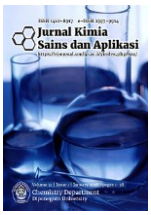
ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 29 (4) (2026): 276-287

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa



Determination of the Activity of Bioactive Compounds from *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth Against Breast Cancer Cells: An *In Silico* Study

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https://doi.org/10.14710/jksa.29.4.276-287

Article Info

Article history:

Received: 07th January 2026
Revised: 05th April 2026
Accepted: 08th April 2026
Online: 25th May 2026

Keywords:
ADMET prediction; *Biancaea sappan*; Breast Cancer; *In Silico*; *Spatholobus ferrugineus*

Abstract

Breast cancer is one of the most prevalent malignancies worldwide, with subtypes expressing hormone receptors such as the Estrogen Receptor (ER), Progesterone Receptor (PR), and HER2 being primary therapeutic targets. This study explores the potential of natural compounds as inhibitors for these key proteins. This research aimed to evaluate the potential of bioactive compounds from sappanwood (*Biancaea sappan* (L.) Tod.), namely Brazilin and its derivative Brazilein, and from the stem of *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth Variabilin, Medicarpin, and its derivative Vestitol, (-)- as candidate inhibitors against the target proteins of breast cancer (ERα, ERβ, PR, and HER2) using a molecular docking methodology. The analysis was performed by comparing the docking scores and interaction patterns of the test compounds against their native ligands and a reference ligand (lapatinib for HER2). Furthermore, *in silico* predictions of their pharmacokinetic (ADMET) profiles and drug-likeness were conducted. The docking results showed that Variabilin exhibited the highest binding affinity for HER2 among the test compounds and demonstrated a high predicted binding affinity for the antagonist conformations of both ERα and ERβ. The other test compounds also showed potential as ERα and ERβ inhibitors; however, none of the five compounds showed potential as PR antagonists. The ADMET analysis predicted that the majority of the compounds possess an acceptable pharmacokinetic profile. Variabilin shows favorable docking to the HER2 kinase site and to ER LBDs. Functional activity requires experimental confirmation.

1. Introduction

Breast cancer is a heterogeneous disease characterized by the uncontrolled growth of abnormal cells in breast tissue. According to data from the Global Cancer Observatory (Globocan) in 2020, breast cancer ranks first as the most commonly diagnosed cancer worldwide, with an estimated 2.3 million new cases (11.7%), and is the fifth leading cause of cancer-related death [1]. The luminal A subtype, characterized by positive estrogen receptor (ER+) and/or progesterone receptor (PR+) expression and low HER2 expression, represents one of the clinically relevant breast cancer groups [2, 3]. The T47D breast cancer cell line belongs to this subtype and is often employed as an in vitro model in hormone-dependent breast cancer studies. HER2 targeted for broader screening, as low-level HER2 signaling contributes to endocrine resistance via ER crosstalk [4].

For HER2-positive breast cancer, targeted therapy commonly involves tyrosine kinase inhibitors (TKIs) such as Lapatinib. Lapatinib functions by blocking the kinase activity of HER2 and epidermal growth factor receptor (EGFR), thereby disrupting critical intracellular signaling cascades, including PI3K/Akt and Ras/MAPK pathways, which regulate proliferation, survival, and metastasis [4, 5]. Although effective, therapeutic limitations such as drug resistance and side effects

highlight the urgency of discovering novel anticancer agents.

Natural products are among the most promising sources of new drug discovery. More than 60% of currently used anticancer agents are derived from natural products or their derivatives, underscoring the importance of exploring biodiversity to identify novel drug candidates with high efficacy and lower toxicity. *Biancaea sappan* (L.) Tod. (commonly known as sappanwood) and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth. are traditional medicinal plants widely utilized in Indonesian ethnomedicine. Sappanwood contains active compounds such as brazilin, sappanchalcone, and protosappanin, which have demonstrated cytotoxic activity against several cancer cell lines, including breast cancer [6, 7]. Meanwhile, *Spatholobus ferrugineus* produces secondary metabolites, including flavonoids, isoflavonoids, pterocarpans, and steroids, which have been reported to exhibit antimicrobial, antioxidant, and anticancer properties [8]. Among these, pterocarpans such as variabilin and medicarpin are of particular interest for their pharmacological potential in cancer therapy [9].

Advancements in computational drug discovery, particularly in silico approaches, have significantly accelerated the identification of novel therapeutic compounds. Molecular docking is a powerful in silico technique used to visualize and quantify ligand interactions with target proteins, as well as to predict binding affinity and stability [9]. In the context of breast cancer, Lapatinib serves as a positive control due to its established mechanism of inhibiting ATP-binding pockets within HER2 and EGFR kinase domains. By employing this approach, bioactive compounds from natural sources, such as brazilin, variabilin, and medicarpin, can be computationally screened for their potential inhibitory effects on breast cancer cells.

Molecular docking provides binding pose hypotheses validated by RMSD (Root Mean Square Deviation) < 2.0 Å reproduction of crystal ligands (pose accuracy), but exhibits scoring uncertainties of 2–3 kcal/mol, limiting absolute affinity predictions [10]. Recent reviews emphasize docking's strength in relative ranking and pharmacophore mapping, while requiring experimental validation for functional activity [11].

Based on this rationale, the present study aims to explore the potential of bioactive compounds from sappanwood and *Spatholobus ferrugineus* as candidate anticancer agents through molecular docking analysis. Additionally, the study evaluates the pharmacokinetic and toxicity (ADMET) profiles of these compounds to provide a strong scientific basis for further drug development.

