MOLECULAR DOCKING OF 2-HYDROXYCHRYSENE AS JAK2 RECEPTOR INHIBITORS IN BREAST CANCER

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Abstract

Laportea aestuans is a plant that has anti-carcinogenic potential. The antioxidant activity in this plant can help protect cells from damage that can cause the development of cancer by activating the apoptosis pathway in cancer cells and inhibiting protein kinase activity, there by inhibiting the signaling pathway from the cell membrane to the cell nucleus. The aim of this research is to find potential anticancer compounds targeting the JAK2 receptors. JAK2 has become a significant target in myeloproliferative disorders and is increasingly relevant in solid tumors like breast cancer. In silico molecular docking was carried out by optimizing the 2 and 3 dimensional chemical structure, validating the method and docking two receptors, namely the JAK2 protein (PDB code 6VGL) with the compound 2-Hydroxychrysene and the comparison as an positive control is ruxolitinib. Based on research results, 2-Hydroxychrysene compounds can inhibit the target proteins of breast cancer. The ΔG binding value of 2-Hydroxychrysene compounds in the JAK2 signaling pathway is -7,77 kcal/mol and the ruxolitinib is -7,36 kcal/mol.

Key words: Breast cancer, JAK2, 2-Hydroxychrysene, Laportea aestuans

INTRODUCTION

One of the most common cancers in women throughout the world is breast cancer. Breast cancer ranks second among all types of cancer with 1.4 million new cases worldwide (Adetunji *et al*., 2021). According to The Global Cancer Observatory (GLOBOCAN) in 2020, the number of new cases of breast cancer reached 68,858 cases (16.6%) out of a total of 396,914 with a death rate of more than 22,000 cases (Kementrian Kesehatan RI, 2016).

Breast cancer is a condition in which normal cells experience mutations in DNA or RNA. This results in uncontrolled cell growth and the formation of tissue masses known as tumors . Mutations in DNA can change normal cells in the breast into cancer cells (Kori, 2018).

Breast cancer treatment has significant long-term toxic effects, thereby reducing the quality of life in breast cancer sufferers (Haidinger & Bauerfeind, 2019). Conventional anticancer drugs cannot specifically differentiate between cancer cells and normal cells, so normal cells are damaged along with cancer cells (Jha *et al*., 2022).

Laportea aestuans is a plant that has anti-carcinogenic potential. Phytochemical screening of itching leaves showed good antioxidant activity in preventing cancer. Laportea aestuans also has the ability to activate the apoptosis pathway in cancer cells and inhibit protein kinase activity, thus inhibiting the signaling pathway from the cell membrane to the cell nucleus (Simaremare *et al*., 2020). 2-Hydroxychrysene is one of the bioactive compounds from Laportea aestuans. However, the effect of 2-Hydroxychrysene compounds on target proteins and their level of toxicity are not yet known, so further research is needed (Adetunji *et al*., 2021).

Janus kinase-2 (JAK2) plays a crucial role in signaling through various cytokine receptors. It is involved in erythropoiesis (via erythropoietin receptors) and mammary differentiation (via prolactin receptors). JAK2 has become a significant target in myeloproliferative disorders and is increasingly relevant in solid tumors like breast cancer. Recent research links JAK2 to IL-6–dependent self-renewal of breast cancer stem cells and the growth of triple-negative breast cancers driven by both IL-6 and IL-8. Additionally, JAK2 signaling may contribute to resistance against targeted breast cancer therapies (Miller *et al*., 2014).

The in silico method is a computer technology that helps reduce initial costs in the process of identifying drug candidates and also increases the opportunity to discover new drugs. This process is generally carried out by predicting the orientation of the ligand molecule in the receptor and then using

a scoring function to describe the interactions between molecules and their bond strengths (Praceka *et al.*, 2022).

Based on the background above, researchers are interested in knowing the anticancer activity of Laportea aestuans in silico. The target proteins involved were selected based on breast cancer pathways according to the Kyoto Encyclopedia of Genes and Genomes (KEGG), and analyzed further to determine binding energy and interaction patterns using the AutoDock Tools application.

