Jurnal Kimia Valensi, Vol 9(1), May 2023, 163-182
Available online at Website: http://journal.uinjkt.ac.id/index.php/valensi

The Virtual Screening of Flavonoid Derivatives on Progesterone, Estrogen, and HER-2 Receptor for Breast Cancer Treatment Candidate

Ade Dwi Septian[1], Gatut Ari Wardani[1], Richa Mardianingrum[2], Ruswanto Ruswanto[1*]

[1]Pharmacy Study Program, Faculty of Pharmacy, Universitas Bakti Tunas Husada
Jl. Cilolohan 36 Tasikmalaya, Indonesia.
[2]Department of Pharmacy, Health Science Faculty, Universitas Perjuangan Tasikmalaya
Jl. PETA No. 177, Indonesia.

**Corresponding author: ruswanto@universitas-bth.ac.id*

Received: March 2023; Revision: June 2023; Accepted: June 2023; Available online: June 2023

Abstract

Cancer is defined as a disease caused by progressive and abnormal cell proliferation in the body. This condition is caused by deoxyribonucleic acid (DNA) changes, which causes cells to lose their normal function. The aim of this study is to find that flavonoid compounds have a more stable interaction than tamoxifen as anti-cancer candidates. Research has been conducted in silico with molecular docking (AutodockTools-1.5.7) and molecular dynamics of 200 flavonoid compounds. Furthermore, pharmacokinetic parameters, toxicity, and the application of the Lipinski Rule of Five were investigated. Based on molecular docking results, the compounds eriocotrin, glabrol, kaempferitrin, linarin, and narirutin have more stable interactions with lower binding energy (ΔG) than tamoxifen. From the results of molecular docking, molecular dynamics, and pharmacokinetic studies, it is predicted that the kaempferitrin compound can be used as an anti-cancer candidate and does not cause toxicity through further research.

Keywords: AutoDock; cancer; flavonoids; molecular docking; molecular dynamics.

DOI: *10.15408/jkv.v9i1.31482*

1. INTRODUCTION

Cancer is defined as a disease caused by progressive and abnormal cell proliferation in the body. This condition is caused by deoxyribonucleic acid (DNA) changes, which causes cells to lose their normal function. These cancer cells spread (metastasize) to other organs in the body, and infiltration can cause progressive loss of organ function and death (Hartini et al., 2020). Breast cancer is the most frequent cancer globally, reaching 2.2 million cases worldwide (11.7%) of the world population. (Globocan, 2020).

Surgery, radiation, and systemic therapy, which includes chemotherapy, targeted therapy, hormone therapy, and immunotherapy, are all common cancer treatments. These targeted therapies are listed in the National Cancer Data Base (NCDB). Chemotherapy combines both targeted therapy and immunotherapy for uniformity and comparability (Miller et al., 2019). However, chemotherapy has limitations, namely exacerbating low-level toxicities such as diarrhea and insomnia can interfere with the patient's Health-Related Quality of Life (HRQOL). Multiple oncologic therapy symptoms, including urination, nausea, night sweats, and digestive symptoms, influence the concurrent effect of chemotherapy on insomnia symptoms. Chemotherapy frequently has negative side effects, including changes to taste and smell. (Binotto et al., 2020).

Plants contain phytochemical components such as phenolic acids, flavonoids, carotenoids, stilbenes, and lignans, which have been shown to prevent the development and spread of breast cancer. (Selvakumar et al., 2020). Flavonoids are natural products and can be used as a prerequisite for preventing breast cancer recurrence in patients and its occurrence in healthy individuals (Ezzati et al., 2020). Flavonoids have been examined *in vitro* and *in vivo* and have shown outstanding anti-cancer action as well as inhibiting drug-target protein

P-ISSN: 2460-6065, E-ISSN: 2548-3013

overexpression in cancer cells (Baksi et al., 2018). Flavonoids have received a great deal of interest due to their numerous biological roles, which include antioxidant, anti-inflammatory, and anticancer properties. They have been proven by clinical trials in patients with different characteristics such as age, sex, and disease stage (Kikuchi et al., 2019). Flavonoids have also been tested in silico against the receptor Cyclin-Dependent Kinase 8 (6T41) 2.45 Å (Rathod et al., 2022), mTOR (5H64) 4.40 Å (Sharma et al., 2020) and it is proven that flavonoids have interactions with cancer receptors. However, some receptors have better resolution including Progesterone (1ZUC) 2.00 Å, Estrogen (5W9C) 1.80 Å, and HER-2 (7PCD) 1.77 Å (Protein Data Bank, n.d.).

The process of discovering and developing new medications is commonly known to be time-consuming and resource-intensive (Surabhi & Singh, 2018). Computational approaches can reduce the number of ligands tested in biological testing, which reduces the cost, time, and effort necessary to produce novel medications (Salman et al., 2021). Based on this knowledge, researchers conducted a study in which they computationally developed anticancer drug candidates utilizing molecular docking methodologies, molecular dynamics, pharmacokinetic, and toxicity studies of flavonoid chemicals in numerous plants. This study has found that flavonoid compounds have a stable interaction with breast cancer receptors, give a better score on parameters of Lipinski drug similarity, pharmacokinetic profile, and toxicity, and achieve better cancer treatment compared to tamoxifen.

2. MATERIALS AND METHODS

Materials

Equipment used in the form of software and hardware. AutodockTools-1.5.7, Discovery Studio version 21.1, Molegro Molecular Viewer, MarvinSketch version 22.19.0, Amber, Desmond academic software, and several web-based programs such as Protein Data Bank (PDB), PubChem, SAVES, Lipinski's Rule of Five and pKCSM. The hardware used is a laptop with AMD Ryzen 5 5600H specifications with Radeon Graphics (12CPU) ~ 3.30 GHz, 8.00 GB of RAM 64-bit Operating System of Windows 11, and a personal computer with Intel® Xeon(R) CPU E5-267 specifications v2 @ 2.70 GHz (24CPU), NVIDIA GeForce RTX 3060, 7.7 GB of RAM and the Linux Ubuntu 18.04.5 LTS.

The materials used for this research were 200 flavonoid compounds obtained from Biopurify and downloaded from PubChem. Progesterone Receptor (1ZUC), Estrogen Receptor (5W9C), HER-2 Receptor (7PCD).

Receptor Analysis

The PDB receptor profile was examined via the website http://www.ebi.ac.uk/pdbsum/ for receptor analysis. Enter the PDB code for the receptor, and profile data for the receptor will be displayed and examined using the Ramachandran plot, with 0.8% prohibited sections (Ruswanto, Garna, et al., 2018). The subsequent analysis is ERRAT with the website https://saves.mbi.ucla.edu/ and the generally accepted range of values is >50 (Elengoe *et al*., 2022).

Receptor Preparation

The initial step in receptor preparation involves dissociating the macromolecular chain that binds the target. Progesterone Receptor (1ZUC), Estrogen Receptor (5W9C), and HER-2 Receptor (7PCD) downloaded from Protein Data Bank (PDB) (Rachmania *et al*., 2018). The Molegro Virtual Docker application is used to separate native ligands, macromolecules, and removing water molecules. The result of this separation is the receptor structure ready for macromolecular optimization. This structure is saved in .pdb format. (Ruswanto et al., 2018).

The receptor's native ligand must also be removed because the ligand binds to the receptor's active site and can prevent other ligands' binding. Water molecules must also be removed to not interfere with the binding and interaction processes formed so that only the protein-ligand interacts (Dermawan et al., 2019).

The following step is to fine-tune the addition of hydrogen atoms to the Discovery Studio Visualizer application (Mardianingrum et al., 2020). The hydrogen atoms are added because some hydrogen atoms are lost during protein-ligand complex separation, particularly when employing the X-ray crystallographic diffraction method (Victory et al., 2018). Then the file is saved in .pdb format.

The gasteiger charge was then applied to the macromolecules using the AutodockTools-1.5.7 software, then a non-polar merge was

performed so that only polar hydrogen atoms appeared (Sari et al., 2020). Then save the structure in .pdbqt format.

Ligand preparation

The PubChem website was used to download 200 flavonoid compounds in total. All flavonoid compounds are protonated at a pH of 7.4 to obtain a structure following the body's physical condition. Geometry is optimized to achieve a stable molecule structure with a low potential energy. The MMFF94 approach is used for optimization. The MMFF94 method is a force field-based method for energy minimization. This method can help improve the accuracy and efficiency of molecular docking results (Hanif et al., 2021). Then the optimized flavonoid compounds are stored in the .pdb file format. Furthermore, the flavonoid compounds were converted into .pdbqt format using the AutodockTools-1.5.7 software.

Docking Validation and Analysis of Docking Results

The docking method was validated by re-tagging the previously separated ligand to the receptor with the AutodockTools-1.5.7 application. The method validation steps must demonstrate the method's validity. If the resulting Root Mean Square Deviation (RMSD) value is less than 2Å, the docking approach is deemed to be good (Leung et al., 2019).

AutoDock and AutoDock Vina are among the fastest and most widely used molecular docking programs (Aziz et al., 2016). AutoDock Vina makes designing and running simultaneous simple and complex docking simulations and multi-ligand docking against a single target easy. The grid box settings used must be the same as during method validation. This is done so that the ligands of the flavonoid compounds interact with the receptors (Eberhardt et al., 2021).

The molecular docking data were investigated by selecting the conformational ligand with the lowest binding energy (ΔG). The results of receptor and ligand molecular docking are then saved in.pdb format. Then, using Discovery Studio software, the interaction between the ligand and the receptor active site was investigated (Utami et al., 2020).

Molecular Dynamics

The docking results are utilized to calculate the stability of the protein-ligand binding relationship in a molecular dynamics simulation. Several factors, including compound conformation, water molecules, ions, cofactors, compound protonation, and changes in solvent entropy, influence the results of molecular dynamics simulations (Ruswanto et al., 2022).

The structures of the best candidate flavonoid compounds for breast cancer receptors were carried out by molecular dynamics simulations using Desmond Schrödinger LLC software (Alnajjar et al., 2020) was first released for free in June 2008 (Chow et al., 2008). The TIP3P water model was used to imitate physiological ion concentrations using 0.15 M NaCl. Molecular dynamics simulations were performed in an orthorhombic box with buffer dimensions of 10 Å × 10 Å × 10 Å at 300 K and 1.01325 bar utilizing ensemble NPT (Number of Atoms, Pressure, and Temperature). Each simulation was ran for 100 ns with a 1.2 ps recording interval (Kumar et al., 2020).

Screening Ligan-Based Drugs Likeness (Drug Scan)

The finest flavonoid compounds are subjected to a drug Scan. The rules of good pharmaceuticals were used to make observations, which include lipophilicity <5, molecular weight <500 g/mol, hydrogen bond donors < 5, refractory molar between 40-130, and hydrogen bond acceptors <10. These parameters can be determined using the website http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp (Ruswanto *et al*., 2020). The Lipinski Rule Of Five was set up to be a conservative predictor in an era where drugs produced too many compounds with very poor physicochemical properties. If a compound fails the Lipinski Rule Of Five test, there is a high probability that problems with oral activity will be encountered. However, passing the Lipinski Rule Of Five test does not guarantee that a compound is like a drug (Lipinski, 2004).

Pharmacokinetic Analysis (ADME and Toxicity)

The pharmacokinetic properties ADME and optimal toxicity prediction of flavonoid

compounds were determined using the pkCSM web tools (Pires et al., 2015). The structure of the best flavonoid compounds is translated into SMILES format. In this SMILES format, compounds are processed using pkCSM online tools(http://biosig.unimelb.edu.au/pkcsm/prediction) to estimate ADME and chemical toxicity (Mvondo et al., 2021).

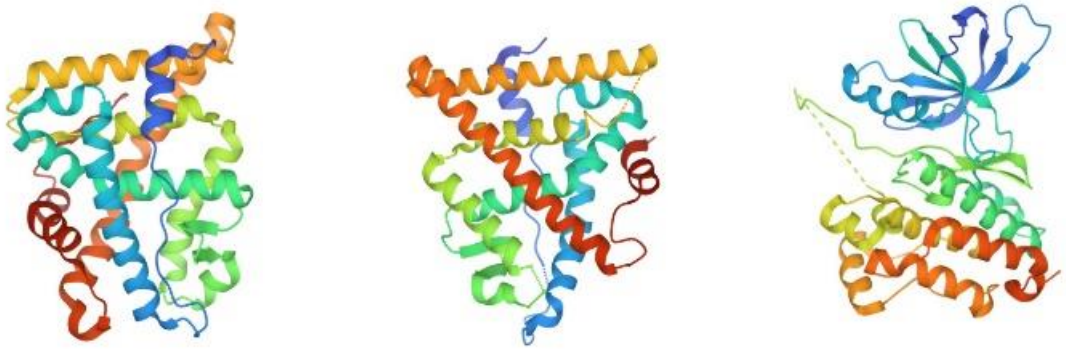
3. RESULTS AND DISCUSSION

Receptor Analysis

Receptor analysis was performed on Progesterone Receptor with pdb code 1ZUC, Estrogen Receptor with pdb code 5W9C, and HER-2 Receptor with pdb code 7PCD for breast cancer. Each receptor's structure (Figure 1) was retrieved from the Protein Data Bank (PDB) and saved in the.pdb file format. Receptor analysis was performed using PROCHECK and ERRAT.

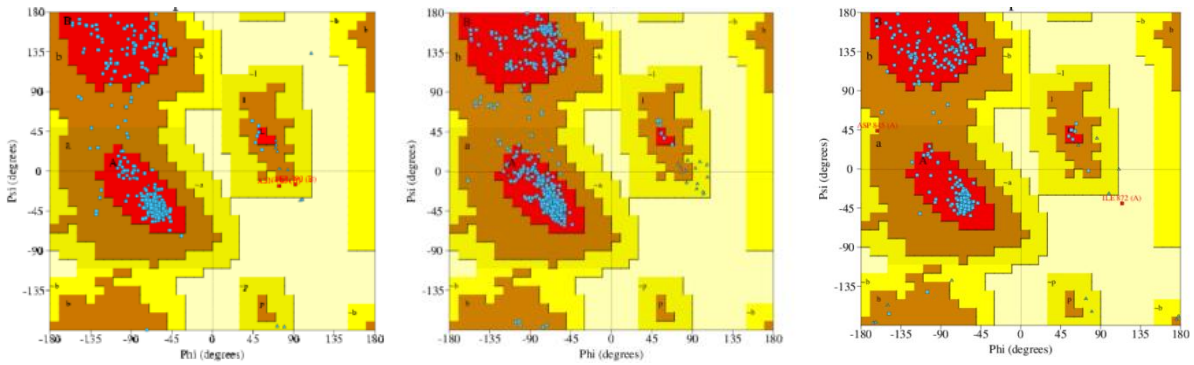
PROCHECK inspection by Ramachandran plots (Figure 2) focuses on specific geometries and allows the overall structure to be evaluated (Puspita et al., 2021). To see if the Ramachandran plot is good for protein structure analysis, it can be checked by plotting non-glycine residues in disallowed regions less than 0.8% (Ruswanto et al., 2018).

According to the Ramachandran plot study of the progesterone receptor (1ZUC), 93% of the amino acid residues are found in the regions that are most favored, while only 0.0% are found in the regions that are forbidden. 95.6% of the amino acid residues of the estrogen receptor (5W9C) are in the most preferred areas, whereas just 0.0% are in the forbidden regions. The number of amino acid residues in the most preferred areas of the HER-2 receptor (7PCD) is 93.6%, whereas the number of residues in the forbidden regions is 0.4%. The results of the analysis showed that progesterone receptor (1ZUC), estrogen receptor (5W9C), and HER-2 receptor (7PCD) have a stable protein structure.



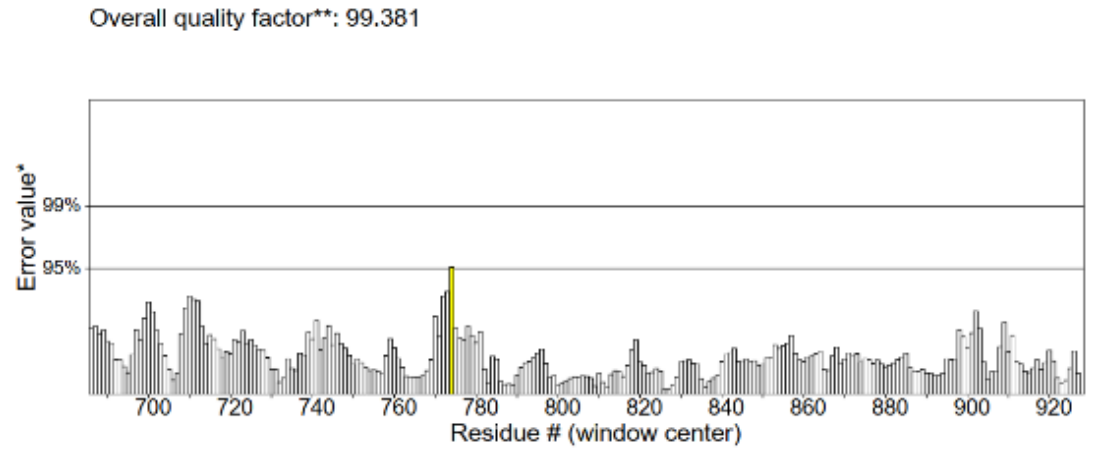
(a) (b) (c)

Figure 1. Structure of (a) progesterone receptor (1ZUC); (b) estrogen receptor (5W9C); and (c) HER-2 receptors (7PCD**)**

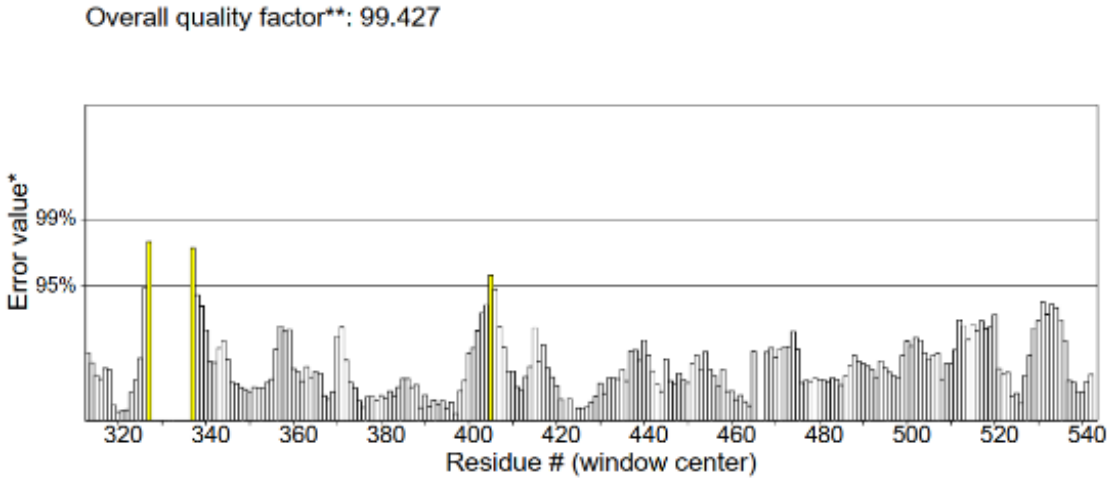


(a) (b) (c)

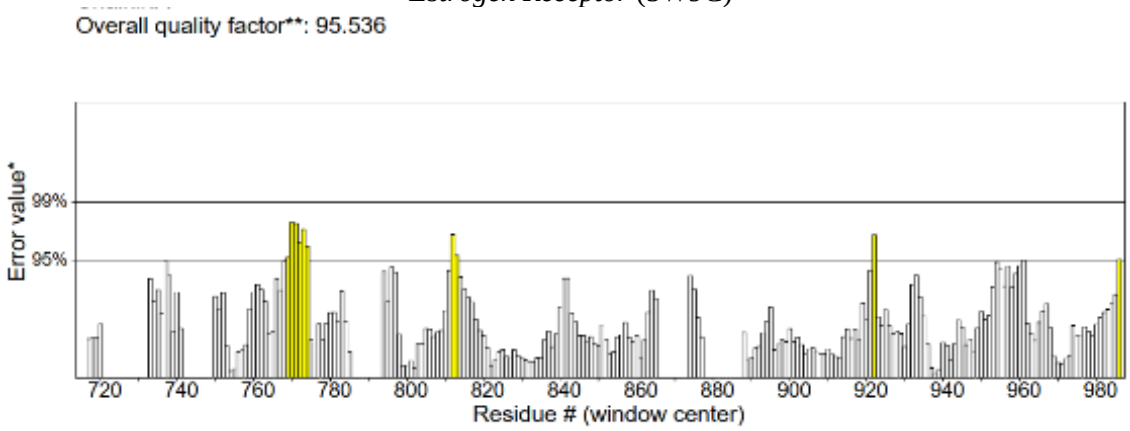
Figure 2. Ramachandran plot of (a) progesterone receptor (1ZUC); (b) estrogen receptor (5W9C); and (c) HER-2 receptors (7PCD)



Progesterone Receptor (1ZUC)



Estrogen Receptor (5W9C)



HER-2 Receptor (7PCD)

Figure 3. ERRAT Analysis Results

ERRAT validates models with statistical relationships of unbound interactions between different types of atoms based on the nature of the atomic interactions, with higher scores indicating higher quality. ERRAT also provides results with significance and overall quality, with a generally accepted score of over 50, constituting a stable protein (Elengoe et al., 2022).

