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# Molecular Docking and Drug-likeness Prediction of New Potent Tubulin Colchicine Binding Site Inhibitors for Potential Antitumor Drug

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**ABSTRACT**

Cancer is a real public health problem that figures among the main causes of morbidity and mortality in the world. The Colchicine Binding Site (CBS) is an important pocket for potential tubulin polymerization destabilizers. Colchicine binding site inhibitors (CBSI) exhibit their biological effects by inhibiting tubulin assembly and suppressing microtubule formation. In order to identify new potent CBSI, molecular docking and drug likeness prediction were performed. In this context, a collection of 850 similar compounds to combretastatinA-4from PubChem database was docked into the CBS. Out of these, compounds S1 and S2 were found to have highest negative binding energy of -9.462 and -9.017 Kcal/mol respectively. Furthermore, these two compounds were predicted to have satisfying drug likeness properties, indicating that they might be promising lead compounds for further antitumor drug research.

***Keywords:*** Cancer, Colchicine binding site, Glide, Molecular docking, Tubulin.

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## Introduction

Cancer is one of the leading causes of morbidity and death in the world [1]. The International Agency for Research on Cancer estimated 19 292 789 new cases and close to 10 million deaths in 2020 alone [2-3]. It is well known that cancer results from erratic cell division. This process is carried by different structures such as the mitotic spindle, which is composed of polymerized tubulin to form microtubules [4-6].

Tubulin is a dimeric protein containing an α and β chain and possessing multiple binding sites. Chemotherapeutic agents targeting vinca alkaloids and taxanes binding sites (Figure 1-A) were used clinically for over 50 years [7]. Although having good potency at first, they often trigger multidrug resistance in patients, which significantly reduces the therapeutic effects of these molecules [8]. Colchicine Binding Site (CBS) targeting agents in tubulin, commonly known as Colchicine Binding Site Inhibitors (CBSI), were recently gaining traction as potential multi-resistance evading drugs for their ability to bind well to different kinds of tubulin isomers [9]. The CBS cavity is located on the β subunit directly facing the α-subunit. This site is composed of three zones. The first zone includes Valα181, Serα178, Valβ313, Metβ257 and Asn β256. The second zone is a hydrophobic pocket composed of Lysβ350, Asnβ348, Ileβ368, Valβ313, Alaβ315, Alaβ314, Leuβ253, Lysβ252, Leuβ250, Alaβ248, Leuβ246, Leuβ240 and Cysβ239. The last zone is situated deeper in the CBS cavity and contains Thrβ237, Valβ236, Tyrβ200, Gluβ198, Pheβ167, Asnβ165, Glnβ134 and Ileβ4 (Figure 1-B) [10].

This word aims at identifying new potent CBSIs using molecular docking approach followed by a computational drug likeness prediction.

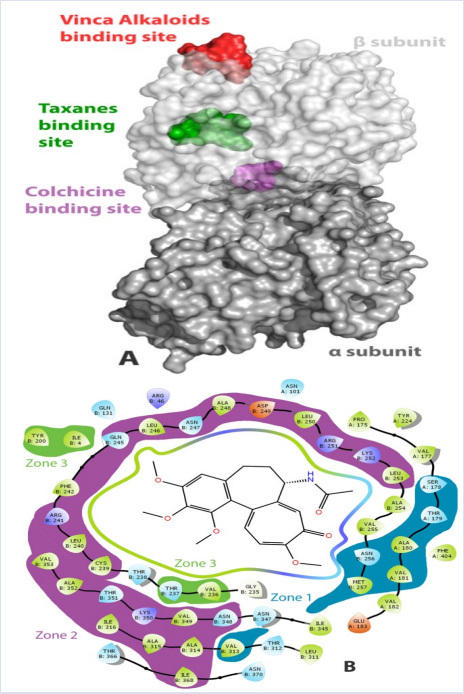


Figure 1. (A) Surface representation of tubuline subunits and its binding sites. (B) Representation of Colchicine binding site amino acids

## Materials and Methods

The identification of new potent CBSIs requires the use of several programs which are:

Schrödinger Suites is one of the most knwon software for computer aided drug design. This program ranks molecules using GlideScore scoring function which is an empirical scoring function that estimates the free binding energy in Kcal/mol. In this study, we used Glide SP [11] for docking calculation. We also used LigPrep for ligand preparation, Epik [12] and Maestro for protein preparation.