METHODS

The hardware used is an HP laptop which has Intel(R) Core(TM) i3-10110U CPU @ 2.10GHz 2.59 GHz specifications, 8.00 GB RAM (7.83 usable) with an Intel(R) UHD graphic card. The software and websites used include Protein Data Bank (PDB) which can be accessed via the Protein Data Bank (PDB) database (https://www.rcsb.org) , MarvinJS online (https://marvinjsdemo.chemaxon.com/) , Autodock tools version 1.5.7, Autodock4, Open Babel, PyMOL Educational, Discovery Studio Visualizer v16.1.0.15350 and ADMETlab 2.0 (https://admetmesh.scbdd.com/service/screening/molecule).

This research uses macromolecule (receptor) data obtained from the Protein Data Bank (PDB) with a unique code called the PDB code. The receptors used are JAK2 (PDB code: 6VGL) and the active compound is 2-Hydroxychrysene. The two-dimensional and three-dimensional structures of the ligands were drawn using the online MarvinJS web server, then saved in .sdf format and converted into .pdb files using Open Babel software.

The macromolecular structures of JAK2 (pdb code: 6VGL) were downloaded from the Protein Data Bank (RCSB PDB) in .pdb format. Macromolecular preparation using the Autodock Tools application. Removing unnecessary chains, removing water molecules, adding hydrogen and adding Kollman charges.

The aim of validating the docking method against the original ligand is to determine the conformation of the original ligand. The Root Mean Square Deviation (RMSD) value used to compare the conformation of the docked ligand with the crystallographic ligand is a common and useful approach to ensure the accuracy of the docking results. PyMOL is software for combining data from docking results with original ligands. If the RMSD value between the structure of the docked ligand and the crystallographic ligand is less than or equal to 2 (Å), it indicates that the structure of the docked ligand is geometrically similar or identical to the crystallographic ligand.

The PDBQT format was used to optimize ligand and receptor binding. The active binding site on the JAK2 receptor is arranged with a gridbox orientation of x = 56.237, y = -5.937, z = 23.506, then saved in GPF format before running autogrid. The molecular docking stage involves selecting the receptor and ligand to be tested and setting the genetic algorithm parameters. The docking process is carried out with autodock output saved in DLG format. AutoDock Tools software is used to analyze docking results with parameters such as binding energy (ΔG) and visualization of interactions between ligands and amino acids.

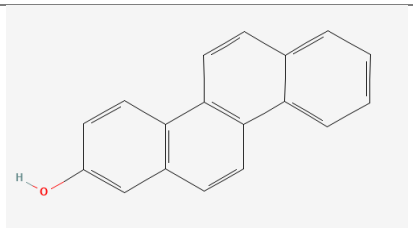
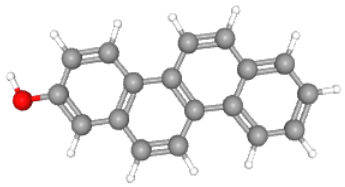
Discovery Studio Visualizer is software used to visualize and analyze molecular structures and protein ligand complexes. The aim is to examine the interaction of the compound with amino acid residues on the target protein. Visualization of ligand-protein complexes can reveal various aspects of interactions, such as hydrogen bonds, cation-π interactions, hydrophobic interactions, and electrostatic interactions between the compound and amino acid residues on the target protein.

RESULTS AND DISCUSSION

In this research, in silico testing involves the use of two-dimensional and three-dimensional structures of the test compound and reference compounds. The test compound in this study was 2-Hydroxychrysene shown in Table 1, while the comparison compound used was the original ligand of the target protein and the positive control Ruxolitinib shown in Table 2.

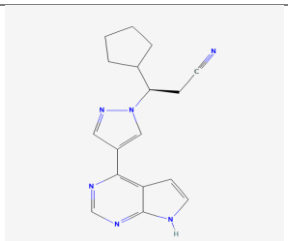
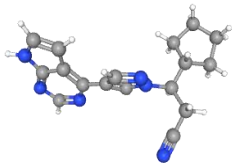
The RMSD value is a parameter that measures how big the deviation or change is between the position of the ligand in the crystal structure and the position of the ligand resulting from the docking process. The RMSD values for the 6VGL and 3PP0 macromolecules are presented in Table 3.

