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Original Article

In Silico Analysis of Sea Cucumber Bioactive Compounds as Anti-Breast Cancer Mechanism Using AutoDock Vina

S. Yasman[a,c*], Arry Yanuar[b], Zeta Tamimi[b], S. Rezi Riadhi[b]

[a]Department of Biology, FMIPA Universitas Indonesia, Kampus UI Depok, 16424, Indonesia, [b]Faculty of Pharmacy, FMIPA Universitas Indonesia, Kampus UI Depok, 16424, Indonesia, [c]Research Cluster of Bioprospecting for Sustaining Nature, Department of Biology, FMIPA Universitas Indonesia, Kampus UI Depok, 16424, Indonesia

Abstract

In recent years, the potential of marine natural products as anticancer agents, specifically for breast cancer, has been examined. The sea cucumber (Holothuroidea: Echinodermata) is known to contain triterpene glycosides, which have shown anticancer or cytotoxic activity. In this research, molecular docking of selected sea cucumber bioactive compounds was conducted on five receptor targets that play an important role in breast cancer: estrogen receptor alpha (ER-α), fibroblast growth factor receptor 1 (FGFR1), vascular endothelial growth factor receptor 2 (VEGFR2), progesterone receptor (PR), and insulin-like growth factor 1 receptor (IGFR1). The purpose of this was to observe the interaction between active compounds and the active site of breast cancer receptor targets. Holothurin A gave the lowest binding energy (-7.1 kcal/mol) and was involved in a hydrogen bond with amino acid His-516 when superposition towards to E4D cocrystal was present. Holothurin A also had a similar posing with raloxifene, in which the hydrogen bond with His-516 with a RMSD value of 3.3 Å was observed with superposition towards to the positive control raloxifene. The analysis and visualization results of 24-dehidroechinoside that was superposed on E4D cocrystal, BMI cocrystal, and positive control raloxifene showed that 24-dehidroechinoside had a hydrophobic interaction with amino-acid residue Leu-346, a strong hydrogen bond to Gln-977, as well as a hydrogen bond to Thr-347 in a distance of 3.7 Å, and a hydrophobic interaction with amino-acid residue Ala-350. The most potent in silico anti-breast cancer compounds in sea cucumbers are holothurin A and 24-dehidroechinoside. Holothurin A is active as an anti-breast cancer agent by inhibiting ER-α, while 24-dehidroechinoside inhibits both ER-α and IGFR1.

Keywords: AutoDock Vina, estrogen receptor alpha, 24-dehidroechinoside, fibroblast growth factor receptor 1, holothurin A, sea cucumber.

Corresponding Authors: S. Yasman, Department of Biology, FMIPA Universitas Indonesia, Kampus UI Depok, 16424, Indonesia; Research Cluster of Bioprospecting for Sustaining Nature, Department of Biology, FMIPA Universitas Indonesia, Kampus UI Depok, 16424, Indonesia
Tel: +62217270163
E-Mail: yasman.si@sci.ui.ac.id
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1. Introduction

The sea cucumber (Holothuroidea: Echinodermata) is a marine invertebrate that provides a source of secondary metabolites with interesting bioactivities [1]. Sea cucumbers are known to contain triterpene glycoside compounds, such as frondoside A, okhotosides B1-B3, intercedensides A-C, holothurin A, and 24-dehydroechinoside. These molecules have been reported to show anticancer or cytotoxic activity [2-5]. In recent years, much attention has been given to examining the in silico potential of sea cucumber active compounds as an anticancer agent [6]. It is known that breast cancer has receptor targets, such as estrogen receptor alpha (ER-α) [7], fibroblast growth factor receptor 1 (FGFR1) [8], vascular endothelial growth factor receptor 2 (VEGFR2) [9], progesterone receptor (PR) [10], and insulin-like growth factor receptor 1 (IGFR1) [11]. The interaction between active compounds and the active site of breast cancer receptor targets can be simulated using the molecular docking method. Optimization of molecular docking analysis can be performed using several methods to get reliable results. Parameter optimization was performed using cocrystal redocking or ligand contained in the crystal macromolecules' structure, as obtained from the protein databank (PDB).

In this research, molecular docking was done by using AutoDock and Vina. Both software programs are the most widely used and easily accessible for the purpose of molecular docking [12]. This in silico research is expected to obtain the optimum method for molecular docking on any related breast cancer enzymes. Furthermore, it could determine the affinity of each sea cucumber compound and analyze the binding mechanism with related macromolecules.