2. Experimental

The research method used was in silico experimental research by conducting physicochemical screening through drug suitability analysis based on Lipinski's Five Rules, molecular docking, and pharmacokinetic and toxicity predictions of active compounds in (*Biancaea sappan* (L.) Tod.) and (*Spatholobus ferrugineus* (Zoll. and Moritzi) Benth), which have the potential to inhibit the target protein of breast cancer cells.

2.1. Materials and Tools

The materials used were seven target protein structures as target proteins consisting of ERα bound to agonist ligands (PDB ID 1QKU) and antagonists (PDB ID 3ERT), ERβ with agonist ligands (PDB ID 5TOA) and antagonists (PDB ID 1QKM), PR with agonist (PDB ID 3D90) and antagonist ligand (PDB ID 2OVM), and HER2 with ligand (PDB ID 3PP0) downloaded in .PDB format from the PDB website (https://www.rcsb.org). The test ligands used were Brazilin, Variabilin, and Medicarpin, which are bioactive compounds in *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth with their derivatives: Brazilein and Vestitol, (-)- obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The reference compounds used were the native ligands for each target protein; specifically, for the HER2 target protein, lapatinib, an HER2 inhibitor, was used as the ligand reference. The tools used in this study were laptops with AMD Ryzen 3 4300U with Radeon Graphics 2.70 GHz processors, 16.0 GB LPDDR4 RAM, Windows 11 Home Single Language 64-bit operating systems, and the software used was Protein Data Bank (PDB), YASARA 24.9.26, MarvinSketch 22.19, PubChem, PLANTS 1.2, Discovery Studio 2021, SwissADME, and pkCSM.

2.2. Preparation of Target Proteins

The target proteins used were ERα bound to agonist ligands (PDB ID 1QKU) and antagonist ligands (PDB ID 3ERT), ERβ bound to agonist ligands (PDB ID 5TOA) and antagonist ligands (PDB ID 1QKM), PR with agonist (PDB ID 3D90) and antagonist ligand (PDB ID 2OVM), and HER2 with ligand (PDB ID 3PP0) downloaded in .PDB format from the PDB website (https://www.rcsb.org) [12]. The target proteins were then prepared using YASARA 24.9.26 software by separating the native ligands and residues, such as water, to leave only the target proteins, hydrogen was added to the proteins. Energy minimization was performed using the AMBER14 force field (steepest descent, 500 steps, ε = 4.0) to resolve clashes. After preparation, the target proteins were saved in mol2 Sybyl Mol2 format with the file "Protein.mol2" [13].

2.3. Preparation of the Native Ligand

The native ligands used were Estradiol, 4OHT, Tetrahydrochrysene, Levonogestrel, Asoprisnil, and 03Q, which bind to the target protein. The native ligands were prepared using YASARA 24.9.26 by separating the protein and other residues, including water molecules, from the native ligands. The prepared ligands were saved in Sybyl Mol2 (*.mol2) format as "ref_ligand.mol2". Preparation was then carried out using MarvinSketch software. The ligand files were arranged in 2D form and protonated at pH 7.4 to simulate physiological conditions. The resulting files were saved as "ligand_2D.mrv". Subsequently, a conformational search was performed by opening the ligand_2D.mrv file and selecting Tools > Conformation > Conformers, followed by determining the number of

conformations and clicking OK. The generated conformers were then saved as "ligand.mol2" [13].

2.4. Preparation of Test Ligands and Reference Ligands

The structure of test ligands, consisting of bioactive compounds from *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth., namely Brazilin, Brazilein, Variabilin, Medicarpin, and Vestitol (-)-, were obtained from PubChem (https://PubChem.ncbi.nlm.nih.gov/). The structures were downloaded by copying the canonical SMILES and then prepared in MarvinSketch. The ligand files were arranged in 2D format and protonated at pH 7.4 to simulate physiological conditions. The prepared structures were saved as "ligand_2D.mrv". Conformational analysis was then performed using the MMFF94 force field with a systematic search algorithm to generate 100 conformers for each ligand, and the lowest-energy conformer was selected for molecular docking. Subsequently, the optimized structures were saved in Mol2 (*.mol2) format as "ligand.mol2".

2.5. Drug-likeness Analysis

Drug-likeness analysis of test compounds was based on Lipinski's rule of five. The analysis used the SwissADME web server by copying the canonical SMILES of bioactive compounds in *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth, namely: Brazilin, Brazilein, Variabilin, Medicarpin, Vestitol, (-)- uploaded to the web server (http://www.SwissADME.chindex.php). Ghose filter was additionally checked for molar refractivity.

2.6. Molecular Docking Method Validation

Validation was performed using YASARA 24.9.26 to determine the RMSD by combining the original ligand file with either the best conformation file or the one with the lowest docking score from the docking results. It was then analyzed using the analyze > RMSD > molecules step. An RMSD value of less than 2.0 Å was considered acceptable, indicating that the docking method is capable of reproducing the experimentally observed binding pose. Conversely, an RMSD value greater than 2.0 Å suggests insufficient accuracy of the docking protocol.

2.7. Molecular Docking with PLANTS

Molecular docking simulations were performed using PLANTS version 1.2 with the ChemPLP scoring function under a Windows operating system. ChemPLP generates positive fitness scores, where higher values indicate stronger predicted binding affinity. For consistency with conventional reporting (kcal/mol scale), the scores were converted to negative values by multiplying by -1 [13].

