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In Silico Analysis of Depsidone as Anticancer

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ABSTRACT

The purpose of this research is to isolate and analysis one compound include in depsidones as anticancer drug via in silico. One compound include in depsidones group is vicanicine had been isolated from acetone extract of Ramalina javanica Nyl. thallus and its structure had determined by spectroscopic methods UV, IR, MS, and ^{1}HNMR. Spectroscopic methods were showed that natural product compound of R. javanica was vicanicine. Vicanicine had been analyzed by in silico via docking with Autodock 4 and it has proven that vicanicine can act as anticancer. Vicanicine bind to β-tubulin from Protein Data Bank (PDB), 1Z2B code, which is resulted in -5.32 kcal/mol of free binding energy and inhibition constant is 126.14 μM. Hydrogen bond occur between vicanicine and amino acids residue of Val177 and Thr221. Van der Waals and electrostatic binding are bound to five amino acids residue, Val177; Tyr21; Phe214; Thr221; and Pro222. In silico analysis result was indicated that vicanicine could bind to β-tubulin as well as vinblastine as the native ligand.

Keywords: Depsidone, Vicanicine, *Ramalina javanica*, Anticancer, Docking

INTRODUCTION

One compound include in depsidones group is vicanicine. In this research this compound was obtained by isolated acetone extract of *Ramalina javanica* Nyl. thallus, and the structure of vicanicine has been determinate by spectroscopic method. Cancer is diseases caused by abnormal dividing cells without stopping [1].

Anticancer drugs natural products are vary, such as anticancer-antibiotic, anticancer plant-product and anticancer animal-product, compounds which act as anticancer plant-product is vinblastine, vincristine and etoposide [2]. Anticancer mechanism interfere the tubulin binding site and inhibits microtubule formed on the mitosis coil, so that the metaphases can be stop [2]. Popular anticancer drug which can inhibit polymerization is taxol [3].

Computational method has developed rapidly to determine the structure target in drug discovery. Structure target determination is based on ligand-protein interaction, ligand-ligand interaction, or protein-protein interaction, by docking two molecules together and studying the binding affinity between two molecules [4]. Ligand is small molecule which is usually known as structure target, docking gives the advantage as virtual screening to sort out the molecules which are predicted having potential activity; virtual screening of compound libraries has become a standard technology in modern drug discovery pipelines [5]. Virtual screening can reduce the hardship and less expensive in determination of structure target [6]. Due to this reason, in this experiment, vicanicine from *R. javanica* which has been isolated was analyzed by *in silico* through docking approach.

MATERIALS AND METHODS

Extraction

Ramalina javanica was taken from Big Botanical Garden Cibodas Indonesia and identified by Herbarium Bogoriense. Powder of *Ramalina javanica* (2 kg) was soaked in hexane during 2 weeks at 30°C and daily stirred. To gain n-hexane extract, the solvent was vapored with rotary evaporator. Following this, the *R. javanica* powder which was free from n-hexane was then soaked in the acetone during 2 weeks at 30°C, and the solvent was vaporized with rotary evaporator to obtain 40 g acetone extract.

Isolation and purification

The compounds in acetone extract were separated by chromatography column with gradient of n-hexane-ethylacetate as mobile phase and silica gel Merck 60 as stationary phase. To purify the compound, separation process was continued with thin layer chromatography aluminum plate silica gel $60F_{254}$. The pure compound structure was then determined by spectroscopic methods (FTIR-Jasco 5300; H^1-NMR Bruker 500 MHZ; MS spectrometer-MAT 800; UV-Shimadzu Pharmaspec 1700) and melting point analysis is determinate by Fisher-John apparatus. Chemical ionization method is used to gain MS spectra with methane as gas carrier. UV, IR, H^1-NMR and MS spectra were then compared to literature [7]. Spot test analysis determinate by iodine vapor, Godin reagent, UV lamp wave length 254 and 366 nm.

Analysis *in silico*

Protein structure of β-tubulin (β1) was obtained from Protein Data Bank with 1Z2B code. *In silico* analysis via docking simulation was using Autodock4. For docking validation, re-docking of native ligand, vinblastine, was used with 1 Å spacing grid box centered position on 89.375; 72.634; -13.426 (x,y,z) and size 19; 19; 19 (x,y,z). Ligand preparation was the first step to do docking process with drawing vicanicine structure using ChemDraw Ultra 9.0. The macromolecule and docking preparation was visualized using AutodockTools 1.5.6 and the results were analyzed using PyMol 1.3 and Accelrys Discovery Studio 2.4 visualizer.

