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RESEARCH ARTICLE

Pinostrobin and its derivatives as novel anti-breast cancer agents against human oestrogen receptor alpha: *In silico* studies of ADMET, docking, and molecular dynamics

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Abstract

Background: Breast cancer is the main cause of cancer death among women. The issue is more complex due to the side effects and resistance of the currently used breast cancer drugs. Pinostrobin, a compound found in *Boesenbergia pandurata*, has anticancer activity. Modifying the structure of pinostrobin can improve drug bioavailability, reduce toxicity, and work more selectively. **Objective:** This study aimed to predict the potentials of pinostrobin and its derivatives as anti-breast cancer agents against human oestrogen receptors by an *in silico* approach. **Methods:** The pkCSM was utilised to predict the ADMET characteristics. The interaction of ligands with the binding site of the human oestrogen receptor alpha with PDB ID 3ERT was used for molecular docking using MOE, and AMBER was then used for molecular dynamic simulation. **Results:** Pinostrobin pentanoate had good pharmacokinetic properties and was neither mutagenic nor hepatotoxic. Based on molecular docking, it was more potent compared to pinostrobin, with binding free energy values of -7.849 kcal/mol. Furthermore, in the interaction stability evaluation using 100 ns molecular dynamic simulation by the MM-GBSA calculation method, pinostrobin pentanoate had a stable interaction with a total bond energy value of -9.943 kcal/mol. **Conclusion:** Pinostrobin pentanoate has the potential as an anti-breast cancer alternative through the human oestrogen receptor alpha.

Introduction

Breast cancer is a condition in which the breast cells proliferate uncontrollably. According to the International Agency for Research on Cancer (IARC) in Global Cancer Observatory (GLOBOCAN, 2020), the new breast cancer cases in Indonesia reached 65,858 people (16.6%) with a death rate of 22,430 people (9.6%). The incidence rate is still increasing in women aged between 40-45 years (Bray *et al.*, 2018). Based on Widiananda and Irawan's research in 2020 regarding information on demographic, clinical, and pathological characteristics of breast cancer patients registered with the Oncology Surgery Division in Indonesia for a period

of five years (2014 to 2020) using a retrospective study, oestrogen receptor alpha (ER- α) plays a major role in breast cancer pathogenesis by activating the PI3K/AKT signalling pathway (Liu *et al.*, 2020) and it was found on immunohistochemical (IHC) examination of 1260 cases of breast cancer (58.6%) with positive oestrogen receptors (ER+).

Doxorubicin belongs to the anthracycline class of antibiotics, which are frequently widely used to treat various cancer types (Childs *et al.*, 2002). Long-term use and high doses of doxorubicin have several side effects that decrease the effectiveness of chemotherapy (Childs *et al.*, 2002). Additionally, stem-like breast

cancer cells are produced by TGF β 1 signalling, which also enhances drug resistance (Bandyopadhyay *et al.*, 2010) and cardiotoxicity (Lovitt *et al.*, 2018), so it is necessary to develop drugs to obtain greater activity, lower toxicity, and working more selective (Siswandono, 2014). Generally, chemopreventive agents are obtained from natural substances such as plants that contain flavonoids and other secondary metabolites. Research by Ikegawa *et al.* proved that flavonoid-derived compounds have anticancer activities and are potential candidates for multidrug-resistance-reversing agents in cancer chemotherapy (Ikegawa *et al.*, 2002). One of the flavonoid derivatives with pharmacological effects as an anticancer is pinostrobin (Fadilah *et al.*, 2018).