The target protein file, native ligand, test ligand, and reference ligand were prepared in a single folder using the PLANTS 1.2 application and its supporting files. Then, the active binding site between the protein and ligand was searched for using the command "PLANTS --mode bind ref_ligan.mol2 protein.mol2". The active site was then saved in Notepad as pc(PDB code).txt. The molecular docking was run with the command: "PLANTS --mode screen pc(PDB code).txt" and waited until the process completed. The molecular docking results were viewed on the command terminal: "cd results" followed by "more bestranking.csv". All ligands, including native ligands, reference compounds, and test ligands derived from *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth., were docked against each target protein. The docking results were ranked according to their binding affinity scores for further analysis.

2.8. Docking Analysis and Visualization

Docking visualization was performed using the Discovery Studio 2021 software. Before visualization, the best ligand docking results were first combined with the target protein file in YASARA 24.9.26 using the edit > join > object command and saved in .PDB format. They were then visualized in Discovery Studio by selecting the receptor ligand interaction > ligand interaction menu. The interaction results were viewed in 2D by pressing the show 2D button, then saved as an image. Amino acid similarity percentage quantifies interaction overlap between test compounds and native ligands. The similarity percentage was calculated using Equation (1).

$$Similarity\ (\%) = \frac{interacting\ residues\ \leq 4.5\ \text{Å}\ with\ native\ ligand}{total\ interacting\ residues\ of\ native\ ligand} \times 100\% \quad (1)$$

2.9. Pharmacokinetic and Toxicity Prediction Results

Predictions were made using the pkCSM web server. The canonical SMILES of the active compounds were copied from the PubChem website and then entered into the pkCSM web server (https://biosig.lab.uq.edu.au/pkCSM/prediction).

3. Results and Discussion

3.1. Results of Drug-Likeness Analysis

Drug-likeness analysis was conducted to evaluate whether the tested compounds possess physicochemical properties consistent with orally active drugs. This analysis was based on Lipinski's Rules, which state that a compound has good absorption or permeability properties if its molecular weight is less than 500 Daltons; LogP value is less than 5; number of hydrogen bond acceptors is less than 10; number of hydrogen bond donors is less than 5 [13]. In addition, the Ghose filter was considered, where acceptable molar refractivity ranges from 40 to 130. The drug-likeness evaluation results for the five bioactive compounds are presented in Tables 1a and 1b.

Table 1a. Results of drug-likeness of the bioactive compound based on Lipinski's rule

Compound	MW (g/mol)	LogP	HBA	HBD	Lipinski violations
Brazilin	286.28	1.49	5	4	0
Brazilein	284.26	1.50	5	3	0
Variabilin	398.54	0.95	5	3	0
Medicarpin	270.28	2.57	4	1	0
Vestitol, (-)-	272.29	0.95	4	2	0

Table 1b. Molar refractivity by the Ghose filter

Compound	MR	Ghose range	Status
Brazilin	75.20	40-130	Accepted
Brazilein	74.39	40-130	Accepted
Variabilin	118.66	40-130	Accepted
Medicarpin	73.17	40-130	Accepted
Vestitol, (-)-	75.62	40-130	Accepted

Note: MW: Molecular Weight; LogP: High lipophilicity; HBA: Hydrogen Bond Acceptors; HBD: Hydrogen Bond Donors; MR: Molar Refractivity

From Tables 1a and 1b, all five bioactive compounds from *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth. showed no violations of Lipinski's Rule of Five. These results suggest that all five compounds have potential as oral drug candidates. If two parameters are assessed to be outside the range, then the compound has poor absorption or permeability. Good absorption and permeability are achieved when a compound meets all parameters and violates a maximum of one criterion [10].

3.2. Validation of Molecular Docking

Method validation is a crucial step in molecular docking studies to ensure that the computational protocol used is capable of accurately reproducing the binding pose of the ligand. The most common validation procedure is redocking. In this method, the native ligand extracted from the protein crystal structure was reattached to the binding pocket of the same protein. The success of the validation was then evaluated using the RMSD parameter. A low RMSD value (< 2.0 Å) indicates that the program successfully predicts the conformation and orientation of the ligand very similarly to the experimental conditions. The smaller the RMSD value, the higher the accuracy and reliability of the docking method used [10, 11].

Based on Figure 1 and Table 2, the RMSD values of all seven PDB codes are less than 2.0 Å. and the resulting poses are close to the original ligand conformations. The validation results show that all seven PDB codes are valid and can be used for the next stage of the docking process.

Table 2. Numeric RMSD values

PDB ID	Target	RMSD value (Å)
1QKU	ERα agonist	0.362
3ERT	ERα antagonist	1.5834
5TOA	ERβ agonist	0.2532
1QKM	Erβ antagonist	1.1512
3D90	PR agonist	1.1594
2OVM	PR antagonist	0.9084
3PPO	HER 2 Kinase	1.3734

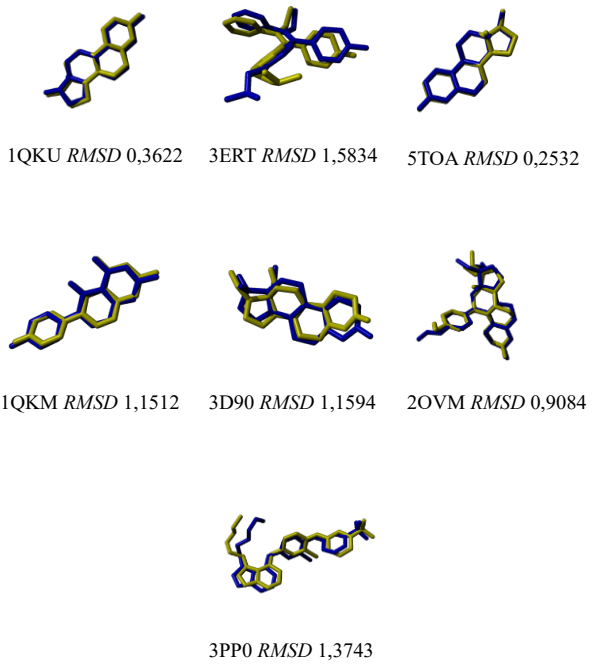

Figure 1. Molecular docking validation results, initial ligand (blue) and re-docked ligand (yellow)

