\gamma\)-tubulin is an essential component of the microtubule organization center (MTOC). It is known to interact with the minus end of microtubules and found to be localised at the centrosome. It plays an extremely crucial role in the nucleation and organization of microtubules [1, 2]. At the MTOC, \(\gamma\)-tubulin forms complexes with other proteins to form \(\gamma\)-tubulin small complex (\(\gamma\)TuSC, around 280 kDa) comprises two molecules of \(\gamma\)-tubulin and one molecule each of GCP (\(\gamma\)-tubulin complex protein) 2 and 3. These proteins structurally associate to form Y-shaped flexible structure with \(\gamma\)-tubulins located on the two arms. Furthermore, \(5\) \(\gamma\)TuSCs associate together to form the larger cone shaped complex known as \(\gamma\)-tubulin-ring complex (\(\gamma\)TuRC), in association with GCP4, GCP5 and GCP6 [3-9]. \(\gamma\)-tubulin form the rim of this cone shaped complex. \(\gamma\)-tubulin interacts directly via one of its longitudinal interface with the minus end of microtubules and via the other longitudinal interface with the GCPs (GCP2, GCP3, GCP4). \(\gamma\)-tubulin interacts laterally with another \(\gamma\)-tubulin.

Many drugs that are currently administered as chemotherapeutic regime like paclitaxel derivatives and vinca-alkaloids, bind to \(\alpha\beta\)-tubulin and target the mitotic spindle dynamics [10]. However, these drugs are plagued with many drawbacks like development of multi-drug resistance over time which often leaves patients with more aggressive tumors and side effects. Indiscriminate and prolonged use of microtubule targeting drugs affects the normal rapidly dividing cells like those of the hair follicles and intestinal lining causing hair loss and nausea. These drawbacks call for continuous progressive research to come up with newer drugs with no or negligible side effects.

In recent studies \(\gamma\)-tubulin has been found to be over-expressed in pre-invasive lesions, carcinomas of the breast and glioblastoma multiforme (GBM) [11-13]. Since \(\gamma\)-tubulin over-expression is associated with tumor progression, a potent inhibitor of \(\gamma\)-tubulin would possibly halt mitosis in cancer cells. In the endeavour to explore if small ligands could bind at the interface and disrupt the \(\gamma\)-\(\gamma\) tubulin lateral interactions, we studied probable binding interaction of noscapinoids (noscapine, amino-noscapine and bromo-noscapine) with \(\gamma\)-tubulin homodimer. In this direction, we first prepared the structure of the \(\gamma\)-tubulin homodimer and ligands. We then performed molecular docking to obtain the protein –ligand
complexes, which were then simulated in Amber 11 for 10 ns and then predicted the binding affinity using the MM-PBSA and MM-GBSA methods.

**Figure 3.1:** Schematic representation of overall methodology followed to study the molecular interaction of noscapinoids with of γ-γ tubulin dimer.

### 3.2 Materials and Methods

#### 3.2.1 Protein structure preparation

In order to study the molecular interaction of noscapinoids at the binding interface of γ-γ tubulin dimer, the structure of γ-tubulin dimer was first prepared as described in chapter 1.

#### 3.2.2 Ligand preparation

The molecular structure of the lead molecule, noscapine and two of its derivatives such as amino-noscapine and bromo-noscapine 1-3 (Figure 3.2) were built using molecular builder of Maestro (version 8.5, Schrodinger LLC). All these structures were energy minimized *in-vacuo* using Impact (version 5.6, Schrodinger, LLC). Appropriate bond orders were assigned to each structure using Ligprep (version 2.4, Schrodinger LLC) and initial
optimization was performed on each structure by employing OPLS 2005 force field using default setting. Furthermore, geometrical optimization of these ligands was performed in Jaguar (version 7.7, Schrodinger, LLC) using hybrid density functional theory with Becke’s three-parameter exchange potential and the Lee–Yang–Parr correlation functional (B3LYP) [14, 15] using basis set 3-21G* level [16-18].