2. Materials and Methods

2.1. Preparation of 3D Structure of Selected Sea Cucumber Compounds

The 2D structure of sea cucumber compounds could be found in the PubChem database. First, the 2D structures of sea cucumber compounds were drawn using the Marvin Sketch program to then generate the 3D structure. The transformation from 2D to 3D was completed using the mmff94 force field and select strict optimization limit. The results were saved in .pdb format. In addition, the 3D structure was rechecked by visualization with the PyMOL program.

2.2. Preparation of Docking Target Macromolecules

Docking target macromolecules were downloaded from the Protein Data Bank (PDB) website (www.rscb.org/pdb) and saved

in .pdbqt format. The macromolecules used in this research are five receptor targets for breast cancer, which include:

- 3C4F (*Fibroblast Growth Factor Receptor I*)
- 2P2I (*Vascular Endothelial Growth Factor Receptor 2)*
- 2OJ9 (*Insulin-Like Growth Factor Receptor I*)
- 1SJ0 (*Estrogen Receptor α*)
- 3KBA (*Progesterone Receptor*)

The macromolecules were sepa Marated from solvents and other nonstandard residues using AutoDock and then saved in .pdb format. The separated macromolecules were then prepared for the docking process.

2.3. Macromolecule Optimization

The 3D structures of the macromolecules were optimized using AutoDock. The optimization includes removing water molecules, adding hydrogen atoms, and repairing the charges by adding a Gasteiger charge, followed by a minimization process. The results were saved as .pdb format.

2.4. Docking Method Validation

The validation of molecular docking was done by redocking the cocrystal. The docking parameter had to be varied in order to generate the lowest free binding energy, a more homogenous cluster distribution, and a lower RMSD value (<2 Å) This validation was done using AutoDock and Vina parameters assisted with PyRx software.

2.5. Sea Cucumber Compounds Docking Towards the Macromolecules Target

The target that was used for this docking method was the positive control (ligand). There are three types of positive ligands that were used in this study; the cocrystal ligand compound from the crystal structure, th cocrystal drawn with Marvin Sketch, and the positive controls that have been recommended by FDA for the treatment of breast cancer (dovinitib (FGFR1 inhibitors); bevacizumab (VEGFR2 inhibitors); anastrazole, tamoxifen, toremifen, fulvestrant, letrozole, raloxifene, everolimus, and exemestane (ER-α inhibitors); onapristone and mifepristone (PR inhibitors); and cixutumumab and figutumumab (IGFR1 inhibitors)).

2.6. Analysis and Visualization of the Protein-Ligand Interaction

The protein-ligand visualization was processed using PyMOL software and LigPlot. The docking results from the AutoDock and Vina parameters must be in .pdb form in order to be visualized and analyzed in PyMOL. The purpose of this visualization was to find the interaction between the ligand and amino-acid residues on macromolecules.

3. Results and Discussion

3.1. AutoDock and Vina Validation Docking Method

Based on the validation results of the cocrystal created with Marvin Sketch and the cocrystal from the crystal structure using the AutoDock parameter, all target receptors except VEGFR2 presented a low binding

energy and the docked ligand to satisfy the RMSD value (< 2.0 Å) In this research we used ER-α and IGFR1 as a docking target based on the lowest RMSD value and the availability of positive controls. Among the targets, the ER- α cocrystal showed the highest binding energy with -13.31 kcal/mol and a 0.81 Å RMSD value, while the Vina binding energy was -11.7 kcal/mol with a 0.427 Å RMSD value (Table 1 and 2).

Validation results of the cocrystal drawn with Marvin Sketch shows a binding energy and RMSD value close to that of the cocrystal obtained from th crystal structure. This showed thah the Marvin Sketch- drawn cocrystal could be a good parameter for seeking lead compounds in future drug development.

The docking results for positive control FDA shows that raloxifene's binding energy using Autodock and Vina was -13.82 kcal/mol and -10.9 kcal/mol, respectively. Other controls show binding energy varying from -2.49 to -11.68 kcal/mol.

Table 1. Validation docking results for ER-α.

