ORIGINAL ARTICLE

Molecular Dynamic of Pinostrobin and Pinocembrin from *Kaempferia pandurata* Roxb. towards Estrogen Receptor Positive (ESR) and Estrogen Receptor Negative (VEGFR) of Breast Cancer

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Abstract

Aim: This research was conducted to simulate the molecular dynamic of pinocembrin and pinostrobin against erythrocyte sedimentation rate and estimated glomerular filtration rate protein. **Materials and Methods:** In this study, the interaction of pinostrobin and pinocembrin as key compounds of *Kaempferia pandurata* toward ER and vascular endothelial growth factor (VEGF) as a molecular marker of estrogen receptor positive and ER negative (ER−) of breast cancer. The simulation was done by molecular docking and dynamic simulation. The molecular docking was conducted using AutoDock 4.2, while the dynamic simulation using AMBER 14 software. **Results:** Analysis of dynamics simulation was done by considering the root mean square deviation (RMSD), Root Mean Square Fluctuation, hydrogen bonding conditions, and MM-PBSA calculation. The dynamic simulation result showed that pinocembrin chalcone compounds have less free energy than pinostrobin. **Conclusion:** Pinostrobin and pinocembrin can interact with ER and VEGF, having a potential for specific ER− treatment.

Key words: Estrogen receptor, molecular docking, molecular dynamic, pinocembrin, pinostrobin

INTRODUCTION

Invasive breast cancer with the amount of 250,000 new cases will be diagnosed each year in women and over 2400 in men, approximately 40,610 women and 460 men die yearly.[1] Breast cancer development and progression are identified with molecular targets. Some of them are estrogen, estrogen receptors (ERs),[2] and non ER estrogens such as vascular endothelial growth factor (VEGF) and human epidermal growth factor receptor 2.[2-4] Molecular targeted therapy in the clinic is capable of delivering clear benefits to patients as evidenced by improvement in progression-free survival, and response rate. The majority of cancer patients relapsed because small cohorts of tumor cells can survive in cryptic anatomic loci and exhibit up to 90% resistance to one or more therapeutic agents for months or year(s). As such, drug resistance has been a major hurdle for classic anticancer medicines,[5] and it still is a great challenge facing the emerged array of targeted therapies.[6] Expression of receptors erythrocyte sedimentation rate (ESR) and other receptors in breast cancer has played a role in the molecular classification of breast cancer as immunohistochemistry marker and has been a constant target of specific drug development.

The rhizome is popular for the treatment; they are used to treat a range of conditions including colic, asthma, cough,

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obesity, rheumatism, and anticancer. Such as *Kaempferia pandurata* that rhizome reported major constituents were chalcones and flavonoids.[7-9] Studies have also shown *K. pandurata* having anticancer effects to many tumor cell lines, including breast cancer and myeloma.[10] *K. pandurata* consists mostly of flavonoids and various essential oils as its bioactive compound, with pinostrobin as its most abundant flavonoid.[11] Pinostrobin itself is known to have an apoptotic and antiaromatase effect on breast cancer cell lines.[12]

Many methods for drug discovery and development have done by molecular simulation. The most common version used of molecular dynamic (MD) simulation, which the trajectories of atoms and molecules are determined by numerically solving for a system of interacting particles, where forces between the particles and their potential energies are often calculated using interatomic potentials or molecular mechanics force fields.[13] MD, in medical biology, is frequently applied to study the motions of macromolecules such as proteins and ligand, which can be useful for interpreting the results of certain biomedical experiments and for modeling interactions with other molecules, as in ligand docking. MD can be used for prediction of protein structure by simulating folding of the polypeptide chain from random coil.[14]

This research conducted the interaction of pinostrobin and pinocembrin chalcone as key compounds of *K. pandurata* toward ER as a molecular marker of ER positive breast cancer, by dynamic simulation and molecular docking. The molecular docking using AutoDock 4.2, while the dynamic simulation using AMBER software.

