Computational Study of Trisindoline 5 Against Overexpressed EGFR Protein on Breast Cancer Stem Cell

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Abstract. The current treatment to control the proliferation rate of breast cancer is still not optimal due to the presence of breast cancer stem cells (BCSCs) which are resistant to several chemotherapy agents. Epidermal Growth Factor Receptor (EGFR) may promote the survival of BCSCs. The existing inhibitory drugs used to treat the EGFR that act as the master regulator of the signaling network still have a limited response in breast cancer. Trisindoline is an indole trimer alkaloid natural compound that provide a cytotoxic effect on cancer cells. In 2021, modification of trisindoline has been synthesized into trisindoline 5. This study aims to analyze the interaction between trisindoline 5 and EGFR through in silico. Data retrieval trisindoline 5 using ChemDraw, doxorubicin as positive control from PubChem, EGFR from RCSB database. Docking was done using AutoDock Vina and the results were visualized using Biovia Discovery Studio. The binding affinity of trisindoline 5 is lower than doxorubicin to the EGFR. Trisindoline 5 can inhibit EGFR binding site on some amino acids and forms hydrogen bonds that predicted to be more stable. This research informed that trisindoline 5 might be potential for developing novel therapeutic drug against BCSCs.

1. Introduction

Breast cancer is a deadly disease that presenting major public issue among women worldwide [1]. The current treatment to control the proliferation rate of breast cancer is still not optimal due to the presence of breast cancer stem cells (BCSCs) which are resistant to several chemotherapy agents [2]. BCSCs are subpopulation of cancer cells and associated with self-renewal, stemness, tumor initiation properties and metastasis [3][4]. The previous study reported that the overexpression of Epidermal Growth Factor Receptor (EGFR) is having critical role in survival of BCSCs [5]. EGFR is a receptor of the tyrosine kinase family that causes uncontrolled proliferation, increases tumor sphere formation and induces epithelial-mesenchymal transition (EMT) correlated with aggressive metastasis [4][6].

Given the evidence for the role of EGFR in breast cancer, there was great hope for targeting EGFR that act as the master regulator of signaling network [7]. The commercial chemotherapy agent that is often used for breast cancer is doxorubicin by DNA-adduct formation, reactive oxygen species (ROS) production and topoisomerase II inhibition [8]. Although it may be kill cancer cells, but also result a side effect in kill healthy cells [9]. Thus, developing a new therapeutic natural agents as anti-BCSCs is a challenging project. The utilization of bioactive natural products from marine sponges has a special

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attention. Sponges are widespread in tropical reef and rich source of novel secondary metabolites that are potential for development of new drugs [10].

Trisindoline is an indole trimer alkaloid natural compound that provide a cytotoxic effect on cancer cells [11]. It was first isolated from *Vibrio* sp. symbiosis with sponge *Hyrtios altum* in Okinawa, Japan [12]. The cytotoxic activity of trisindoline have shown in several types of cancer cells, such as uterine sarcoma MES-SA with IC_{50} 3.51 ± 0.03 µM, lung cancer A549 with IC_{50} 18.4 µM, liver cancer HepG2 with IC_{50} 20.4 µM and normal human lung cells MRC-5 with IC_{50} >100 µM [11]. Previous research also showed the synthesis of trisindoline into trisindoline 1, 3 and 4 by adding the nitro group, bromo group and chloro group respectively as anti-cancer candidate. Based on the cytotoxicity result against breast cancer MCF-7, trisindoline 1 and 3 classified as compounds with good activity with lower IC_{50} 2.059 µM and 3.9759 µM respectively compared to trisindoline 4 with IC_{50} 15.46 µM [13]. The activity of trisindoline 1 against BCSCs MDA-MB-231 has been tested. The IC_{50} value was 57.72 µg/ml and the percentage of apoptotic cells was 12.6 ± 0.96 % lower than doxorubicin as a positive control which is 98.2 ± 0.88% [14].

