UNPAD

Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/

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

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Submitted 15 January 2019; Revised 14 February 2019; Accepted 27 February 2019; Published 30 April 2019
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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

Ligan aktif cenderung memiliki bobot molekul (BM) rendah. Paclitaxel adalah antikanker alami dengan BM besar. Beberapa senyawa (BM = 750-1000 dalton) dari database TCMSP dalam Sea cucumber telah terbukti sebagai antikanker. Studi ditujukan untuk mendapatkan profil docking senyawa terpilih dibandingkan taxol sebagai native. Protein target, human kinesin-8 diunduh dari database RCSB yang telah dipreparasi dengan penambahan atom hidrogen dan muatan menggunakan Dock Prep dalam program Chimera. Berkas protein hasil dan senyawa terpilih diperlakukan sesuai permintaan PyRx. Vina-score (kkal/mol) telah diperoleh berdasarkan nilai pusat massa dan volume grid-box dari penelitian sebelumnya. Data docking dan konformasi tiga dimensinya telah dianalisis dengan PyMOL, PoseView, dan PLIP. Nilai RMSD penjajaran native hasil docking adalah kurang dari satu dan hanya satu yang memiliki kesamaan konformasi ruang terhadap posisi awalnya. Secara berurutan, holothurinosida D, desholothurin B, dan 2,4-dehidroechinosida B didapatkan afinitas ikatan -9,7; -9.4; dan -9,3 kkal/mol. Hasil ini memperlihatkan nilai yang lebih baik atau sama dengan native (-9,3 kkal/mol). Interaksi hidrofobik terjadi pada residu F272, V23, dan P360. Penelitian ini membuktikan bahwa mekanisme antikanker ketiga senyawa dapat melalui penghambatan kinesin-8.
Kata Kunci: antimitotic, 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® 4th-generation i5-4460 as processor with 16 GB RAM. All steps were accomplished using Chimera 1.12[12], Marvin Sketch 16.5.16[13], Openbabel 2.4.1, PyRx 0.9.8[14], PLIP 1.4.2[15], PyMOL 2.1.1[16]. It also used OSRA 2.1.0[17] and PoseView[18] 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[19] 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

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

Parameter	5oam	5ocu	5ogc
Center of mass			
x	204.520	204.384	204.779
y	303.864	304.327	303.668
z	265.100	266.224	266.019
Grid-Box size (Å)			
X	28.955	27.892	30.928
Y	35.534	35.484	38.543
Z	33.397	32.650	32.871

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

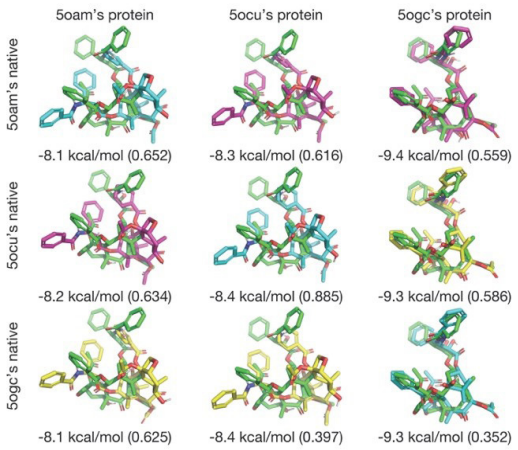

Figure 1 Docked ligand of native compared to its original view with their vina score and RMSD (in parentheses).

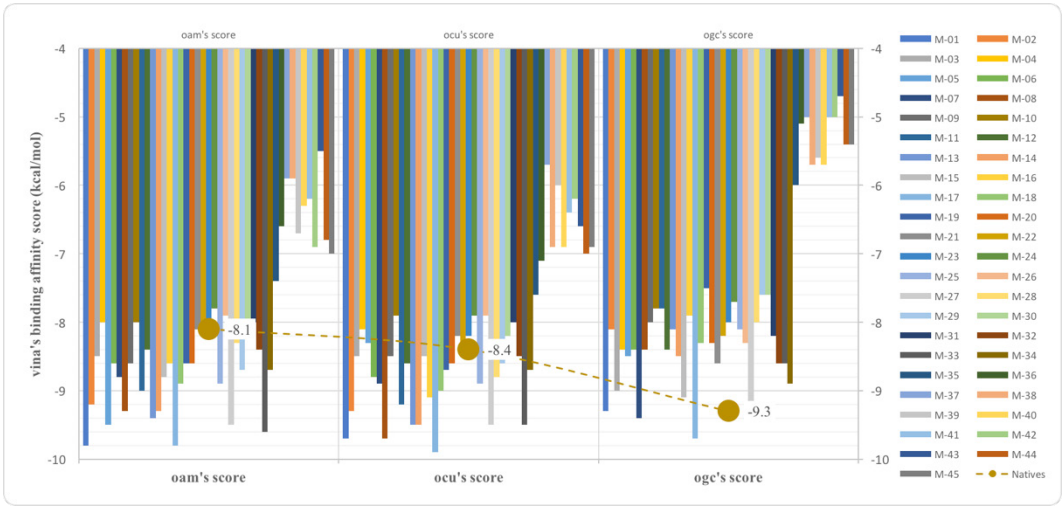

Figure 2 .Vina score of molecular docking results of target molecules and native (different chart type).