ADMETlab2.0 (http://admet.scbdd.com/) is a web server that provides ADMET predictions based on a comprehensive database from 288967 entries [13]. It was used to predict physicochemical, pharmacokinetic and toxicity properties of the most promising compounds from molecular docking.

PyMOL is an open-source software for 3D visualization. Tubulin surface generation and visualization of 3D poses of the docked compounds were performed using PyMOL 2.4.0.

PDBaser is an open-source python tool that we designed on top of Biopython [14] and Openbabel [15] to provide a fast and intuitive way to separate ligands and chains from protein PDB files [16]. We used this tool in order to facilitate the preparation process.

### *Validation Of Docking Protocol*

The performance of Glide protocol was evaluated before starting docking study on CBS. The Root Mean Square Deviation (RMSD) was calculated for 100 protein-ligand crystal structures from Protein Data Bank (PDB). It is a metric that measures average distances between the docking binding mode and the experimental conformation of a ligand. A docking protocol reproduce correctly the experimental conformation of a ligand in its binding site when RMSD value is less than or equal to 2Å [17].

### *Protein Preparation*

A Tubulin-CBSI complex was downloaded from PDB (ID: 6BR1). This protein was prepared for docking study using Schrödinger's protein preparation wizard. This program serves to define the protonation state of some CBS's residues, to control their side chain orientation and to all hydrogen atoms [18]. The Colchicine binding site was defined using a grid box which was generated from position of the co-crystallized ligand occupying the CBS (E3Y) using default settings.

### *Ligand Preparation*

850 analogs compounds to combretastatin A-4 (CA-4), a potent CBSI (Figure S1, Supplimentary file), were downloaded from PubChem database in sdf format. These molecules were prepared for docking using LigPrep. It is one of Schrödinger's modules that serve to prepare and optimize the structures of ligand to meet the requirements of the simulation programs without necessitating any further user intervention. In our study, LigPrep was used to generate for each compound a number of structures (up to 32) with various tautomers, protonation states at pH 7.0 ± 2 and enantiomers (when undefined). After this preparation, the geometries of each ligand were optimized and the final chemical library contained 1151 molecules [19].

### *Molecular Docking Calculations*

Docking calculations of the prepared molecules against CBS were done with the default parameters of Glide Standard Precision (SP). The resulting poses were clustred according to their GlideScore, which were given as a binding free energy, ΔG (kcal/mol).

### *Drug Likeness and Toxicity Prediction*

The physicochemical, pharmacokinetic and toxicity properties of the best CBSI obtained in this study were predicted using ADMETLab at http://admet.scbdd.com/. These properties consist of Lipinski and Veber's Rule, GastroIntestinal absorption (GI), Blood-Brain Barrier permeability (BBB), cell permeability (CACO-2), Cytochrome P450 (CYP) inhibition, and toxicity (hERG inhibition, Ames test, carcinogenicity). The same parameters of Colchicine were also studied for comparison.

## Results and Discussion

### *Validation of Docking Protocol*

Before carrying out molecular docking study, docking protocol was evaluated by calculating the RMSD value of 100 protein-ligand complexes from PDB. The predicted binding mode was considered correct if the RMSD was below 2.0 Å. In our results, Glide reproduced well the experimental data. Indeed, 86 % of RMSD values were less than 2Å (Table 1) which indicates that this program can reproduce correctly the binding mode of a co-crystal ligand. For example, Figure 2 shows that there was a negligible deviation between experimental pose (in green) and docked conformation (in blue) of the ligand E3Y from the complex 6BR1 in the PDB.

