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Original Research Paper

In Silico Study of Brazilin from *Secang* Wood (Caesalpinia Sappan L) as a Candidate for Splenomegaly Therapy

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Abstract
Brazilin is a flavonoid found in *secang* (sappan) wood extract (Caesalpinia sappan L) currently undergoing clinical trials in phase 2 for the treatment of thalassemia patients. It is recognized for its antioxidant effects and its efficacy as a strong iron chelator, facilitating the binding and excretion of excess iron in the bloodstream of patients with thalassemia. This flavonoid compound may serve as a Janus kinase 2 (JAK2) inhibitor through the EPO/EPOR/JAK2/STAT5 pathway, which is responsible for splenomegaly (enlarged spleen). This study aims to investigate the mechanism by which sappan wood metabolite chemicals (brazilin) inhibit JAK2 in silico. This inhibition is expected to reduce splenomegaly in thalassemia patients and serve as an alternative to ruxolitinib (conventional medications). The pharmacokinetic profile of the ligand is predicted according to Lipinski's rule, while the binding energy (ΔG), initiation constant, and chemical bonds are examined using molecular docking with AutoDock v.4.25. This study successfully determined that brazilin, with a binding energy of -8.37 kcal/mol, is comparable to ruxolitinib, which has a binding energy of -8.71 kcal/mol. This finding shows that brazilin derived from sappan wood contains bioactive chemicals with potential JAK2 inhibitory activities. This finding establishes a foundation for further research aimed at developing new therapeutic agents for the treatment of splenomegaly in β-thalassemia and associated disorders.

Keywords: *Caesalpinia sappan; in silico; JAK2 inhibitor; splenomegali; thalassemia*

1. Introduction

Splenomegaly, or spleen enlargement, frequently occurs in non-transfusion dependent thalassemia (NTDT) patients, particularly in children, with an occurrence rate of 30% and a mortality rate reaching 15% (Suttorp & Classen, 2021). Splenomegaly may impair circulating blood cells, leading to lowered white blood cell counts that increase infection risk, reduced red blood cell counts that worsen anemia, and reduced platelet counts that increase bleeding risk (Aldulaimi & Mendez, 2021). If untreated, the spleen will persist in enlarging and is susceptible to rupture, resulting in hemorrhage and mortality. Furthermore, splenomegaly results in an increased need for red blood cell transfusions, as certain circulating red blood cells are annihilated by the impaired spleen. This may result in further complication, including the accumulation of alloantibodies and iron overload (Khan et al., 2023; Mizher, 2024).

The treatment methods for splenomegaly in β-thalassemia include splenectomy, radiotherapy, transfusion therapy, hydroxyurea, and bone marrow transplantation (Yadav & Singh, 2022). Chronic blood transfusion is the common treatment for patients with β-thalassemia and splenomegaly to increase

hemoglobin levels and prevent tissue hypoxia (Subahi et al., 2022). However, red blood cell transfusion would significantly increase iron overload due to disruptions in iron homeostasis and subsequent iron deposition in organs, being a primary factor of morbidity in patients with β-thalassemia (Brissot, 2016; De Sanctis et al., 2017; Narahari et al., 2024). Furthermore, splenectomy and bone marrow transplantation are prevalent treatment methods for splenomegaly. However, both options present certain disadvantages, including the potential for infectious stroke, blood clots and pulmonary hypertension associated with splenectomy. Patients with splenomegaly may exhibit immunocompromised status and typically receive lifelong prophylactic oral antibiotics following splenectomy (Buzelé et al., 2016). The risk associated with splenectomy is particularly increased during the first 4 years post-procedure of splenectomy, especially in pediatric patients under the age of 5 (Amid & Merkeley, 2023). Conversely, 60% of bone marrow transplants lack an appropriate donor, consequently increasing the risk of complications (Inamoto & Lee, 2017; Peffault de Latour, 2016).