From the results of the ERRAT analysis (Figure 3), the quality factor values were 99.381% for Progesterone Receptor (1ZUC), 99.247% for Estrogen Receptor (5W9C), and 95.536% for HER-2 receptor (7PCD). The results of these calculations show that the

protein structures of the progesterone receptor (1ZUC), estrogen receptor (5W9C), and HER-2 receptor (7PCD) receptors are of high quality and high-resolution protein structures are neglected. It shows that the model is modeled with a possible error. Standards for amino acid residues in binding proteins.

Receptor and Ligand Preparation

The ligand preparation was protonated in accordance with the blood pH, which was 7.4. The ligand was conformed so that it could engage with the receptor in the most stable location possible. The conformation with the lowest energy was chosen from among the ten conformations created for use in the following stage. Additionally, receptor preparation was performed to remove water molecules from the receptor so that the interaction between the receptor and the ligand would be unaffected.

Method Validation and Docking

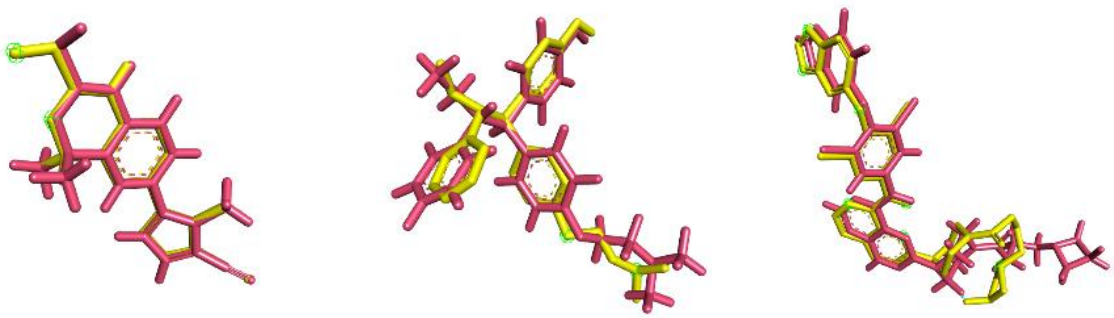
Software called AutodockTools-1.5.7 was used for validation. The active site of the co-crystalline ligands was validated using the redocking approach, namely 5-(4,4-dimethyl-2-thioxo-1,4-dihydro-2h-3,1-benzoxazin-6-yl)-1-methyl-1h-pyrrole-2-carbonitrile for Progesterone Receptor (1ZUC), 4-hydroxytamoxifen for Estrogen Receptor (5W9C) and 1-[4-[4-[[3,5-bis(chloranyl)-4-([1,2,4]triazolo[1,5-a]pyridine-7-yloxy)phenyl]amino]pyrimido[5,4-d]pyrimidin-6-yl]piperazine-1-yl]-4-(3 -fluoranylasethidine-1-yl)butan-1-one for HER-2 Receptor (7PCD) on crystallographic results (Table 1). If the root mean square deviation (RMSD) is less than 2, the approach is considered justifiable (Ramadhan et al., 2020).

The validation findings for each receptor demonstrate that the RMSD value for each receptor is ≤ 2.0, indicating that the docking method's validation is valid (Table 1). After obtaining RMSD ≤ 2Å, a virtual screening process was carried out using AutoDock Vina on the test compound using a grid box with the same settings in the validation process. In this study, virtual screening were performed 20 times for each ligand to obtain the best binding energy (ΔG).

Results of molecular docking of 200 flavonoid compounds using AutoDock Vina showed that all flavonoid compounds interacted with Progesterone Receptor (1ZUC), Estrogen Receptor (5W9C), and HER-2 Receptor (7PCD). However, only the five best compounds were taken, which had the potential to treat breast cancer, and the strongest interactions were towards the HER-2 Receptor (7PCD), namely the compounds eriocitrin, glabrol, kaempferitrin, linarin, and narirutin. The results of molecular docking using AutoDock Vina can be seen in Table 2.

Table 1. Method Validation Results

PDB Code	Grid Box			RMSD (Å)
	X	Y	Z	
1ZUC	27.623	-7.733	9.371	0.53
5W9C	15.045	-10.616	-26.4	0.63
7PCD	8.413	-8.996	-13.532	1.73



Progesterone Receptor (1ZUC) Estrogen Receptor (5W9C) HER-2 Receptor (7PCD)

Figure 4. Overlay Results for Each Receptor

Next, the HER-2 Receptor (7PCD) was docked using AutodockTools-1.5.7 software because the binding energy (ΔG) on the HER-2 Receptor (7PCD) is smaller than the Progesterone Receptor (1ZUC) and Estrogen Receptor (5W9C).

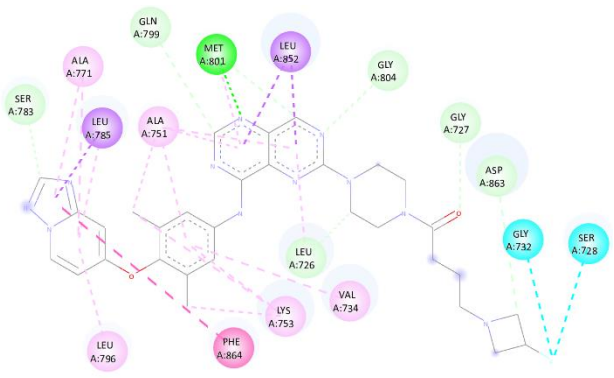
Visualization and Analysis of Docking Results

Docking results were displayed in 2D (Figure 5) and 3D (Figure 6) using Discovery Studio Software. Interactions formed between ligands with the lowest binding energies and amino acid residues of the progesterone receptor (1ZUC), estrogen receptor (5W9C), and HER-2 receptor (7PCD) were observed.

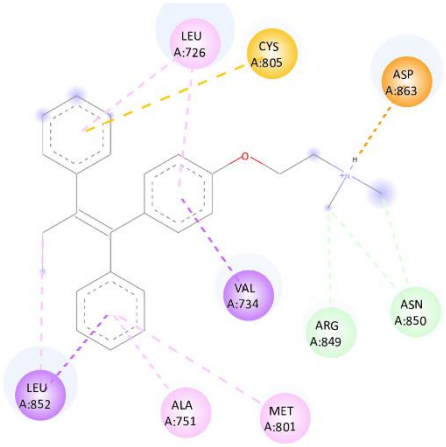
Based on the docking results (Figure 5), the bonds between the ligand and HER-2 Receptor (7PCD) are known to be conventional hydrogen bonds, Van der Waals bonds, hydrophobic bonds, Pi-Sigma bonds, and other bonds.

Table 2. Results of molecular docking of flavonoid compounds against progesterone receptor (1zuc), estrogen receptor (5W9C), and HER-2 receptor (7PCD) using AutoDock Vina

Receptors	Compound	Binding Energy (kcal/mol)
1ZUC	Baicalin	-10.3
	Chrysin-7-o-glucuronide	-10.2
	Eriodictyol	-10.1
	Oroxylin A 7-o-beta-d-glucoronide	-10.1
	Oroxylin A glucoronide	-10.1
5W9C	Apigenin-7-O-glucuronide	-9.9
	Baicalin	-9.8
	Chrysin-7-O-glucuronide	-9.7
	Luteolin 7 glucuronide	-9.9
	Scutellarin	-9.9
7PCD	Eriocitrin	-10.4
	Glabrol	-10.5
	Kaempferitrin	-10.3
	Linarin	-10.2
	Narirutin	-10.4



Native Ligand



tamoxifen

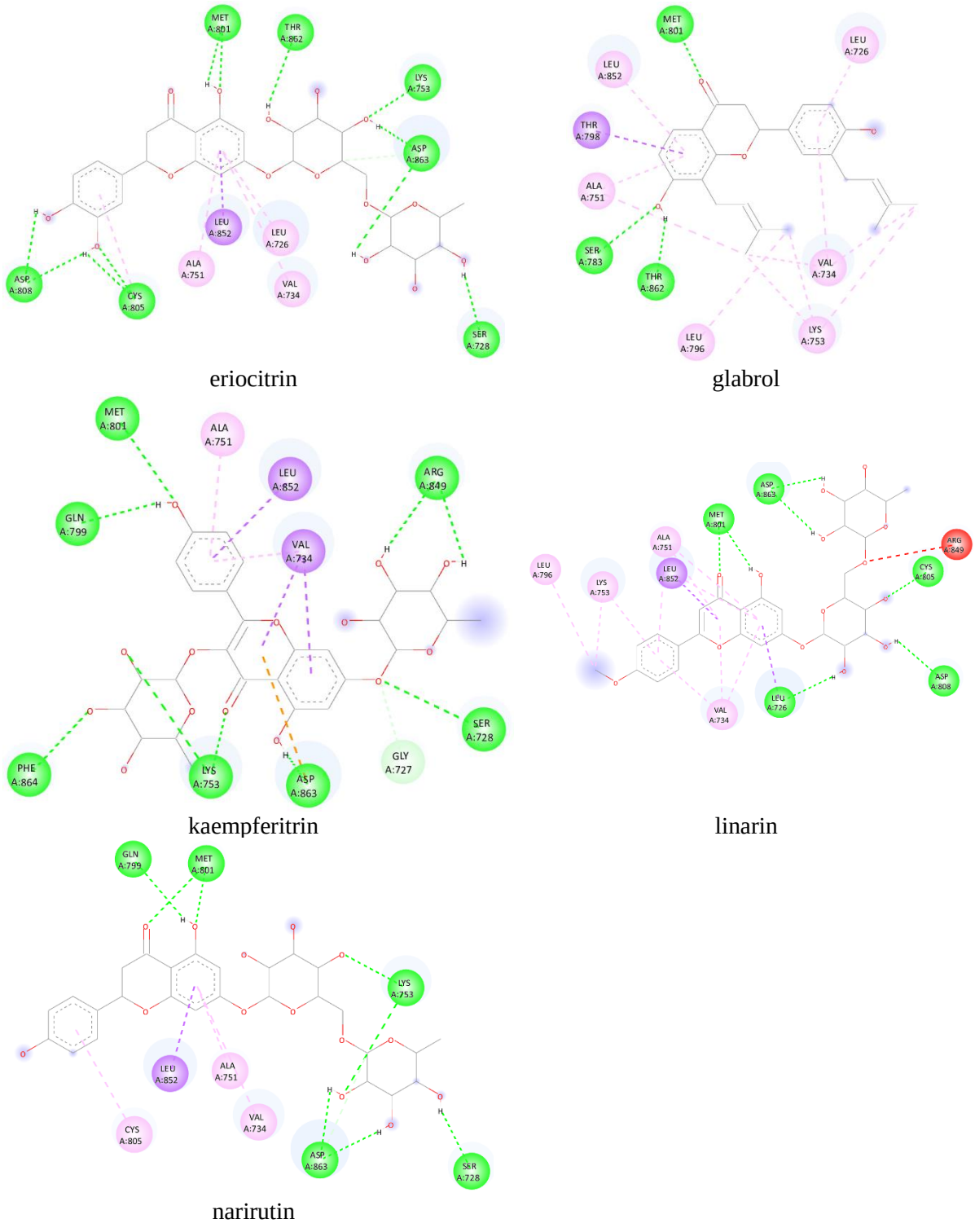


Figure 5. 2D Visualization of Native ligands, tamoxifen, eriocitrin, glabrol, kaempferitrin, linarin, and narirutin

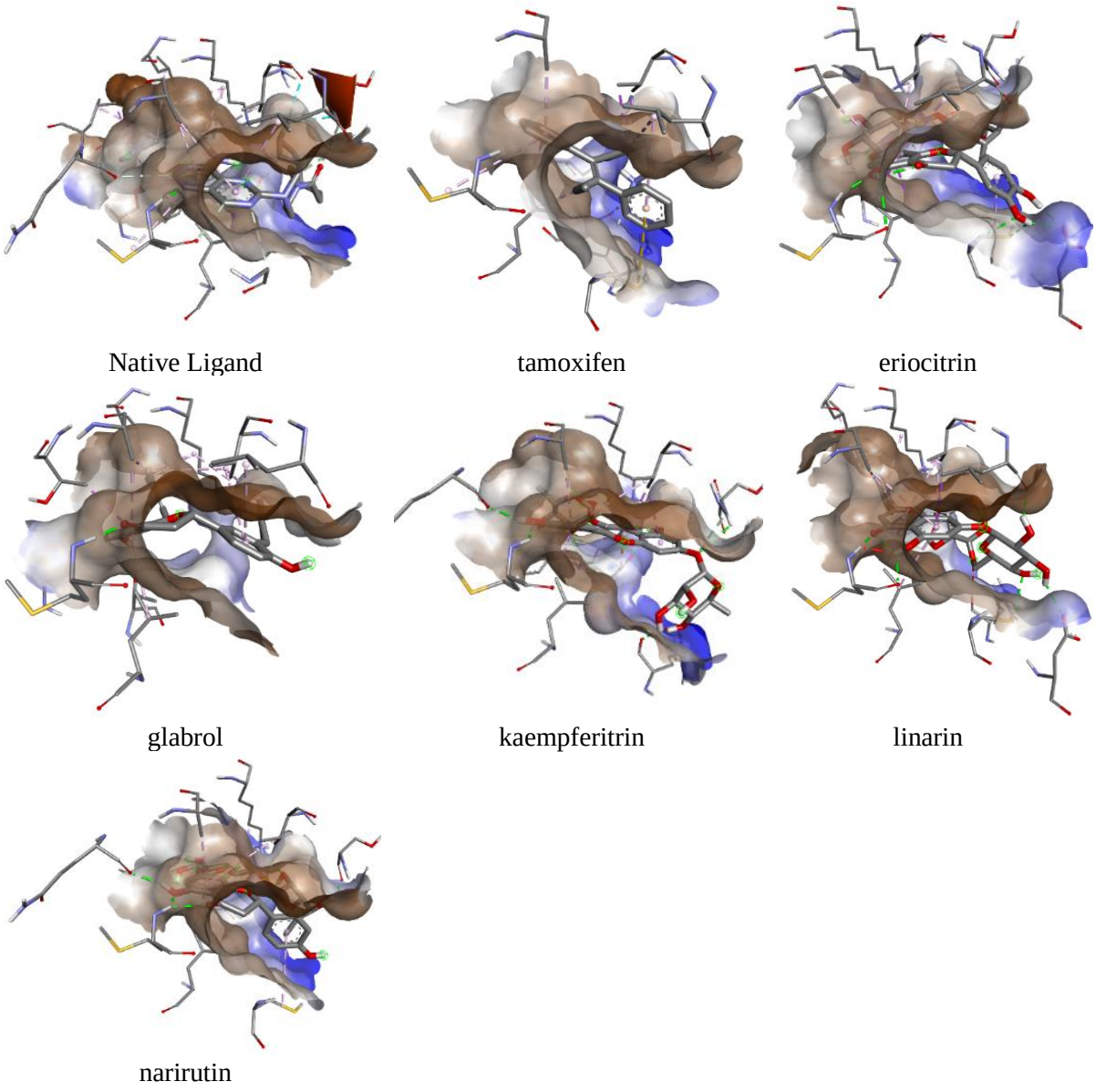


Figure 6. 3D Visualization of Native ligands, tamoxifen, eriocitrin, glabrol, kaempferitrin, linarin, and narirutin

Based on Table 3, it can be seen that the binding affinity between the ligand and the receptor. All of the best flavonoid compounds have a lower free energy value (ΔG) compared to the anticancer comparator compound on the market, namely tamoxifen. Free energy value (ΔG) of narirutin with a ΔG value of -8.97 kcal/mol and a Ki value of 268.02 μM, glabrol with a ΔG value of -8.72 kcal/mol and a Ki value of 407.83 μM, eriocitrin with a ΔG value of -8.71 kcal /mol and a Ki value of 411.52 μM, linarin with a ΔG value of -8.52 kcal/mol and a Ki value of 570.51 μM, kaempferitrin with a ΔG value of -8.27 kcal/mol and a Ki value of 873.62 μM. The free energy value (ΔG) of tamoxifen is -7.77 kcal/mol with a Ki value of 2.02 μM, so the affinity of the compounds narirutin, glabrol, eriocitrin, linarin, and kaempferitrin for HER-2 receptors is higher than the comparator compound, tamoxifen. This shows that the compounds narirutin, glabrol, eriocitrin, linarin, and kaempferitrin are predicted to have stable interactions and have more potential as anti-breast cancer drugs than the compounds on the market, namely tamoxifen because they have lower binding energy (ΔG) and inhibition constant (Ki). (Qasaymeh et al., 2019).

In the interaction between HER-2 Receptor (7PCD) and native ligands, comparator compounds (tamoxifen) and test compounds (eriocitrin, glabrol, kaempferitrin, linarin, narirutin) showed that there were interactions between native ligands and amino acid residues of 17 bonds and a total of 1

hydrogen bonds (MET A:801). With the presence of hydrogen bonds between flavonoids and receptors it will improve the interactions that occur. Then the interaction between the comparator compound (tamoxifen) with amino acid residues of 9 bonds and has no hydrogen bonds. Then in the test ligand, eriocitrin interacts with amino acid residues for 11 bonds and a total of 7 hydrogen bonds (MET A: 801, THR A: 862, LYS A: 753, ASP A: 863, SER A: 728, CYS A: 805, ASP A:808), glabrol interacts with 10 bonds of amino acid residues and a total of 3 hydrogen bonds (MET A:801, SER A:783, THR A:862), kaempferitrin interacts with 10 bonds of amino acid residues and a total of 6 bonds hydrogen (MET A:801, GLN A:799, ARG A:849, SER A:728, ASP A:863, LYS A: 753), linarin interacts with amino acid residues for 11 bonds and a total of 5 hydrogen bonds (META A:801, ASP A:863, CYS A:805, ASP A:808, LEU A:726), narirutin interacts with amino acid residues for 9 bonds and a total of 5 hydrogen bonds (MET A:801, GLN A:799, LYS A:753, ASP A:863, SER A:728). If the active molecule binds strongly through hydrogen bonds and can bind to one of the same amino acid residues from the active side, it is considered to have a strong connection with the target receptor (Frimayanti et al., 2021). This shows that the compounds narirutin, glabrol, eriocitrin, linarin, and kaempferitrin have strong bonds with target receptors because they have the same bonds in hydrogen bonds, namely (META A: 801).