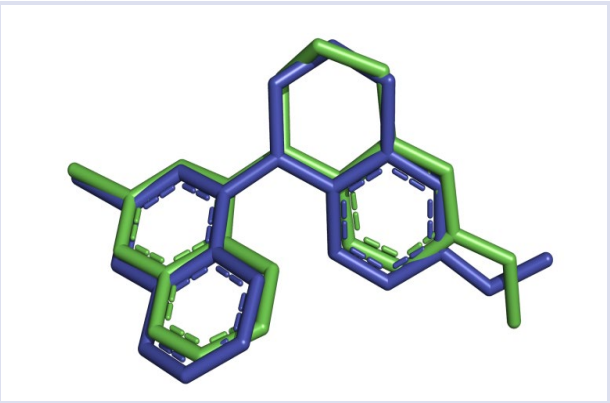

Figure 2. Superposition of the crystal conformation of the ligand extracted from 6BR1 (colored in green) against the best predicted pose (colored in blue).

Table 1. List of 100 complexes protein-ligand used to test the reliability of the docking protocol

| Protein | Ligand | RMSD | Protein | Ligand | RMSD | Protein | Ligand | RMSD |
|---|---|---|---|---|---|---|---|---|
| 6OHS | MJY | 1.876 | 2OLE | KR2 | 1.7138 | 4E00 | 0F1 | 0.4476 |
| 2XF0 | 4UB | 0.4289 | 2OS3 | BB2 | 1.9188 | 4E01 | 0F1 | 0.8993 |
| 3C56 | PH4 | 1.459 | 2P98 | YE7 | 0.5281 | 4EY7 | E20 | 0.9358 |
| 5IX0 | 6EZ | 0.3958 | 2P9A | YE6 | 2.6817 | 4FLL | YZ6 | 1.663 |
| 6MD7 | JE1 | 0.7884 | 2Q95 | A05 | 0.8423 | 4IKR | PVP | 1.3035 |
| 1CEB | AMH | 0.8469 | 2Q96 | A18 | 0.2668 | 4IKS | TFD | 4.8465 |
| 1G27 | BB1 | 1.4265 | 2QT9 | 524 | 0.8796 | 4IU6 | FZ1 | 1.9144 |
| 1G2A | BB2 | 1.1678 | 2QTB | 474 | 1.345 | 4JE7 | BB2 | 2.2446 |
| 1G36 | R11 | 0.9716 | 2R3O | 2SC | 1.0854 | 4O09 | 2R6 | 0.4272 |
| 1LOX | RS7 | 0.772 | 2R4B | GW7 | 0.3121 | 4PES | 2PJ | 0.8403 |
| 1LQY | BB2 | 1.2246 | 2RGU | 356 | 1.8357 | 4U69 | Q07 | 1.2069 |
| 1LRY | BB2 | 1.9926 | 2FZD | TOL | 0.943 | 4WY1 | 3VO | 0.2048 |
| 1N7I | LY1 | 0.4849 | 2RJP | 886 | 0.5776 | 5AUV | AGI | 0.4577 |
| 1N8Q | DHB | 0.1285 | 2RJQ | BAT | 1.7085 | 5CA1 | NZO | 0.4506 |
| 1Q1Y | BB2 | 0.9882 | 2V0Z | C41 | 1.7057 | 5CVK | 56E | 6.6885 |
| 1QZF | CB3 | 7.5903 | 3C43 | 315 | 1.0281 | 5DC5 | B3N | 2.6629 |
| 1QZY | TDE | 1.7746 | 3D01 | PG5 | 4.2033 | 5EDH | 5MF | 0.5759 |
| 1RBP | RTL | 1.1126 | 3D4L | P6G | 1.1565 | 5F00 | 5T8 | 0.2391 |
| 1RTI | HEF | 0.8429 | 3EBH | BES | 1.0669 | 5JF1 | BB2 | 1.7818 |
| 1S17 | GNR | 3.0068 | 3F8S | PF2 | 2.2045 | 5LB6 | UN9 | 0.5594 |
| 1SZZ | BB2 | 1.7339 | 3G0B | T22 | 1.0045 | 5OR6 | A4K | 1.0859 |
| 1X7J | GEN | 0.2432 | 3G0D | XIH | 0.3508 | 5PZQ | 93V | 1.1727 |
| 1YVM | TMG | 0.5978 | 3HAB | 677 | 0.7651 | 5S1O | WQA | 0.7486 |
| 1ZVX | FIN | 1.5694 | 3IU8 | T03 | 0.6359 | 5UMW | RBF | 5.072 |
| 2ABI | 1CA | 0.3414 | 3IU9 | T07 | 0.9728 | 6HOT | CIY | 0.4124 |
| 2ADU | R20 | 0.9403 | 3K6L | 2BB | 1.0032 | 6IND | AKO | 1.2239 |
| 2AI7 | SB7 | 0.4472 | 3KWF | B1Q | 0.4655 | 6M8C | IRH | 0.7043 |
| 2AIA | SB8 | 1.2518 | 3L0L | HC3 | 0.4431 | 5LYJ | 7BA | 1.4671 |
| 2AIE | SB9 | 0.8716 | 3M6P | BB2 | 2.4957 | 6BR1 | E3Y | 0.5614 |
| 2AJ8 | SC3 | 0.9461 | 3OAP | 9CR | 1.0484 | 6PZ0 | FMN | 0.8904 |
| 2BUC | 008 | 1.1211 | 3PKC | Y08 | 1.4013 | 6PZR | P7D | 1.2664 |
| 2EW5 | Y12 | 2.3255 | 3PN4 | BB2 | 1.2453 | 6THZ | NB5 | 1.3521 |
| 2EW6 | Y13 | 1.4993 | 4D09 | 788 | 4.0803 | | | |
| 2NQ7 | HM5 | 1.1931 | 4DR9 | BB2 | 2.3316 | | | |