Pinostrobin is a flavanone compound in the rhizome of *Boesenbergia pandurata* (Roxb.) Schlecht (Purwantiningsih *et al.*, 2020). Siswandono *et al.* () have researched the analgesic effect on 5-O-Acylpinostrobin derivatives by molecular modelling, synthesis and Quantitative Structure-Activity Relationship (QSAR), and the study's finding yielded four derivative compounds, including pinostrobin acetate, pinostrobin propionate, pinostrobin butyrate, and pinostrobin pentanoate (Siswandono *et al.*, 2020). Two of its derivative compounds, pinostrobin propionate and pinostrobin butyrate were examined in T47D breast cancer cells for their cytotoxic efficacy and selectivity against breast cancer (Widiandani *et al.*, 2023). The result revealed that both derivatives showed greater activity and selectivity than pinostrobin, so these compounds are promising to be further developed as anticancer candidates (Widiandani *et al.*, 2023). Therefore, pinostrobin and other derivatives can be predicted to have potential as anti-breast cancer agents. This study uses an *in silico* approach to predict the anti-breast cancer potential of pinostrobin and its derivatives against human oestrogen receptors by ADMET prediction, docking, and molecular dynamics.

Methods

Instruments and materials

The instruments used were hardware and software. The software used were Discovery Studio 2021, MOE 2022.02 for an academic license (K22CCG679), and AMBER 16. Meanwhile, the hardware used were laptop 11th Gen Intel Core i3-1115G4 RAM 4 GB (Discovery studio 2021 and MOE 2022) and CPU 12th Gen Intel[®] Core™ i9-12900F RAM 32 GB (AMBER 16). This study used structures of pinostrobin (A) and its derivatives designed by Siswandono *et al.* (2020), pinostrobin acetate (B), pinostrobin propionate (C), pinostrobin

butyrate (D), and pinostrobin pentanoate (E) (Figure 1), and oestrogen receptor alpha (PDB ID 3ERT).

Target receptor analysis

The analysis of the target receptor with PDB ID 3ERT was carried out by Ramachandran plot that can be accessed freely at <http://www.ebi.ac.uk/pdbsum/> (Ruswanto *et al.*, 2018).

Prediction of physicochemical, pharmacokinetic and toxicity properties

Physicochemical, pharmacokinetic, and toxicity prediction uses a computational approach accessible at <https://biosig.lab.uq.edu.au/pkcsim/> (Pires *et al.*, 2015).

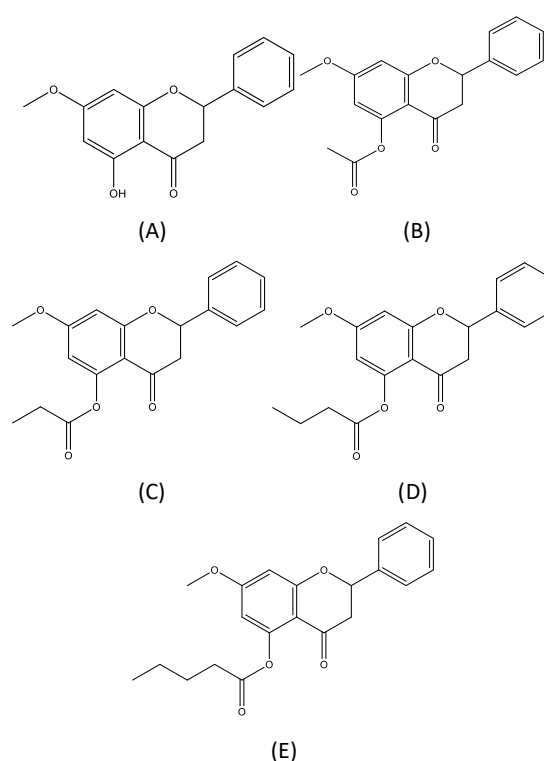


Figure 1: The structure of pinostrobin (A), pinostrobin acetate (B), pinostrobin propionate (C), pinostrobin butyrate (D), and pinostrobin pentanoate (E)

Molecular docking

Validation of the method was done before carrying out molecular docking by re-docking the native ligand against the binding site receptor using the MOE program. The Root Mean Square Deviation (RMSD) value was regarded as valid if they were $\leq 2\text{\AA}$ (Ruswanto *et al.*, 2018). Protein stability was evaluated, and their three-dimensional coordinates were seen using a Ramachandran plot (Ho & Bresseur, 2005). The good-

quality model of protein would be expected to have over 90% in the most favoured regions and below 15% in the disallowed regions (Ruswanto *et al.*, 2020). Molecular Operating Environment (MOE) 2022.02 software was used for molecular docking. The ligand conformation with the lowest bond energy (S) from the best cluster was used for the next analysis. The visualization results obtained were in the form of the best free energy value (smallest S) with the MOE database format (.mdb).