Janus Kinase 2 (JAK2) inhibitors have recently been extensively studied as an effective approach for preventing the development of splenomegaly, because of their ability to obstruct the JAK2-signal transducer and activator of transcription 5 (JAK2-STAT5) pathway, which is related to splenomegaly (Casu et al., 2020). Various therapeutic strategies have been developed to inhibit JAK2; for instance, preliminary studies in β-thalassemia models in mice have demonstrated that JAK2 inhibitors (JAK2i) effectively treat splenomegaly by enhancing the balance of proliferation and differentiation of erythroid progenitor cells (Fahmideh et al., 2022; Oikonomidou & Rivella, 2018; Taher et al., 2023). Understanding the molecular alterations in splenomegaly is the key factors for its prevention and treatment. Numerous research has been undertaken to identify new substances with the potential to act as JAK2 inhibitors (Casu et al., 2016; Cui et al., 2023). One of the JAK2i molecules accepted by the Food and Drug Administration (FDA) is ruxolitinib. Ruxolitinib reduced spleen volume by 26.8% over a 30-week duration in a phase-2 clinical trial (Casu et al., 2018). Ruxolitinib did not demonstrate clinical significance in increasing pre-transfusion hemoglobin levels or in reducing serum hepcidin levels and iron overload parameters; thus, the chemical did not proceed to phase 3 clinical trials (Casu et al., 2018). The selection of herbs for treating splenomegaly was based on their reduced side effects and lower cost compared to synthetic medications (Intan & Silvia, 2021; Margono & Sumiati, 2019).

Brazilin is the predominant metabolite identified in Caesalpinia sappan L, commonly referred to *secang* (sappan) in Indonesia (Mekala & Radha, 2015; Syamsunarno et al., 2021; Vij et al., 2023). This plant has been widely used in several countries and is considered to help reduce iron overload in thalassemia because of its antioxidant and chelating effects (Maskoen & Safitrie, 2018; Pitaloka et al., 2022; Purnama et al., 2022; Safitri et al., 2018; Sari & Suhartati, 2016). *Secang* may serve as a potential JAK2 inhibitor candidate due to its chemical components, which originated from natural sources and have notable and effective bioactivity, enriched in flavonoids(Nirmal & Panichayupakaranant, 2015; Wang et al., 2021). The unpaired electrons and hydrogen bonds can bind to amino acids, leading to ineffective erythropoiesis and splenomegaly (Aldosari et al., 2023; Vázquez-Jiménez et al., 2024). Moreover, its capacity to chelate excess iron and its antioxidant properties that reduce reactive oxidative stress in thalassemia patients will enhance its therapeutic efficacy in treating thalassemia (Galy et al., 2024; Khalili & Ebrahimzadeh, 2015; Mobarra et al., 2016; Safitri et al., 2017; Yadav et al., 2016; Yadav & Singh, 2022).

Vaziri-Amjad et al. (2024) highlighted the potential of natural substances as natural JAK2 inhibitors, encompassing flavonoids, anthraquinones, and cinnamic acid. This method is employed in this study to investigate and generate potential medicines that can be used as JAK2 inhibitors from sappan wood core constituents in silico. These constituents have an important role in the pathophysiology of splenomegaly and can predict a compound's activity in target cells. This study also examined pharmacokinetic features to facilitate the development and discovery of orally administered

medications in accordance with Lipinski's rule of five. This research may facilitate the discovery of potential compounds for JAK2 inhibitors in future studies.

2. Research Method

Ligand preparation starts with designing of a 2D structure by copying SMILES from the website https://pubchem.ncbi.nlm.nih.gov into the ChemDraw Ultra 8.0 software within the ChemOffice v.8.0 package, followed by optimizing the most stable energy structure using the Chem 3D 18.0 program, and saving the file in the .pdb format. Online application available at the pkCSM website: https://biosig.lab.uq.edu.au/pkcsm/ was additionally employed to evaluate data about the drug-like characteristics of bio-inhibitors utilizing Lipinski's criteria (Lipinski, 2016).

The Tryrosine-janus kinase 2 receptor in the ruxolitinib complex (PDB: 6VGL, resolution: 1.9 Å) (Figure 1) was utilized as a reference protein and ligand obtained from the Protein Data Bank archive at https://www.csb.org/. The best protein (chain D) and ruxolitinib were separated utilizing the Discovery Studio Visualizer software, and both receptor-ligands were then saved as *.pdb files (Kamble et al., 2022). Water and cofactors were eliminated, and polar hydrogens were added. The partial charges of each atom were calculated using Kollman addition for the receptor and Gasteiger computation for the ligand, as included in the AutoDock Tools v.4.25 program package. The chain and ligand structures were subsequently stored as *.pdbqt files (Asnawi et al., 2023; Siregar et al., 2020).