### *Molecular Docking Calculations*

In order to identify new potent CBSI, 1151 analogs compounds to combretastatin-a-4 were docked into the CBS using Glide SP. The results show that 795 compounds were found to exhibit a higher CBS inhibitory potency than that of CA-4, the reference molecule, whose score is -5.173 Kcal/mol (Table S1 in supplementary file). Out of these, compounds S1 and S2 were found to have highest negative binding energy of -9.462 and -9.017 Kcal/mol respectively (Table 2).

Table 2. PubChem ID, Docking scores, ranking, Hbond, eModel and Ligand efficiency of the most promising CBSI (S1 and S2). The same data for CA-4 were done for comparison.

| Compound | PubChem ID | Glide Score (Kcal/mol) | Hbond | eModel | Ligand efficiency | Ranking |
|---|---|---|---|---|---|---|
| S1 | 145336937 | -9.462 | -0.330 | -68.181 | -0.411 | 1 |
| S2 | 138585723 | -9.017 | -0.307 | -59.575 | -0.474 | 2 |
| CA-4 | …….. | -5.173 | -0.110 | -16.433 | -0.255 | 796 |

### *Poses Analysis*

The binding mode of these potent inhibitors into the CBS was predicted using the poses given by Glide. As shown in figure 3, compounds S1 and S2 cover the entire CBS in a rational orientation, thus leading to an important inhibitory potency. In addition, compound S1 makes two hydrogen bonds with Thrα179 whereas S2 and CA-4 make one such bond with the same residue. However, an additional hydrogen bond is observed between S2 and Alaβ315, whereas S1 and CA-4 have bare contacts with this residue.

It should be noted that both S1 and S2 form a hydrogen bond with a structural water molecule, which connects theses inhibitors to Valβ236. The difference of the inhibitory potency between these two compounds and CA-4 may be explained by the different number of hydrogen bonds between them and the protein. Indeed, whereas S1 and S2 are involved in three such bonds, CA-4 is involved in only one (Figure 4).