3.3. Molecular Docking

Molecular docking was performed to evaluate the binding affinity and interaction patterns of the test ligands with the target proteins. The primary output of the docking simulation is the docking score, which reflects the predicted stability of the protein–ligand complex. In this study, more negative docking scores indicate stronger binding affinity and greater interaction stability between the ligand and the target protein [10].

Interacting residues were defined as amino acids located within 4.5 Å of any ligand heavy atom, as identified using Discovery Studio (2021) based on the docked protein–ligand complexes. Based on the docking results in Table 3, Variabilin consistently exhibited the most favorable docking scores among all tested compounds across the target proteins (1QKU, 3ERT, 5TOA, 1QKM, 3D90, 2OVM, and 3PP0). Notably, in the 3D90 protein, Variabilin (-102.209) showed a more favorable score than the native ligand (levonorgestrel: -89.2104) and demonstrated complete hydrophobic residue overlap (100% similarity), indicating a comparable binding environment.

For the HER2 target (3PP0), the crystallographic ligand (03Q) yielded a docking score of -121.047, while the reference inhibitor lapatinib showed the most favorable score (-135.108), consistent with its established clinical efficacy. Variabilin (-116.832) ranked between the native ligand and the reference compound, indicating strong binding potential, although not exceeding that of the clinical inhibitor. These results suggest that Variabilin is a promising natural compound with significant binding affinity, but further optimization and experimental validation are required to achieve clinical-level potency [14].

Table 3. Docking scores of native ligands and test compounds against target proteins

PDB ID	Ligand	Docking score	Interacting residues (≤ 4.5 Å)
1QKU	Native ligand (estradiol)	-87.7767	9
	Brazilin	-75.0958	9
	Brazilein	-79.913	7
	Variabilin	-97.4835	11
	Medicarpin	-78.5269	6
	Vestitol, (-)-	-77.0049	8
3ERT	Native ligand (4-Hydroxytamoxifen)	-99.5912	5
	Brazilin	-79.493	6
	Brazilein	-73.7802	3
	Variabilin	-104.624	3
	Medicarpin	-74.4804	7
	Vestitol, (-)-	-76.5301	5
5TOA	Native ligand (Estradiol)	-94.0731	7
	Brazilin	-75.2569	6
	Brazilein	-78.4171	6
	Variabilin	-88.5905	12
	Medicarpin	-72.693	8
	Vestitol, (-)-	-73.3588	5
1QKM	Native ligand (Genistein)	-95.0057	9
	Brazilin	-77.6889	4
	Brazilein	-81.5565	6
	Variabilin	-87.0888	15
	Medicarpin	-70.9333	9
	Vestitol, (-)-	-78.1741	8
3D90	Native ligand (levonogestrel)	-89.2104	8
	Brazilin	-77.3572	7
	Brazilein	-78.6225	7
	Variabilin	-102.209	10
	Medicarpin	-80.1713	8
	Vestitol, (-)-	-79.5601	6
2OVM	Native ligand (Asoprisnil)	-102.896	5
	Brazilin	-76.2248	5
	Brazilein	-76.4835	5
	Variabilin	-104.28	17
	Medicarpin	-76.8233	9
	Vestitol, (-)-	-79.6356	6
3PP0	Lapatinib	-135.108	10
	Native ligand (03Q)	-121.047	10
	Brazilin	-73.3986	7
	Brazilein	-86.0285	7
	Variabilin	-116.832	13
	Medicarpin	-76.1545	10
	Vestitol, (-)-	-77.1869	7

Variabilin shows a preference for the ERα antagonist conformation, with a more favorable docking score in 3ERT (-104.624) compared to 1QKU (-97.4835). This trend suggests a potential tendency to stabilize the inactive receptor state associated with antagonist binding, which is typically characterized by helix-12 displacement [15]. A similar pattern was observed for ERβ, where binding affinities for 1QKM (-87.0888) and 5TOA (-88.5905) indicate comparable interactions across conformational states [16]. Although molecular docking predicts binding affinity rather than functional activity, these findings suggest that Variabilin may exhibit

characteristics consistent with selective estrogen receptor modulators (SERMs). Therefore, it can be considered a potential SERM candidate for Luminal A breast cancer, pending further experimental validation through reporter gene assays and co-activator recruitment studies [17].

3.4. Docking Analysis and Visualization

Visualization was performed using software such as Discovery Studio to display the protein-ligand complex in 3D. The aim was to observe in detail the binding pose of the ligand and identify specific non-covalent interactions formed between the ligand and amino acid residues in the protein binding pocket [13]. For estrogen receptor (ER) and progesterone receptor (PR) targets, additional structural considerations are required, as these receptors adopt distinct agonist and antagonist conformations depending on ligand binding. In the context of ER/PR-positive breast cancer, inhibition of receptor activity is desired; therefore, compounds that preferentially bind to the antagonist conformation are of particular interest.