In 2021, the latest modification of trisindoline has been successfully synthesized into trisindoline 5-fluoro-3,3-di((methylindole-5-carboxylate)-3-yl)-2-indolon or trisindoline 5 [15]. It combines the isatin with fluoro group and indole with methyl ester group. Based on the comparison of the group substitution of trisindoline, it was able to increase toxicity in cancer cells and has the highest cytotoxic activity compared to the variation from the other groups [11][15]. However, the potential of trisindoline 5 as EGFR inhibitor in BCSCs is still unrevealed. In this study, we aims to investigate and analyze the interaction between trisindoline 5 and EGFR through bioinformatics approach using molecular docking.

2. Material and Methods

2.1. Ligand and Receptor Preparation

The ligand structure of the trisindoline 5 and trisindoline 1 compound was obtained through ChemDraw Ultra 12.0 as SDF format, the 3D ligand structure of the doxorubicin (CID: 31703) compound as a positive control was obtained through PubChem database (http://pubchem.ncbi.nlm.nih.gov/) as 3D Conformer SDF format, while the structure of the target protein or receptor, EGFR (ID: 3POZ) was obtained from the RSCB PDB database (http://www.rscb.org/pdb/) as PDB format. Receptor preparation was carried out using Biovia Discovery Studio 2021 software to remove the contaminant molecules such as water molecule, hetero atom and ligands. Ligands were prepared using Open Babel in PyRx 0.8 software to minimize the conformation energy. Then files were saved to PDB format.

2.2. Docking of Ligand and Receptor

Docking was done by PyRx 0.8 software which integrated with AutoDock Vina program to predict the possible interaction and binding energy of trisindoline 5-EGFR, trisindoline 1-EGFR and doxorubicin-EGFR that expressed as affinity (kcal/mol). The specific docking method is used by set up the grid box in the ATP binding pocket of EGFR protein by the coordinates (center x= 16.732; center y=33.121; and center z= 12.166) with the size of dimension is 40 x 40 x 40 Å [16]. It aimed to identify of trisindoline 5 compound as inhibitors.

2.3. Validation and Visualization of Docking Results

The validation of the docking results is done by determining the Root Mean Square Deviation (RMSD) value of conformation bearing the lowest docking score. The RMSD value <2 Å represented as the success criteria for the docking method. The visualization was conducted by using Biovia

Discovery Studio 2021 software to analyze the hydrogen and hydrophobic interaction formed between ligand and receptor. A molecular complex is stable if it has low unfavorable interactions [17].

3. Results and Discussion

The result of molecular docking show that trisindoline 5 compound bind to the ATP binding pocket or the active site of EGFR protein. Trisindoline 5 binds to the active site of EGFR with lower binding affinity value (-10.5 kcal/mol) compared than trisindoline 1 (-9.9 kcal/mol) and doxorubicin (-9.4 kcal/mol) with RMSD value 0 (<2 Å) (Table 1). Binding affinity (ΔGbind) can be defined as the strength of interaction between two molecules, which is ligand and the target protein that bind reversibly. It also predicted whether interactions can form between two molecules or not. The lower the binding affinity value, the less energy and easier of the compound to bind with receptor [18][19].

While RMSD values <2 Å are used as a criteria for the success of docking method and considered the docking accuracy [20]. Another study used a higher affinity small molecule EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib that can bind to the intracellular catalytic domain of EGFR, inhibit EGFR autophosphorylation and downstream gene target signaling [21]. Previous research also reported using natural compound carvacrol (-6.2 kcal/mol) and gefitinib (-8.2 kcal/mol) as EGFR inhibitor [22]. Therefore, it indicates that trisindoline 5 has high potential as EGFR inhibitor. It can bind more stable to the protein target with lower binding affinity value than the others.

Table 1. The position and types of chemical interaction between EGFR with the ligands.