![Molecular structures](image)

Figure 3.2: Molecular structure of Noscapine (1), Amino-noscapine (2), Bromo-noscapine (3)

### 3.2.3 Molecular docking

After ensuring that protein and ligands are in correct form, molecular docking of the optimized ligands onto the γ-tubulin dimer was performed using Glide (version 4.5, Schrodinger, LLC). The receptor-grid file was generated using grid receptor generation program with van der Waals scaling of 0.4 Å. A grid box size of 10 Å each for the bounding and enclosing boxes were generated at the centroid of the predicted binding sites (using SiteMap, version 2.4, Schrodinger, LLC) [19, 22]. The ligands were first docked using the “standard precision” method and further refined using “extra precision” Glide algorithm [22-24] (Table 3.1). Single best conformation for each ligand–protein complex was selected for further molecular modeling calculations.

Table 3.1: Molecular docking evaluation of binding sites. All the three ligands were docked onto the 10 predicted binding sites of γ-tubulin dimer complex. The binding site that showed better docking score with all the ligands was selected as the probable binding site of interaction of these ligands on γ-γ tubulin dimer. On the basis of docking score, site 6 was selected.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Site1</th>
<th>Site2</th>
<th>Site3</th>
<th>Site4</th>
<th>Site5</th>
<th>Site6</th>
<th>Site7</th>
<th>Site8</th>
<th>Site9</th>
<th>Site10</th>
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<tbody>
<tr>
<td>Amino</td>
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<td>-2.9</td>
<td>-3.48</td>
<td>-3.36</td>
<td>-3.1</td>
<td>-3.55</td>
<td>-1.88</td>
<td>-2.54</td>
<td>-3.08</td>
<td>-3.61</td>
</tr>
<tr>
<td>Bromo</td>
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<td>-3.09</td>
<td>-2.7</td>
<td>-2.42</td>
<td>-4.23</td>
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<td>-2.48</td>
<td>-2.36</td>
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</tr>
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<td>Noscapine</td>
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<td>-3.97</td>
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<td>-2.74</td>
<td>-2.14</td>
<td>-1.93</td>
<td>-3.88</td>
</tr>
</tbody>
</table>
3.2.4 Molecular dynamics simulation and binding affinity calculation

The protein ligand complexes obtained after molecular docking were then simulated for 10 ns in Amber11 using the parameters as described in chapter 2. Before simulation, the missing parameters for all three ligands were estimated with the antechamber program [22, 25] implemented in Amber 11. AM1-BCC charge model was used to calculate the atomic point charges [23]. Missing hydrogens were added and FF99SB forcefield was employed to assigned parameters to the complex of GCP4 and γ-tubulin, while GAFF forcefield was used to assigned the parameters to each ligand using tleap module available in Amber 11. Each system was neutralized using sodium ions and solvated using TIP3 water model in a truncated octahedron with distance of at least 15 Å between the wall of the box and the closest atom of the complex [24]. All systems were simulated for 10 ns keeping the parameters consistent with those employed for γ-tubulin dimer in chapter 2. A total of 10,000 frames were obtained after 10 ns simulation. Binding affinity of noscapinoids at the γ-γ tubulin binding interface was then calculated using MM-PBSA and MM-GBSA methods [25, 26]. Free energy of binding was calculated as the ensemble average of the binding free energy of a total of 500 snapshots, extracted every 10 ps from the last 5 ns of the MD simulation trajectory.

\[ \Delta G_{\text{bind}} = \Delta G_{\text{complex}} - [\Delta G_{\text{Rec}} + \Delta G_{\text{lig}}] \]

\[ G = E_{\text{gas}} + G_{\text{sol}} - TS. \]

\[ E_{\text{gas}} = E_{\text{int}} + E_{\text{ele}} + E_{\text{vdw}} \]

\[ G_{\text{sol}} = G_{\text{PBI(GB)}} + G_{\text{sol-np}} \]

\[ G_{\text{sol-np}} = \gamma \text{SAS} \]