In docking analysis, this preference is evaluated by comparing binding affinities across receptor conformations. Compounds exhibiting more favorable docking scores (i.e., more negative values) in antagonist structures (e.g., 3ERT and 1QKM) relative to agonist structures (e.g., 1QKU and 5TOA) may indicate a tendency toward antagonist-like binding behavior. However, it is important to note that docking predicts binding affinity and pose, but cannot definitively distinguish between agonistic and antagonistic functional outcomes. Therefore, experimental validation, such as reporter gene assays or cell proliferation studies, is required to confirm receptor inhibition [18].

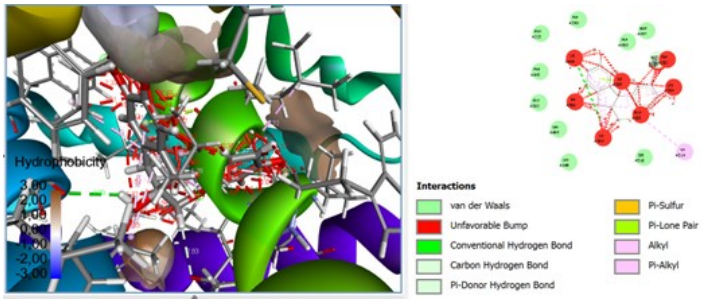

Figure 2. Visualization of Brazilin on protein ERα code 1QKU

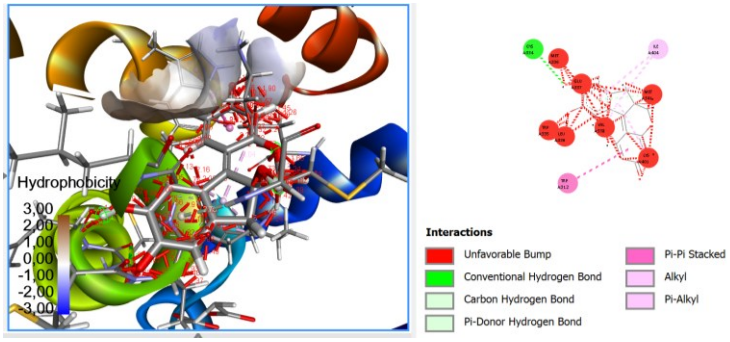

Figure 3. Visualization of Brazilein on protein Erβ code 1QKM

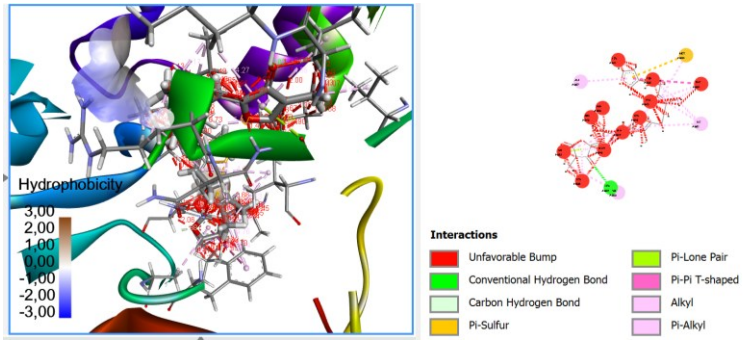

Figure 4. Visualization of Variabilin on protein HER2 code 3PP0

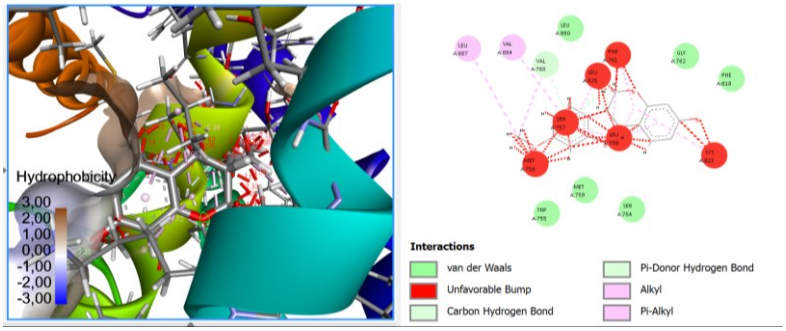

Figure 5. Visualization of Medicarpin on protein PR code 3D90

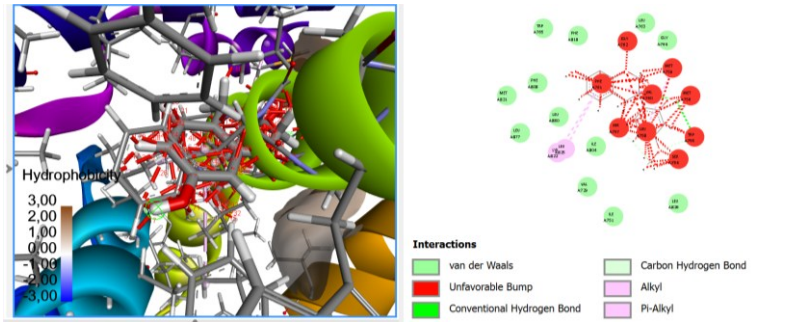

Figure 6. Visualization of Vestitol, (-)- on protein HER2 code 2OVM

Based on Figures 2, 3, 4, and 5, the docking poses indicate that hydrophobic interactions were dominant in stabilizing most complexes, while hydrogen bond formation varied depending on the protein target and ligand. Based on Figure 6, hydrogen bonds are relatively strong and directional non-covalent interactions that play an important role in binding stability and specificity of protein–ligand complexes. Most of the tested compounds (Brazilin, Brazilein, Variabilin, Medicarpin, and Vestitol, (-)-) were able to form hydrogen bonds with target proteins, although variability was observed depending on the binding site. Variabilin and Vestitol, (-)- showed limited hydrogen bond formation in several targets (e.g., Variabilin in 1QKU, 5TOA, 1QKM, and 3PP0; Vestitol, (-)- in 1QKU and 3PP0), suggesting that their binding is not primarily driven by polar interactions.