Table 3. Molecular docking results analysis of tamoxifen and selected compounds against HER-2 receptors

Compounds	ΔG (kcal/mol)	KI (μM)	Hydrogen Bonds	Hydrophobic Bonds
Native ligands	-10.01	45.79	**MET A:801**	SER A:783, GLN A:799, GLY A:804, GLY A:727, ASP A:863, LEU A:726, GLY A:732, SER A:728, LEU A:785, LEU A:852, PHE A:864, ALA A:771, ALA A:751, VAL A;734, LYS A:753, LEU A:798
Tamoxifen	-7.77	2.02	-	CYS A:805, ASP A:863, ASN A:850, ARG A:849, VAL A:743. LEU A;852, LEU A:726, MET A:801, ALA A:751
Eriocitrin	-8.71	411.52	**MET A:801**, THR A:862, LYS A:753, ASP A:863, SER A:728, CYS A:805, ASP A:808	LEU A:852, LEU A:726, ALA A:751, VAL A:734
Glabrol	-8.72	407.84	**MET A:801**, SER A:783, THR A:862	THR A:798, LEU A:852, LEU A:726, VAL A:734, LYS A:753, LEU A:796, ALA A:751,
Kaempferitrin	-8.27	873.62	**MET A:801,** GLN A:799, ARG A:849, SER A:728, ASP A:863, LYS A: 753	ALA A:751, LEU A:752, VAL A:734, GLY A:727
Linarin	-8.52	570.51	**MET A:801,** ASP A:863, CYS A:805, ASP A:808, LEU A:726	LEU A:796, LYS A:753, ALA A:751, LEU A:852, ARG A: 849, VAL A:734
Narirutin	-8.97	268.02	**MET A:801,** GLN A:799, LYS A:753, ASP A:863, SER A:728	CYS A:805, LEU A:852, ALA A:751, VAL A:734

Note: Words in bold indicate the same interaction with native ligands.

Molecular Dynamic

Molecular dynamics simulations were conducted for the best binding compound, tamoxifen (comparison), and the native ligand compound against the 7PCD receptor (Table 3). This molecular dynamics simulation was performed during a 100 ns simulation by examining the RMSD (Figure 7) and RMSF (Figure 8) graphs. RMSD measures the average change in atomic displacement to measure how much the shape of the protein changes. RMSF measures the variation of amino acid residues in a protein chain (Ruswanto et al., 2022).

Tamoxifen, eriocitrin, glabrol, kaempferitrin, linarin, narirutin, and native ligand showed RMSD ranging from 0 to 30 ns, according to the RMSD graph (Figure 7). From 30 ns to the completion of the experiment, the RMSD of 100 ns was reasonably consistent. The RMSD graph demonstrates that, for a 100 ns molecular dynamics simulation, kaempferitrin has the most stable contact stability when compared to the other chemicals. During the 100 ns simulation, the RMSD average value supports this result (Table 4).

Kaempferitrin compounds can be utilized to illustrate the stability of the interaction based on the RMSF plot (Figure 8). Residual variation has been shown to have the same range of variation. However, the kaempferitrin compound has a lower fluctuation than the native ligand when viewed from the average RMSF of the kaempferitrin compound (1.129 Å) and the native ligand (1.363 Å). The graph proves that the kaempferitrin compound has a more stable interaction than native ligands.

Table 4. Average RMSD and RMSF values

Compounds	Average	
	RMSD (Å)	RMSF (Å)
Tamoxifen	3.447	1.174
Eriocitrin	4.168	1.676
Glabrol	4.531	1.561
Kaempferitrin	3.317	1.129
Linarin	4.027	1.463
Narirutin	3.974	1.427
Native ligands	3.400	1.363

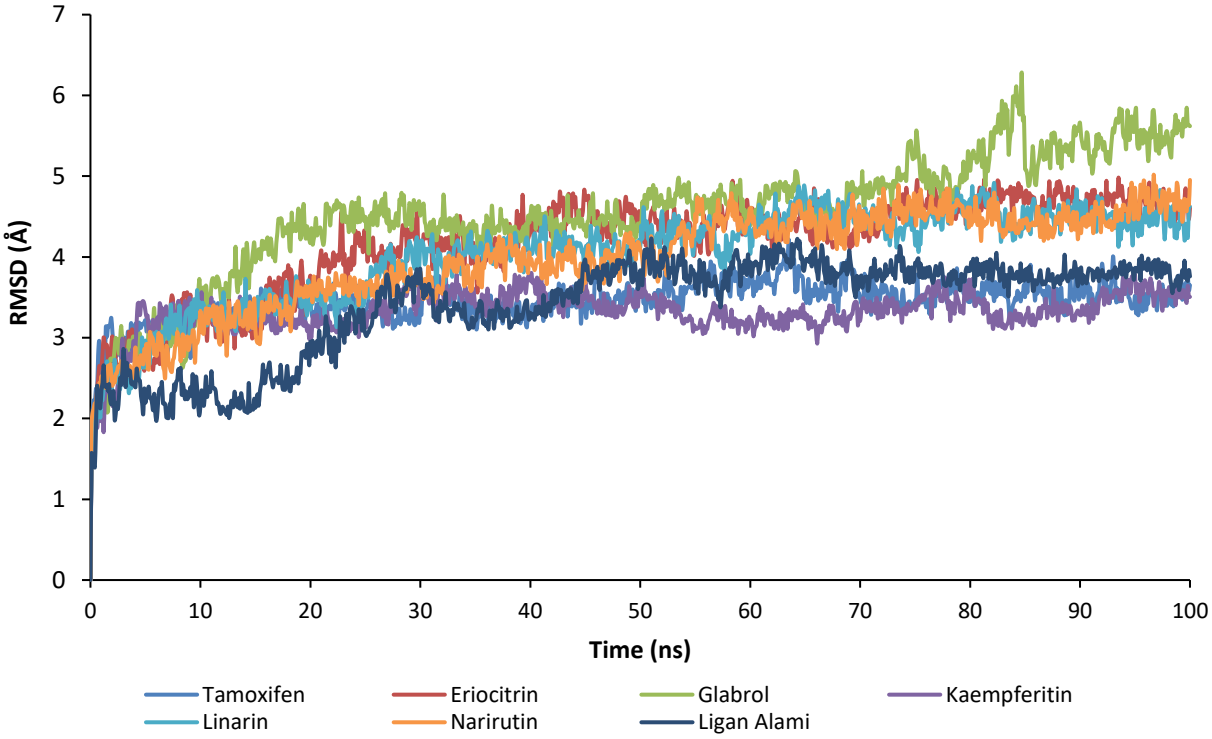


Figure 7. RMSD graph: Tamoxifen (dark blue), Eriocitrin (orange), Glabrol (gray), Kaempferitrin (yellow), Linarin (light blue), Narirutin (green), Native ligand (dark blue)

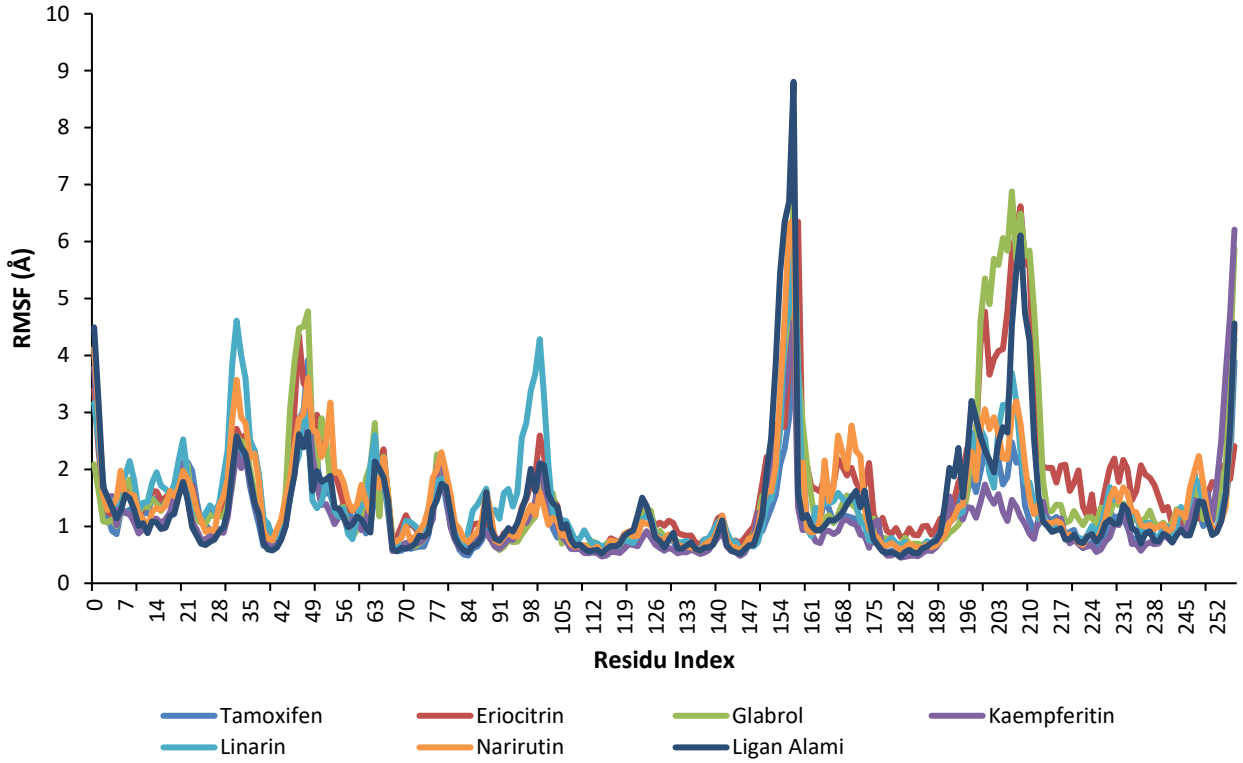


Figure 8. RMSF graph: Tamoxifen (dark blue), Eriocitrin (orange), Glabrol (gray), Kaempferitrin (yellow), Linarin (light blue), Narirutin (green), Native ligand (dark blue).

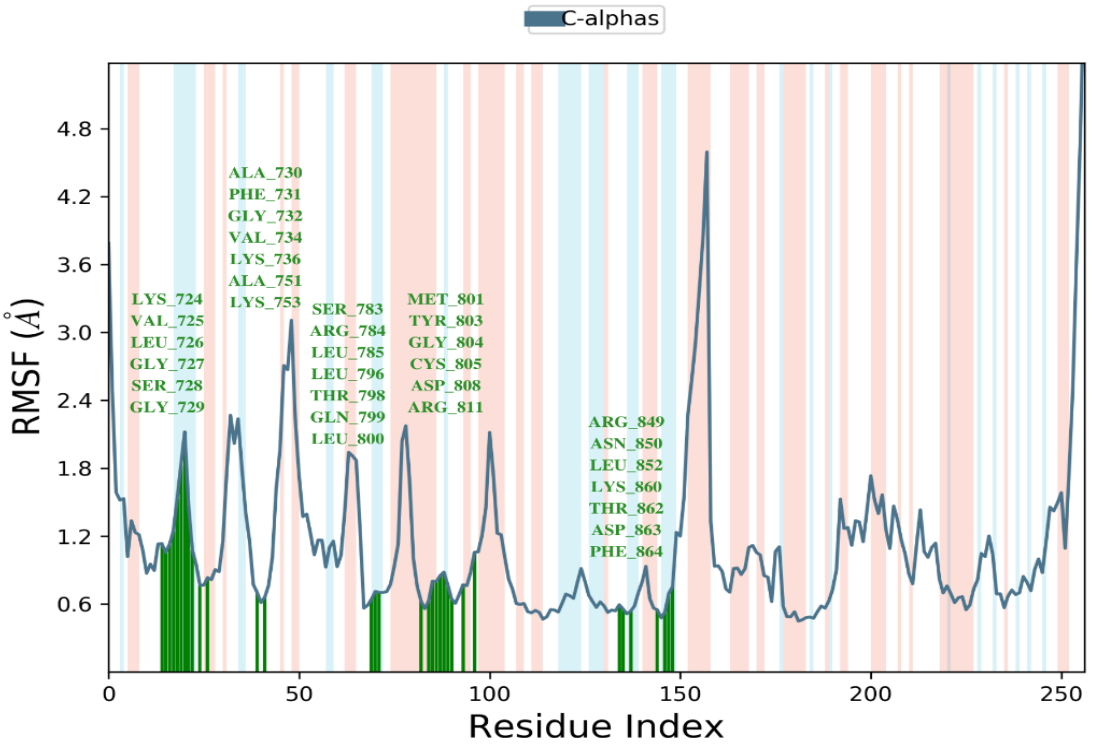


Figure 9. Graph of RMSF contact and Kaempferitrin residue in 100 ns molecular dynamics simulation

According to Figure 9, the residues that interact with the kaempferitrin compound are LYS:724, VA:725, LEU:726, GLY:727, SER:728, GLY:729, ALA:730, PHE:731, GLY:732, VAL:734, LYS:736, ALA:751, LYS:753, SER:783, ARG:784, LEU:785, LEU:796, THR:798, GLN:799, LEU:800, MET:801, TYR:803, GLY:804, CYS:805, ASP:808, ARG:811, ARG:849, ASN:850, LEU:852, LYS:860, THR:862, ASP:863, PHE:864 (33 residual contacts) Both interact via hydrogen bonds, hydrophobic bonds, ionic bridges, and water, as illustrated in (Figures 9 and 10).

Figure 11 demonstrates how the residues and ligands interact on each pass. A darker orange color indicates protein residues that make multiple specific contacts with the ligand. As shown in Figure 12, a total of six variables were examined to describe the stability of the kaempferitrin molecule at the 7PCD receptor during the 100 ns molecular dynamics simulation.

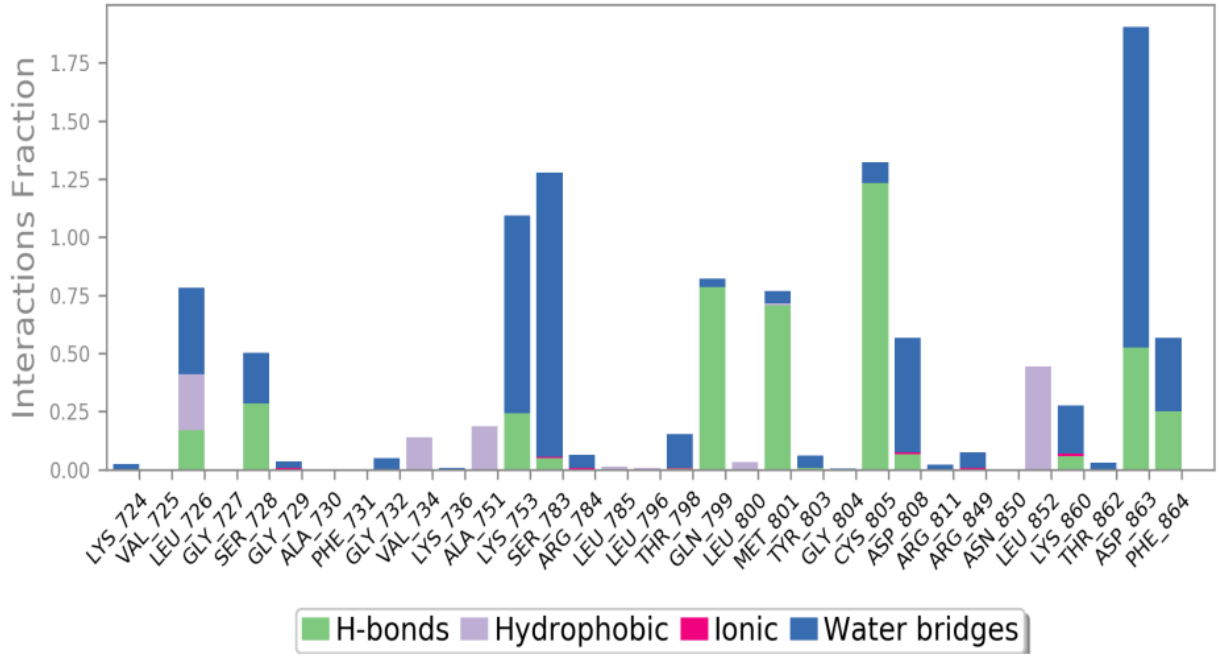


Figure 10. Kaempferitrin residue contact diagram

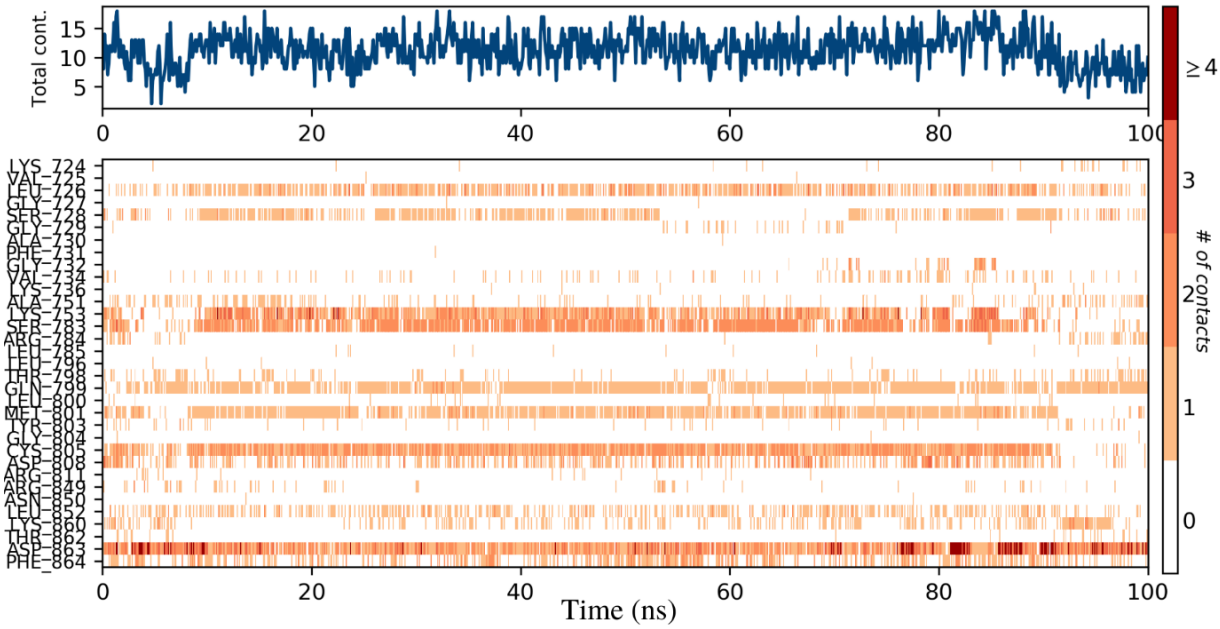


Figure 11. Representation of contact residues and interactions of kaempferitrin compounds for 100 ns