MATERIALS AND METHODS

Protein and ligands preparation

The crystal structure of VEGF and ESR can be obtained from the protein data bank (PDB code: 3U6J and 3ERT, respectively) in a complex form with a natural ligand, so VEGF, ESR receptor, and natural ligand were isolated from the complex. The protein extracted from the complex was treated by removing all of the substructures, removing all of the water molecules and adding hydrogen atoms.

The ligand structures (two-dimensional and three-dimensional) of pinostrobin (pins) and pinocembrin (pinc) were created using marvinsketch software then performed the determination of physicochemical parameters and geometric optimization (*Gaussian 09)*.

Molecular docking

Molecular docking was performed using AutoDock Tools v.4.2.3 software. The molecular docking method was validated by re-docking the natural ligand against ES receptor.

MDs simulation

The MDs simulations were carried out using the Amber 14 software package.[15] MD is a commonly used methodology in exploring the interaction between ligand and protein. For the ligand pins and pinc, the general atomic force field parameter assignments[16] were made using antechamber program and the partial charges were assigned using the AM1-BCC method[16] Amber 14 package and the Amberff03 force field were used for all MDs simulations. Sander program was carried out for the energy minimization and equilibration protocol.[17] First step of the MD simulation process was create of topology and coordinates of ligand, ESR receptor creates of complexes with a ligand. After the preparation, then the complex between the test compounds with the receptor was made in a vacuum atmosphere and a water solvent. At this stage also, the addition to made the system neutral. The next step is equilibration to make the whole system at constant temperature and pressure. The last stage of the MD process is the production of 30 ns.

Validation methods the molecular dynamic simulations of ESR. We calculated the binding free energy by MM/GBSA method between the four ligands pins, pinc, and ESR to validate the reliability of the MD simulation. Table 7 lists the binding free energy and all of the energy terms for the two compounds.

RESULTS AND DISCUSSION

Determination of physicochemical parameters

Physochemical parameters are used to aid the interpretation of molecular docking and MDs simulation of the pins and pinc compound. The physicochemical parameter determination was performed on Molsoft programs. Physicochemical parameters determined include lipophilicity of the test compound expressed as C logP and the compound test effectiveness denoted as molar refractivity shown in Table 1.

Lipophilicity of the test compound was calculated to illustrate the ability of the test compound to arrive at its work target. The lipophilicity or hydrophobicity index is expressed as Log P. Where the higher the Log *P* value, the ligand more easily soluble in the fat or ligand the easier to penetrate the membrane layer, or it can be stated, if the Log *P* value becomes negative the test compound is hydrophilic. From the research obtain that the test compounds have a positive ClogP value means the compounds were hydrophobic.

Molecular docking of VEGFR

Molecular docking simulation was done with the same grid point Grid Center X = 5.254, Y = −4.426, Z = 17.339 with

Table 1: Physicochemical parameters of pinostrobin and pinocembrin

Test compound	MW	HBD	HB4A	Log P	TPSA
Pinostrobin	270.09	1	4	2.91	44.29
Pinocembrin chalcone	257.11	1	3	1.93	43.86
Pinocembrin	256.07	2	4	2.56	54.37

Table 2: Docking results of pinostrobin and pinocembrin with VEGFR

Test compound	ΔG (kcal/mol)	Constant of inhibition (k_i)
Pinostrobin	−8.92	287.96 nM
Pinocembrin chalcone	−6.55	15.68 uM
Pinocembrin	−8.90	300.09 nM
Native ligand	−11.74	84.81 pM

VEGFR: Vascular endothelial growth factor receptor

box size as validation 40 × 40 × 40. Based on the results of the study, it is known that the test compound has the value of free energy bond (ΔG) and low inhibition constant (k_i) shown at Table 2. Small ΔG values prove that the test compound has a stable interaction with the SSR and VEGF. The low k_i value indicates a high affinity or strong ligand attachment between the test compounds to the receptor. However, compared to natural ligands, the free energy bond (ΔG) and inhibition constant (k_i) of the test compound were much greater [Figure 1]. Sequences of amino acid as binding sites that have been prepared were Phe1047, Asp1046, Cys1045, Leu1035, Leu1019, Thr916, Val899, Val898, Lys868. This docking result shown that there is an interaction between the ligands and the binding pocket of VEGFR. The complexs of VEGFR between pins, pinc, and native ligand have shown at Table 3, interactions the compounds of pins, pinc and native ligand to the binding site of VEGFR have 11, 10 and 22 respectivly. This proves the interaction of all test compounds that have bonds to one or more amino acid residues found in VEGFR bindings pocket.