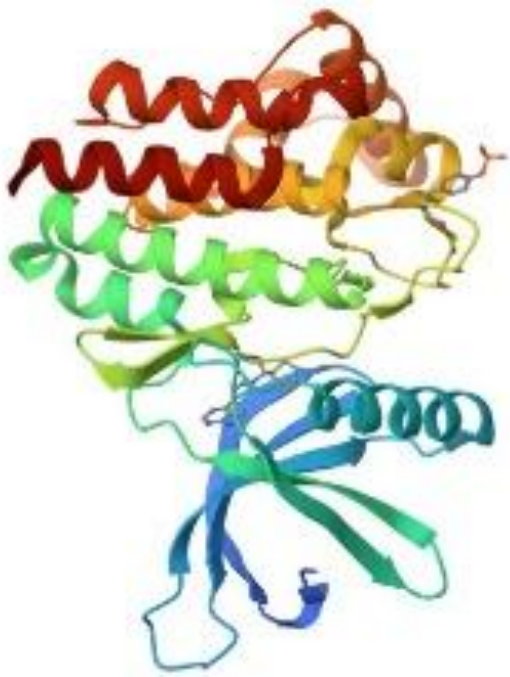

Figure 1. JAK2 receptor complexed with ruxolitinib ligand, constructed with AutoDock Tools version 4.25.

The docking procedure was executed with AutoDock Tools v.4.25 software by entering the target protein and the original ligand structure. The grid box is configured to facilitate the binding of all ligands to the receptor through the central region of the ligand. The number of x, y, and z dimension points, along with their spacing and central grid box, are recorded and utilized for validation in further docking procedures. The Lamarckian Genetic Algorithm is employed to calculate the algorithm, which is executed 100 times for docking. Validation is achieved by re-docking the natural ligand into the receptor's active site Docking is conducted using the default software settings, without modifications to the run or grid parameters. The comparative data analysis of the values is regarded as good and reliable

if the resulting Rate Mean Square Deviation (RMSD) value is less than or equal to 2 Å (Ruswanto et al., 2024).

The AutoDock v.4.25 software was utilized to examine the inhibitory affinity of the brazilin compound on the Tyrosine-janus kinase 2 protein through the empirical free energy function integrated with the Lamarckian Genetic Algorithm (LGA). A grid was established with appropriate dimensions, utilized during the validation procedure to encompass all amino acid residues involved in ligand binding with Tyrosine-janus kinase 2 (6VGL): Dimensions of 40 x 40 x 40 Å with dots separated 0.375 Å apart, with the grid center established at coordinates 8.07, -27.559, and 52.372 for X, Y, and Z (Asnawi et al., 2023; Siregar et al., 2020). Moreover, molecular docking studies were employed to determine the best possible docking results for protein receptors and ligands derived from sappan wood by identifying the ligand conformation with the lowest free binding energy (ΔG). The assessed parameters included ligand structural orientation, hydrophobic interactions, and hydrogen bonds established by each ligand. The ligand-receptor complex was displayed in 2D and 3D formats using Discovery Studio Visualizer version 17.2.0.1634 software. The binding affinity of each ligand, together with its physicochemical, pharmacokinetic, and toxicological predictions, was presented in tabular format. The ligand exhibiting the most negative binding affinity is selected for comparison with the native ligand.

3. Results and Discussion

3.1. Drug-Likeness Prediction

Lipinski's rule of five (R05) serves as a guideline for assessing drug-likeness and oral bioavailability in the pharmaceutical industry during the initial phases of drug development. The criteria include a molecular weight of ≤ 500 Dalton, a maximum of 5 hydrogen bond donors and 10 hydrogen bond acceptors, a lipophilicity (Plog) of ≤ 5, and a molar refractivity ranging from 40 to 130 (Adianingsih et al., 2022; Agosto, 2024). Potential drug ligands must adhere to Lipinski's criterion, and brazilin satisfies all drug-likeness requirements to be considered for additional analysis (Kusuma et al., 2022) (Table 1).

Table 1. Results of Stability Analysis of Ruxolitinib and Brazilin from Sappan Wood Extract using Lipinski's Rule

No	Parameter	Criteria	Result	
			Brazilin	Ruxolitinib
1	Molecular weight	≤ 500 Da	286,283	306,373
2	LogP	≤ 10	1,615	3,466
3	Molar refractivity	40-100	73,87	98,01
4	Hydrogen proton donors	≤ 5	4	1
5	Hydrogen proton acceptors	≤ 10	5	5