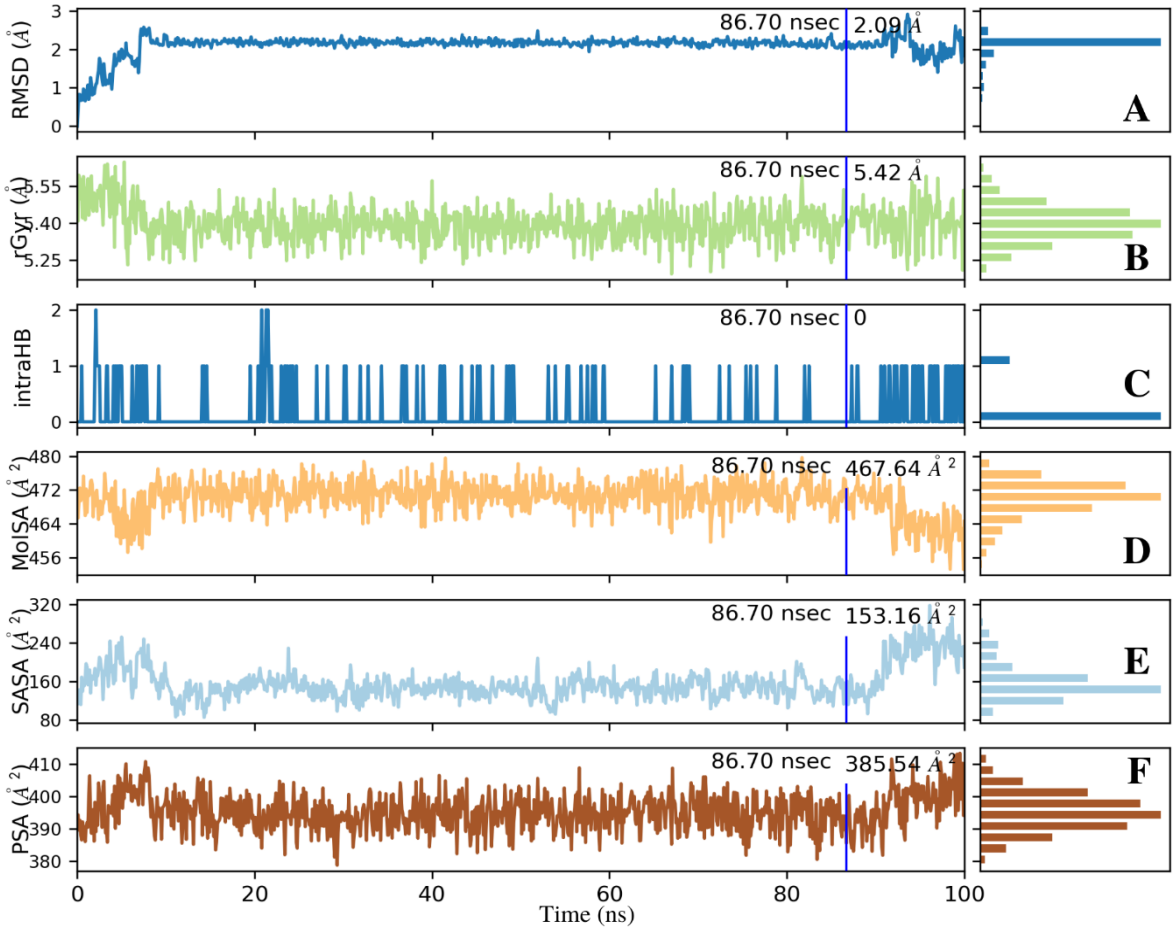


Figure 12. Characterization of the kaempferitrin compound during the 100 ns molecular dynamics simulation: (A) RMSD ligand, (B) gyration radius (rGyr), (C) NS34, (D) molecular surface area (MolSA), (E) surface area solvent accessible (SASA), and (F) polar surface area (PSA)

The kaempferitrin compound's RMSD simulation process fluctuates, as shown in Figure 12. A steady RMSD was seen throughout the entire simulation session after the initial stage of oscillations from 0 to 15 ns. Up until 15 ns, the gyration radius fluctuated. A stable conformation was then achieved until the simulation was completed. The SASA plot exhibits a fluctuation pattern for 15 ns before becoming stable for the duration of the simulation. The MolSA and PSA plots of the kaempferitrin compound varied but remained constant across the 100 ns simulation. At the same time, the intramolecular hydrogen bond plot of kaempferitrin from start to finish shows how intramolecular hydrogen bonds stabilize after protein contact.

The conformational variations of the kaempferitrin complex were also shown in the molecular dynamics simulation data (Figure 13), which demonstrate the conformational changes seen in the tracks through the 100-ns MD simulation, starting at 20, 40, 60, 80, and 100 ns. Conformational changes occur in the visualization from the beginning to the end of the 100 ns Molecular Dynamics simulation (kaempferitrin, tamoxifen, and native ligand). However, the 7PCD protein's binding site still exhibits the conformational change. Therefore, it is thought to interact similarly with Tamoxifen in its anti-cancer activity.

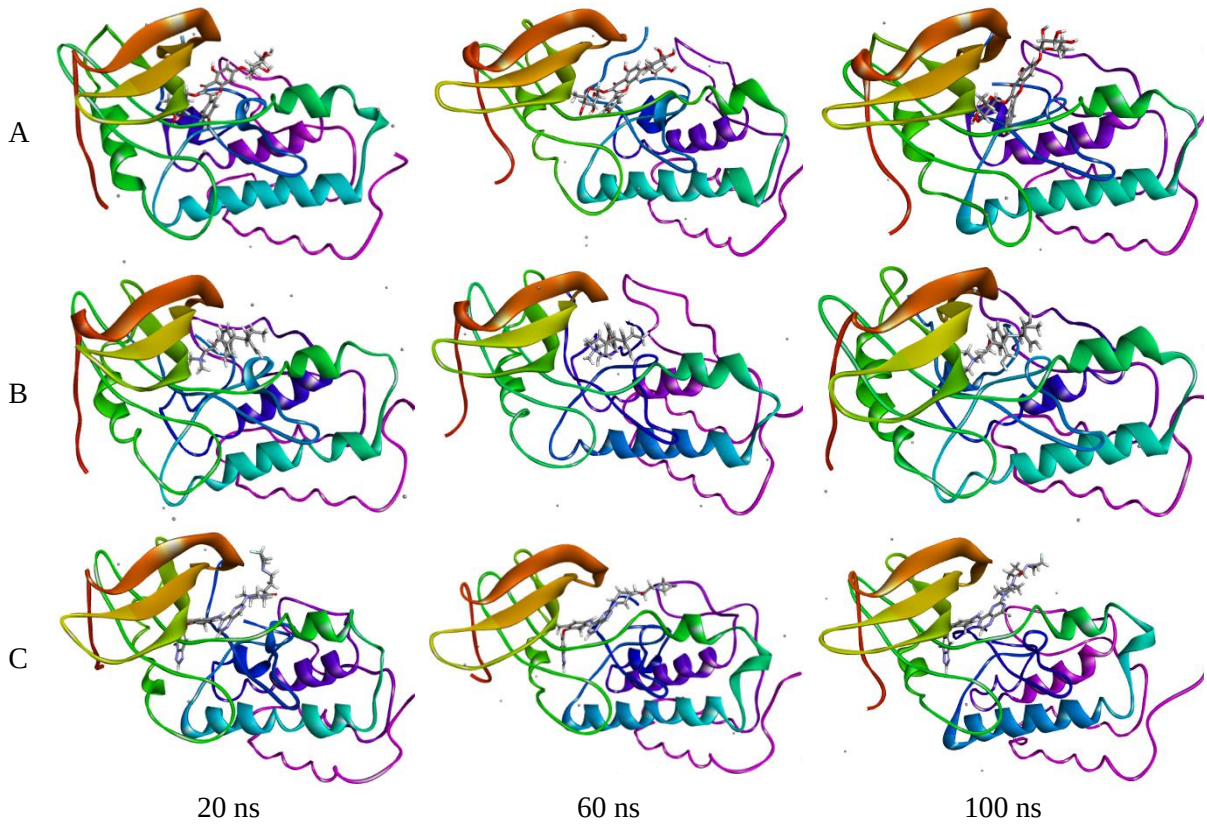


Figure 13. Conformational changes of kaempferitrin (A), Tamoxifen (B), and Native ligands (C) in 100 ns molecular dynamic simulation

Screening Ligan-Based Drugs Likeness (Drug Scan)

Lipinski rule of five states that the ideal medicine molecule follows the standards for physicochemical qualities. Lipinski rule of five forecasts a compound's chemical resemblance to a medicine with a certain biologic activity designed for oral delivery (Chen et al., 2020). Lipinski rule of five includes lipophilicity < 5, molecular weight < 500 g/mol, hydrogen bond donors < 5, refractory molar between 40-130, and hydrogen bond acceptors < 10 (Lipinski, 2004).

The size of the molecular weight affects drug dispersion. Due to the large molecular size of drugs, the larger the molecular weight, the more difficult it is for the drug to penetrate biological membranes (Ruswanto, 2015). LogP indicates the solubility and lipophilicity absorbed by the human body (Villa et al., 2021). The term "lipophilicity" refers to the ability of a substance to dissolve in lipids, oils, fats and non-polar solvents and is defined as the logarithm of the drug ratio. A drug must be sufficiently hydrophobic, but not too hydrophobic, to be absorbed and cross the lipid bilayer (Ivanović et al., 2020).

The ratio of hydrogen bond donors to acceptors shows that the energy required for the absorption process increases with hydrogen bond capacity (Weni et al., 2020). Molar refraction measures the total polarization of a substance, which depends on temperature, index of refraction, and pressure (Putra et al., 2020). Molar refraction describes the steric properties of a compound that can affect drug-receptor interactions. The greater the Calculated Molar Refraction (CMR), the greater the steric properties obtained, and the worse the drug interaction with the receptor (Gyebi et al., 2021). The results of the Drug Scan (Table 5) show that only the glabrol and kaempferitrin compounds fulfill at least two of the five Lipinski rule of five requirements. So that only the glabrol and kaempferitrin compounds can be used as oral preparations.

Table 5. Analysis of drug scan

No	Compounds	Parameter				
		Molecular Weight	Hydrogen Donor	Hydrogen Acceptor	Log P/Lipopility	Molar Reaction
		< 500 g/mol	< 5	< 10	< 5	40 - 130
1	Eriocitrin	596	9	15	2.53	134.40
2	Glabrol	392	2	4	4.46	114.73
3	Kaempferitrin	578	8	14	2.42	130.63
4	Linarin	592	7	14	3.14	135.85
5	Narirutin	580	8	14	2.62	132.85

Pharmacokinetic Analysis (ADME and Toxicity)

In testing the pharmacokinetic and toxicity profiles using the pkCSM website (Table 5). Compounds with an absorption value of >80% have a good absorption value and compounds have a poor absorption value if the absorption value is <30% and for good Skin Permeability values have values ranging from -2.7 to -3.4 (Pires et al., 2015). Based on the findings in (Table 5), the Human Intestinal Absorption (HIA) value of the Glabrol compound is known to have a good absorption value with an absorption value of 90.39%, and the Eriocitrin compound is known to have a poor absorption value with an absorption value of 26.97%. Meanwhile, the Skin Permeability test results showed that all compounds had good Skin Permeability.

Vdss, BBB, and CNS predictions are the findings of the distribution test. The volume of distribution at steady state (VDss) is a theoretical volume that indicates how evenly a drug's complete dose must be spread in order for its concentration to be the same as in blood plasma. A high VD value indicates that more drug is distributed to the tissues than plasma (Pires et al., 2015). The Volume of Distribution (VD) is high if the Log VD > 0.45, it can be concluded that only the Glabrol compound can be distributed evenly at the same concentration as in blood plasma with a VDss value of -0.055. A compound can penetrate the central nervous system if it has a Log PS value > -2 (Pires et al., 2015). According to the CNS permeability test results, only the Glabrol molecule has a CNS permeability value in the central nervous system of -2, namely -1.635. This causes the glabrol compound not to penetrate the central nervous system.

The body has important enzymes for the detoxification process. This enzyme is often called cytochrome P450 and is found in the liver. Cytochrome P450 works by oxidizing organic substances that enter the body and contains inhibitors that can affect drug metabolism (Pratama et al., 2022). To determine whether a drug compound or molecule can be used, it can be seen from the results of CYP3A4 inhibitor testing, the active compounds glabrol, kaempferitrin, linarin, and narirutin do not inhibit the action of the P450 enzyme.

The Total Clearance test can predict the rate of excretion of the active substances examined. The results of the Total Clearance test show that the glabrol compound has the highest Total Clearance, with a value of 0.691, while Kaempferitrin has the lowest Total Clearance, with a value of -0.102. Total Clearance is connected to bioavailability, which is a mix of metabolism in the liver and bile, and chemicals with the highest Total Clearance can be eliminated quickly (Yeni & Rachmania, 2022). The AMES Toxicity test is used to determine a compound's toxicity, which is a method for analyzing a compound's mutagenesis potential using bacteria (Halder *et al*., 2022). AMES Toxicity test results, all compounds are not hepatotoxic with an LD_{50} between 2,273-2,587 mg/kg and are not carcinogenic. Based on this, all test compounds are predicted to be safe and non-toxic (Ferrari & Mario, 2022).

Table 6. Pharmacokinetic profile analysis results (ADME and Toxicity)

Pharmacokinetic Profile	Parameter	Compounds				
		Eriocitrin	**Glabrol**	**Kaempferitrin**	**Linarin**	**Narirutin**
Absorption	CaCO2	-1.101	1.16	0.225	0.357	0.521
	HIA	26.972	90.398	35.385	36.679	36.625
	Skin Permeability	-2.735	-2.795	-2.735	-2.735	-2.735
Distribution	VDss	1.529	-0.055	1.487	0.919	1.295
	BBB	-1.831	-0.161	-1.823	-1.636	-1.594
	SSP	-4.859	-1.635	-4.673	-4.674	-4.708
Metabolism	CYP 2D6 *substrate*	No	No	No	No	No
	CYP 2D6 *inhibitor*	No	No	No	No	No
	CYP 3A4 *substrate*	No	Yes	No	No	No
	CYP 3A4 *inhibitor*	No	Yes	No	No	No
	CYP 2C9 *inhibitor*	No	Yes	No	No	No
	CYP 2C19 *inhibitor*	No	Yes	No	No	No
Excretion	*Total Clearance*	0.115	0.691	-0.102	0.103	0.307
Toxicity	LD50	2.514	2.273	2.587	2.521	2.519
	AMES Toxicity	No	No	No	No	No
	Hepatoksisitas	No	No	No	No	No

4. CONCLUSION

The flavonoid chemicals eriocitrin, glabrol, kaempferitrin, linarin, and narirutin have been identified with anticancer properties based on molecular docking and pharmacokinetic studies. The kaempferitrin outperforms the other compounds when the interaction stability outcomes of the docking molecules are assessed using 100 ns molecular dynamics simulations. Kaempferitrin has the lowest RMSD and RMSF values when compared to other drugs. As a result, more research on kaempferitrin compounds can be done in order to discover and develop novel molecules as prospective therapeutic options for breast cancer treatment.

ACKNOWLEDGEMENT

The authors thank the Universitas Bakti Tunas Husada and Universitas Perjuangan Tasikmalaya for the facilities in research.

REFERENCES

Alnajjar, R., Mostafa, A., Kandeil, A., Al-Karmalawy, A. A. (2020). Molecular docking, molecular dynamics, and in vitro studies reveal the potential of angiotensin II receptor blockers to inhibit the COVID-19 main protease. *Heliyon*, *6*(12), e05641. https://doi.org/10.1016/j.heliyon.2020.e05641

Aziz, F. K., Nukitasari, C., Oktavianingrum, F. A., Aryati, L. W., Santoso, B. (2016). Hasil In Silico Senyawa Z12501572, Z00321025, SCB5631028 dan SCB13970547 dibandingkan Turunan Zerumbon terhadap Human Liver Glycogen Phosphorylase (1l5Q) sebagai Antidiabetes. *Jurnal Kimia VALENSI*, *2*(2), 120–124. https://doi.org/10.15408/jkv.v2i2.4170

Baksi, R., Singh, D. P., Borse, S. P., Rana, R., Sharma, V., Nivsarkar, M. (2018). In vitro and in vivo anticancer efficacy potential of Quercetin loaded polymeric nanoparticles. *Biomedicine and Pharmacotherapy*, *106*(July), 1513–1526. https://doi.org/10.1016/j.biopha.2018.07.106

Binotto, M., Reinert, T., Werutsky, G., Zaffaroni, F., Schwartsmann, G. (2020). Health-related quality of life before and during chemotherapy in patients with early-stage breast cancer. *Ecancermedicalscience*, *14*, 1–11. https://doi.org/10.3332/ECANCER.2020.1007

Chen, X., Li, H., Tian, L., Li, Q., Luo, J., Zhang, Y. (2020). Analysis of the Physicochemical Properties of Acaricides Based on Lipinski's Rule of Five. *Journal of Computational Biology*, *27*(9), 1397–1406. https://doi.org/10.1089/cmb.2019.0323

Chow, E., Rendleman, C. A., Bowers, K. J., Dror, R. O., H, D., Gullingsrud, J., Sacerdoti, F. D., Shaw, D. E. (2008). Desmond Performance on a Cluster of Multicore Processors. *Simulation*, *July*, 1–14.

Dermawan, D., Sumirtanurdin, R., Dewantisari, D. (2019). Molecular Dynamics Simulation Estrogen Receptor Alpha againts Andrographolide as Anti Breast Cancer. *Indonesian Journal of Pharmaceutical Science and Technology*, *6*(2), 65. https://doi.org/10.24198/ijpst.v6i2.18168

Villa, F. X. D, Durán-Iturbide, N. A., Ávila-Zárraga, J. G. (2021). Synthesis, molecular docking, and in silico ADME/Tox profiling studies of new 1-aryl-5-(3-azidopropyl)indol-4-ones: Potential inhibitors of SARS CoV-2 main protease. *Bioorganic Chemistry*, *106*(October). https://doi.org/10.1016/j.bioorg.2020.104497

Eberhardt, J., Santos-Martins, D., Tillack, A. F., Forli, S. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *Journal of Chemical Information and Modeling*, *61*(8), 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203

Elengoe, A., Kumar, Selvam, S., Selvarajah, M., Manikam, H., Vijaya, R. (2022). Sequence analysis and structure prediction of malaysia sars-cov-2 strain's structural and accessory proteins. In *Biointerface Research in Applied Chemistry*, 12(3). https://doi.org/10.33263/BRIAC123.32593304

Ezzati, M., Yousefi, B., Velaei, K., Safa, A. (2020). A review on anti-cancer properties of Quercetin in breast cancer. *Life Sciences*, *248*(September 2019). https://doi.org/10.1016/j.lfs.2020.117463

Ferrari, I. V., Mario, M. D. (2022). Comparison and Prediction of toxicity parameters of principal Aflatoxins , antimicrobial compounds and Antifungal Drugs by pKCSM server. *International Journal of Scientific Research in Biological Sciences*, *9*(1), 48–52.