Table 3: Amino acid interaction of pinostrobin and pinocembrin with VEGFR

Amino acid	Native ligand	Pinocembrin	Pinocembrin chalcone	Pinostrobin
Leu840	v			
Lys920	v			
Leu1035	v		v	v
Ala866	v		v	
Glu917	v		v	
Cys1045	v	V	v	v
His1026	v	V		v
Asp1046	v (HB)	v (HB)	v	v (HB)
Ile1044	v	V		v
Val898	v	V		v
Ile892	v			
Leu1019	v	V		v
Leu889	v	V		v
Ile888	v			
Glu885	v			
Val848	v		v	
Lys868	v (HB)	v (HB)		v (HB)
Thr916	v	v (HB)	v (HB)	v (HB)
Phe918	v		v	
Gly841	v			
Phe1047	v		v	
Cys919	v (HB)		v (HB)	
Val899		V	v	v
Val914				
Val867				
Ile1025				
Interaction	22	10	11	11

VEGFR: Vascular endothelial growth factor receptor

Molecular docking of ESR

Molecular docking simulation was done with the same grid point Grid Center X = 3.173, Y = 33.766, Z = 17.175 with box size as validation 50 × 50 × 50. Based on the results of the study, it is known that the test compound has the value of free energy bond (ΔG) and low inhibition constant (k_i) shown in Table 4 [Figure 2]. Docking simulation of ESR with pins, pinc and native ligand shown at Table 5. The binding site sequences of ESR are site Phe1045, Cys1043, Leu1033, Leu1017, Asp1044, Cys 917, Phe916, Glu915, Val914, Val846. The docking results shown that there were interactions of pins, pinc and native ligand compounds with binding pocket from ESR have 14, 11 and 20 interaction respectivly. This shows that the compounds have bonds to amino acid residues in the ESR binding pocket. The interaction of the docking results will strengthen the initial analysis that Pins or pinc can be used as anti-ESR ligand compounds.

Table 4: Docking results of pinostrobin and pinocembrin with ESR receptor

Test compound	ΔG (kcal/mol)	Constant of inhibition (k_i)
Pinostrobin	−8.73	397.86 nM
Pinocembrin chalcone	−7.66	2.45 uM
Pinocembrin	−8.75	386.61 nM
Native ligand	−12.38	989.8 pM

ESR: Erythrocyte sedimentation rate

MD simulation

The MD simulation between VEGF receptor (VEGFR) and the test compound was performed with the Amber 14 program. The purpose of the MD simulation is to observe the bonding stability that occurs over space and time, analyzing the dynamics of the interaction of inhibition and

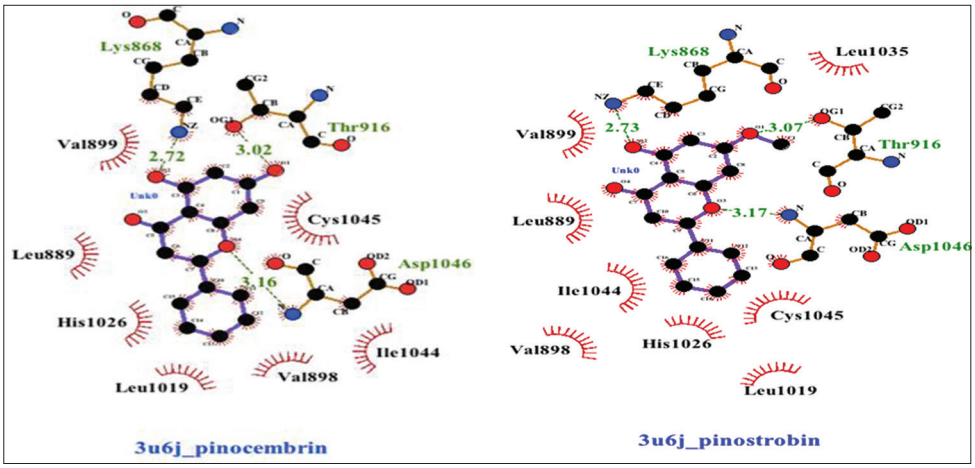

Figure 1: Complex of pinostrobin and pinocembrin with vascular endothelial growth factor receptor