Molecular dynamics simulations

Molecular dynamics simulations were carried out on the best pinostrobin derivatives complexes from the docking results with oestrogen receptor alpha (PDB ID 3ERT), using AMBER 16 to study its stability. The molecular dynamics simulation began with the ligand parameterization step by adding the ff14SB force field. Subsequently, choosing the system's initial coordinate point and minimising while including ions and solvation. Then, a steady heating from 0°K to 310°K was used to equilibrate the system, along with a decrease in resistance and a continual change of resistance for 100 ns (Kumar *et al.*, 2020).

Results

Target receptor analysis

Based on the Ramachandran plot, the oestrogen receptor alpha with PDB ID 3ERT had 91.2% in the most favoured regions and 0.0% in the disallowed region, and it was stable for use in the next step.

Prediction of physicochemical, pharmacokinetic and toxicity properties

The requirements of Lipinski's Rule of Five are lipophilicity (less than five), molecular weight <500 g/mol, hydrogen acceptors (less than ten), and hydrogen donors (less than five). The result in Table I showed that pinostrobin and its derivatives were appropriate with Lipinski's Rules of Five, and, therefore, they were suitable for oral administration. In alignment with the physicochemical properties, the pharmacokinetic and toxicity properties also showed good results. Pinostrobin and pinostrobin pentanoate were predicted not to be carcinogenic and mutagenic based on the AMEST toxicity.

Table I: Prediction of physicochemical, pharmacokinetic and toxicity properties

Compound	Log P <5	Physicochemical			HBD <5	AMES Test	Toxicity	
		M _w <500 g/mol	HBA <10				LD ₅₀ (mol/kg)	Hepatotoxicity
1	2.28	270.09	5	1	No	2.168	No	
2	2.26	312.10	6	0	Yes	2.377	No	
3	2.91	326.12	6	0	Yes	2.363	No	
4	3.33	340.13	6	0	Yes	2.369	No	
5	3.75	354.15	6	0	No	2.374	No	

Compound	Pharmacokinetic					CI (ml/minutes/kg)
	Caco-2	HIA (%)	BBB	CYP3A4 substrate	CYP2D6 inhibitor	
1	1.271	94.656	-0.076	No	No	0.136
2	1.344	97.959	-0.164	Yes	No	0.322
3	1.355	97.951	-0.486	Yes	No	0.344
4	1.369	97.655	-0.547	Yes	No	0.352
5	1.230	97.064	-0.469	Yes	No	0.303

HIA: Human Intestinal Absorption; BBB: Blood-Brain Barrier; CI: Clearance; Log P: lipophilicity; M_w: molecular weight; HBA: Hydrogen Bond Acceptors; HBD: Hydrogen Bond Donors; LD₅₀: Lethal dosage 50%

Molecular docking

The result showed the RMSD of 3ERT was 0.221Å. Therefore, the method was valid and could be used for docking test compounds. Table II shows the binding affinity energy for pinostrobin and its derivatives. The

lower the binding affinity energy value, the more stable ligand and receptor interaction. According to the result, pinostrobin pentanoate has a lower binding affinity energy value (S) compared to pinostrobin and other derivatives.