Frimayanti, N., Djohari, M., Khusnah, A. N. (2021). Molekular Docking Senyawa Analog Kalkon sebagai Inhibitor untuk Sel Kanker Paru-Paru A549. *Jurnal Ilmu Kefarmasian Indonesia*, *19*(1), 87. https://doi.org/10.35814/jifi.v19i1.765

Globocan. (2020). Cancer Incident in Indonesia. *International Agency for Research on Cancer*, *858*, 1–2. https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf

Gyebi, G. A., Ogunro, O. B., Adegunloye, A. P., Ogunyemi, O. M., Afolabi, S. O. (2021). Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): an in silico screening of alkaloids and terpenoids from African medicinal plants. *Journal of Biomolecular Structure and Dynamics*, *39*(9), 3396–3408. https://doi.org/10.1080/07391102.2020.1764868

Halder, S. K., Ahmad, I., Shathi, J. F., Mim, M. M., Hassan, M. R., Jewel, M. J. I., Dey, P., Islam, M. S., Patel, H., Morshed, M. R., Shakil, M. S., Hossen, M. S. (2022). A Comprehensive Study to Unleash the Putative Inhibitors of Serotype2 of Dengue Virus: Insights from an In Silico Structure-Based Drug Discovery. *Molecular Biotechnology*, *0123456789*. https://doi.org/10.1007/s12033-022-00582-1

Hanif, A. U., Lukis, P. A., Fadlan, A. (2021). Pengaruh Minimisasi Energi MMFF94 dengan MarvinSketch dan Open Babel PyRx pada Penambatan Molekular Turunan Oksindola Tersubstitusi. *ALCHEMY : JOURNAL OF CHEMISTRY*.

Hartini, S., Winarsih, B. D., Galih, E., Nugroho, Z., Studi, P., Ners, P. (2020). *Peningkatan Pengetahuan Perawat Untuk Perawatan Anak Penderita Kanker*. *3*(2), 141–149.

Ivanović, V., Rančić, M., Arsić, B., Pavlović, A. (2020). Lipinski's rule of five, famous extensions and famous exceptions. *Popular Scientific Article*, *3*(1), 171–177.

Kikuchi, H., Yuan, B., Hu, X., Okazaki, M. (2019). Chemopreventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. *American Journal of Cancer Research*, *9*(8), 1517–1535. http://www.ncbi.nlm.nih.gov/pubmed/31497340%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6726994

Kumar, S., Sharma, P. P., Shankar, U., Kumar, D., Joshi, S. K., Pena, L., Durvasula, R., Kumar, A., Kempaiah, P., Poonam, Rathi, B. (2020). Discovery of New Hydroxyethylamine Analogs against 3CLproProtein Target of SARS-CoV-2: Molecular Docking, Molecular Dynamics Simulation, and Structure-Activity Relationship Studies. *Journal of Chemical Information and Modeling*, *60*(12), 5754–5770. https://doi.org/10.1021/acs.jcim.0c00326

Leung, S. H., Bodkin, M. J., von Delft, F., Brennan, P. E., Morris, G. M. (2019). SuCOS is better than RMSD for evaluating fragment elaboration and docking poses. *ChemRxiv*, 1–47.

Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Mardianingrum, R., Ruswanto, R., Agustien, G. S., Nuraisah, A. (2020). The Active Compound of Bangle Essential Oil as Cyclooxygenase-2 (Cox-2) Inhibitor: In Silico Approach. *Jurnal Kimia Valensi*, *6*(2), 156–168. https://doi.org/10.15408/jkv.v6i2.16943

Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., Jemal, A., Kramer, J. L., Siegel, R. L. (2019). Cancer treatment and survivorship statistics, 2019. *CA: A Cancer Journal for Clinicians*, *69*(5), 363–385. https://doi.org/10.3322/caac.21565

Mvondo, J. G. M., Matondo, A., Mawete, D. T., Bambi, S.-M. N., Mbala, B. M., Lohohola, P. O. (2021). In Silico ADME/T Properties of Quinine Derivatives using SwissADME and pkCSM Webservers. *International Journal of TROPICAL DISEASE & Health*, *42*(11), 1–12. https://doi.org/10.9734/ijtdh/2021/v42i1130492

Pires, D. E. V., Blundell, T. L., Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, *58*(9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104

Pratama, P. R., Isman, F., Fadlan, A. (2022). Penyelidikan Aktivitas Antikanker Payudara Oleh Minyak Atsiri Bunga Michelia Alba Secara in Silico. *Jurnal Ilmu Kimia Dan Terapan*, *9*(1).

Protein Data Bank. (n.d.). *Enabling Breakthroughs in Scientific and Biomedical Research and Education*. https://www.rcsb.org/

Puspita, P. J., Ambarsari, L., Adiva, A., Sumaryada, T. I. (2021). In Silico Analysis of Glucose Oxidase H516r and H516d Mutations for an Enzymatic Fuel Cell. *Jurnal Kimia Valensi*, *7*(2), 83–93. https://doi.org/10.15408/jkv.v7i2.20733

Putra, P. P., Fauzana, A., Lucida, H. (2020). Analisis Sifat Fisika-Kimia, Potensi Target dan Toksikologi Senyawa Isolat Murni dari Bahan Alam dengan Metode In Silico. *Indonesian Journal of Pharmaceutical Science and Technology Journal Homepage*, *7*(3), 107–117. http://jurnal.unpad.ac.id/ijpst/UNPAD

Qasaymeh, R. M., Rotondo, D., Oosthuizen, C. B., Lall, N., Seidel, V. (2019). Predictive binding affinity of plant-derived natural products towards the protein kinase g enzyme of mycobacterium tuberculosis (Mtpkng). *Plants*, *8*(11), 1–14. https://doi.org/10.3390/plants8110477

Rachmania, R. A., Hariyanti, H., Zikriah, R., Sultan, A. (2018). Studi In Silico Senyawa Alkaloid Herba Bakung Putih (Crinum Asiaticum L.) pada Penghambatan Enzim Siklooksigenase (COX). *Jurnal Kimia VALENSI*, *4*(2), 124–136. https://doi.org/10.15408/jkv.v4i2.7686

Ramadhan, D. S. F., Fakih, T. M., Arfan, A. (2020). Activity Prediction of Bioactive Compounds Contained in Etlingera elatior Against the SARS-CoV-2 Main Protease: An In Silico Approach. *Borneo Journal of Pharmacy*, *3*(4), 235–242. https://doi.org/10.33084/bjop.v3i4.1634

Rathod, S., Shinde, K., Porlekar, J., Choudhari, P., Dhavale, R., Mahuli, D., Tamboli, Y., Bhatia, M., Haval, K. P., Al-Sehemi, A. G., Pannipara, M. (2022). Computational Exploration of Anti-cancer Potential of Flavonoids against Cyclin-Dependent Kinase 8: An In Silico Molecular Docking and Dynamic Approach. *ACS Omega*. https://doi.org/10.1021/acsomega.2c04837

Ruswanto, Mardianingrum, R., Siswandono, Kesuma, D. (2020). *Reverse Docking, Molecular Docking, Absorption, Distribution, and Toxicity Prediction of*

Jurnal Kimia Valensi, Vol 9(1), May 2023, 163-182
Available online at Website: http://journal.uinjkt.ac.id/index.php/valensi

The Virtual Screening of Flavonoid Derivatives on Progesterone, Estrogen, and HER-2 Receptor for Breast Cancer Treatment Candidate

Ade Dwi Septian[1], Gatut Ari Wardani[1], Richa Mardianingrum[2], Ruswanto Ruswanto[1*]

[1]Pharmacy Study Program, Faculty of Pharmacy, Universitas Bakti Tunas Husada
Jl. Cilolohan 36 Tasikmalaya, Indonesia.
[2]Department of Pharmacy, Health Science Faculty, Universitas Perjuangan Tasikmalaya
Jl. PETA No. 177, Indonesia.

**Corresponding author: ruswanto@universitas-bth.ac.id*

Received: March 2023; Revision: June 2023; Accepted: June 2023; Available online: June 2023

Abstract

Cancer is defined as a disease caused by progressive and abnormal cell proliferation in the body. This condition is caused by deoxyribonucleic acid (DNA) changes, which causes cells to lose their normal function. The aim of this study is to find that flavonoid compounds have a more stable interaction than tamoxifen as anti-cancer candidates. Research has been conducted in silico with molecular docking (AutodockTools-1.5.7) and molecular dynamics of 200 flavonoid compounds. Furthermore, pharmacokinetic parameters, toxicity, and the application of the Lipinski Rule of Five were investigated. Based on molecular docking results, the compounds eriocotrin, glabrol, kaempferitrin, linarin, and narirutin have more stable interactions with lower binding energy (ΔG) than tamoxifen. From the results of molecular docking, molecular dynamics, and pharmacokinetic studies, it is predicted that the kaempferitrin compound can be used as an anti-cancer candidate and does not cause toxicity through further research.

Keywords: AutoDock; cancer; flavonoids; molecular docking; molecular dynamics.

DOI: *10.15408/jkv.v9i1.31482*

1. INTRODUCTION

Cancer is defined as a disease caused by progressive and abnormal cell proliferation in the body. This condition is caused by deoxyribonucleic acid (DNA) changes, which causes cells to lose their normal function. These cancer cells spread (metastasize) to other organs in the body, and infiltration can cause progressive loss of organ function and death (Hartini et al., 2020). Breast cancer is the most frequent cancer globally, reaching 2.2 million cases worldwide (11.7%) of the world population. (Globocan, 2020).

Surgery, radiation, and systemic therapy, which includes chemotherapy, targeted therapy, hormone therapy, and immunotherapy, are all common cancer treatments. These targeted therapies are listed in the National Cancer Data Base (NCDB). Chemotherapy combines both targeted therapy and immunotherapy for uniformity and comparability (Miller et al., 2019). However, chemotherapy has limitations, namely exacerbating low-level toxicities such as diarrhea and insomnia can interfere with the patient's Health-Related Quality of Life (HRQOL). Multiple oncologic therapy symptoms, including urination, nausea, night sweats, and digestive symptoms, influence the concurrent effect of chemotherapy on insomnia symptoms. Chemotherapy frequently has negative side effects, including changes to taste and smell. (Binotto et al., 2020).

Plants contain phytochemical components such as phenolic acids, flavonoids, carotenoids, stilbenes, and lignans, which have been shown to prevent the development and spread of breast cancer. (Selvakumar et al., 2020). Flavonoids are natural products and can be used as a prerequisite for preventing breast cancer recurrence in patients and its occurrence in healthy individuals (Ezzati et al., 2020). Flavonoids have been examined *in vitro* and *in vivo* and have shown outstanding anti-cancer action as well as inhibiting drug-target protein

P-ISSN: 2460-6065, E-ISSN: 2548-3013

overexpression in cancer cells (Baksi et al., 2018). Flavonoids have received a great deal of interest due to their numerous biological roles, which include antioxidant, anti-inflammatory, and anticancer properties. They have been proven by clinical trials in patients with different characteristics such as age, sex, and disease stage (Kikuchi et al., 2019). Flavonoids have also been tested in silico against the receptor Cyclin-Dependent Kinase 8 (6T41) 2.45 Å (Rathod et al., 2022), mTOR (5H64) 4.40 Å (Sharma et al., 2020) and it is proven that flavonoids have interactions with cancer receptors. However, some receptors have better resolution including Progesterone (1ZUC) 2.00 Å, Estrogen (5W9C) 1.80 Å, and HER-2 (7PCD) 1.77 Å (Protein Data Bank, n.d.).

The process of discovering and developing new medications is commonly known to be time-consuming and resource-intensive (Surabhi & Singh, 2018). Computational approaches can reduce the number of ligands tested in biological testing, which reduces the cost, time, and effort necessary to produce novel medications (Salman et al., 2021). Based on this knowledge, researchers conducted a study in which they computationally developed anticancer drug candidates utilizing molecular docking methodologies, molecular dynamics, pharmacokinetic, and toxicity studies of flavonoid chemicals in numerous plants. This study has found that flavonoid compounds have a stable interaction with breast cancer receptors, give a better score on parameters of Lipinski drug similarity, pharmacokinetic profile, and toxicity, and achieve better cancer treatment compared to tamoxifen.

2. MATERIALS AND METHODS

Materials

Equipment used in the form of software and hardware. AutodockTools-1.5.7, Discovery Studio version 21.1, Molegro Molecular Viewer, MarvinSketch version 22.19.0, Amber, Desmond academic software, and several web-based programs such as Protein Data Bank (PDB), PubChem, SAVES, Lipinski's Rule of Five and pKCSM. The hardware used is a laptop with AMD Ryzen 5 5600H specifications with Radeon Graphics (12CPU) ~ 3.30 GHz, 8.00 GB of RAM 64-bit Operating System of Windows 11, and a personal computer with Intel® Xeon(R) CPU E5-267 specifications v2 @ 2.70 GHz (24CPU), NVIDIA GeForce RTX 3060, 7.7 GB of RAM and the Linux Ubuntu 18.04.5 LTS.

The materials used for this research were 200 flavonoid compounds obtained from Biopurify and downloaded from PubChem. Progesterone Receptor (1ZUC), Estrogen Receptor (5W9C), HER-2 Receptor (7PCD).

Receptor Analysis

The PDB receptor profile was examined via the website http://www.ebi.ac.uk/pdbsum/ for receptor analysis. Enter the PDB code for the receptor, and profile data for the receptor will be displayed and examined using the Ramachandran plot, with 0.8% prohibited sections (Ruswanto, Garna, et al., 2018). The subsequent analysis is ERRAT with the website https://saves.mbi.ucla.edu/ and the generally accepted range of values is >50 (Elengoe *et al*., 2022).

Receptor Preparation

The initial step in receptor preparation involves dissociating the macromolecular chain that binds the target. Progesterone Receptor (1ZUC), Estrogen Receptor (5W9C), and HER-2 Receptor (7PCD) downloaded from Protein Data Bank (PDB) (Rachmania *et al*., 2018). The Molegro Virtual Docker application is used to separate native ligands, macromolecules, and removing water molecules. The result of this separation is the receptor structure ready for macromolecular optimization. This structure is saved in .pdb format. (Ruswanto et al., 2018).

The receptor's native ligand must also be removed because the ligand binds to the receptor's active site and can prevent other ligands' binding. Water molecules must also be removed to not interfere with the binding and interaction processes formed so that only the protein-ligand interacts (Dermawan et al., 2019).

The following step is to fine-tune the addition of hydrogen atoms to the Discovery Studio Visualizer application (Mardianingrum et al., 2020). The hydrogen atoms are added because some hydrogen atoms are lost during protein-ligand complex separation, particularly when employing the X-ray crystallographic diffraction method (Victory et al., 2018). Then the file is saved in .pdb format.

The gasteiger charge was then applied to the macromolecules using the AutodockTools-1.5.7 software, then a non-polar merge was

performed so that only polar hydrogen atoms appeared (Sari et al., 2020). Then save the structure in .pdbqt format.

Ligand preparation

The PubChem website was used to download 200 flavonoid compounds in total. All flavonoid compounds are protonated at a pH of 7.4 to obtain a structure following the body's physical condition. Geometry is optimized to achieve a stable molecule structure with a low potential energy. The MMFF94 approach is used for optimization. The MMFF94 method is a force field-based method for energy minimization. This method can help improve the accuracy and efficiency of molecular docking results (Hanif et al., 2021). Then the optimized flavonoid compounds are stored in the .pdb file format. Furthermore, the flavonoid compounds were converted into .pdbqt format using the AutodockTools-1.5.7 software.

Docking Validation and Analysis of Docking Results

The docking method was validated by re-tagging the previously separated ligand to the receptor with the AutodockTools-1.5.7 application. The method validation steps must demonstrate the method's validity. If the resulting Root Mean Square Deviation (RMSD) value is less than 2Å, the docking approach is deemed to be good (Leung et al., 2019).

AutoDock and AutoDock Vina are among the fastest and most widely used molecular docking programs (Aziz et al., 2016). AutoDock Vina makes designing and running simultaneous simple and complex docking simulations and multi-ligand docking against a single target easy. The grid box settings used must be the same as during method validation. This is done so that the ligands of the flavonoid compounds interact with the receptors (Eberhardt et al., 2021).

The molecular docking data were investigated by selecting the conformational ligand with the lowest binding energy (ΔG). The results of receptor and ligand molecular docking are then saved in.pdb format. Then, using Discovery Studio software, the interaction between the ligand and the receptor active site was investigated (Utami et al., 2020).

Molecular Dynamics

The docking results are utilized to calculate the stability of the protein-ligand binding relationship in a molecular dynamics simulation. Several factors, including compound conformation, water molecules, ions, cofactors, compound protonation, and changes in solvent entropy, influence the results of molecular dynamics simulations (Ruswanto et al., 2022).

The structures of the best candidate flavonoid compounds for breast cancer receptors were carried out by molecular dynamics simulations using Desmond Schrödinger LLC software (Alnajjar et al., 2020) was first released for free in June 2008 (Chow et al., 2008). The TIP3P water model was used to imitate physiological ion concentrations using 0.15 M NaCl. Molecular dynamics simulations were performed in an orthorhombic box with buffer dimensions of 10 Å × 10 Å × 10 Å at 300 K and 1.01325 bar utilizing ensemble NPT (Number of Atoms, Pressure, and Temperature). Each simulation was ran for 100 ns with a 1.2 ps recording interval (Kumar et al., 2020).

Screening Ligan-Based Drugs Likeness (Drug Scan)

The finest flavonoid compounds are subjected to a drug Scan. The rules of good pharmaceuticals were used to make observations, which include lipophilicity <5, molecular weight <500 g/mol, hydrogen bond donors < 5, refractory molar between 40-130, and hydrogen bond acceptors <10. These parameters can be determined using the website http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp (Ruswanto *et al*., 2020). The Lipinski Rule Of Five was set up to be a conservative predictor in an era where drugs produced too many compounds with very poor physicochemical properties. If a compound fails the Lipinski Rule Of Five test, there is a high probability that problems with oral activity will be encountered. However, passing the Lipinski Rule Of Five test does not guarantee that a compound is like a drug (Lipinski, 2004).

Pharmacokinetic Analysis (ADME and Toxicity)

The pharmacokinetic properties ADME and optimal toxicity prediction of flavonoid

compounds were determined using the pkCSM web tools (Pires et al., 2015). The structure of the best flavonoid compounds is translated into SMILES format. In this SMILES format, compounds are processed using pkCSM online tools(http://biosig.unimelb.edu.au/pkcsm/prediction) to estimate ADME and chemical toxicity (Mvondo et al., 2021).

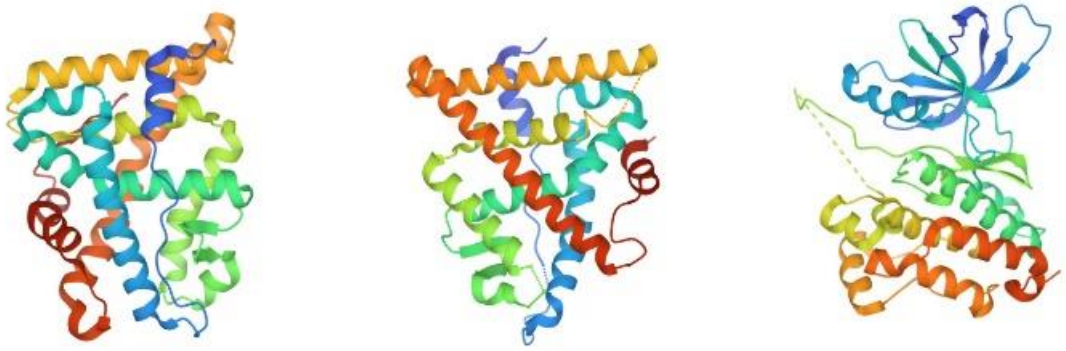
3. RESULTS AND DISCUSSION

Receptor Analysis

Receptor analysis was performed on Progesterone Receptor with pdb code 1ZUC, Estrogen Receptor with pdb code 5W9C, and HER-2 Receptor with pdb code 7PCD for breast cancer. Each receptor's structure (Figure 1) was retrieved from the Protein Data Bank (PDB) and saved in the.pdb file format. Receptor analysis was performed using PROCHECK and ERRAT.