Macro-molecule	**Ligand**	**Autodock**				**Vina**			
		Grid box	Binding energy (ΔG)	Inhibitory Constant (Ki)	RMSD (Å)	Grid box	Binding energy (ΔG)	Inhibitory Constant (Ki)	RMSD (Å)
ER-α	Cocrystal (E4D)	60x60x60	-13.31	174.56 pM	0.81	22.5	-11.70	2.47 nM	0.42
	Cocrystal (Marvin Sketch)	60x60x60	-14.05	50.53 pM	1.13	22.5	-11.05	7.44 nM	0.71
	Anastrazole	60x60x60	8.26	8.40 uM		22.5	-8.30	7.84 uM	
	Everolimus		-2.49	14.70 mM			-4.10	9.64 mM	
	Exemestane		-10.10	3.72 nM			-10.40	2.24 nM	
	Fulvestrant		-11.68	2.56 nM			-10.10	3.72 nM	
	Letrozole		-8.62	4.56 uM			-8.70	3.98 uM	
	Raloxifene		-13.82	6.80 pM			-10.90	9.59 nM	
	Tamoxifen		-10.19	3.19 nM			-9.60	8.68 nM	
	Toremifen		-10.16	3.36 nM			-9.30	1.44 uM	

Table 2. Validation docking results for IGFR1.

Macro-molecule	**Ligand**	**Autodock**				**Vina**			
		Grid box	Binding energy (ΔG)	Inhibitory Constant (Ki)	RMSD (Å)	Grid box	Binding energy (ΔG)	Inhibitory Constant (Ki)	RMSD (Å)
IGFR1	Cocrystal (BMI)	50x50x50	-8.74	391.62 nM	1.40	18.8	-9.50	1.02 uM	1.37
	Cocrystal (Marvin Sketch)	50x50x50	-8.80	353.71 nM	2.42	18.8	-9.50	1.02 uM	1.18

3.2. Docking Results for Sea Cucumber Bioactive Compounds

Based on the docking results in both parameters, AutoDock could not obtain binding energy low enough for sea cucumber bioactive compounds to affect the receptor target. It is suggested that AutoDock could not run the program with a high-torsion structure because the maximum torsion allowed in AutoDock is 32. Therefore, for the purposes of this research, the binding energy is obtained solely from Vina.

The macromolecule-ligand binding that provided the lowest energy in Vina were ER-α and IGFR1. ER-α and IGFR1 exhibited the lowest binding energy with frondoside A (-5.8 kcal/mol) and holothurin A (-7.1 kcal/mol). Therefore, both ligand interactions with both amino-acid residues were analyzed and visualized.

3.3. Visualization of Sea Cucumber Active Compound Interactions with ER-α and IGFR1

Based on binding energy and an analysis of superposition towards E4D cocrystal, compounds that were considered anti-breast cancer were 24-dehidroechinoside, holothurin A, intercedenside A and B, and okhotoside B1 and B2. Amino acids involved in the hydrogen bond as the hydrogen bond donor are His-516 (holothurin A, okhotoside B1) and Arg-394 (intercedenside B); both give weak hydrogen bond (Figure 1: A, B, C). As for 24-dehidroechinoside, okhotoside B2, and intercedenside A, the visualization only shows a hydrophobic interaction involving amino-acid residues Leu-346 (24-dehidroechinoside, okhotoside B2) and Met-343 (intercedenside A) (Figure 1: D, E, F).