Table 1. Structure of the test compound 2-Hydroxychrysene.

Compound Name	
Structure Image *2-Hydroxychrysene*	
Two Dimensional	**Three Dimensional**
	

The molecular docking process begins by preparing macromolecules (target proteins) which are predicted using the Swiss Target Prediction web server. After prediction, the obtained macromolecules are downloaded from the Protein Data Bank (PDB) via the website address http://www.rcsb.org/. The macromolecules used are the JAK2 receptor. The macromolecular structure in PDB contains solvent molecules in the form of water (H2O) and other residues, so it is necessary to remove water molecules to maximize the interaction between the test compound and the desired target protein. This leaves only amino acids in the target protein without any interference from water molecules. Hydrogen bonds are a common type of interaction that occurs between ligands and receptors, so it is necessary to add hydrogen to optimize the interactions that occur.

Table2. Structure of the native ligand JAK2 (PDB code: 6VGL)

Compound Name	
Structure Image *Ruxolitinib (RXT)*	
Two Dimensional	**Three Dimensional**
	

Data analysis of molecular docking results includes RMSD (Root Mean Square Deviation) measurements, binding energy, ligand-receptor interaction analysis, and toxicity assessment of the compound 2-Hydroxychrysene on the target protein 6VGL. The interactions observed include hydrogen bonds between the 2-Hydroxychrysene and amino acids in the target protein, as well as bonds formed between the reference compound and the drug Lapatinib and amino acids in the target protein.

The molecular docking process produces interaction data between the 2-Hydroxychrysene and the native ligand of the target protein. The docking results have been analyzed and presented in Table 4, Table 5 and Table 6.

Table 4. Docking results for the compound 2-Hydroxychrysene and Ruxolitinib with JAK2 Receptor

Ligand	**Affinity (Binding energy) (kcal/mol)**	**Interaction of Amino Acids via Hydrogen bonds**	**Other Amino Acids**
Ruxolitinib	-7,36	LEU932	LEU983, ALA880, MET929, LEU855, VAL863, VAL911
2Hydroxychrysene	-7,77	LEU932, GLU930	LEU983, ALA880, MET929, LEU855, VAL863

In the development and discovery of a candidate medicinal substance, the criteria for a compound must fulfill Lipinski's Law of Five (Praceka *et al.*, 2022). The results of the analysis with Lipinski's Five Laws are shown in Table 5.

Table 5. Results of Analysis of Lipinski's Rules for LA Compounds

Compound Name	Molecular Weight (<500 Dalton)	H-Bond acceptors (<10)	H-bond donors (<5)	logP (<5)
2-Hydroxychrysene	244,090	1	1	5,037

The parameters tested in the toxicity test are Toxicophore Rules predictions which include Acute Toxicity Rule, Carcinogenicity (genotox and nongenotox) and In vitro mutagenicity (Ames test). Toxicity prediction results are shown in Table 6.

Table 6. Prediction results for 2-Hydroxychrysene toxicity

Compound	Parameters			
	Acute Toxicity Rule	***Genotoxic***	***NonGenotoxic***	***In vitro Mutagenicity***
2-Hydroxychrysene	0 alert(s)	3 alert(s)	0 alert(s)	+++

Binding energy is the energy needed to release two molecules that are bonded together. The best binding energy is the smallest, the smaller the binding energy value, the stronger the interaction between ligand and target protein (Aziz *et al.*, 2016). Based on the results of molecular docking carried out on the JAK2 receptor with the PDB code 6VGL, ΔGbinding value of -7.77 kcal/mol was obtained, the 2-Hydroxychrysene shows good affinity for the JAK2 receptor (6VGL), this ΔGbinding value is better than the native ligand (-7.36 kcal/mol).