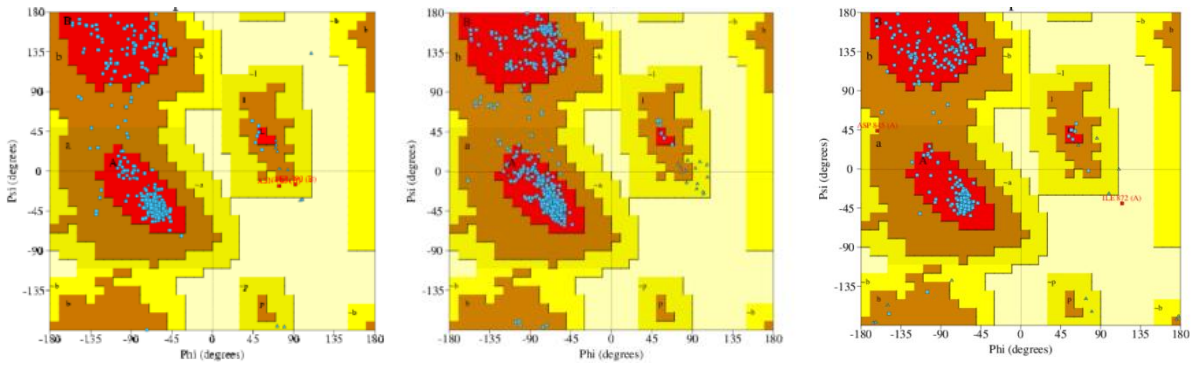
PROCHECK inspection by Ramachandran plots (Figure 2) focuses on specific geometries and allows the overall structure to be evaluated (Puspita et al., 2021). To see if the Ramachandran plot is good for protein structure analysis, it can be checked by plotting non-glycine residues in disallowed regions less than 0.8% (Ruswanto et al., 2018).

According to the Ramachandran plot study of the progesterone receptor (1ZUC), 93% of the amino acid residues are found in the regions that are most favored, while only 0.0% are found in the regions that are forbidden. 95.6% of the amino acid residues of the estrogen receptor (5W9C) are in the most preferred areas, whereas just 0.0% are in the forbidden regions. The number of amino acid residues in the most preferred areas of the HER-2 receptor (7PCD) is 93.6%, whereas the number of residues in the forbidden regions is 0.4%. The results of the analysis showed that progesterone receptor (1ZUC), estrogen receptor (5W9C), and HER-2 receptor (7PCD) have a stable protein structure.



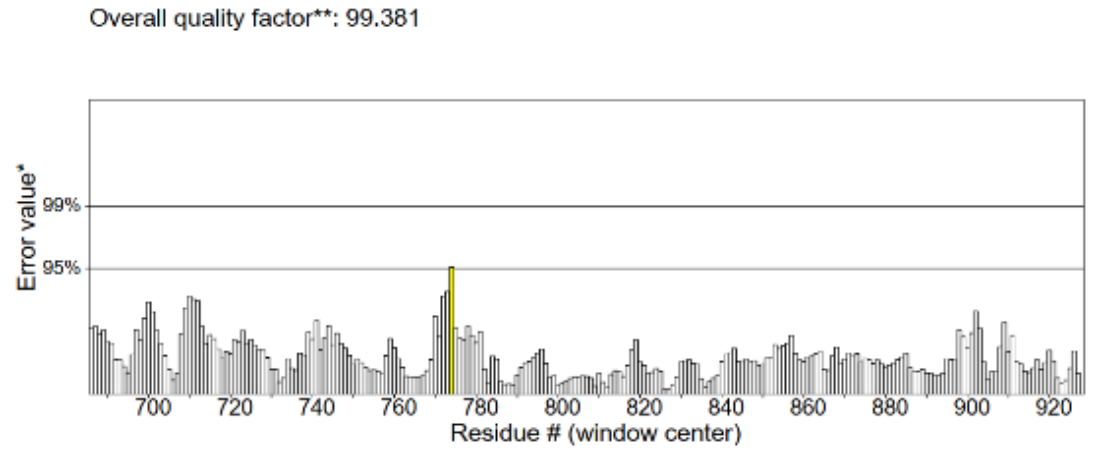
(a) (b) (c)

Figure 1. Structure of (a) progesterone receptor (1ZUC); (b) estrogen receptor (5W9C); and (c) HER-2 receptors (7PCD**)**

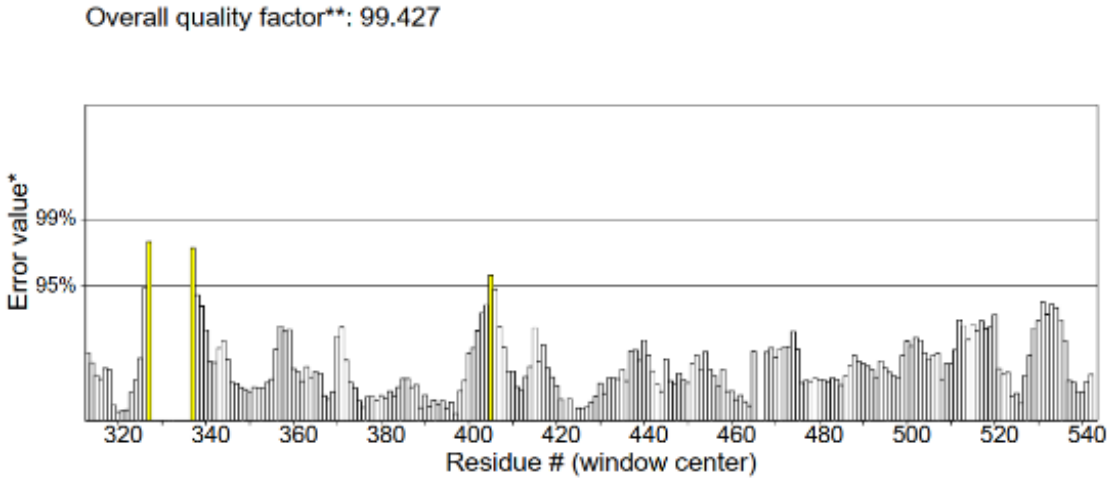


(a) (b) (c)

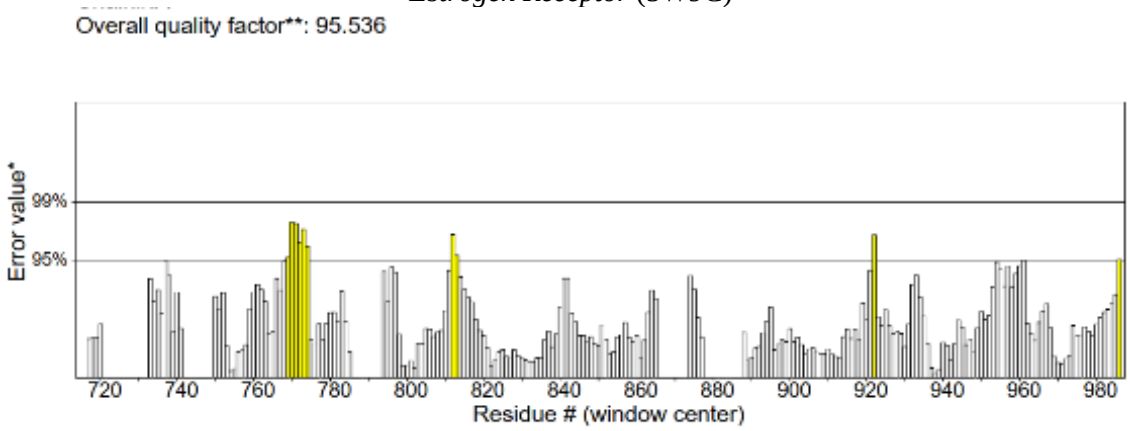
Figure 2. Ramachandran plot of (a) progesterone receptor (1ZUC); (b) estrogen receptor (5W9C); and (c) HER-2 receptors (7PCD)



Progesterone Receptor (1ZUC)



Estrogen Receptor (5W9C)



HER-2 Receptor (7PCD)

Figure 3. ERRAT Analysis Results

ERRAT validates models with statistical relationships of unbound interactions between different types of atoms based on the nature of the atomic interactions, with higher scores indicating higher quality. ERRAT also provides results with significance and overall quality, with a generally accepted score of over 50, constituting a stable protein (Elengoe et al., 2022).

From the results of the ERRAT analysis (Figure 3), the quality factor values were 99.381% for Progesterone Receptor (1ZUC), 99.247% for Estrogen Receptor (5W9C), and 95.536% for HER-2 receptor (7PCD). The results of these calculations show that the

protein structures of the progesterone receptor (1ZUC), estrogen receptor (5W9C), and HER-2 receptor (7PCD) receptors are of high quality and high-resolution protein structures are neglected. It shows that the model is modeled with a possible error. Standards for amino acid residues in binding proteins.

Receptor and Ligand Preparation

The ligand preparation was protonated in accordance with the blood pH, which was 7.4. The ligand was conformed so that it could engage with the receptor in the most stable location possible. The conformation with the lowest energy was chosen from among the ten conformations created for use in the following stage. Additionally, receptor preparation was performed to remove water molecules from the receptor so that the interaction between the receptor and the ligand would be unaffected.

Method Validation and Docking

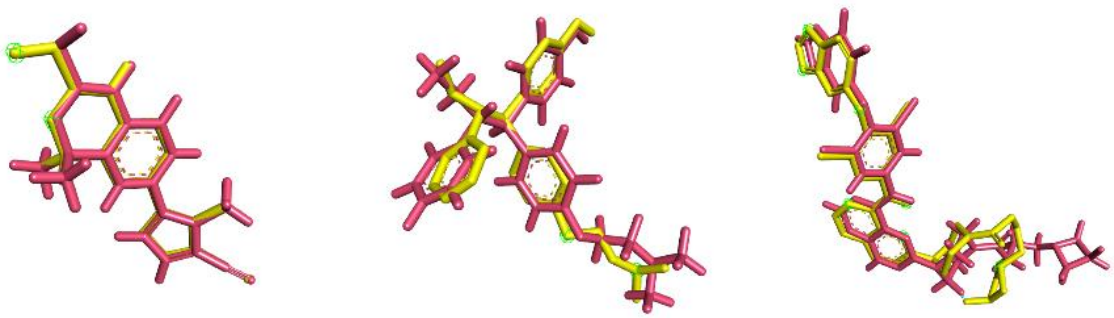
Software called AutodockTools-1.5.7 was used for validation. The active site of the co-crystalline ligands was validated using the redocking approach, namely 5-(4,4-dimethyl-2-thioxo-1,4-dihydro-2h-3,1-benzoxazin-6-yl)-1-methyl-1h-pyrrole-2-carbonitrile for Progesterone Receptor (1ZUC), 4-hydroxytamoxifen for Estrogen Receptor (5W9C) and 1-[4-[4-[[3,5-bis(chloranyl)-4-([1,2,4]triazolo[1,5-a]pyridine-7-yloxy)phenyl]amino]pyrimido[5,4-d]pyrimidin-6-yl]piperazine-1-yl]-4-(3 -fluoranylasethidine-1-yl)butan-1-one for HER-2 Receptor (7PCD) on crystallographic results (Table 1). If the root mean square deviation (RMSD) is less than 2, the approach is considered justifiable (Ramadhan et al., 2020).

The validation findings for each receptor demonstrate that the RMSD value for each receptor is ≤ 2.0, indicating that the docking method's validation is valid (Table 1). After obtaining RMSD ≤ 2Å, a virtual screening process was carried out using AutoDock Vina on the test compound using a grid box with the same settings in the validation process. In this study, virtual screening were performed 20 times for each ligand to obtain the best binding energy (ΔG).

Results of molecular docking of 200 flavonoid compounds using AutoDock Vina showed that all flavonoid compounds interacted with Progesterone Receptor (1ZUC), Estrogen Receptor (5W9C), and HER-2 Receptor (7PCD). However, only the five best compounds were taken, which had the potential to treat breast cancer, and the strongest interactions were towards the HER-2 Receptor (7PCD), namely the compounds eriocitrin, glabrol, kaempferitrin, linarin, and narirutin. The results of molecular docking using AutoDock Vina can be seen in Table 2.

Table 1. Method Validation Results

PDB Code	Grid Box			RMSD (Å)
	X	Y	Z	
1ZUC	27.623	-7.733	9.371	0.53
5W9C	15.045	-10.616	-26.4	0.63
7PCD	8.413	-8.996	-13.532	1.73



Progesterone Receptor (1ZUC) Estrogen Receptor (5W9C) HER-2 Receptor (7PCD)

Figure 4. Overlay Results for Each Receptor

Next, the HER-2 Receptor (7PCD) was docked using AutodockTools-1.5.7 software because the binding energy (ΔG) on the HER-2 Receptor (7PCD) is smaller than the Progesterone Receptor (1ZUC) and Estrogen Receptor (5W9C).

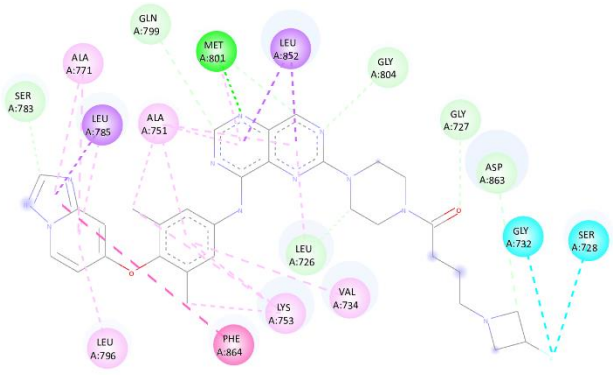
Visualization and Analysis of Docking Results

Docking results were displayed in 2D (Figure 5) and 3D (Figure 6) using Discovery Studio Software. Interactions formed between ligands with the lowest binding energies and amino acid residues of the progesterone receptor (1ZUC), estrogen receptor (5W9C), and HER-2 receptor (7PCD) were observed.

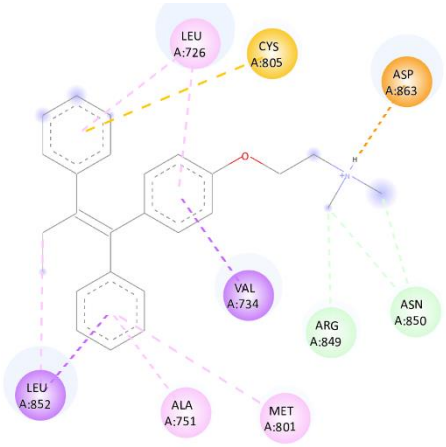
Based on the docking results (Figure 5), the bonds between the ligand and HER-2 Receptor (7PCD) are known to be conventional hydrogen bonds, Van der Waals bonds, hydrophobic bonds, Pi-Sigma bonds, and other bonds.

Table 2. Results of molecular docking of flavonoid compounds against progesterone receptor (1zuc), estrogen receptor (5W9C), and HER-2 receptor (7PCD) using AutoDock Vina

Receptors	Compound	Binding Energy (kcal/mol)
1ZUC	Baicalin	-10.3
	Chrysin-7-o-glucuronide	-10.2
	Eriodictyol	-10.1
	Oroxylin A 7-o-beta-d-glucoronide	-10.1
	Oroxylin A glucoronide	-10.1
5W9C	Apigenin-7-O-glucuronide	-9.9
	Baicalin	-9.8
	Chrysin-7-O-glucuronide	-9.7
	Luteolin 7 glucuronide	-9.9
	Scutellarin	-9.9
7PCD	Eriocitrin	-10.4
	Glabrol	-10.5
	Kaempferitrin	-10.3
	Linarin	-10.2
	Narirutin	-10.4



Native Ligand



tamoxifen

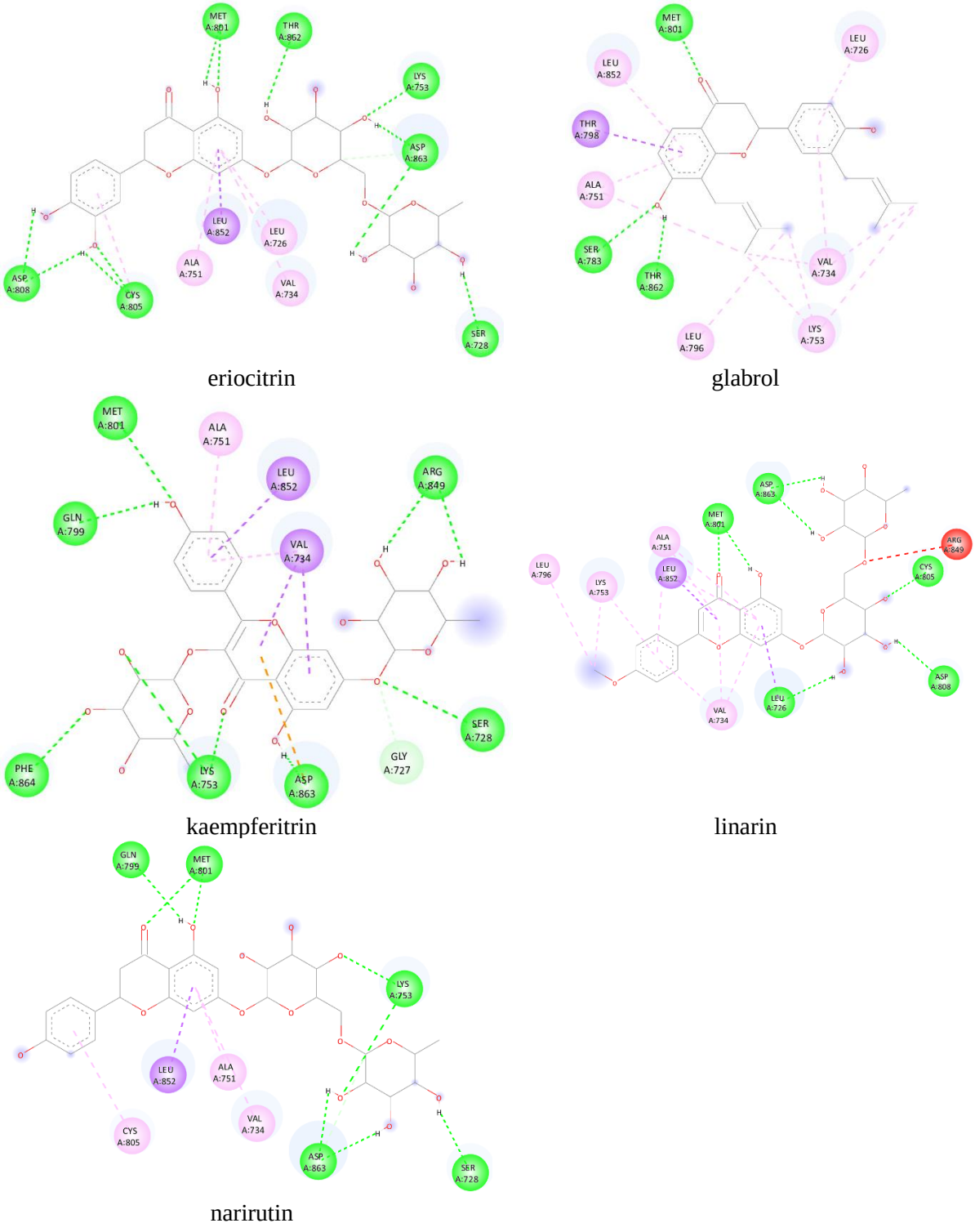


Figure 5. 2D Visualization of Native ligands, tamoxifen, eriocitrin, glabrol, kaempferitrin, linarin, and narirutin