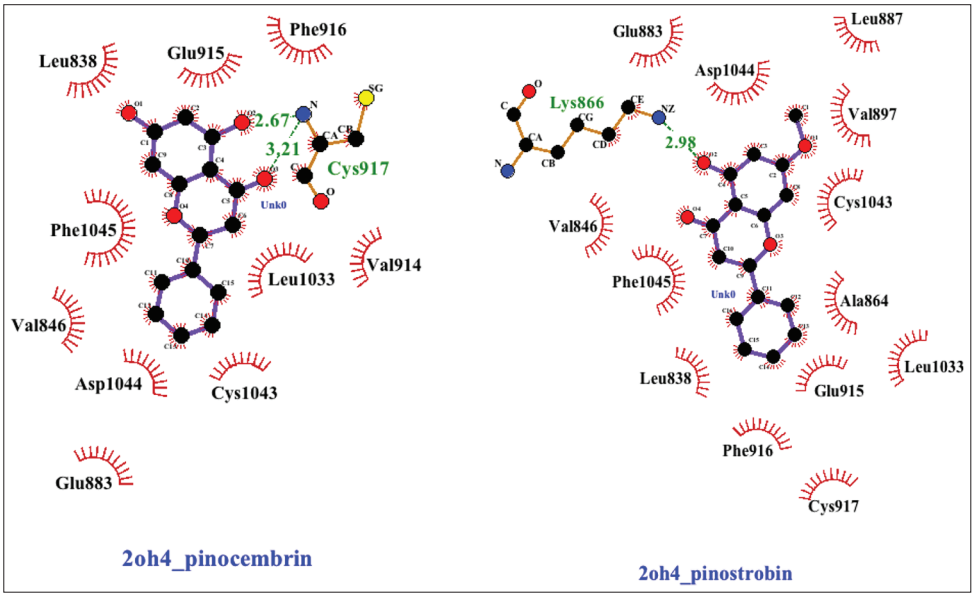

Figure 2: Complex of pinostrobin and pinocembrin with erythrocyte sedimentation rate receptor

observing the stability of the bonds that occur as well as the interaction further. MD simulation for VEGF and pinostrobin and pinocembrin runs for 30 ns with two conditions of temperature 300°K (27°C). The system was checked first in minimization level to make sure the ligand binds to the protein (macromolecule). The equilibration state includes checking stabilization of temperature, pressure, and energy will be optimized to make sure that the system has already to continue for 30 ns production.

Root mean square deviation (RMSD) and root mean square fluctuation

To verify whether the studied systems reach equilibrium, the RMSDs of all the backbone atoms of the protein, the Cα atoms for the residues of the active site (residues within 5Å around ligand), and the heavy atoms of ligand from the initial structure were monitored to examine the dynamic stability of the systems shown in Figure 3. In Figure 3, the pinostrobin, pinocembrin, and native ligand compounds having stable RMSD values at 10 ns.

As shown in Figure 4, RSMF of the amino acid residues such as Lys868, Leu889, Thr916, and Asp1046 is the key of active sites of VEGF, at these residues fluctuation showed relatively low and the temperature of 300 K.