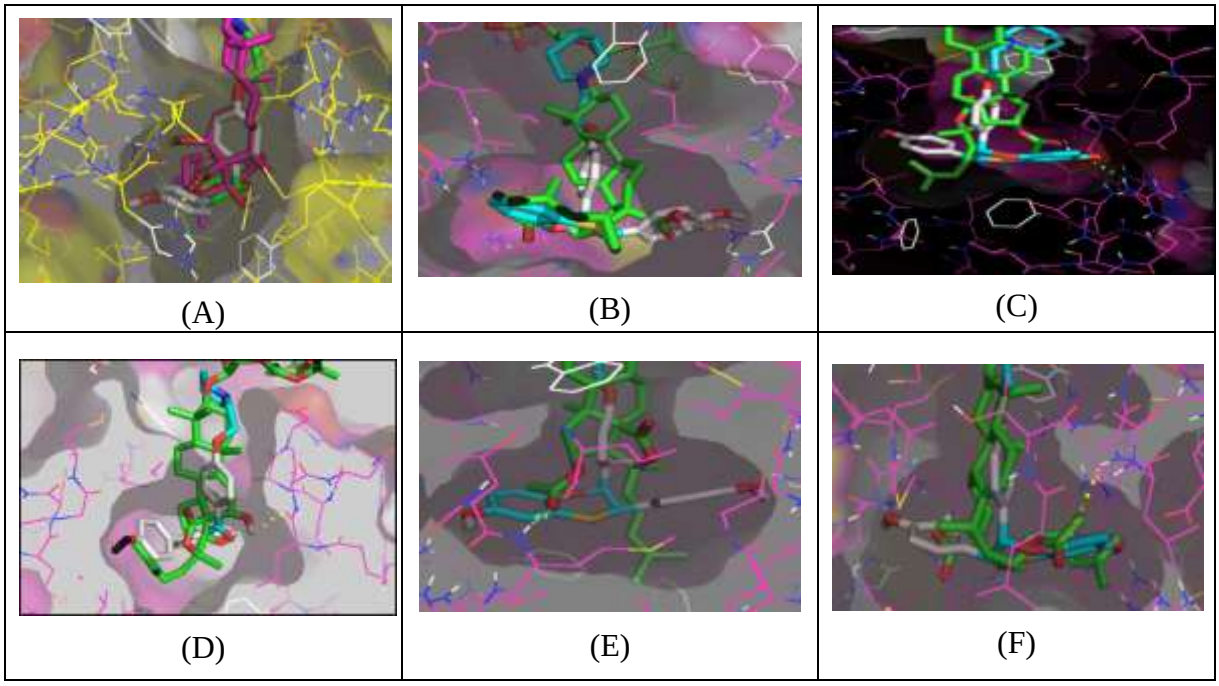

Figure 1. Visualization results of compond superposition between active compounds: (A) holothurin A; (B) okhotoside B1; (C) intercedenside B; (D) 24-dehidroechinoside; (E) intercedenside A; and (F) okhotoside B2 and E4D cocrystal with ER-α.

The analysis and visualization results of sea cucumber compounds that were superposed to positive-control raloxifene showed that compounds with posing similar to raloxifene were 24-dehidroechinoside, holothurin A, and okhotoside B1 and B2. The results show that the posing mainly due to their aromatic ring property. A hydrogen bond was observed between amino-acid residue His-516 with okhotoside B1 and B2, as well as with holothurin A with a RMSD value of 3.6 Å, 4.1 Å, and 3.3 Å (Figure 2: A, B, C). In addition, 24-dehidroechinoside showed a hydrogen bond with Thr-347 with a distance of 3.7 Å, as well as a hydrophobic interaction with amino-acid residue Ala-350 (Figure 2: D).

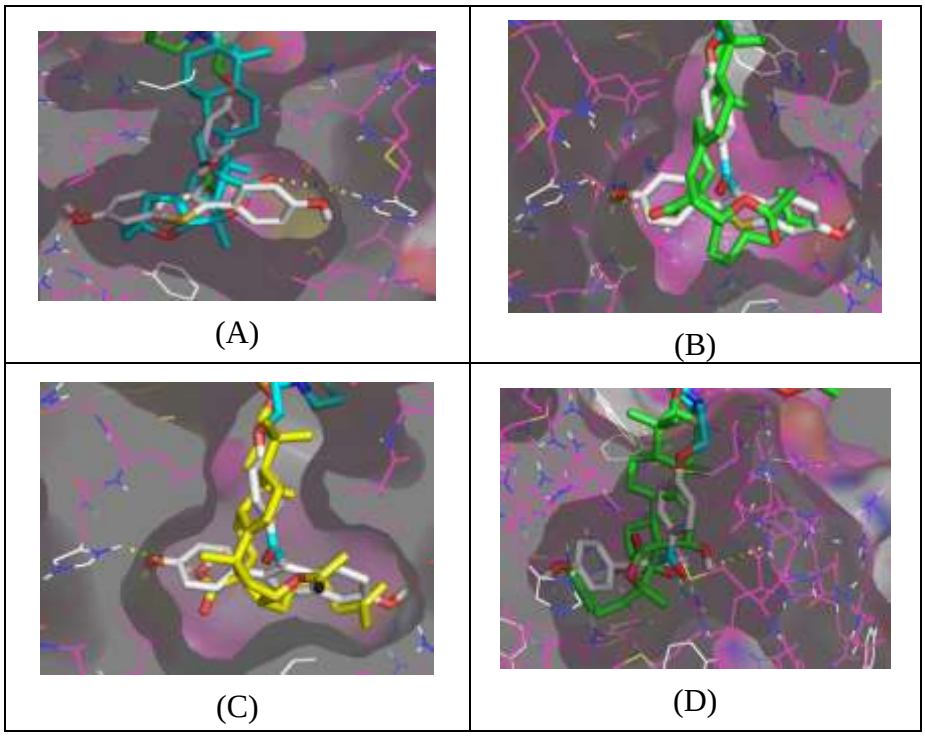

Figure 2. Visualization results of compound superposition between active compounds: (A) okhotoside B1; (B) okhotoside B2; (C) holothurin A; and (D) 24-dehidroechinoside and raloxifene with ER-α.