Ligands	ΔGbind (kcal/mol)	RMSD (Å)	Chemical interaction		
			Hydrogen Bonds	Hydrophobic Bonds	Electrostatic
Trisindoline 5	-10.5	0	Arg841, Leu718	Leu844, Phe723, Val726	Lys745
Trisindoline 1	-9.9	0	Arg841, Asn842, Thr854, Asp 855, Lys745	Leu718, Ala743, Leu844, Val726	Lys745
Doxorubicin	-9.4	0	Lys745, Asp837	Leu844, Ala743, Leu718, Leu1001, Phe997, Val726	-

_: Same position of interactions.

The visualization of trisindoline 5-EGFR, trisindoline 1-EGFR and doxorubicin-EGFR are shown in Figure 1. Trisindoline 5 interacts with EGFR by forming 2 hydrogen bonds, 7 hydrophobic bonds and 1 electrostatic. Trisindoline 1 interacts with EGFR by forming 5 hydrogen bonds, 12 hydrophobic bonds and 3 electrostatic. While doxorubicin interacts with EGFR by forming 2 hydrogen bonds, 10 hydrophobic bonds and 1 unfavorable acceptor-acceptor. Hydrogen bonds have an important role in both ligand-receptor interaction. If there are more intermolecular hydrogen bonds, their effect on the formation of the complex will be stronger and the docking result would be more accurate [23]. It also facilitates the stability of protein conformation [24]. EGFR have an ATP binding pocket, which is part of the protein kinase where ATP binds to perform its phosphorylation activity. It is located near the C-helix and A-loop structures [25]. Using competitive ATP inhibitor compound is one of the strategy in order to inhibit ATP from binding to this protein. Taken together, these results confirm that the more hydrogen bonds, the more stable the intermolecular interactions.