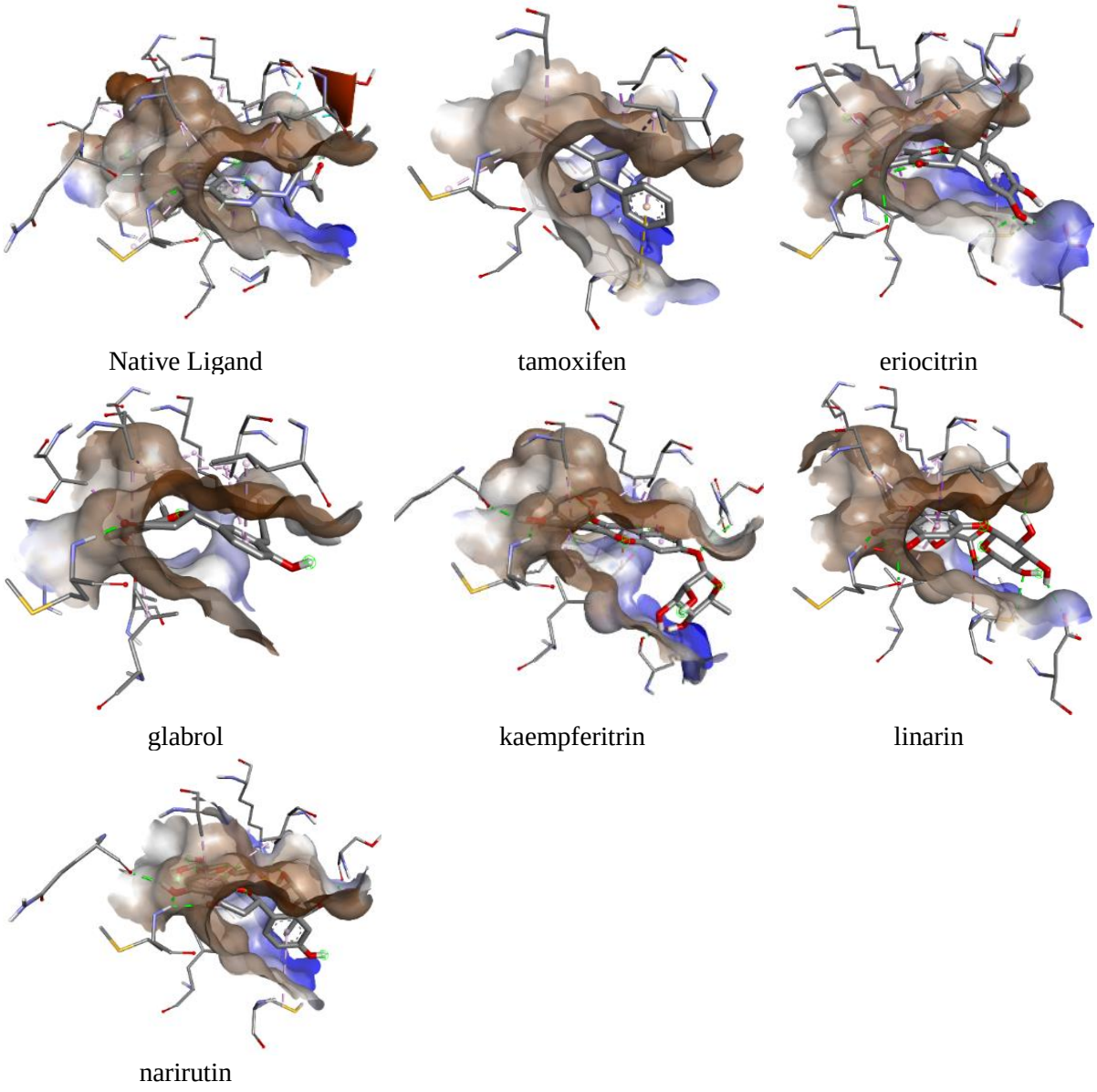


Figure 6. 3D Visualization of Native ligands, tamoxifen, eriocitrin, glabrol, kaempferitrin, linarin, and narirutin

Based on Table 3, it can be seen that the binding affinity between the ligand and the receptor. All of the best flavonoid compounds have a lower free energy value (ΔG) compared to the anticancer comparator compound on the market, namely tamoxifen. Free energy value (ΔG) of narirutin with a ΔG value of -8.97 kcal/mol and a Ki value of 268.02 μM, glabrol with a ΔG value of -8.72 kcal/mol and a Ki value of 407.83 μM, eriocitrin with a ΔG value of -8.71 kcal /mol and a Ki value of 411.52 μM, linarin with a ΔG value of -8.52 kcal/mol and a Ki value of 570.51 μM, kaempferitrin with a ΔG value of -8.27 kcal/mol and a Ki value of 873.62 μM. The free energy value (ΔG) of tamoxifen is -7.77 kcal/mol with a Ki value of 2.02 μM, so the affinity of the compounds narirutin, glabrol, eriocitrin, linarin, and kaempferitrin for HER-2 receptors is higher than the comparator compound, tamoxifen. This shows that the compounds narirutin, glabrol, eriocitrin, linarin, and kaempferitrin are predicted to have stable interactions and have more potential as anti-breast cancer drugs than the compounds on the market, namely tamoxifen because they have lower binding energy (ΔG) and inhibition constant (Ki). (Qasaymeh et al., 2019).

In the interaction between HER-2 Receptor (7PCD) and native ligands, comparator compounds (tamoxifen) and test compounds (eriocitrin, glabrol, kaempferitrin, linarin, narirutin) showed that there were interactions between native ligands and amino acid residues of 17 bonds and a total of 1

hydrogen bonds (MET A:801). With the presence of hydrogen bonds between flavonoids and receptors it will improve the interactions that occur. Then the interaction between the comparator compound (tamoxifen) with amino acid residues of 9 bonds and has no hydrogen bonds. Then in the test ligand, eriocitrin interacts with amino acid residues for 11 bonds and a total of 7 hydrogen bonds (MET A: 801, THR A: 862, LYS A: 753, ASP A: 863, SER A: 728, CYS A: 805, ASP A:808), glabrol interacts with 10 bonds of amino acid residues and a total of 3 hydrogen bonds (MET A:801, SER A:783, THR A:862), kaempferitrin interacts with 10 bonds of amino acid residues and a total of 6 bonds hydrogen (MET A:801, GLN A:799, ARG A:849, SER A:728, ASP A:863, LYS A: 753), linarin interacts with amino acid residues for 11 bonds and a total of 5 hydrogen bonds (META A:801, ASP A:863, CYS A:805, ASP A:808, LEU A:726), narirutin interacts with amino acid residues for 9 bonds and a total of 5 hydrogen bonds (MET A:801, GLN A:799, LYS A:753, ASP A:863, SER A:728). If the active molecule binds strongly through hydrogen bonds and can bind to one of the same amino acid residues from the active side, it is considered to have a strong connection with the target receptor (Frimayanti et al., 2021). This shows that the compounds narirutin, glabrol, eriocitrin, linarin, and kaempferitrin have strong bonds with target receptors because they have the same bonds in hydrogen bonds, namely (META A: 801).

Table 3. Molecular docking results analysis of tamoxifen and selected compounds against HER-2 receptors

Compounds	ΔG (kcal/mol)	KI (μM)	Hydrogen Bonds	Hydrophobic Bonds
Native ligands	-10.01	45.79	**MET A:801**	SER A:783, GLN A:799, GLY A:804, GLY A:727, ASP A:863, LEU A:726, GLY A:732, SER A:728, LEU A:785, LEU A:852, PHE A:864, ALA A:771, ALA A:751, VAL A;734, LYS A:753, LEU A:798
Tamoxifen	-7.77	2.02	-	CYS A:805, ASP A:863, ASN A:850, ARG A:849, VAL A:743. LEU A;852, LEU A:726, MET A:801, ALA A:751
Eriocitrin	-8.71	411.52	**MET A:801**, THR A:862, LYS A:753, ASP A:863, SER A:728, CYS A:805, ASP A:808	LEU A:852, LEU A:726, ALA A:751, VAL A:734
Glabrol	-8.72	407.84	**MET A:801**, SER A:783, THR A:862	THR A:798, LEU A:852, LEU A:726, VAL A:734, LYS A:753, LEU A:796, ALA A:751,
Kaempferitrin	-8.27	873.62	**MET A:801,** GLN A:799, ARG A:849, SER A:728, ASP A:863, LYS A: 753	ALA A:751, LEU A:752, VAL A:734, GLY A:727
Linarin	-8.52	570.51	**MET A:801,** ASP A:863, CYS A:805, ASP A:808, LEU A:726	LEU A:796, LYS A:753, ALA A:751, LEU A:852, ARG A: 849, VAL A:734
Narirutin	-8.97	268.02	**MET A:801,** GLN A:799, LYS A:753, ASP A:863, SER A:728	CYS A:805, LEU A:852, ALA A:751, VAL A:734

Note: Words in bold indicate the same interaction with native ligands.

Molecular Dynamic

Molecular dynamics simulations were conducted for the best binding compound, tamoxifen (comparison), and the native ligand compound against the 7PCD receptor (Table 3). This molecular dynamics simulation was performed during a 100 ns simulation by examining the RMSD (Figure 7) and RMSF (Figure 8) graphs. RMSD measures the average change in atomic displacement to measure how much the shape of the protein changes. RMSF measures the variation of amino acid residues in a protein chain (Ruswanto et al., 2022).

Tamoxifen, eriocitrin, glabrol, kaempferitrin, linarin, narirutin, and native ligand showed RMSD ranging from 0 to 30 ns, according to the RMSD graph (Figure 7). From 30 ns to the completion of the experiment, the RMSD of 100 ns was reasonably consistent. The RMSD graph demonstrates that, for a 100 ns molecular dynamics simulation, kaempferitrin has the most stable contact stability when compared to the other chemicals. During the 100 ns simulation, the RMSD average value supports this result (Table 4).

Kaempferitrin compounds can be utilized to illustrate the stability of the interaction based on the RMSF plot (Figure 8). Residual variation has been shown to have the same range of variation. However, the kaempferitrin compound has a lower fluctuation than the native ligand when viewed from the average RMSF of the kaempferitrin compound (1.129 Å) and the native ligand (1.363 Å). The graph proves that the kaempferitrin compound has a more stable interaction than native ligands.

Table 4. Average RMSD and RMSF values

Compounds	Average	
	RMSD (Å)	RMSF (Å)
Tamoxifen	3.447	1.174
Eriocitrin	4.168	1.676
Glabrol	4.531	1.561
Kaempferitrin	3.317	1.129
Linarin	4.027	1.463
Narirutin	3.974	1.427
Native ligands	3.400	1.363

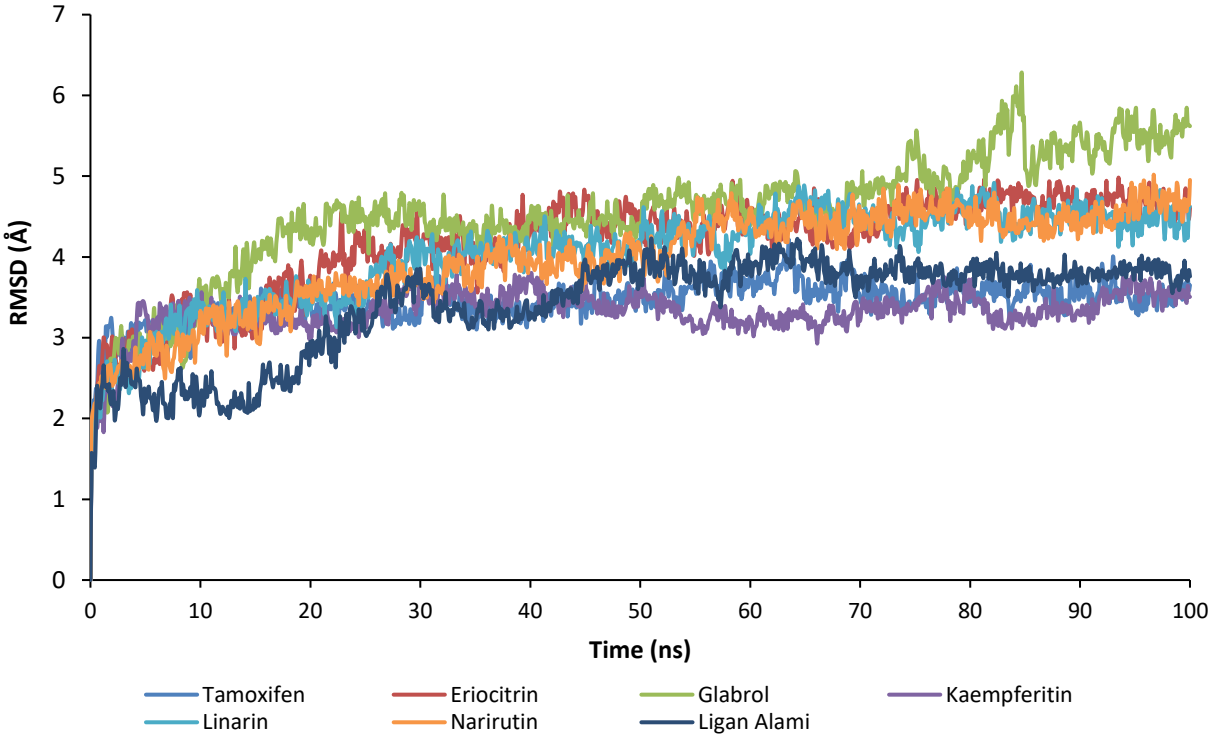


Figure 7. RMSD graph: Tamoxifen (dark blue), Eriocitrin (orange), Glabrol (gray), Kaempferitrin (yellow), Linarin (light blue), Narirutin (green), Native ligand (dark blue)

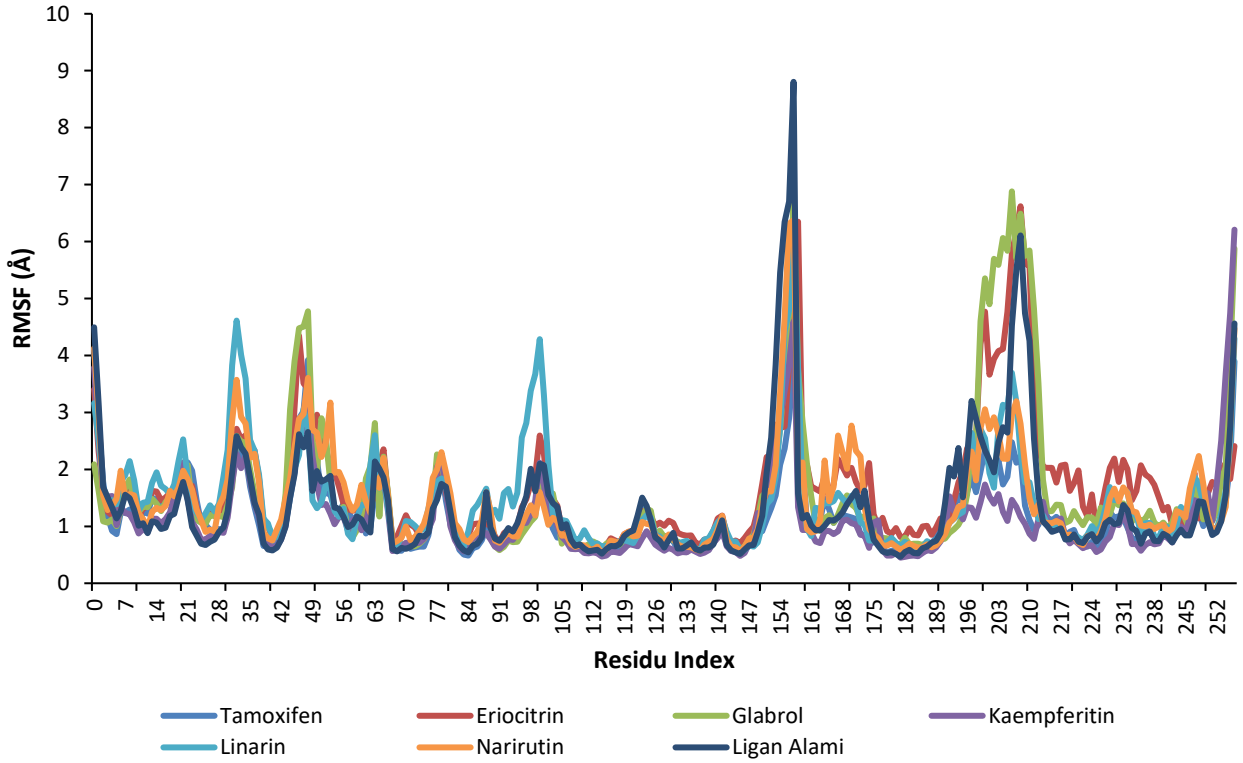


Figure 8. RMSF graph: Tamoxifen (dark blue), Eriocitrin (orange), Glabrol (gray), Kaempferitrin (yellow), Linarin (light blue), Narirutin (green), Native ligand (dark blue).

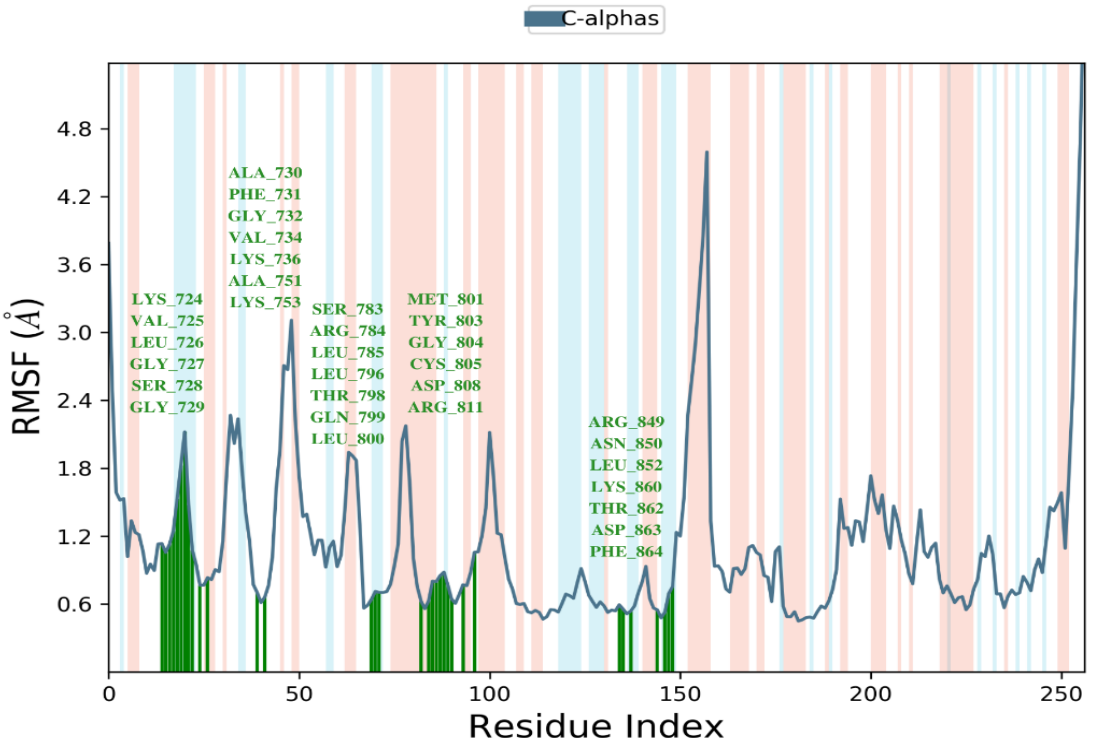


Figure 9. Graph of RMSF contact and Kaempferitrin residue in 100 ns molecular dynamics simulation

According to Figure 9, the residues that interact with the kaempferitrin compound are LYS:724, VA:725, LEU:726, GLY:727, SER:728, GLY:729, ALA:730, PHE:731, GLY:732, VAL:734, LYS:736, ALA:751, LYS:753, SER:783, ARG:784, LEU:785, LEU:796, THR:798, GLN:799, LEU:800, MET:801, TYR:803, GLY:804, CYS:805, ASP:808, ARG:811, ARG:849, ASN:850, LEU:852, LYS:860, THR:862, ASP:863, PHE:864 (33 residual contacts) Both interact via hydrogen bonds, hydrophobic bonds, ionic bridges, and water, as illustrated in (Figures 9 and 10).