Where, \( G \) is Gibbs free energy, \( E_{\text{gas}} \) is the gas phase energy calculated as the sum of internal energy (\( E_{\text{int}} \)), energy generated as a result of the electrostatic interaction (\( E_{\text{ele}} \)) and the van der Waals interaction(\( E_{\text{vdw}} \)). \( G_{\text{sol}} \) is the solvation free energy calculated as the sum of polar(\( G_{\text{PBI(GB)}} \)) and nonpolar contributions (\( G_{\text{sol-np}} \)). Polar interaction contribution (\( G_{\text{PBI(GB)}} \)) was calculated as the summation of electrostatic contribution (\( E_{\text{ele}} \)) and polar solvation contribution (\( G_{\text{PBI(GB)}} \)). The nonpolar solvation contribution (\( G_{\text{sol-np}} \)) is approximated as
linearly dependent on the solvent accessible surface area (SAS) and \( \gamma \) is the surface tension constant that was set to 0.0072 kcal mol\(^{-1}\) Å\(^{-2}\) \cite{26}\cite{30}.

### 3.2.5 Per residue energy decomposition

The contribution of each amino acid residue of the \( \gamma \)-tubulin dimer was calculated to identify those residues which show strong interaction with noscapinoids. These calculations were performed using MM-GBSA method implemented in Amber 11 over 500 frames obtained every 10 ps from last 5 ns trajectory.

### 3.3 Results and discussions

#### 3.3.1 Molecular dynamics simulation

The \( \gamma \)-tubulin dimer complex bound to drugs (1-3) were simulated for 10 ns to obtain a trajectory of 10,000 frames, each frame recorded every 1 ps. Root mean square deviations (RMSD) of \( \text{Ca} \)-atoms during the entire duration of simulation were calculated for all the frames to monitored the stability of the system. The molecular systems were observed to get stabilized after 4 ns of simulation (Figure 3.3), since the relative fluctuation in the RMSD of \( \text{Ca} \)-carbon atoms (\( \text{Ca-rmsd} \)) is very small after equilibration. The overall RMSD ranges from 0 to 2.2 Å. Furthermore, root mean square fluctuations (RMSF) of \( \text{Ca} \)-atoms were also calculated for all four molecular systems to find any changes in the residue flexibilities. The RMSF values were plotted against residue numbers as shown in Figure 3.4. The residues with higher RMSF tend to show more flexibility (Figure 3.4).
Figure 3.3: Root mean square deviation. The root mean square deviation (RMSD) of Ca atoms of the γ-tubulin dimer in the free form and bound form with different ligands (noscapine, amino-noscapine and bromo-noscapine) during the entire duration of MD simulation.

![Graph showing RMSD values over time](image)

Figure 3.4: Root mean square fluctuation (RMSF) of Ca atoms. RMSF of residues in γ-tubulin dimer in the free form and in the bound form with different ligands (noscapine, amino noscapine, bromo noscapine) during the entire duration of MD simulation. The residues with higher RMSF tend to show more flexibility. The residues in the bound form show a small degree of flexibility when compared with free γ-tubulin dimer.

3.2 Theoretical binding affinity calculation

The binding affinity of all the three noscapinoids (1-3) was calculated using MM-PBSA and MM-GBSA methods as described in chapter 1. All three noscapinoids displayed stable interaction throughout simulations (Figure 3.3 and 3.4). Among all the three noscapinoids, bromo-noscapine showed the best binding affinity with the value of -37.6 kcal/mol followed by noscapine with -29.85 kcal/mol and amino noscapine with the -23.99 kcal/mol using the MM-PBSA method (Table 3.2). Using the MM-GBSA method also, bromo-noscapine showed highest binding affinity with the value of -43.64 kcal/mol followed by amino-noscapine with -37.56 kcal/mol and noscapine with the value of -34.57 kcal/mol. This could be attributed to the formation of hydrogen bonds between bromo-noscapine and surrounding amino acid residues like Met249, Asn250 and Gly247 in chain A (Figure 3.5).
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Noscapine also forms hydrogen bonds with Met249 and Asn250 of chain B. No significant hydrogen bonding was observed for amino-noscapine.