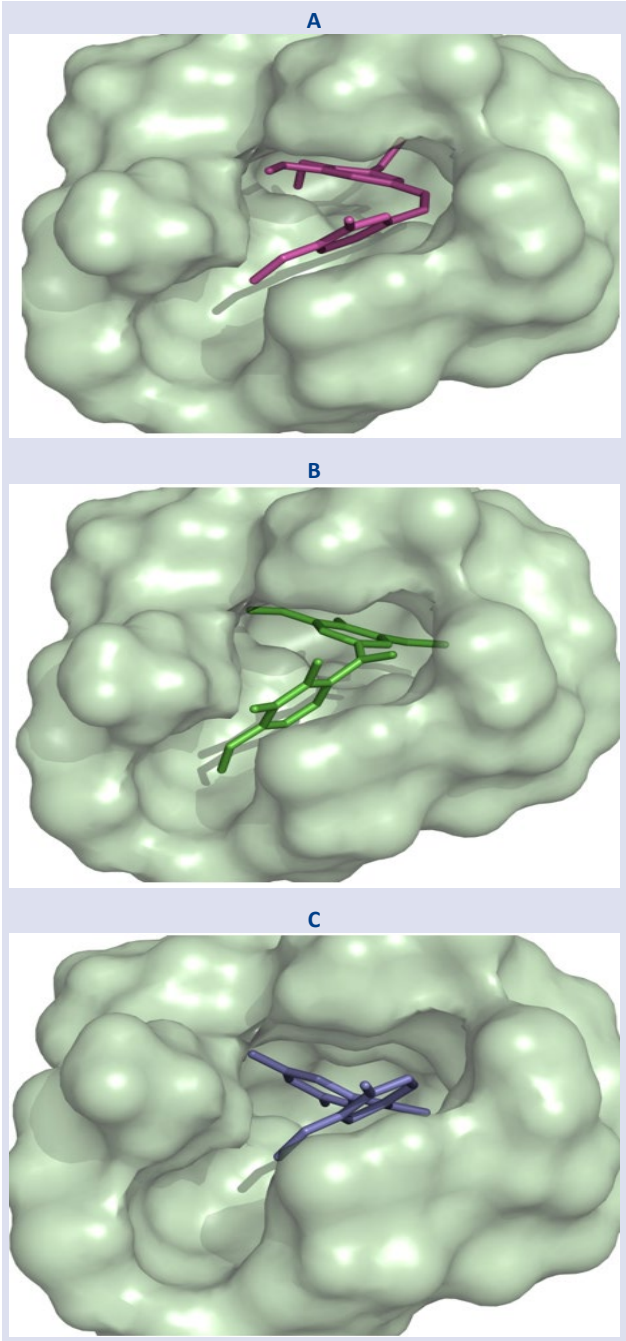


Figure 3. Positioning of CA-4 (A), S1 (B) and S2 (C) into the Tubuline Colchicine Binding Site.