RESULTS AND DISCUSSION

Isolation of *Ramalina javanica* acetone extract was obtained as pure compound with characteristics shown in Table 1, is shown that the isolated compound of acetone extract of *R. javanica* is vicanicine, because of physical properties and spectra Fourier Transform Infrared Spectroscopy (FTIR), Ultra-violet (UV), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Mass Spectroscopy (MS) of both compounds (pure compound isolated as result research and vicanicine in are same. Hydroxyl group in vicanicine in was not showed, because vicanicine in ^{1}H-NMR 90 MHz instrument was used, whereas in this research compound was analyzed with ^{1}H-NMR 500 MHz, which is more sensitive. Vicanicine in MS spectra showed that molecule ion M^+ at m/z=382, but in this research molecule ion shown as (M^++1) at m/z=383, because in this research MS spectra determinate by chemical ionization method. The structure of vicanicine is shown in Figure 1.

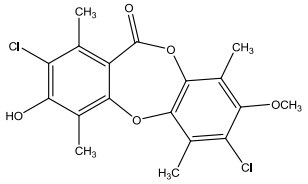

Figure 1: Vicanicine structure of *Ramalina javanica*

Table 1: Physical properties and spectra compound in this research

Melting point (°C)	248-250
MS (m/z) 383 (M^++1)	385 (M^{++}2)+1
	387 (M^++4)+1
	349
UV ($\lambda_{max,}$ nm) ($CHCl_3$)	264
IR (υ, cm^{-1}) (KBr)	3432; 2935; 1736
^{1}H-NMR (δ, ppm)	($CDCl_3$, 500 MHz)
	6.21 (s, ^{1}H, -OH)
	3.79 (s, 3H, -OMe)
	2.53 (s, 3H, -Me)
	2.52 (s, 3H, -Me)
	2.45 (s, 3H, -Me)
	2.33 (s, 3H, -Me)

To analysis the vicanicine inhibition activity, *in silico* analysis via docking with Autodock4 was performed. Binding of β-tubulin-vicanicine complex was represented free energy binding and inhibition constant. Macromolecule of β-tubulin (β1) was obtained from Protein Data Bank (PDB), 1Z2B code, with vinblastine as native ligand. Validation method of β-tubulin was re-docking the native ligand, vinblastine, to gain the grid box position (Figure 2). 1 Å spacing grid box centered position on 89.375; 72.634; -13.426 (x,y,z) and size 19; 19; 19 (x,y,z) was used to docking vicanicine with β-tubulin.

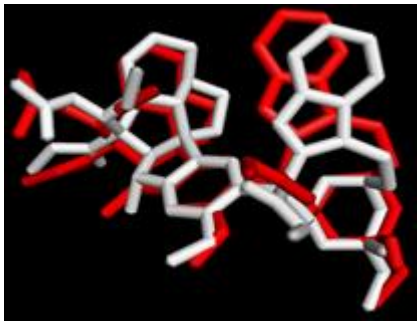

Figure 2: Re-docking result of vinblastine (white is the vinblastine native position and red is vinblastine re-docking position)

β-tubulin-vicanicine complex results free binding energy and inhibition constant (Ci) are -5.32 kcal/mol and 126.14 µM respectively. Negative value from free binding energy shows the spontaneous process. Vicanicine interacts with amino acids in active site of β-tubulin through hydrogen bond, Van der Waals, and electrostatic binding. Hydrogen bond occurred between vicanicine and amino acids of Val177 which is 2.2 Å and Thr221 which is 2.4 Å (Figures 3 and 4). Van der Waals and electrostatic binding are bound to 5 amino acids, Val177, Tyr21, Phe214, Thr221 and Pro222 (Figures 3-5; Table 2).