Table II: Binding affinity energy and amino acid interaction of pinostrobin and its derivatives by molecular docking

Compound	S (kcal/mol)	Amino acid interaction	
		Hydrogen bond	Hydrophobic bond
(1)	-6.646	Met528	Arg394, Glu353, Leu349, Phe404, Leu346, Leu384, Thr347, Leu525, His524, Glu419, Gly420, Gly521, Met388, Met421, Lys520
(2)	-7.188	-	Phe404, Met388, Leu384, Met421, Glu419, Ile424, Gly420, Lys520, His524, Asp351, Thr347, Trp383, Leu349, Glu353, Arg394
(3)	-7.729	-	Arg394, Phe404, Met388, Leu384, Leu428, Ile424, Gly521, His524, Gly420, Met421, Glu419, Met528, Thr347, Trp383, Leu349, Glu353
(4)	-7.688	-	Thr347, Met528, Leu384, Leu346, Glu419, Gly521, His524, Gly420, Met421, Phe404, Glu353, Arg394, Trp383, Asp351
(5)	-7.849	-	Asp351, Trp383, Gly420, Gly521, Lys520, Met 421, Met388, Leu428, Leu387, Arg394, Cys530, Thr347, Met528, Leu346, Leu384, Phe404, Glu353

S: binding affinity energy value

Molecular dynamics simulation

Protein enzyme stability, conformational changes, structure folding of protein, and ion transport can all be examined using molecular dynamics. The parameters examined were Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Molecular Mechanics-Generalised Born Surface Area (MM-GBSA). According to the result in Figure 2, the RMSD of pinostrobin pentanoate and pinostrobin with oestrogen receptor alpha (3ERT) has stable interaction with the average RMSD value of 1.02Å and 1.39Å, respectively. Meanwhile, the RMSF of pinostrobin and pinostrobin with oestrogen receptor alpha (3ERT) had stable fluctuations. These fluctuations and instability

are due to the residues' interaction with the enzyme. Therefore, the protein tends to maintain its structure. Complexes of ligand and protein reach their maximal or stable conformation once they have bonded with the position-maintaining proteins. The low fluctuation of pinostrobin pentanoate and pinostrobin residues is shown in Figure 2, namely Leu448 and Lys449, respectively. Those residues are stable residues because they did not take a lot of their position changes during the simulation. Furthermore, the energy calculation between pinostrobin pentanoate and oestrogen receptor alpha showed a lower Gibbs free energy (ΔG) than the interaction between pinostrobin and oestrogen receptor alpha with value -9.943 kcal/mol and -0.856 kcal/mol, respectively.

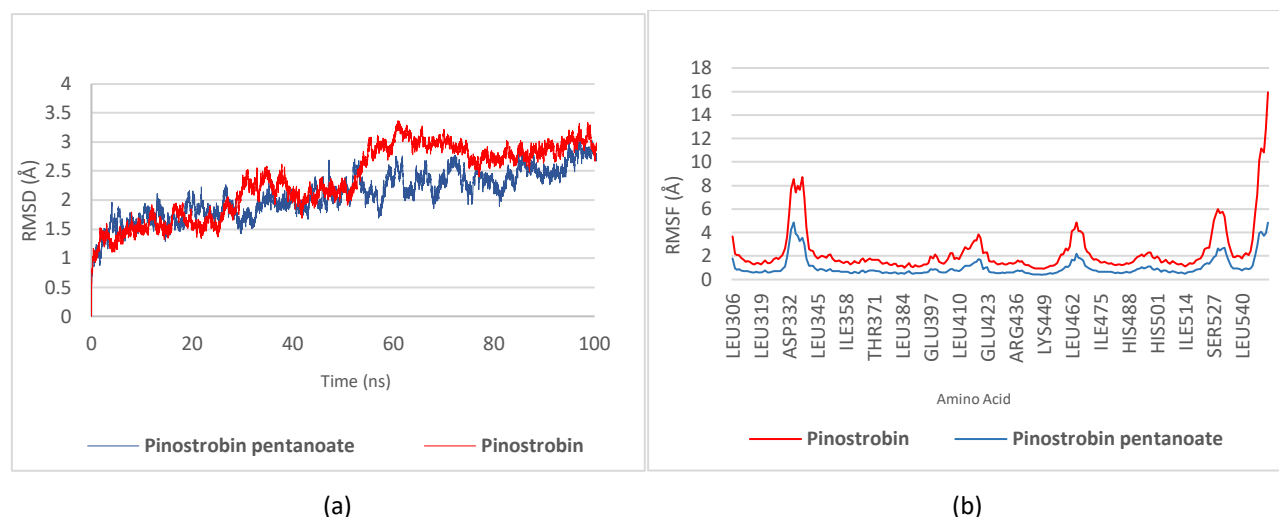


Figure 2: (a) Root Mean Square Deviation RMSD and (b) Root Mean Square Fluctuation (RMSF) curves of molecular dynamics in 100 ns