In contrast, Brazilin and Brazilein consistently formed hydrogen bonds, particularly in 3ERT and 1QKM, while Medicarpin also exhibited hydrogen bonding interactions in multiple targets, including 1QKM, 3ERT, and 3PP0. For the HER2 target (3PP0), Medicarpin formed a hydrogen bond with the hinge residue THR862, similar to the reference ligand lapatinib, indicating a comparable binding feature. In contrast, Variabilin did not form the typical hinge hydrogen bond with THR862 but exhibited a greater number of hydrophobic interactions (13 residues compared to 10 for lapatinib). This different binding suggests a potential new mechanism.

Target inclusion (ERα/β, PR, and HER2) reflects an exploratory multi-target strategy beyond the T47D subtype. Although T47D cells are HER2-negative, evaluation of HER2 binding remains relevant for heterogeneous breast cancer cases and resistance mechanisms. Notably, Variabilin demonstrated relatively strong binding to HER2 (3PP0; docking score -116.832), suggesting potential activity that warrants further validation in HER2-positive cell models.

For PR, potential as antagonists requires superior affinity in the antagonist-bound conformation (PDB 2OVM; Asoprisnil native) compared to agonist (PDB 3D90) [19]. All test compounds showed weaker binding vs Asoprisnil (e.g, Variabilin -104.28 vs -102.90). With fewer interactions (5-10 bound residues vs native 5), this model indicates no competitive PR antagonism potential.

Variabilin exhibited the most favorable docking scores across nearly all target proteins, indicating strong predicted binding affinity. Notably, in 1QKU, Variabilin showed a highly favorable docking score (-97.4835) despite the absence of hydrogen bonds, suggesting that hydrophobic and van der Waals interactions may play a dominant role in stabilizing the complex in this binding environment. The amino acid residues involved in hydrogen bonding vary depending on the protein and ligand. For example, residues such as GLU323 and LYS449 were frequently observed in interactions within 1QKU and 3ERT. Additional specific interactions included MET388 (3ERT) and CYS334 (1QKM), which were involved in hydrogen bonding with Brazilein, as well as GLY762 (3D90) and SER757 (2OVM), which interacted with Variabilin and Brazilin/Brazilein.

Hydrophobic interactions (as seen from the Number of interacting residues (≤4.5 Å)) are often the main contributor to total binding energy, especially if hydrogen bonds are not formed. The Variabilin ligand consistently shows the highest number of hydrophobic amino acids bound (11 in 1QKU, 13 in 3ERT, 12 in 5TOA, 15 in 1QKM, 10 in 3D90, and 17 in 2OVM). This high number of interactions correlates with its best docking scores (most negative), such as -104.624 in 3ERT and -104.28 in 2OVM, indicating that Variabilin tends to bind through extensive hydrophobic interactions. Other ligands, such as Brazilin, Brazilein, Medicarpin, and Vestitol (-)- show fewer hydrophobic bonds (around 3 to 9 residues).

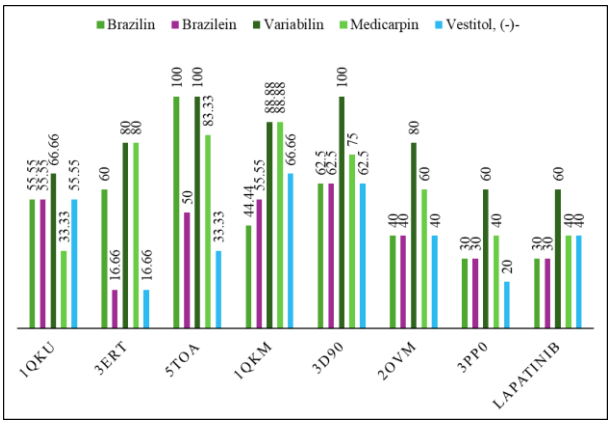

Figure 7. Graph of amino acid similarity percentage of test compounds vs. native ligands

Figure 7 shows the extent to which the test compound interacts with amino acid residues that are the same as natural ligands. A high percentage of similarity is a strong indicator that the compounds may have a similar mechanism of action. Brazilin exhibited 100% similarity in 5TOA, interacting with hydrophobic residues (VAL338, LYS401, ILE404, and MET340) similar to those involved in estradiol binding. Variabilin showed 100% similarity in 3D90 and 80% in 3ERT, indicating a relatively strong overlap with native ligand interaction patterns in these receptors.

Overall, Variabilin demonstrated consistently strong binding affinity across most target proteins. This behavior is primarily associated with extensive hydrophobic interactions, as reflected by a high number of interacting residues and relatively high similarity percentages with native ligands. These interaction profiles suggest that Variabilin binds effectively within or near the native ligand binding pocket.

Molecular docking predicts binding affinity but cannot assess functional consequences (agonist vs. antagonist activity) [20]. Although preferential binding to antagonist conformations may serve as a useful screening indicator, it does not directly confirm receptor modulation. Therefore, all lead compounds require further experimental validation using cell-based assays to evaluate their effects on receptor activity and downstream biological responses, including T47D cell proliferation.

