In silico Study of Selected Molecules of Sea Cucumber as Antimitotic Using PyRx-Vina Program

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Abstract

Active ligands usually have low molecular weights (MW). However, paclitaxel, a natural anticancer, has high MW. Some compounds (MW = 750-1000 dalton) in sea cucumber from TCMSP database have anticancer activity. The objective of study is to obtain docking profile of selected compounds compared to taxol as native. Three of human kinesin-8 were collected from the RCSB database and regenerated by adding hydrogen atoms and charge using the Dock Prep in Chimera. These proteins and selected compounds were prepared to fulfil PyRx's requirement. Molecular docking was performed based on mass center value and grid-box volume from previous studies and resulted vina-score (kcal/mol). Docking data and their 3D conformation were analyzed using PyMOL, PoseView, and PLIP. The native alignment results between docked and original conformation showed that their RMSD value is less than one and only one that has the same three-dimensional conformation. Holothurinoside D, desholothurin B, and 2,4-dehydroechinoside B have a binding affinity of -9.7, -9.4, and -9.3 kcal/mol, respectively. Their values are better or at least the same as the native (-9.3 kcal/mol). Hydrophobic interactions between proteins and ligands occurred at residue of F272, V23, and P360. These results confirm that the anticancer mechanism of these compounds may be through inhibition of kinesin-8.

Keywords: antimitotic, in silico, PyRx-vina, sea cucumber.

Kajian in silico Senyawa Terpilih Dari Sea Cucumber Sebagai Agen Antimitotik Menggunakan Program PyRx-Vina

Abstrak


Kata Kunci: antimitotik, in silico, sea cucumber, PyRx-vina.
1. **Introduction**

An online portal for the largest database of compounds on earth, ZINC has managed to record more than 1 billion compounds that can be used as a resource for drug discovery. Unfortunately, the number of compounds with MW of more than 500 daltons is only recorded around 0.13%.\(^1\) This is based on the rules of Lipinski. Rule of five, in another name, has given the limitation that a ligand can have drug-like properties and ability in absorption, distribution, metabolism and excretion (ADME) if the Lipinski rule is enforced.\(^2\) Paclitaxel and other eleven natural compounds from 19 natural compounds that have been proven as an anticancer have MW out of range of the Lipinski rule.\(^3\)

Some compounds from sea cucumber have been studied to have anticancer abilities for the MW range between 750-1000 daltons, especially the triterpene glycoside group.\(^4\) The TCMSP database shows that there is 11% MW of compounds in sea cucumber that meets Lipinski rule. Some types of glycosides that can be attached to the core compound make natural compounds have a large MW.\(^5\) In silico model, large MW compounds have proven to be difficult in the virtual screening for drugs from our previous studies.\(^6\) Taxol, for example, has a large volume binding site pocket (around 796 Å)\(^37\) and produces 400 conformers from 64 isomers.\(^8\) The target protein with binding site pocket of taxol, did not provide precise results if this pocket will be applied for molecular docking of small molecular weight compounds, its value is less than that of taxol.\(^6\)

Previous molecular screening with PLANTS and DOCK6 obtained the different results of validation and molecular binding affinity profiles for the same group of selected test compounds.\(^6,9,10\) The difference in grid shape is one of the factors besides the type of calculation algorithm used.

Kinesin-8 is an ATP-dependent motor of microtubule (MT) that affects multiple roles in mitosis. Ligands that can inhibit mitotic kinesin-8 will interfere cell division and have benefits as an antimitotic agent. Recent study shows that BTB-1 or 4-chloranyl-2-nitro-1-(phenylsulfonyl) benzene can inhibit function of kinesin-8 which taxol was bound to it as an MT-binding agent. This inhibitor will increase the antimitotic effect of the kinesin-MT complex which also blocks activity of other motors in the MTs’ spindle.\(^11\) In addition, the target protein with taxol as an active ligand bound to microtubule is not extensively acquired in the RCSB database. The 5ogc is one of these protein that recommended for use by both of program.\(^6,9,10\) The research’s objective is to gain docking scoring and interaction of protein-ligands profiles from selected compounds using PyRx-vina.

2. **Methods**

2.1. **Instrument and Computational Software**

The study was completed using customized personal computer with Ubuntu 14.04 as its operating system. It has Intel® 4\(^{th}\)-generation i5-4460 as processor with 16 GB RAM. All steps were accomplished using Chimera 1.12, Marvin Sketch 16.5.16, Openbabel 2.4.1, PyRx 0.9.8, PLIP 1.4.2, PyMOL 2.1.116. It also used OSRA 2.1.0 and PoseView online version.

2.2. **Collection and Preparation**

Tested compounds, native molecules, and target protein used result of previous research. They have been downloaded from TCMSP and RCSB database. Each target proteins’ ligand (referred to as native) was separated and prepared by hydrogen atoms addition and charge adjustment using Chimera tools: Dock Prep. Target ligands was treated properly to get PyRx compatible files.

2.3. **System Validation**

Docking of native ligands is carried out before the test ligand docking. Details on how to do this can be seen in the next molecular docking subtitle. The results obtained were used to ensure that molecular screening remained within the binding site pocket and approached the native ligand conformation. The docking system testing phase used the center of mass and selected grid-box dimensions from previous studies as presented in Table 1.\(^6,7,8\) The valid system is
marked with all atoms from docked and native molecules located in the binding site pocket with conformational spaces that resemble. Both molecules are aligned using PyMOL and an RMSD value was obtained, but this value did not describe the real conformation of them.