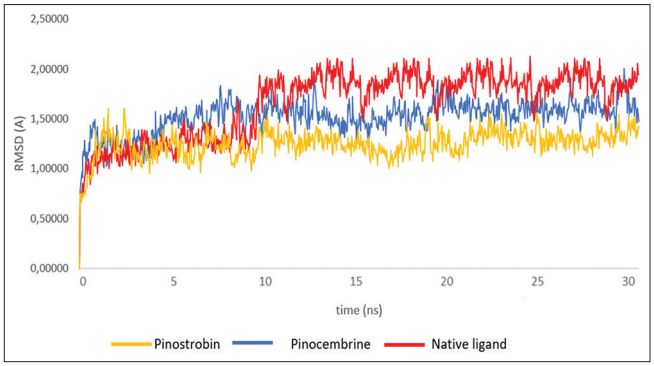

Figure 3: Root mean square deviation of pinostrobin and pinocembrin with erythrocyte sedimentation rate receptor for 30 ns

Hydrogen bond

The occupancy percentage of the hydrogen bond is divided into three categories: Weak (25–50%), strong (50–75%), and very strong (75–100%).[8] Hydrogen bonding formation is important in ligand-residue interaction,[9] the ligand pinostrobin interacted with Lys866 and Leu887 (NH-O) 2.92 Å; Cys917-Cys1043(NH-O) 7.90 Å which is key role for enzyme inhibition. These interaction also discovered in native ligand. This implying strong binding between the amino acids which altered the inhibit of enzyme.

In Table 6, Thr916 amino acid residues in native ligand compounds have the highest hydrogen bond occupancy percentages reaching 28.90, in pinostrobin 25.90%. Hydrogen bonding occupancy the amino acid Lys868 forms a hydrogen acceptor bond with an O group in the main structure. Amino acid interactions in pinostrobin compounds have hydrogen bond occupancy against lys868, Thr916, and Phe1047 with consecutive distances of 25.90, 15.80Å, 18.30 Å, and 16.20 Å.

To obtain the detailed interaction between pinostrobin and pinocembrin and VEGF, the decomposition of binding free energy, which is calculated by MM/GBSA method,[18] was executed to identify. This study analyzed the value of MMGB and PBSA by taking the first 50 frames (1–50 frames) and then taking the frame (maximum 100 frames) at the time of the stable point of the RMSD graph. The results obtained using the MM-PBSA calculation, the most important subsite for binding substrates. Table 7, the values of GB and PB of the pinostrobin and pinocembrin compounds are consistent at frames 100–150, the values of GB and PB are lower than the frames taken when the point is stable. Overall, the GB value is lower than the PB value. The results of GB and PB energy calculations, pinostrobin compounds have GB values

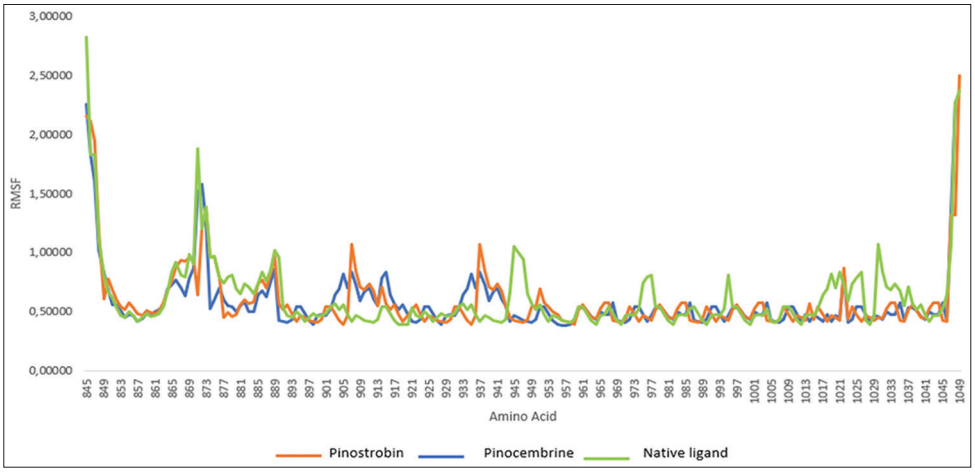

Figure 4: Root mean square fluctuation of pinostrobin and pinocembrin with vascular endothelial growth factor receptor receptor