Source: Primary Data, 2025

Compounds with a molecular mass exceeding 500 Daltons encounter challenges in penetrating the cell membrane due to their inability to diffuse into the cell. In contrast, compounds with low molecular mass may penetrate the cell through diffusion(Fakhruri & Rahmayanti, 2021). Donor and acceptor hydrogen can bind to targets, influencing the chemical-physical properties of compounds, including melting and boiling points, as well as water solubility for chelates. Excess hydrogen may hinder cell membrane permeability and is associated with higher toxicity levels (Indratmoko et al., 2023). The log P value correlates positively with hydrophobicity, defined as the capacity of a chemical compound to dissolve in oil, fat, and non-polar solvents. A compound exhibits a favourable log P value when it falls within the range of -2 to 5, indicating its ability to effectively penetrate membranes (Mustarichie et al.,

2017). The drug should possess enough hydrophobicity to penetrate the lipid bilayer; however, excessive hydrophobicity may hinder membrane penetration and lead to toxicity from prolonged accumulation in the body(Mardianingrum et al., 2021). A higher logP value indicates increased hydrophobicity of the molecule, facilitating its retention within the lipid bilayer of the cell membrane. The widespread distribution of the compound will consequently diminish its selectivity in binding to the target protein (Weni et al., 2020). Molar refractivity correlates with molecular shape and relative molecular mass, usually leading to an increase in electron number (Mardianingrum et al., 2021). A higher drug similarity score indicates a higher possibility that a candidate molecule will possess all the physiochemical parameters (Prottoy et al., 2019).

3.2. Validation of Docking Method

The docking process was validated by re-docking the native ligand ruxolitinib into the active site of Janus kinase 2, conducted over 100 repetitions using the grid box reported in Table 2. This analytical approach aims to compare the positioning of the native ligand on the target protein with that of the test ligand. Figure 2 shows the visualization results, indicating that ruxolitinib has the same conformation as the native ligand before redocking. The configuration overlap results for ruxolitinib, before and after validation, indicate that the RMSD value of the native ligand 6VGL is 1.41 Å. The results indicate that the redocking ligand shows similarities with the native ligand, as evidenced by an RMSD value of less than 2Å. This suggests that the configuration of the test ligand is valid and closely resembles that of the native ligand. Furthermore, the atomic positions in the redocking ligand are not significantly different from those observed in the crystallographic results (Ruswanto et al., 2024).

Table 2. Docking Validation Grid Box Values

Space	Grid box			RMSD	Dimension Size x,y,z
	X	Y	Z		
0,375	8,07	-27,559	52,372	1,41	40 x 40 x 40

Source: Primary Data, 2025

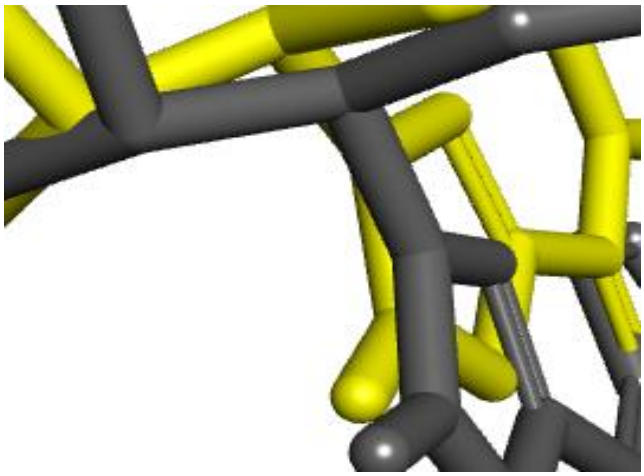

Figure 2. Illustration of overlapping natural ligands before (yellow) and after redocking (black)

3.3. Visualization and Analysis of Docking Results

The results of the docking process were visualized to determine the interactions between ligands and the amino acid residues of the anus kinase 2 receptor. The interaction of ligands with receptors is illustrated in Table 3.

Table 3. Molecular docking results of ruxolitinib and brazilin to Janus kinase 2 protein

Ligan	(ΔG) (kcal/mol)	Ki	Amino Acid Residues Hydrogen Bond	Distance (Å)	Hydrophobic Interactions
Ruxolitinib	-8,71	413,4 nM	Glu930 (H) Gly993 (H) Asp994 (H) Gly993 (C)	2,09; 2,17 2,12 3,41 3,48	Leu983, Val863, Leu855, Ala880, Leu932
Brazilin	-8,37	728,3 nM	Leu932 (H) Asp994 (H) Asp939 (H)	1,92 1,62 2,43; 2,03	Leu855, Val863, Leu983