The JAK2 (Janus tyrosine kinase 2) pathway plays an important role of breast cancer. Activation of this pathway will increase the tumor viability and metastasis, promotes cancer cell transition (cancer stem cells), and contributes to resistance to chemotherapy by enchancing epithelialmesenchimal (EMT)

From the results of molecular docking carried out on the JAK2 receptor with the PDB code 6VGL, it was found that the ΔG binding value produced from the 2-Hydroxychrysene compound was -7.77 kcal/mol. This value shows that the ΔGbinding value of the 2-Hydroxychrysene compound is lower than the native ligand 6VGL (RXT). The difference in binding between the ligand for amino acids and the receptor can affect the binding energy value. The lower binding energy value, the stronger the interaction between the ligand and receptor. This shows that the molecular complex formed is more stable and stronger for interactions (Frimayanti *et al.*, 2021).

Ruxolitinib is a JAK2 inhibitor in the treatment of breast cancer with JAK2 overexpression. ruxolitinib is a drug that has been approved by the FDA for patients with cancer. ruxolitinib was developed as a triple-negative breast cancer drug that works by inhibiting JAK2 over expression (Blako, 2017). Therefore, components that can inhibit the kinase activity of HER-2 can be developed as potential anticancer drug candidates. Ruxolitinib from the results of molecular docking on the JAK2 receptor is known to have a ΔGbinding value -7.36 kcal/mol (Table 4).

These results indicate that 2-Hydroxychrysene has potential activity as an anticancer agent based on the binding affinity and similarity of amino acid residues between the original ligand and the compound (Figure 1).

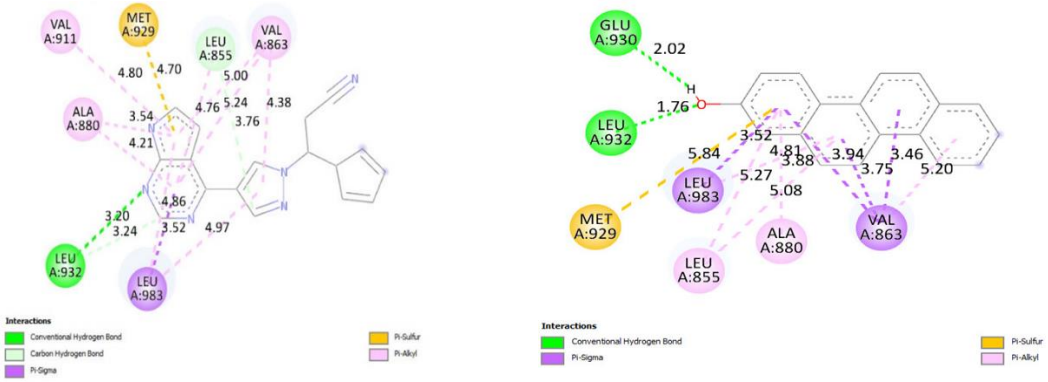


Figure 1. Molecular Docking of the JAK2 Receptor with 2-Hydroxychrysene and Ruxolitinib (a) Molecular interaction JAK2 with 2-Hydroxychrysene (b) Molecular interaction of JAK2 with the native ligand (Ruxolitinib) of 6VGL protein

In the development and discovery of a candidate medicinal substance, the criteria for a compound must fulfill Lipinski's rules (Praceka *et al*., 2022). Rule's of Five (ROF) is a set of empirical rules used in drug research to evaluate whether a drug candidate compound has the potential to be an effective oral drug Generally, Lipinski's rule describes the solubility of certain compounds to penetrate cell membranes by passive diffusion (Garai *et al*., 2012)This shows that the 2-Hydroxychrysene compound meets the requirements of Lipinski's Rule of Five (Table 5).
The parameters looked at in the toxicity test are Toxicophore Rules predictions which include Acute Toxicity Rule, Carcinogenicity (genotox and nongenotox) and In vitro mutagenicity (Ames test). Based on toxicity predictions, 2-Hydroxychrysene compounds are known to tend to be non-toxic (Table 6).

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the 2-Hydroxychrysene has a high affinity for the target protein receptors JAK2. This is shown by the binding free energy value between the 2-Hydroxychrysene and the target protein JAK2 with ΔGbinding value is -7.77. So that the molecular complex formed becomes stable. The toxicity of LA compounds is known to tend to be toxic which will need to be considered for the development of this compound as a drug candidate.

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