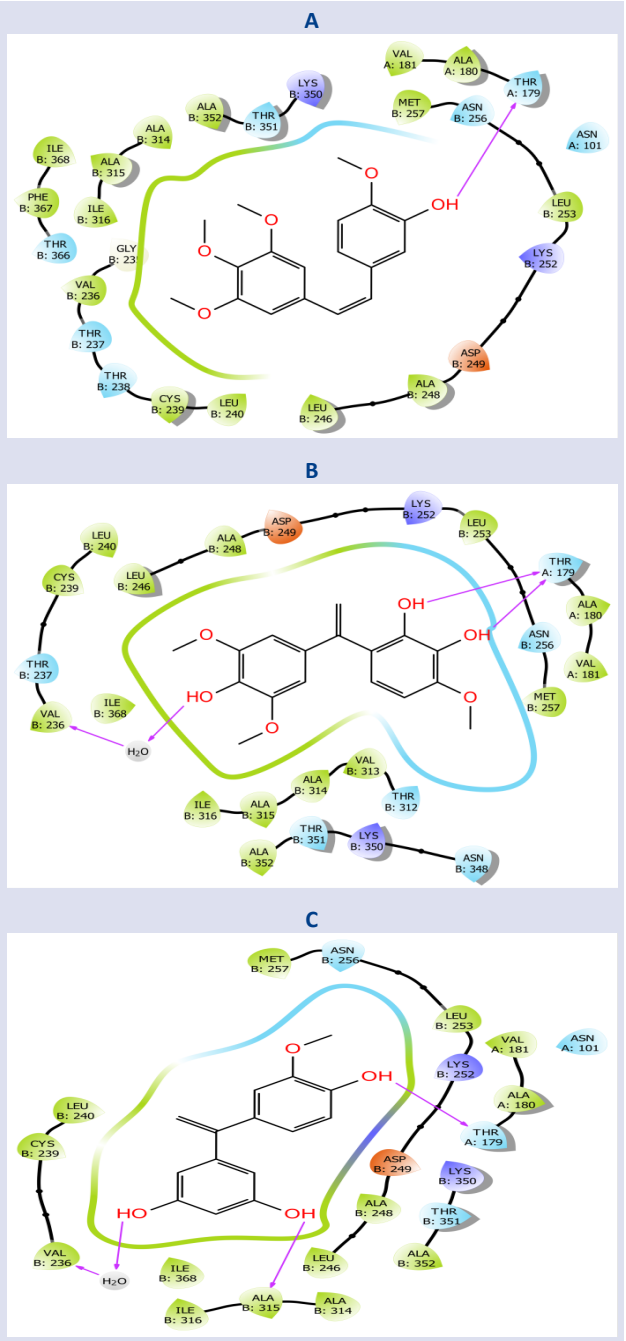


Figure 4. Binding mode prediction of CA-4 (A), S1 (B) and S2 (C) into the Tubuline Colchicine Binding Site. Purple arrows head from the donor to the acceptor of hydrogen bonds.

### *Drug Likeness and Toxicity Prediction*

Physicochemical, pharmacokinetic and toxicity parameters of the most promising CBSI, S1 and S2 were predicted using ADMETLab. The same properties of Colchicine were also predicted for comparison. As shown in Table 3, compounds S1 and S2 are predicted to have a higher BBB penetration than that of colchicine. In addition, they have a high CACO-2 cell permeability and intestinal absorption, which allows them to reach the bloodstream. With no Lipinski and Veber's rule violation, both S1 and S2 follow the criteria for orally available drugs. Furthermore, these two promising compounds did not show potential toxicity, which guarantees their use in vivo. However, these two compounds inhibit same CYP which are important for the metabolism of numerous drugs in the liver. It should be noted that this problem can be resolved during their optimization.

Table 3. Drug likeness prediction and toxicity of S1, S2 and Colchicine.

| Properties | Colchicine | S1 | S2 |
|---|---|---|---|
| BBB permeability | Suitable average | Suitable | Suitable |
| GI absorption | High | High | High |
| CYP inhibition | None | CYP1A2 | CYP3A4, CYP2D6, CYP1A2 |
| CACO-2 | -4.712 | -5.110 | -5.023 |
| Lipinski | Suitable | Suitable | Suitable |
| Veber | Suitable | Suitable | Suitable |
| Toxicity | None | None | None |

## Conclusion

In brief, molecular docking approach using Glide was employed to predict the binding energies and the interaction modes of 1150 compounds from the PubChem database. After the validation of docking protocol, compounds S1 and S2 were identified as new potent CBSI. Still more remarkably, these two compounds were predicted to have good drug likeness and toxicity properties indicating that they might be promising lead candidates for further anticancer drug discovery.

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## Conflicts of interest

The authors state that did not have conflict of interests

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