The analysis and visualization superposition between sea cucumber active compounds and BMI cocrystal with IGFR1 showed that the only compound which contains potent anti-breast cancer properties is 24-dehidroechinoside. The amino-acid residue involved in a strong hydrogen bond is Gln-977 with a RMSD value of 2.1 Å (Figure 3).

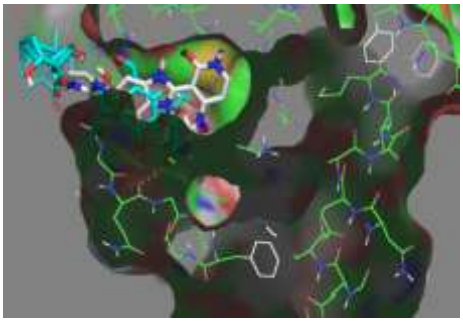

Figure 3. Visualization of superposition between 24-dehidroechinoside (blue) and BMI cocrystal (white) with IGFR1 to show the hydrogen bond in Gln-977.

3.4. Miscellaneous Discussion

In silico examination of sea cucumber cucumariaosides in prior research has shown potent anticancer activity in inhibiting human DNA topoisomerase II alpha [6]. It is of interest that holothurin A, which was shown to be active in inhibiting human DNA topoisomerase II alpha [6], was also found in the present research to be an active anti-breast cancer agent by inhibiting ER-α. Holothurin A had the lowest binding energy (-7.1 kcal/mol) of all the compounds selected for this research, and was involved in a hydrogen bond with amino acid His-516 when superposed to E4D cocrystal. Holothurin A also had a similar posing to raloxifene, in which a hydrogen bond with His-516 at a distance of 3.3 Å was observed while superposed towards it.

This research also found that 24-dehidroechinoside has an increased potential to act as anti-breast cancer agent by inhibiting both ER-α and IGFR1. The ER-α has proven to be the single most important target in breast cancer prevention [7], while the disruption of IGFR1 signaling has been shown to be promising in breast cancer treatment [11]. In comparison with holothurin A, 24-dehidroechinoside has also been reported to be more potent in preventing metastases [13]. Superposition between 24-dehidroechinoside and E4D cocrystal with ER-α suggested that 24-dehidroechinoside showed a hydrophobic interaction with amino-acid residue Leu-346. In addition, superposition between 24-dehidroechinoside and raloxifene with ER-α suggested that 24-dehidroechinoside had posing similarity with positive control raloxifene and showed a hydrogen bond with Thr-347 with a distance of 3.7 Å, as well as a hydrophobic interaction with amino-acid residue Ala-350. The analysis and visualization superposition between 24-dehidroechinoside and BMI cocrystal with IGFR1 showed that amino-acid residue Gln-977 with a RMSD value of 2.1 Å was involved in a strong hydrogen bond.

Both holothurin A and 24-dehidroechinoside have been reported to exhibit significant inhibition of metastases in vitro and in vivo [3]. Zhao *et al.* found that holothurin A and 24-dehidroechinoside significantly decreased the expression of MMP-9 by enhancing the expression level of tissue inhibitors in TIMP-1, an important regulator of MMP-9 activation. It is interesting to note that while Zhao et al. confirmed that both holothurin A and 24-dehidroechinoside remarkably abolished the expression of VEGF [13], the present research showed that VEGFR2 was not active, as shown by inadequate binding energy and RMSD value in the Autodock Vina validation process. This may suggest that even though Autodock Vina has improved the speed and accuracy of docking in silico screening [12], it still has limitations in predicting natural products as anticancer agents in several target enzymes.

4. Conclusion

The most potent in silico anti-breast cancer sea cucumber compounds are holothurin A and 24-dehidroechinoside. Holothurin A is active as an anti-breast cancer agent by inhibiting ER-α, while 24-dehidroechinoside was shown to inhibit both ER-α and IGFR1.

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