Table 3.2: Calculated binding free energy between GCP4 and γ-tubulin. Binding free energy calculated using MM-GBSA and MM-PBSA to ascertain the strength of interaction between GCP4 and γ-tubulin for both dimer 1 and dimer 2. The major energy components like van der Waals, electrostatic, polar solvation and non-polar solvation, contributing to the binding free energy were also estimated.

<table>
<thead>
<tr>
<th>Contribution</th>
<th>Noscapine (kcal/mol)</th>
<th>Amino-noscapine (kcal/mol)</th>
<th>Bromo-noscapine (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔE_{INT}</td>
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<td>0.00</td>
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<tr>
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<td>-139.56</td>
</tr>
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<tr>
<td>H_{TOT, GB}</td>
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<td>-37.56</td>
<td>-43.64</td>
</tr>
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</table>

(A)
Figure 3.5: 2-D ligplot of noscapinoids. A 2-D representation of the binding mode of noscapinoids (A: Noscapine, B: Amino-Noscapine and C: Bromo-Noscapine) with the γ-tubulin dimer. ‘A’ and ‘B’ denote the chains in γ-tubulin dimer. Hydrogen bonding residues are shown in green and red for chain A and chain B, respectively. The Dashed lines denote hydrogen bonds, and numbers indicate hydrogen...
bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. The figures were made using Dimplot [27].

All identified hot spot amino acids were observed to lie at the interaction interface of γ-tubulin dimer (Figure 3.6). For all complexes, the binding energy was decomposed into its various energy components (the electrostatic, van der Waals and solvation). Both van der Waals (ΔE_{vdw}) and the electrostatic component (ΔE_{ele}) were observed to make very significant contributions to the free energy of binding. However, the net polar contribution (ΔG_{ele,polar} = ΔE_{ele} + ΔG_{polar}) was rendered unfavourable due to very large penalty imposed by the desolvation component (ΔG_{desolv}) while the net nonpolar component ΔE_{vdw} and ΔG_{solv} were observed to make highly favourable contribution to the binding free energy (Figure 3.7).

Figure 3.6 A: Binding mode of noscapinoids. Noscapinoids (Noscapine, Amino-noscapine and Bromo-noscapine) docked into γ-tubulin dimer. The zoomed views show the binding mode of the noscapinoids alone. The residues which show contribution of less than -1 kcal/mol to the binding free energy (ΔG_{bind}) are only labeled.
Figure 3.7: Energy contribution of hot spot amino acids. Non-polar, polar as well as total energy contributions of the amino acid residues that contribute most to the stability of the protein ligand (A: Noscapine, B: Amino-noscapine and C: Bromo-noscapine) complex. Polar interactions were calculated as sum of electrostatic (ΔE_\text{ele}) and polar solvation (ΔG_\text{sol,GR}) energy components while the non-polar interactions were calculated as sum of van der Waals (ΔE_\text{vdW}) and non-polar solvation component (ΔG_\text{sol,app}).
**Figure 3.8: Multiple sequence alignment of γ-tubulin.** γ-tubulin sequence show high level of homology across different species. The residues in the cyan region are identical. The residues enclosed with blue rectangle were identified as hot spot amino acids at the γ-γ tubulin dimer while those enclosed in magenta rectangle were observed to make strong contribution while binding with noscapinoids.

**Conclusion**

In this study, the binding modes of the three noscapinoids with the γ-γ tubulin dimer were illustrated using MD simulation and binding free energy calculations. All the three ligands lodged themselves in the pockets located very close to the binding interface of the γ-tubulin dimer. All three ligands showed stable interaction throughout the simulations however, the best binding affinity was calculated for bromo-noscapine. The binding modes of noscapine and bromo-noscapine are quite similar with both the drugs showing strong interaction with Tyr248, Met249, Asn250 of γ tubulin. Multiple sequence alignment analysis showed the amino acid residues which interact with noscapinoids were observed to lie in the highly conserved regions (Figure 3.8). Therefore, if these drugs can interfere with a subset of the hot spot amino acids they might be able to perturb some of the interactions between γ-tubulin units in the γTuRC and further destabilize the γTuRC. Nevertheless, our results offer noscapinoids an important possible chemical framework for the further design of more potent compounds.
REFERENCES


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