Table 5: Amino acid interaction of pinostrobin and pinocembrin with ESR receptor

Amino acid	Native ligand	Pinocembrin	Pinocembrin chalcone	Pinostrobin
Leu1017	v			
Asp1044	v (HB)	v	V	v
Val897	v			v
Val914	v	v	V	
Phe1045	v	v	V	v
Val846	v	v	V	v
Leu1033	v	v	V	v
Gly920	v			
Ala864	v		V	v
Phe919	v			
Lys918	v			
Glu915	v	v	v (HB)	v
Leu838	v	v		v
Cys917	v (HB)	v (HB)	v (HB)	v
Cys1043	v	v	V	v
Glu883	v (HB)	v		v
Ile1042	v			
Leu887	v			v
Phe916	v	v	V	v
Asn921				
Lys866				v (HB)
Interaction	20	11	10	14

ESR: Erythrocyte sedimentation rate

Table 6: Percentation of hydrogen bonds occupantion of MD result

Amino acid	Pinostrobin	Pinocembrin	Native ligand
Leu1035	11.80; 5.10		17.00; 12.40; 16.40; 8.10
Ala866		9.20; 18.30	
Glu917			9.90
Cys1045			19.40
Thr916	25.90; 15.80	6.80	28.90; 18.00
Lys868	10.90	5.00; 25.70	29.20; 18.30
Glu885	7.90; 9.90	17.40	6.90; 5.80
Phe918		6.10	
Gly841	9.50	9.50; 19.70	12.60; 25.40
Phe1047	2.92; 5.80; 9.20; 18.30	21.60; 9.20; 18.30; 10.50	37.90; 10.90
Cys919	16.20	6.00; 5.75	29.90

MD: Molecular dynamic

of −27.6076 kcal/mol and PB −24.1256 kcal/mol lowest compared to pinocembrin compounds.

However, only two key residues Lys866 dan Cys917 were the major energy contributions to compound pinostrobin and pinocembrin binding as shown in Table 6. As mentioned previously, the vast majority of key residues of pinostrobin and pnocembrin were nonpolar; it was reasonable to speculate that these residues can form greater Van der Waals interactions with hydrophobic ligand and exhibit more favorable nonpolar interaction contribution to the binding free energy.

Analysis of hydrogen bond interactions from dynamic simulations of ESR. According to the calculated binding free energy, the strongest binding affinity; on the contrary,

Table 7: The MM-PBSA calculation of pinostrobin and pinocembrin toward VEGFR

Compounds	Frame	GB	PB
Pinostrobin	103–154	−27.6076	−24.1256
	266–317	−23.1305	−20.0519
	335–446	−22.8048	−19.4907
	698–714	−23.2107	−22.7286
	760–771	−21.9979	−20.5520
Pinocembrin	159–168	−21.5370	−21.3553
	290–317	−20.1026	−16.3473
	378–410	−25.2065	−22.2261
	637–648	−21.7419	−13.7215
	882–898	−22.6086	−16.9521

VEGFR: Vascular endothelial growth factor receptor

compound pins and pinc have the lowest binding affinity. As can be seen from Table 7, the nonpolar interactions (Δ*G* nonpolar) including Van der Waals (*E*vdw) and nonpolar solvation (Δ*G* np) terms are the driving force for the binding of the two ligands to VEGFR, and the total polar contributions (Δ*G*p) are unfavorable for their binding. The MD simulation, together with the docking results, confirmed that the newly discovered chemicals pinostrobin and pinocembrin share similar binding mode with the reported compound native ligand, and the *in silico* inhibit VEGFR activities of them are higher through the calculated binding free energy and decomposition of binding free energy.

CONCLUSION

VEGF induced colonization of the lymph node, skeletal tissues, lungs, and brain, with all clones, as described for breast cancer cell line. From *in silico* indicated that *K. pandurata* Roxb. hexane extract very potential as anti-breast cancer with VEGF expression. The flavonoids compound dominates the composition of the *K. pandurata* Roxb. and pinocembrin and pinostrobin potent as anticancer activities to breast cancer cell line.

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