Discussion

Drug development is widely used in the discovery of new drugs to acquire greater pharmacological activity, lower toxicity, and work more selectively. *In silico* is one of the methods that could be used for drug development. It aims to increase the physicochemical, pharmacokinetic, and toxicity properties and activity of the drugs (Praditapuspa *et al.*, 2021). Drug scan studies were carried out to determine the physicochemical properties of the tested ligands using parameters based on Lipinski's Rule of Five by analysing the similarity of the molecules with an oral drug (Pratama *et al.*, 2022). Physicochemical properties are relatable to water solubility, intestinal permeability, and oral bioavailability (Lipinski, 2004). Drugs with lower molecular weight and log P could be related to the mechanism of distribution. Furthermore, a hydrogen bond acceptor value lesser than ten and a hydrogen bond donor value lesser than five would affect the lipophilicity and absorption of the molecules (Pratama *et al.*, 2022). Based on the result, pinostrobin and its derivatives conform to Lipinski's Rules of Five and are acceptable as oral medicines.

In addition, pharmacokinetic (absorption, distribution, metabolism, and excretion) and toxicity properties determine the therapeutic effectiveness of the compounds (Agustin *et al.*, 2022). According to the results, pinostrobin and its derivatives have good pharmacokinetics. While the toxicity, pinostrobin pentanoate is the only compound that was predicted not to have mutagenic and carcinogenic properties based on the AMEST test.

The prediction of pinostrobin and its derivatives activity shows that pinostrobin pentanoate has a lower binding affinity than other derivatives. Ligands with the lowest binding free energy have a stable interaction with the receptor (Pratama *et al.*, 2022). Besides, Van der Waals, as the hydrophobic bonds also contribute the most bond energy between pinostrobin pentanoate and receptor interaction, which means the active site of the receptor becomes dominated by interaction with hydrophobic amino acid (Ruswanto *et al.*, 2018). Hydrophobic bonds minimise contact between non-polar residues and water. Hydrophobic interactions can aid in the stability of the interaction between ligands and receptors. The stability conformation of pinostrobin pentanoate with oestrogen receptors has been proven by molecular dynamics simulation Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) could be used to see the trajectory of conformational changes during 100ns simulation. RMSD predicts the time required for a compound to reach a stable conformation on the active site of the receptor and compares the position and distance of the

ligand to the active site of the receptor after production and before production. Molecular dynamics is a computational method used to gather information about ligands and proteins' interaction in a flexible state. Hence, it can be considered an advanced step of molecular docking (Santos *et al.*, 2019). RMSF predicts amino acid flexibility by analysing their fluctuations during molecular dynamics simulations. Low flexibility amino acid residues have low fluctuations, indicating more stable bonding interaction and predicting the residues have a role play at the active site of ligand-receptor binding. Meanwhile, amino acids with high flexibility have substantial fluctuations, indicating less stable bond interaction due to their positions varying frequently during the molecular dynamic simulation (Renadi *et al.*, 2023). MM-GBSA calculates the Gibbs free energy (ΔG) of the ligand and the receptor bond interaction. This ΔG value can be used to measure the affinity of ligand binding at the receptor active site; the lower the ΔG value, the tighter the binding affinity. In the future, pinostrobin and its derivatives can be further analysed *in vitro*.

Conclusion

Based on the prediction of physicochemical, pharmacokinetic, and toxicity properties, molecular docking and molecular dynamics simulation of pinostrobin pentanoate is predicted to have potential as an anti-breast cancer alternative through the human oestrogen receptor alpha.

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