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 5ogc'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

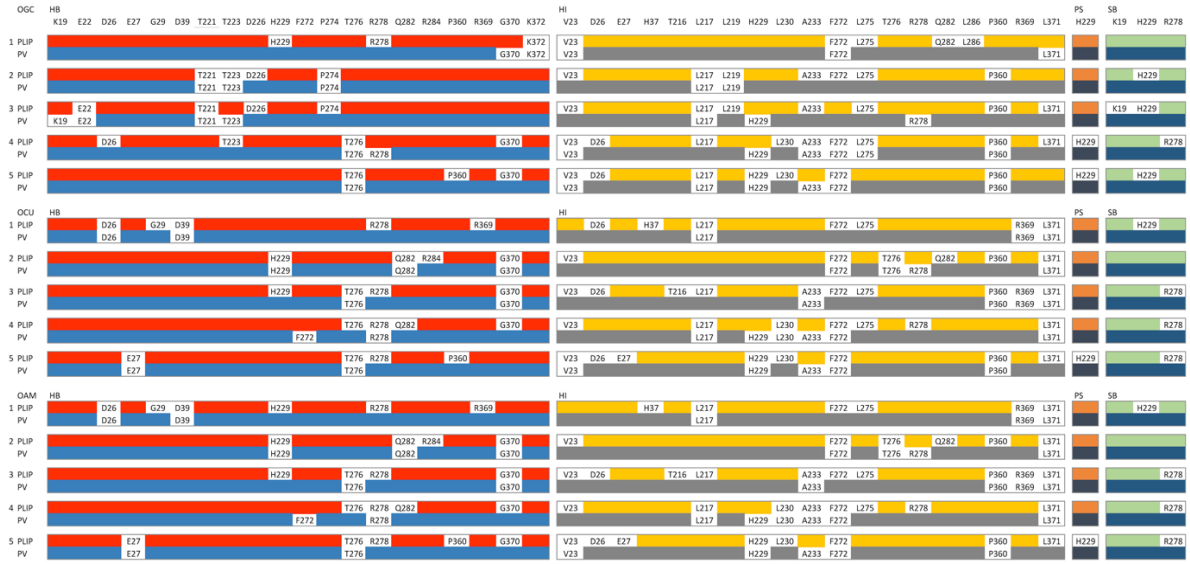

Figure 3. Compilation profile of protein residue to ligand in four different interaction types: hydrogen bonding (HB, red-blue), hydrophobic interaction (HI, yellow-grey), phi-stack (PS, orange-black), and salt bridge (SB, light-dark green). Interaction profiles of protein-ligand were generated using PLIP and PoseView (PV) against holothurinoside D (1), desholothurin B (2), and 2,4-dehydroechinoside B (3), docked (4) and native (5) ligand of protein.

compounds' group have been studied and expressed to have anticancer potential.[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

1. Aminin DL, Menchinskaya ES, Pisliagin EA, Silchenko AS, Avilov SA, Kalinin VI. Anticancer Activity of Sea Cucumber Triterpene Glycosides. Marine Drugs. 2015; 13(3): pp 1202–1223. http://doi.org/10.3390/md13031202.
2. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Research. 2000; 28: pp 235-242.
3. Filippov IV, Nicklaus MC. Optical Structure Recognition Software to Recover Chemical Information: OSRA — An Open Source Solution. J. Chemical Information and Modeling. 2009; 49(3): pp 740–743. doi: 10.1021/ci800067r.
4. Fricker P, Gastreich M, Rarey M. (2004) Automated Generation of Structural Molecular Formulas under Constraints. J Chemical Information and Computer Sciences. 2004; 44: pp 1065-1078.
5. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies. 2014; 1(4): pp 337–341. doi:10.1016/j.ddtec.2004.11.007.
6. Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 16.5.16, 2016, ChemAxon (http://www.

chemaxon.com).
7. Moraes DFC, de Mesquita LSS, do Amaral FMM, de Sousa Ribeiro MN, Malik S. Anticancer Drugs from Plants. In: Malik S. (eds) Biotechnology and Production of Anti-Cancer Compounds. Springer, Cham; 2017.
8. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem. 2004; 25(13): pp 1605-12.
9. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminformatics. 2014; 6(1): pp 13.
10. Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M. PLIP: fully automated protein-ligand interaction profiler. Nucl Acids Res. 2015; 43 (W1): W443-W447. doi: 10.1093/nar/gkv315
11. Santoso B. In silico Cytotoxicity of Taxol-like Weighted Compounds Contained in Sea cucumber using PLANTS against Protein of Human Kinesin-8. Have been presented at International Conference on Applied Science and Engineering in Syariah Hotel Solo, Universitas Veteran Bangun Nusantara; October 6-7th, 2018b and published at Proceedings of the International Conference on Applied Science and Engineering (ICASE 2018); pp 14-16. doi:10.2991/icase-18.2018.4
12. Santoso B. Validasi Ruang Komputasi Senyawa Native Tidak Memenuhi Aturan Lipinski Menggunakan PLANTS, have been presented at the 8th University Research Colloquium, Universitas Muhammadiyah Purwokerto; September 4th, 2018a.
13. Santoso B. Virtual Screening of Selected Sea Cucumber Compounds as Inhibitor Human Kinesin-8, will be presented at 1st International Conference on Innovative Sciences and Technologies for Research and Education, Universiti Tekhnologi Mara, Kuching, Serawak; December 18-20th, 2018c.
14. Sterling T, Irwin JJ. ZINC 15 – Ligand Discovery for Everyone. J Chem Inf Model. 2015; 55 (11), pp 2324–2337. http://pubs.acs.org/doi/abs/10.1021/acs.jcim.5b00559.
15. Stierand K, Maaß P, Rarey M. Molecular Complexes at a Glance: Automated Generation of two-dimensional Complex Diagrams. Bioinformatics. 2006; 22: pp 1710-1716.