Note: Gibbs free energy (binding affinity) (ΔG); Inhibition constant (Ki); Bolded amino acid residues indicate those shared with the reference ligand

Source: Primary Data, 2025

This molecular study examined binding affinity (ΔG value) and inhibition constant (Ki) to assess drug efficacy, which is depending upon the binding strength between the ligand and the protein (Kalontong et al., 2022; Tuyen et al., 2023). A more negative binding affinity indicates a stronger receptor-ligand interaction (Xue et al., 2022). The negative sign signifies that the binding between the receptor and the ligand occurs spontaneously and naturally, allowing the ligand to associate with the targeted protein without the requirement for external energy (Agosto, 2024). An optimal Ki value ranges from 10^-6 to 10^-12 M, indicating that a ligand with a strong bond can be difficult to excrete from the body and may hinder activity. Conversely, weak ligand binding allows for easier release prior to inhibition(Mustarichie et al., 2017).

Donor-acceptor hydrogen bonds and hydrophobic interactions significantly influence the strength of the interaction between the ligand and the receptor. Hydrogen binding is assessed based on its ionization potential, which reflects the energy needed to remove an electron from the highest molecular orbital, facilitating donor-acceptor electron transfer between the native ligand and the test ligand. Additionally, hydrophobic interactions generate electrostatic interactions due to differences in polarity (Indratmoko et al., 2023). Their role is important to creating a stable receptor-ligand complex and contributing the binding affinity of the ligand (Ekowati et al., 2023).

Brazilin from C. sappan may inhibit the target protein JAK2, as indicated by its negative binding free energy value and low Ki. This suggests that the test ligand inhibits both the target receptor and the native ligand, thereby reinforcing its role as a JAK2 inhibitor. The docking simulation results shown in Table 3 demonstrate that brazilin inhibits the activity of JAK2 (6VGL) in silico. The native ligand showed a binding energy interaction of -8.71 kcal/mol with the 6VGL protein, whereas brazilin demonstrated a binding energy of -8.37 kcal/mol with the JAK2 protein. Hydrogen bond interactions are present at the amino acid residues Leu932, Asp994, and Asp939 of the 6VGL protein, involving 5 hydrogen bond donors and 4 hydrogen bond acceptors, with bond distances ranging from 1.62 to 3.48 Å.

Nguyen Vo (Nguyen Vo et al., 2016) asserts that compounds possessing high binding capacities have binding energies below -8 kcal/mol. This indicates that brazilin exhibits a high binding energy of -8.37 kcal/mol for the selected protein target. Ligands having highly negative binding affinities are typically regarded as possessing significant inhibitory potential, because their strong binding to an enzyme's active site is often associated with effective inhibition of enzymatic activity by preventing its normal function (Agosto, 2024).