Table 2: Docking result of vicanicine–β-tubulin complex

Free binding energy (Kcal/mol)	Inhibition constant (µM)	Hydrogen bond	Van der Waals and electrostatic binding
- 5.32	126.14	Val177	Val177
-	-	Thr221	Thr221
-	-	-	Phe214
-	-	-	Thr221
-	-	-	Pro222

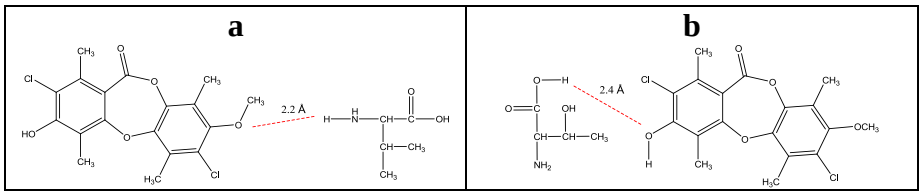

Figure 3: Hydrogen bond between vicanicine-amino acids, Val177 (a) and Thr221 (b)

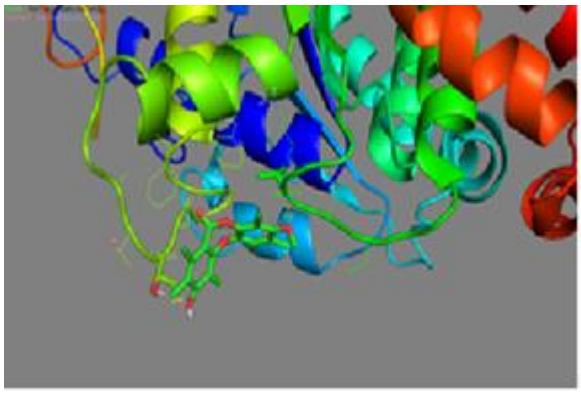

Figure 4: Hydrogen bond between vicanicine and amino acids, Val177 and Thr221

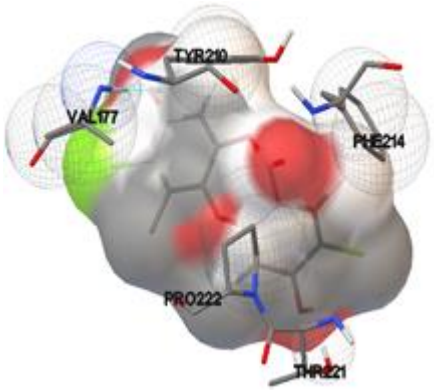

Figure 5: Van der Waals and electrostatic binding vicanicine–β-tubulin complex

Docking visualization of β-tubulin and vicanicine is shown at Figure 6, shows that vicanicine (green) is in the same grid box position with the native ligand, vinblastine (pink). Ligands have different position, vicanicine can be fold up to form two dimensions (2D) by bending and vinblastine position is planar position. However, it can be said that vicanicine can act as anticancer drug as well as vinblastine. *In silico* analysis result could be a good achievement for further experiment to discover anticancer drugs. Wet laboratory experiment, such as *in vitro* and *in vivo*, could be conducted to analyze the inhibition activity of vicanicine from *R. javanica* to β-tubulin.

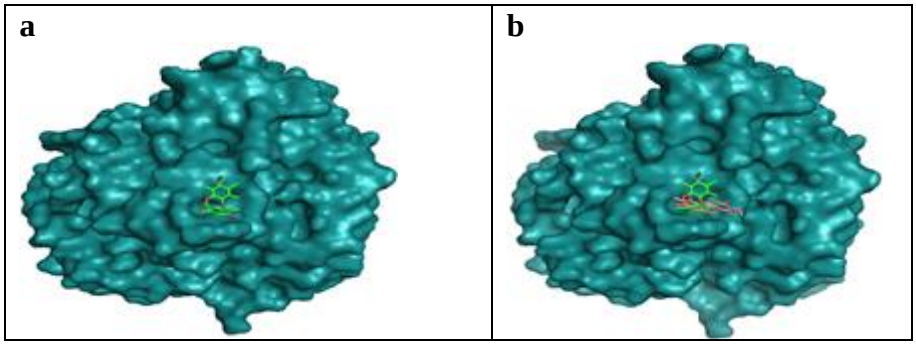

Figure 6: Docking pose visualization of (a) vicanicine (green), (b) vicanicine (green) and the native ligand vinblastine (pink)

CONCLUSION

In silico analysis of one compound include in depsidones groups (vicanicine) was indicated that this compound has biological activity as anticancer. This compound binds with β-tubulin enzyme by spontaneously process and it can form hydrogen bond with residue of amino acids Val177 and Thr221. It can also form van der Waals-electrostatic binding with residue of amino acids Val177, Tyr 21, Phe214, Thr221 and Pro222.

ACKNOWLEGEDMENT

We thank to Director Botanical Big Garden Cibodas for giving permission to take a *R. javanica*.

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