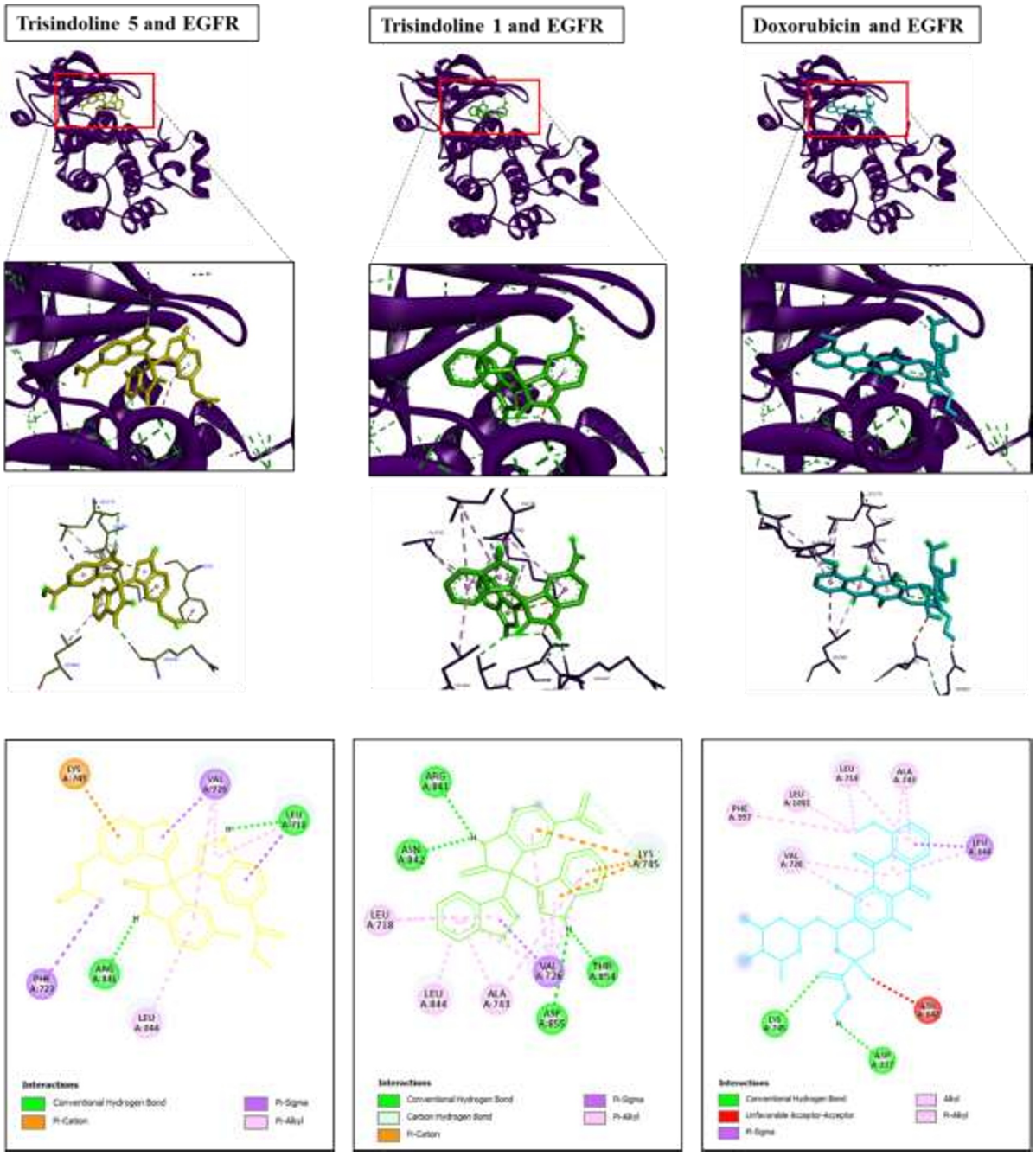

Figure 1. Chemical bonds formed by the interaction between EGFR and the ligands. Trisindoline 5: yellow, trisindoline 1: green and doxorubicin: light blue.

EGFR is a member of the erbB family of receptor tryrosine kinase proteins. It composed of an extracellular ligand-binding domain, a transmembrane lipophilic domain and an intracellular tyrosine kinase domain. Phosphorylation of the tyrosine kinase domain followed by homodimerization or heterodimerization between different receptors of the same family leads to protein activation [26]. The phosphorylation then activates downstream signaling pathways, including the phosphatidylinositol 3-kinase (PI3K)/Akt, the Ras/Raf/mitogen-activated protein kinase (MAPK) (extracellular signal-regulated kinase (ERK) 1/2), the signal transduction and activator of transcription (STAT), c-Jun N-terminal kinase (JNK) and Phospholipase C Gamma (PLCγ) [27]. ERK1 and ERK2 regulate cell

growth and proliferation, whereas PI3K/Akt as well as STAT rather specifically regulate cell survival and apoptosis [26]. The upregulation of EGFR expression may be associated with resistance of doxorubicin [28]. Thus, the survival of BCSCs that promoted by master regulator EGFR is being evaluated.

Previous research report curcumin and phyllanthin exhibit a mechanism of action on several BCSCs-associated genes, including EGFR [4]. Another study also support that treatment using gefitinib as EGFR kinase inhibitor, results in loss of tumorsphere-forming ability [29]. Interestingly, based on molecular docking, trisindoline 5 can inhibit EGFR binding site on some amino acids such as Arg841, Lys745, Val726 and Leu844. Beside, with its lower binding affinity value than others, it is predicted that it will affect the conformation and function of the target protein. It also indicated that the interaction between trisindoline 5 and EGFR could inhibit EGFR activation pathway which impact on inhibited aggressive metastasis. Trisindoline compound had the potential to become anti-cancer drugs because able to trigger the apoptosis process via intrinsic (BH-3 and BAX) and also extrinsic ((Tumor Necrosis Factor receptor 1 (TNFR1), Fas, Death Receptor-5 (DR5)) by multiple signaling pathways [13]. In addition, inhibiting signaling pathway of EGFR resulting in the reduced higher motility and survival of BCSCs [4].

4. Conclusion

Trisindoline 5 might be potential for developing novel therapeutic drug against BCSCs. This compound could bind to the active site of EGFR protein using hydrogen bond with the binding affinity is more negative than trisindoline 1 and doxorubicin, which indicate more stable. However, further studies such as the drug-likeness prediction, molecular dynamics simulation and in vitro assay are necessary to support the results from this study.

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