3.5. ADMET Prediction

In silico ADMET analysis was performed to accurately predict the in vivo pharmacokinetic properties of prospective therapeutic compounds in humans using only virtual structures, thereby reducing the time and resources required for the rational design of new drug candidates [21]. This approach enables rapid evaluation of drug-likeness and helps prioritize compounds with favorable pharmacokinetic profiles.

Key pharmacokinetic parameters were predicted using pkCSM, including Caco-2 permeability, human intestinal absorption, skin permeability, steady-state volume of distribution (VDss), blood–brain barrier (BBB) permeability, central nervous system (CNS) permeability, and total clearance [22]. These descriptors were used to assess the absorption, distribution, and elimination characteristics of the candidate compounds.

Computational (*in silico*) absorption evaluation aims to predict the extent to which a compound can be absorbed by the body, especially through the gastrointestinal tract after oral administration. This process is very important because poor absorption is one of the main reasons drug candidates fail in clinical development. There are seven absorption predictors predicted using pkCSM [21].

Table 4. Results of bioactive compound absorption assessment

Parameter	Compounds					Requirements
	Brazilin	Brazilein	Variabilin	Medicarpin	Vestitol (-) -	
Solubility in water (log mol/L)	-2.94	-2.424	-6.846	-3.912	-3.573	> -6
Caco-2 permeability (log Papp in 10^{-6} cm/sec)	0.169	0.576	1.155	1.318	1.291	> 0.90
Human intestinal absorption (%)	89.97	59.621	90.785	96.515	93.858	> 30
Skin permeability (log Kp)	-3.735	-2.772	-2.97	-3.185	-3.293	> -2.5
P-glycoprotein substrate	Yes	No	No	Yes	Yes	-
P-glycoprotein inhibitor I	No	No	Yes	No	No	-
P-glycoprotein inhibitor II	No	No	No	No	No	-

The absorption analysis results in Table 4 show that all five test compounds (Brazilin, Brazilein, Variabilin, Medicarpin, and Vestitol, (-)-) generally meet the predefined acceptance criteria for key oral absorption parameters. However, all compounds exhibit suboptimal skin permeability values, indicating limited suitability for transdermal delivery.

Among the tested compounds, Variabilin demonstrates the most favorable overall absorption profile, supported by acceptable Caco-2 permeability (1.155×10^{-6} cm/s) and moderate predicted human intestinal absorption (59.621%). Therefore, formulation strategies such as salt formation, use of co-solvents, or amorphous solid dispersions may be required to improve its solubility and enhance oral delivery performance [23].

Drug distribution describes the transport of compounds from systemic circulation to tissues and target sites, influencing both pharmacological efficacy and potential side effects. In silico distribution models estimate the extent of tissue distribution, plasma protein binding, and compound localization after absorption. Once in circulation, drugs exist either in free form or bound to plasma proteins, which directly affects their bioavailability and therapeutic activity [20]. There are four distribution predictors predicted using pkCSM [21].

Based on the prediction results in Table 5, the distribution prediction analysis of the five test compounds indicates that Brazilein has the best distribution value. The metabolic process aims to convert foreign compounds (xenobiotics), including drugs, into water-soluble (polar) molecules that can be more easily excreted from the body. Metabolism is a major determining factor that affects the duration of action, efficacy, and potential toxicity of a drug [20].

Based on Table 6, the compounds Brazilin, Variabilin, and Medicarpin tend to be substrates of CYP3A4 and inhibitors of the CYP1A2 enzyme isoform. Meanwhile, Vestitol, (-)- is only an inhibitor of CYP2C19, while Brazilein is not a substrate for CYP2D6 and CYP3A4 and is also not an inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4.

Prediction of the excretion rate of a compound is an important component in pharmacokinetic evaluation. This process, primarily carried out by the kidneys through urine and also via the biliary/fecal pathway, determines how long a drug remains in the body. This elimination rate is quantitatively described by the Total Clearance parameter. Total Clearance (CL_{tot}) represents the volume of blood plasma cleared of a drug per unit time (usually mL/min/kg). This parameter combines all elimination pathways, including hepatic metabolism and renal excretion. There are two excretion predictors predicted by pkCSM [21], which are presented in Table 7.

Table 5. Results of bioactive compound distribution assessment

Parameter	Compounds					Requirements
	Brazilin	Brazilein	Variabilin	Medicarpin	Vestitol, (-)-	
VDss (human) (log L/kg)	-0.527	0.614	0.268	-0.351	-0.427	> 0.45
Unbound fraction (human) (log Fu)	0.272	0.466	0	0.161	0.189	The higher, the better
BBB permeability (log BB)	-0.614	-0.593	-0.154	0.19	0.053	< -1 indicates poor CNS penetration. > -1 suggest possible CNS entry
SSP permeability (log SP)	-2.322	-2.531	-2.005	-1.422	-2.052	< -3

Table 6. Results of bioactive compound metabolism assessment

Parameter	Compounds				
	Brazilin	Brazilein	Variabilin	Medicarpin	Vestitol, (-)-
CYP2D6 Substrate	No	No	No	No	No
CYP3A4 Substrate	Yes	No	Yes	Yes	No
CYP1A2 Inhibitor	Yes	No	Yes	Yes	Yes
CYP2C19 Inhibitor	No	No	No	Yes	Yes
CYP2C9 Inhibitor	No	No	No	No	No
CYP2D6 Inhibitor	No	No	No	No	No
CYP3A4 Inhibitor	No	No	No	No	No

Table 7. Results of bioactive compound excretion assessment

Parameter	Compounds				
	Brazilin	Brazilein	Variabilin	Medicarpin	Vestitol, (-)-
Total Clearance (log mL/min/kg)	0.151	0.262	1.435	0.221	0.302
OCT2 Renal Substrate	No	No	No	No	No

