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In Vitro Test and Molecular Docking of Alkaloid Compound in Marine Sponge *Cinachyrella anomala* against T47D Cell Cycle

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Abstract

The compound 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol (SA2014) was isolated from the marine sponge *Cinachyrella anomala*. *In vitro* assay for SA2014 compound was found to be able to induce cell-cycle arrest at the sub-G_1 and G_2/M phases of T47D cancerous cell. A combined dosage between of SA2014 compound and of doxorubicin was able to induce cell-cycle arrest at sub-G_1 and G_2/M phases. Molecular docking approach showed that SA2014 compound inhibited cdk2 enzyme. The strength of interaction between SA2014 and cdk2 (docking score = -65,43) was more stable than the interaction between doxorubicin and cdk2 (-36,59).

Keywords: *C. anomala*; SA2014; cdk2 inhibitor; T47D cell

Introduction

Sponge is a member of Metazoa, which has been successfully through millions of years of evolution. This is evident from the fact that it is widely distributed all over the world, both in the salt water and the fresh water [1]. Sponges that live around the coral reefs are able to produce high-level toxic secondary metabolite as the consequence of extreme water pressure, competition, self-defense mechanism against nudibranch, gastropods, carnivorous fishes [2]. They are also found to have pharmacological potentials [3].

Around 20,000 types of active compounds are produced by sponges. They have wide-ranging chemical class diversity, including sterol, terpenoid, isoprenoid, nonisoprenoid, quinone, brominates, nitrogen heterocyclic, and heterocyclic nitrogen sulfur [4], amino acids, porphyrin and peroxide [5]. There are varied groups of functional compounds from the sponge (OH, OCH_3, OAc, OSO_3, Na^+) [6]. Every shift in the functional groups can potentially shift polarity of each component in a dramatic way. An oncological study on marine sponges found that they could interact with essential components in the cell cycle, enzymes, and other targets [7]. Research conducted by Holland et al showed steroid compounds derived from *Cinachyrella sp* for anticancer activity through inhibition of aromatase for specific targets. In this study the cytotoxic activity against T47D cells of alkaloids *Cinachyrella sp.* The first natural 6-hydroximino-4-en-3-one steroids were isolated from *Cinachyrella* spp and are examples of molecules that can be deployed against a specific type of cancer. They displayed high affinity to aromatase, which is the rate-limiting enzyme that catalyzes the conversion of androgens to estrogens [8].

The development of modern medicine is oriented for specific targets. They are mostly macromolecules, particularly proteins directly involved in the biological activities. Molecular docking is a technique, which provides high accuracy for drug interaction with specific receptors. This way, the technique can be used to predict activities of a molecule (compounds) [9]. The problem discussed in this research is how 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound in the marine sponge *C. anomala* interacts with specific receptors participate in the cell cycle.

Materials and Methods

Sponge *C. anomala* was taken from the intertidal area of Kukup Beach, Kemadang Village, Tanjungsari Sub-District, Gunung Kidul Regency, DIY using direct collection technique [6]. The sponge samples were put into plastic bags and stored in an Icebox under a temperature of 5°C until the extraction time. Isolation and identification of alkaloid compound of marine sponge *C anomala* was conducted by Nurhayati [10]. The alkaloid compound from *C. anomala* is cinachyramine derivative, using molecular formula $C_{10}H_{13}N_3O$ 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol [10].

Analysis of cell cycle using flow cytometry method

Cell cycle analysis was conducted using flow cytometry method [11], by distributing 10^6 cell/well, which is distributed into a 6-well plate. T47D cell was treated using 4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol and incubated for 24 hours. The cells were harvested using trypsin-EDTA, then centrifuged (at 2000 rpm, 30 seconds at 4°C). The cell suspension was homogenated and transferred to flowcyto-tube. Flow Cytometry data were analyzed using Modfit LT 3,0 program to find out the cell distribution in each phase of G1, S and G2/M.

Molecular docking of 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound with cdk2 inhibitor receptor

Molecular docking was conducted using the software

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PLANTS (Protein-Ligand ANT-System). The compound 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol was given SA2014 code. Ligand native of the receptor was NS9 (2s,3s)-3-{[7-(benzylamino)-3-(1-methylethyl))(pyrazolo[1,5-a]pyrimidin-5-yl] amino}butane-1,2,4-triol) – an inhibitor of cdk2. Cdk2 inhibitor is a checkpoint component of cell cycle at G_1 phase [12]. The comparative compound was Doxorubicin, with code receptor 3NS9.PDB in the Protein Data Bank (PDB). Protein and reference ligand (ref ligand) were prepared using YASARA it is computer programe, while ligand preparation used marvinSketch.

Results and Discussion

The 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol test against T47D cell cycle

Flow Cytometry analysis for T47D cell showed that 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound could arrest cell cycle in various phases. At a dosage of 31,25 µg/mL, the compound could induce cell cycle arrest in sub-G_1 5,87% and G_2/M 50,50% phases. A combined dosage between 12,25 µg/mL of SA2014 compound and 5 µg/mL of doxorubicin could induce cell cycle arrest in sub-G_1 5,75 and G_2/M 36,89% phases (Table 1). Doxorubicin is a potent anticancer agent for the treatment of breast cancer.