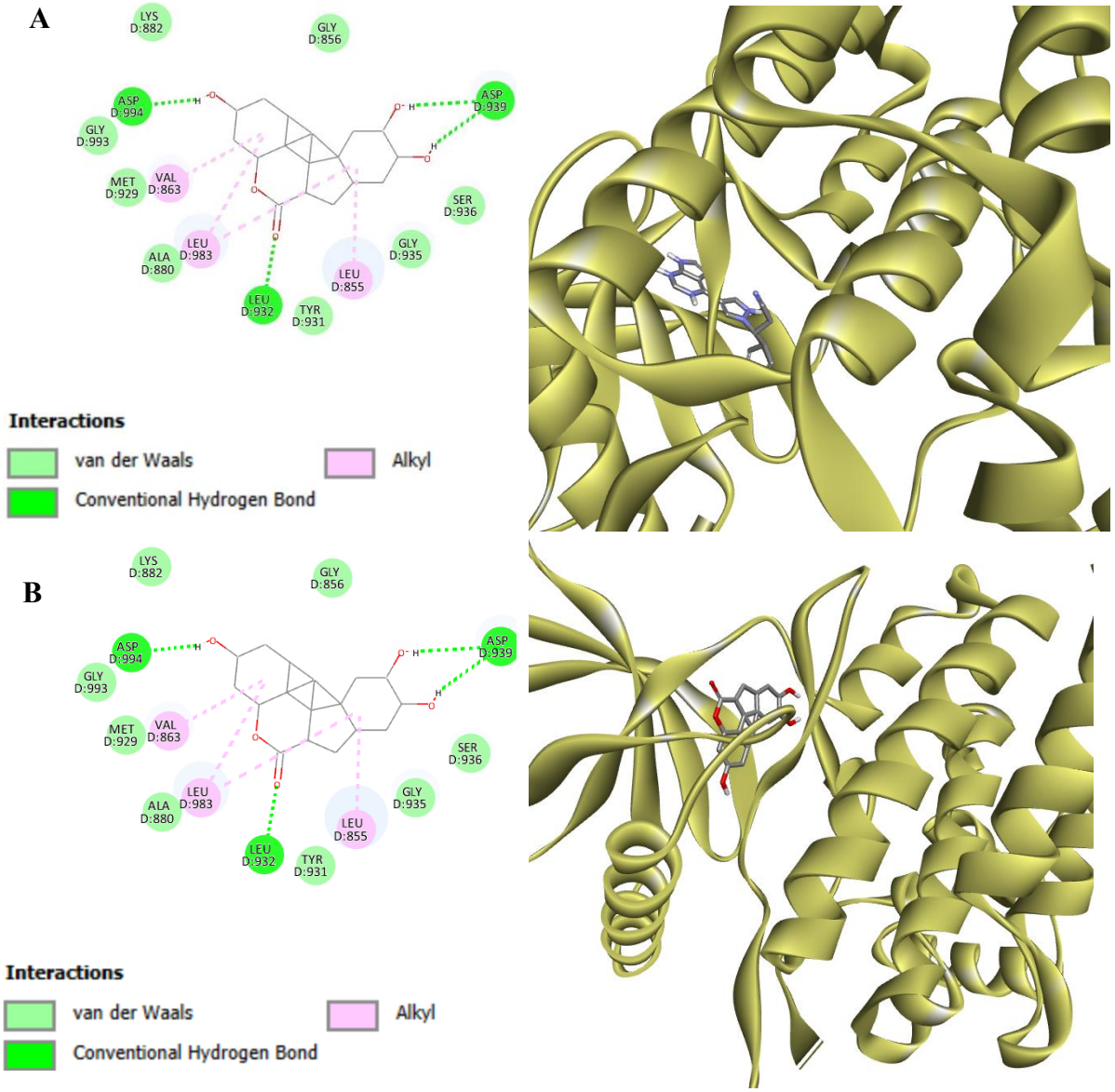

Figure 3. 2D and 3D visualization of the reference ligand ruxolitinib (A) and the test ligand brazilin (B)

Brazilin can inhibit JAK2 due to its binding free energy, which is identical to that of the native ligand, as well as through hydrogen bonds and hydrophobic interactions with the same amino acids as the reference ligand. Active compounds with strong binding to target receptors are characterized by the presence of hydrogen bonds and the same amino acid residues as the control drug. The interaction similarities between ligand amino acid residues and the control drug suggest that brazilin can inhibit the target protein's activity and could potentially act as a replacement for the control drug (Chamata et al., 2020).

This compound has formed hydrogen bonds with two amino acid residues from the native ligand: Leu932 and Asp994. In the docking analysis, brazilin has different versions from the native ligand, specifically at the amino acid Asp939. The lack of similar interactions does not imply that the compound is inactive; rather, it may suggest a new kind of interaction involving different amino acid residues. Consequently, in vitro experiments are required to ascertain this compound's efficacy. Additionally,

brazilin shows hydrophobic bonding similar to that of the native ligand at Leu855, Val863, and Leu983, characterized by π-alkyl interactions. Interestingly, the amino acid Leu932 in the docking ligand forms a hydrogen bond, whereas the native ligand shows a hydrophobic interaction. This occurs because hydrogen and hydrophobic bonds have differing distances. Amino acids separated by 4-6 Å will build Van der Waals interactions. The quantity of these bonds serves as a significant binding factor, despite their weak nature, particularly in high molecular weight compounds due to their influence on the lipid solubility of ligands (Deshmukh et al., 2024). Increased hydrophobic interactions facilitate the solubility of the ligand in the lipid environment, thereby enhancing its ability to penetrate the cell membrane and reach the receptor.

4. Conclusion

According to the results from in silico studies, the brazilin compound derived from sappan wood extract demonstrates potential as a JAK2 inhibitor, effectively inhibiting tyrosine-janus kinase 2 with a PDB code of 6VGL, showing a binding affinity value of -8.37 kcal/mol. The results indicate that the brazilin compound demonstrates efficacy similar to that of the reference ligand ruxolitinib, with a binding energy of -8.71 kcal/mol.

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