2.4. Molecular Docking
First, native ligand and target protein was loaded to PyRx workspace window. They were transformed to Autodock Ligands or Macromolecules using right click of mouse on target name of file and selected AutoDock then Make Ligand or Make Macromolecule. Target ligands was translated to PyRx’s Autodock ligand (pdbqt) file using openbabel tab which has been provided by software it selves. Target ligand file was opened and selected one of ligand’s names on table. By clicking the right mouse and selecting Convert All to Autodock Ligand (pdbqt).

Vina was chosen as docking algorithm. PyRx version 0.9.8 is offered normal or reverse or combine docking mode. Molecular docking was finished by following the wizard’s stage.

2.5. Data Analysis
Vina score data presented as kcal/mol were compared between result of target and native ligands. Three-dimensional conformation of docking results in pdbqt format were merged, analyzed, dan visualized using PyMOL. Profiles of protein ligand interaction were achieved using PoseView and PLIP program.

3. Results
3.1. Validation results
The docking results of all natively to all target proteins are shown in Figure 1. Their

Table 1. Center of mass and grid-box size (Å) of target protein for docking.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5oam</th>
<th>5ocu</th>
<th>5ogc</th>
</tr>
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<tbody>
<tr>
<td>Center of mass</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>204.520</td>
<td>204.384</td>
<td>204.779</td>
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<tr>
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<td>303.864</td>
<td>304.327</td>
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<tr>
<td>z</td>
<td>265.100</td>
<td>266.224</td>
<td>266.019</td>
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<tr>
<td>Grid-Box size (Å)</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>28.955</td>
<td>27.892</td>
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<tr>
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</tr>
<tr>
<td>Z</td>
<td>33.397</td>
<td>32.650</td>
<td>32.871</td>
</tr>
</tbody>
</table>

Figure 1 Docked ligand of native compared to its original view with their vina score and RMSD (in parentheses).
conformations were distinguished by their different color. The green and cyan molecules were native ligand in original and docked form, respectively. Conversely, other native ligands were displayed in pink and yellow.

3.2. Docking Score
Combination of column and line chart were adopted to present docking results of target compounds (Figure 2). The forty-five molecules were shown their vina score (kcal/mol) and differentiated by color in column graph. In other side, native’s binding affinity were represented in line graph. These scores were only showed for each protein’s native ligand.

3.3. Interaction Profile of Protein Ligand
Protein ligand interaction profiling was done by running Protein Ligand Interaction Profiler (PLIP) and PoseView (PV) program against best of three of target molecules which their binding affinity values were lower than 5ogc6’s native (Figure 3). These bests of molecules are holothurinoside D, desholothurin B, and 2,4-dehydroechinoside B versus each of docked and native of protein. The interaction types discovered are hydrogen bonding (HB), hydrophobic interaction (HI), phi-stack (PS), and salt bridge. There are 20, 20, 1, and 3 residue that exhibited protein ligand interaction of HB, HI, PS, and SB, respectively.

4. Discussion
The results of validating the docking system using PyRx-vina reinforce previous findings that antimitotic target proteins, human kinesin-8 are more represented by 5ogc6. In addition to the proximity of the 3D-conformation from docked ligand to its native, the binding affinity value of docked ligand is better than the other two target proteins. The RMSD value obtained from the results of comparing the conformations of the two molecules can also be used to assess the closeness of their conformation. Unfortunately, PyRx-vina did not calculate RMSD value like DOCK6 did, but PyRx-vina did validation better than PLANTS,6,8

The percentage of molecules with a vina score is lower and better than native's affinity score for all three proteins; 5oam, 5ocu, and 5ogc are 75.7, 67.6, 8.1%, respectively. These results provide the same profile as the results of docking using PLANTS and DOCK6. Protein of 5oam and 5ocu are not the first choice for human kinesin-8 target proteins. The docking results for both proteins can be biased and did not approach the actual value to be achieved. In contrast, for three molecules, holothurinoside D (-9.7 kcal/mol), desholothurin B (-9.4 kcal/mol), and 2,4-dehydroechinoside B (-9.3 kcal/mol) have binding affinity value are slightly better or equal to when compared to binding affinity of 5ogc’s native (-9.3 kcal/mol). These
compounds’ group have been studied and expressed to have anticancer potential.\textsuperscript{4}

Hydrophobic interactions between all proteins and three ligands appeared at residue of F272, V23, and P360 (Figure 3). Other interactions were not discovered in all proteins. These results confirm that the anticancer mechanism of these compounds may be through inhibition of kinesin-8 which regulates the role of microtubules.

5. Conclusion

Holothurinoside D, desholothurin B, and 2,4-dehydroechinoside B have a binding affinity of -9.7, -9.4, and -9.3 kcal/mol, respectively. Their value is better or at least the same as the native (-9.3 kcal/mol). Hydrophobic interactions between proteins and ligands occurred at residue of F272, V23, and P360. These results confirm that the anticancer mechanism of these compounds may be through inhibition of kinesin-8.

References


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