Interaction of 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound in marine sponge *C. anomala* and cdk2 enzyme

The results of in silico molecular docking test for the interaction between 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound of marine sponge *C. anomala* and cdk2 protein showed a more stable docking score, compared to that of doxorubicin (Table 2 and Figure 1).

Molecular docking approach showed that 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound of maringe sponge *C. anomala* (SA2014) against cdk2 enzyme showed that SA2014 was related to amino acids of leucine[128], lysine[129], Prolin[130], asparagin[132], leucin[133], and Isoleucin[192], while doxorubicin was related to histidin[125], arginin[126], asparagin[127], valine[184], asparagin[185], and serin[188]. In terms of molecular size, 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound is smaller than doxorubicin, and its binding to cdk2 enzyme is more stable than doxorubicin. Stability of compound binding with negative docking score showed that its binding to ligand is more stable [13]. In silico assay of molecular docking supported the result of in vivo assay that 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound of marine sponge *C. anomala* could induce cell cycle arrest in G_0/G_1 phase of T47D cell.

No	Treatment	Sub-G_1 (%)	G_0/G_1 (%)	S (%)	G_2/M (%)
1	T47D cell as control	3,04	43,83	25,60	30,83
2	12,25 µg/mL of SA2014	3,99	47,51	23,20	29,44
3	31,25 µg/mL of SA2014	5,87	30,53	18,90	50,50
4	5 µg/mL of doxorubicin	5,35	44,07	25	30,87
5	12,25 µg/ml of doxorubicin	6,60	42,71	26,30	30,80
6	Combination of 12,25 µg/mL of SA2014 and 5 µg/mL of doxorubicin	5,75	43,14	19,90	36,89

Table 1: 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol(SA2014) test against T47D cell cycle.

Compounds	*Docking* score against cdk2
Ligand Native (NS9)	-91.31
doxorubicin	-36.59
SA2014	-65.43

Note: NS9=compound of cdk2 inhibitor, SA2014=structure of,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound of maringe sponge *C. anomala*

Table 2: Docking score of 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound of marine sponge *C. anomala* against Cell Devision Protein kinase2 (cdk2).

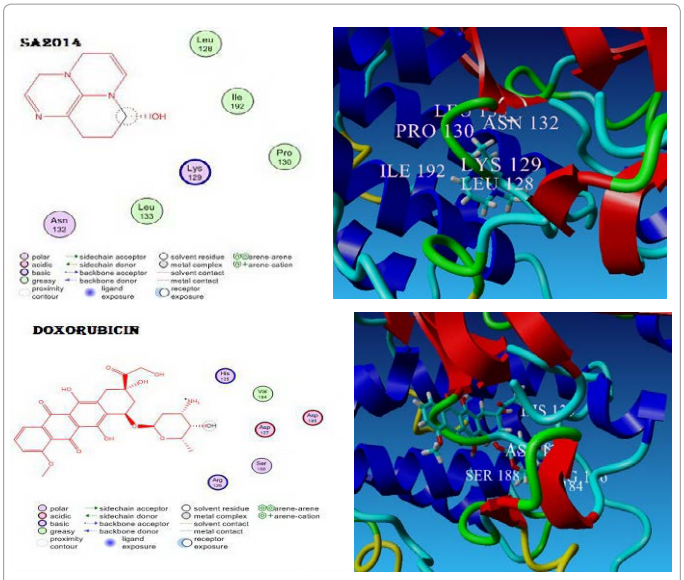

Figure 1: Visualization of *docking* for SA2014 [1,4,9triazatricyclo[7,3,1,0] trideca-3,5(13), 10-trien-8-ol] compound of maringe sponge *C. anomala* and doxorubicin against cdk2 enzyme.

Inhibition of 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound in terms of proliferation and T47D cell cycle in sub-G_1 and G_2/M phases was assumed to play a role as an inhibitor of cdk2. Cdk2 inhibitor could lead to phosphorylation of Thr-14 and Tyr-15 and degenerate cyclin/cdk2 and cyclin A/Cdk2 complexes, thus interfering with the progress of G_1 to S [14].

Cells that are interrupted during the first to the mid-G_1 phase would be postponed in the checkpoint G_1. Checkpoint G_1 depended upon the increasing expression and activation of gene p53 [15]. Gene p53 plays an important role in maintaining genome stability, since p53 serves as a conductor [16]. Gene p53 promoted expression of downstream effector genes, such as p21, gadd45, mdm2 and Bcl-2 associated X protein (Bax) [17] to arrest the cell cycle and improve DNA or apoptosis [18]. The response was due to the fact that effector gene has a certain place in the regulator to recognize P53. After induction, P53 would activate transcription of a number of genes, such as P21 [19]. Gene p21 is related to the inhibitor cyclin A/Cdk2 activity [20]. Over-expression of P21 led to cell arrest at G_1 phase [21].

Conclusion

Molecular docking of 1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol compound of maringe sponge *C. anomala* (SA2014) is smaller than doxorubicin, and its binding to cdk2 enzyme is more stable than doxorubicin.

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Conflicts of Interest

The authors declare no conflict of interest.

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