Table 8. Results of bioactive compound toxicity assessment

Parameter	Compounds					Requirement
	Brazilin	Brazilein	Variabilin	Medicarpin	Vestitol, (-)-	
AMES toxicity	Yes	Yes	No	Yes	Yes	No
Maximum tolerated dose (human) (log mg/kg/day)	0.818	-0.247	0.051	1.001	1.186	≤ 0.477
hERG Inhibitor I	No	No	No	No	No	No
hERG Inhibitor II	Yes	No	No	Yes	Yes	No
Acute oral toxicity in rats (LD_{50}) (mol/kg)	2.166	2.103	2.421	2.274	2.181	-
Chronic toxicity in rats. oral (LOAEL) (log mg/kg/day)	2.186	2.075	2.468	2.076	2.161	-
Hepatotoxicity	No	No	Yes	No	No	No
Skin sensitization	No	No	No	No	No	No
T. pyriformis toxicity (log µg/L)	0.628	0.343	1.511	1.276	1.422	< -0.5
Minnow toxicity (log mM)	1.859	3.031	-1.428	0.695	0.908	> -0.3

Total clearance prediction results (Table 7) show that Variabilin has the highest total clearance value compared to Brazilin, Brazilein, Medicarpin, and Vestitol, (-)-. This indicates that Variabilin is relatively more rapidly excreted from the body compared to the other compounds. Fast clearance may limit exposure despite favorable docking results [24].

Toxicity evaluation is one of the most crucial stages in the drug discovery and development cycle. Before a candidate compound can be tested in humans, its safety profile must be confirmed through a series of rigorous toxicity tests. The main purpose of these tests is not only to determine the safe threshold of a substance but also to identify and characterize potential toxic effects on various organ systems [14]. Ten toxicity predictors were generated using pkCSM [21] to determine the toxicity threshold of a compound.

Table 9. Results of bioactive compound toxicity assessment

Compound	Molecular weight (g/mol)	LD_{50} (mol/kg)	LD_{50} (mg/kg)	Classification toxicity
Brazilin	286.28	2.166	620.082	Slightly toxic
Brazilein	284.26	2.103	597.798	Slightly toxic
Variabilin	398.5	2.421	964.768	Slightly toxic
Medicarpin	270.28	2.274	614.616	Slightly toxic
Vestitol, (-)-	272.29	2.181	593.864	Slightly toxic

Based on Table 8, the AMES test indicates that all test compounds exhibit mutagenic potential and potential carcinogenic risk, except Variabilin, which shows a negative result. Variabilin emerges as a lead candidate with a non-mutagenic profile (AMES negative) and the best docking performance, despite requiring solubility optimization. In the maximum tolerated dose test, Brazilin, Medicarpin, and Vestitol, (-)- show lower tolerance values because their results exceed the acceptable threshold (log ≤ 0.477 mg/kg/day), whereas Brazilein and Variabilin meet the requirement (log ≤ 0.477 mg/kg/day).

In hERG inhibitor predictions, none of the five compounds is predicted as hERG I inhibitors. However, Brazilin, Medicarpin, and Vestitol, (-)- are predicted as hERG II inhibitors. Therefore, the compounds with lower potential for cardiotoxicity related to hERG inhibition are Brazilein and Variabilin. In hepatotoxicity prediction, only Variabilin is predicted to be hepatotoxic, while the other four compounds are non-hepatotoxic. Although pkCSM classifies Variabilin as hepatotoxic, this represents a preliminary screening alert from a predictive model without associated confidence scoring. Therefore, experimental hepatotoxicity evaluation is recommended for prioritized candidates [25].

In chronic toxicity prediction, Variabilin shows the highest LOAEL value among the five compounds. This value represents the lowest dose threshold associated with potential toxicity after repeated exposure. A higher LOAEL indicates a lower potential for chronic toxicity at equivalent doses [15, 16]. In the overall toxicity prediction, all compounds from *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth are indicated to have certain toxicity risks depending on the parameter evaluated. In minnow toxicity prediction, only Variabilin is classified as toxic, while the other four compounds are predicted as non-toxic.

From Table 9, the five bioactive compounds from *Biancaea sappan* (L.) Tod. and *Spatholobus ferrugineus* (Zoll. and Moritzi) Benth are classified as slightly toxic. Among the compounds, Variabilin shows the highest predicted LD_{50} value, while the other four compounds show lower LD_{50} values.

4. Conclusion

Among the five bioactive compounds tested, Variabilin shows preferential binding to antagonist conformations of ERα/ERβ and strong affinity toward HER2, indicating potential for further experimental validation regarding its functional antagonism and anticancer activity. This is supported by consistently favorable (lowest) docking scores across most target proteins, including ERα, PR, and HER2. Brazilin and Medicarpin show amino acid interaction similarities comparable to those of the reference ligands, while Variabilin demonstrates the highest overall similarity to native ligand interaction patterns. All tested compounds satisfy Lipinski's Rule of Five, indicating good drug-likeness properties. Based on ADMET analysis, Brazilin and Variabilin show relatively promising pharmacokinetic profiles; however, Variabilin may require further evaluation due to predicted hepatotoxicity and low aqueous solubility.

Acknowledgement

The author would like to thank the Directorate of Research, Technology, and Community Services (DRTPM), Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, for providing research funding under the Penelitian Dosen Pemula (PDP) 2025 scheme. This research was supported under Grant No. 0917/LL3/AL.04/2025 and 007/SPK-HibahDikti/P/V/2025.

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