Figure 11 demonstrates how the residues and ligands interact on each pass. A darker orange color indicates protein residues that make multiple specific contacts with the ligand. As shown in Figure 12, a total of six variables were examined to describe the stability of the kaempferitrin molecule at the 7PCD receptor during the 100 ns molecular dynamics simulation.

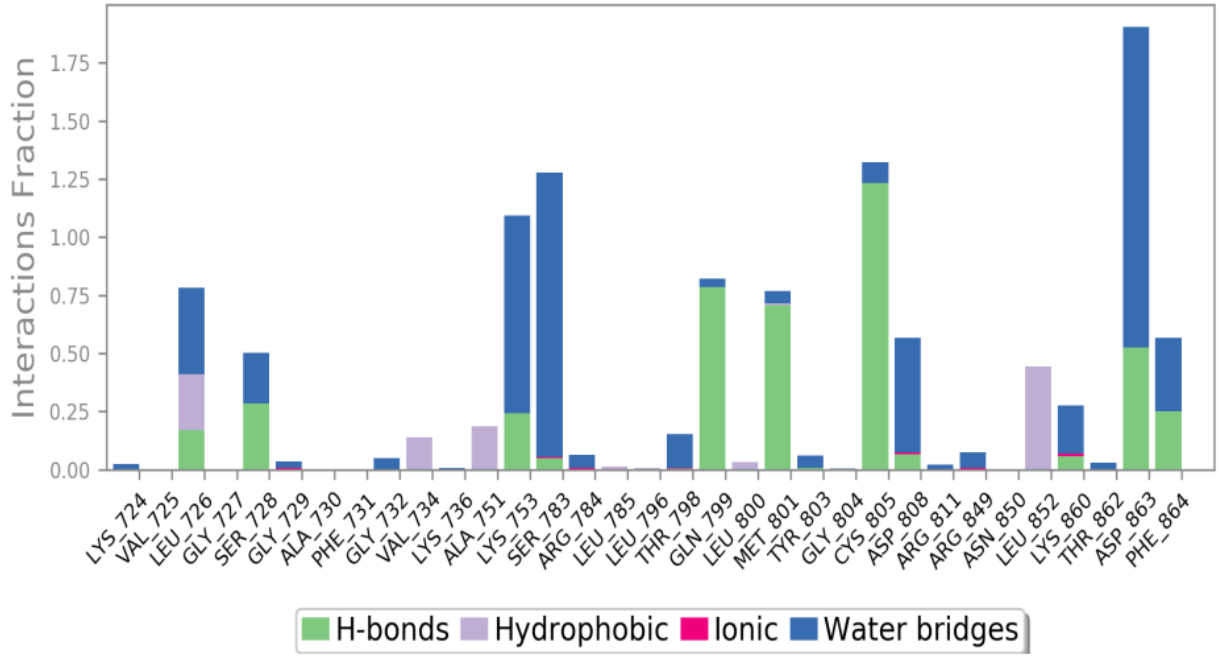


Figure 10. Kaempferitrin residue contact diagram

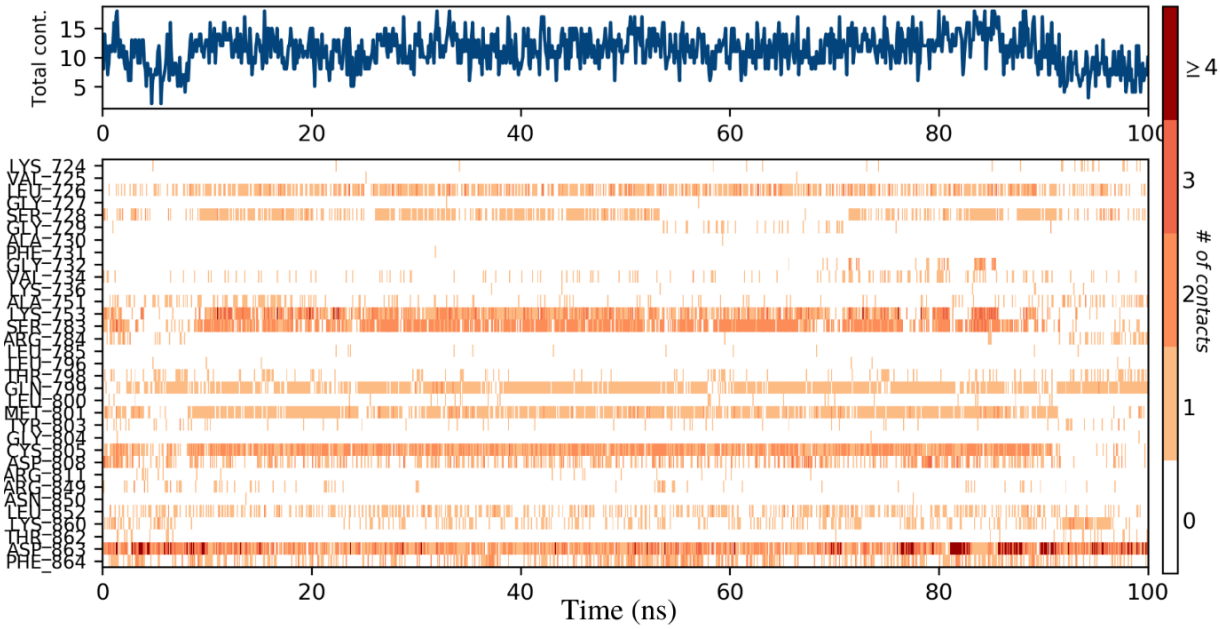


Figure 11. Representation of contact residues and interactions of kaempferitrin compounds for 100 ns

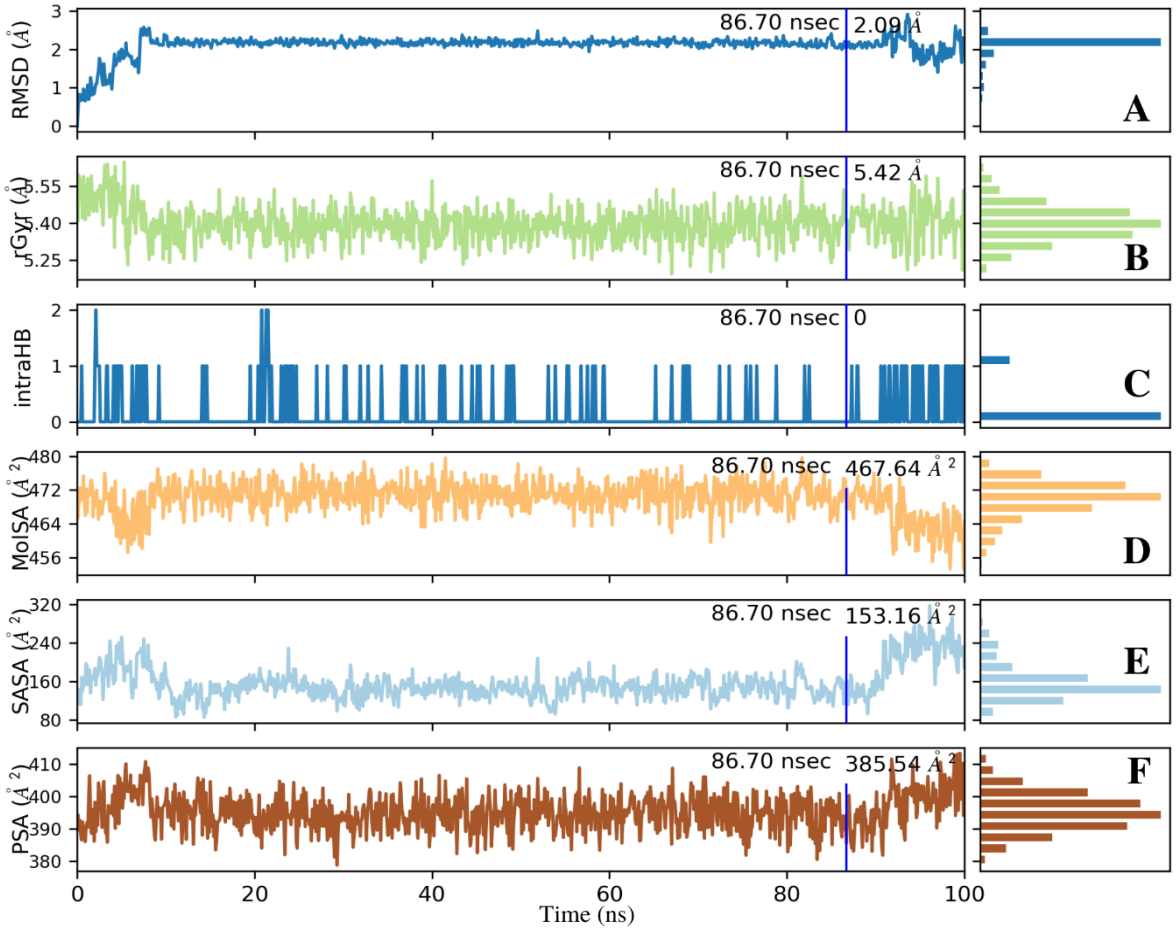


Figure 12. Characterization of the kaempferitrin compound during the 100 ns molecular dynamics simulation: (A) RMSD ligand, (B) gyration radius (rGyr), (C) NS34, (D) molecular surface area (MolSA), (E) surface area solvent accessible (SASA), and (F) polar surface area (PSA)

The kaempferitrin compound's RMSD simulation process fluctuates, as shown in Figure 12. A steady RMSD was seen throughout the entire simulation session after the initial stage of oscillations from 0 to 15 ns. Up until 15 ns, the gyration radius fluctuated. A stable conformation was then achieved until the simulation was completed. The SASA plot exhibits a fluctuation pattern for 15 ns before becoming stable for the duration of the simulation. The MolSA and PSA plots of the kaempferitrin compound varied but remained constant across the 100 ns simulation. At the same time, the intramolecular hydrogen bond plot of kaempferitrin from start to finish shows how intramolecular hydrogen bonds stabilize after protein contact.

The conformational variations of the kaempferitrin complex were also shown in the molecular dynamics simulation data (Figure 13), which demonstrate the conformational changes seen in the tracks through the 100-ns MD simulation, starting at 20, 40, 60, 80, and 100 ns. Conformational changes occur in the visualization from the beginning to the end of the 100 ns Molecular Dynamics simulation (kaempferitrin, tamoxifen, and native ligand). However, the 7PCD protein's binding site still exhibits the conformational change. Therefore, it is thought to interact similarly with Tamoxifen in its anti-cancer activity.

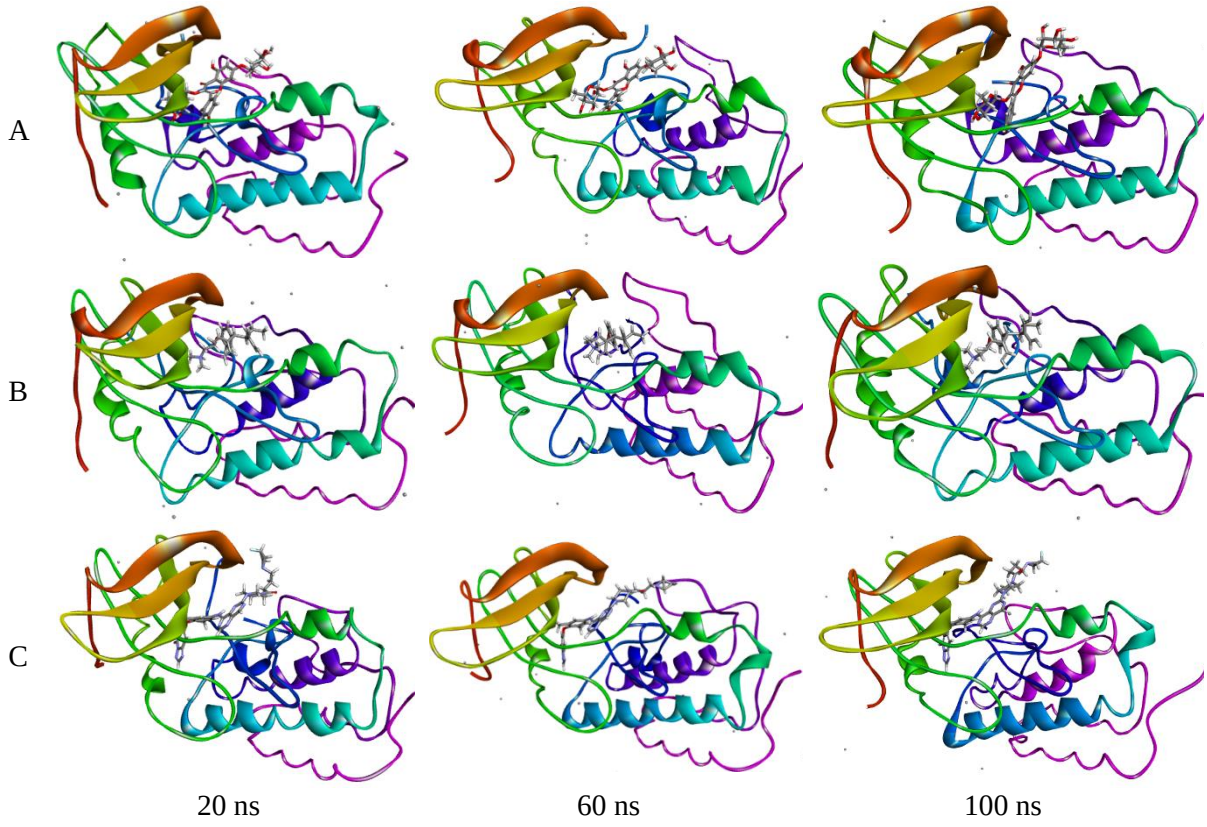


Figure 13. Conformational changes of kaempferitrin (A), Tamoxifen (B), and Native ligands (C) in 100 ns molecular dynamic simulation

Screening Ligan-Based Drugs Likeness (Drug Scan)

Lipinski rule of five states that the ideal medicine molecule follows the standards for physicochemical qualities. Lipinski rule of five forecasts a compound's chemical resemblance to a medicine with a certain biologic activity designed for oral delivery (Chen et al., 2020). Lipinski rule of five includes lipophilicity < 5, molecular weight < 500 g/mol, hydrogen bond donors < 5, refractory molar between 40-130, and hydrogen bond acceptors < 10 (Lipinski, 2004).

The size of the molecular weight affects drug dispersion. Due to the large molecular size of drugs, the larger the molecular weight, the more difficult it is for the drug to penetrate biological membranes (Ruswanto, 2015). LogP indicates the solubility and lipophilicity absorbed by the human body (Villa et al., 2021). The term "lipophilicity" refers to the ability of a substance to dissolve in lipids, oils, fats and non-polar solvents and is defined as the logarithm of the drug ratio. A drug must be sufficiently hydrophobic, but not too hydrophobic, to be absorbed and cross the lipid bilayer (Ivanović et al., 2020).

The ratio of hydrogen bond donors to acceptors shows that the energy required for the absorption process increases with hydrogen bond capacity (Weni et al., 2020). Molar refraction measures the total polarization of a substance, which depends on temperature, index of refraction, and pressure (Putra et al., 2020). Molar refraction describes the steric properties of a compound that can affect drug-receptor interactions. The greater the Calculated Molar Refraction (CMR), the greater the steric properties obtained, and the worse the drug interaction with the receptor (Gyebi et al., 2021). The results of the Drug Scan (Table 5) show that only the glabrol and kaempferitrin compounds fulfill at least two of the five Lipinski rule of five requirements. So that only the glabrol and kaempferitrin compounds can be used as oral preparations.

Table 5. Analysis of drug scan

No	Compounds	Parameter				
		Molecular Weight	Hydrogen Donor	Hydrogen Acceptor	Log P/Lipopility	Molar Reaction
		< 500 g/mol	< 5	< 10	< 5	40 - 130
1	Eriocitrin	596	9	15	2.53	134.40
2	Glabrol	392	2	4	4.46	114.73
3	Kaempferitrin	578	8	14	2.42	130.63
4	Linarin	592	7	14	3.14	135.85
5	Narirutin	580	8	14	2.62	132.85

Pharmacokinetic Analysis (ADME and Toxicity)

In testing the pharmacokinetic and toxicity profiles using the pkCSM website (Table 5). Compounds with an absorption value of >80% have a good absorption value and compounds have a poor absorption value if the absorption value is <30% and for good Skin Permeability values have values ranging from -2.7 to -3.4 (Pires et al., 2015). Based on the findings in (Table 5), the Human Intestinal Absorption (HIA) value of the Glabrol compound is known to have a good absorption value with an absorption value of 90.39%, and the Eriocitrin compound is known to have a poor absorption value with an absorption value of 26.97%. Meanwhile, the Skin Permeability test results showed that all compounds had good Skin Permeability.

Vdss, BBB, and CNS predictions are the findings of the distribution test. The volume of distribution at steady state (VDss) is a theoretical volume that indicates how evenly a drug's complete dose must be spread in order for its concentration to be the same as in blood plasma. A high VD value indicates that more drug is distributed to the tissues than plasma (Pires et al., 2015). The Volume of Distribution (VD) is high if the Log VD > 0.45, it can be concluded that only the Glabrol compound can be distributed evenly at the same concentration as in blood plasma with a VDss value of -0.055. A compound can penetrate the central nervous system if it has a Log PS value > -2 (Pires et al., 2015). According to the CNS permeability test results, only the Glabrol molecule has a CNS permeability value in the central nervous system of -2, namely -1.635. This causes the glabrol compound not to penetrate the central nervous system.

The body has important enzymes for the detoxification process. This enzyme is often called cytochrome P450 and is found in the liver. Cytochrome P450 works by oxidizing organic substances that enter the body and contains inhibitors that can affect drug metabolism (Pratama et al., 2022). To determine whether a drug compound or molecule can be used, it can be seen from the results of CYP3A4 inhibitor testing, the active compounds glabrol, kaempferitrin, linarin, and narirutin do not inhibit the action of the P450 enzyme.

The Total Clearance test can predict the rate of excretion of the active substances examined. The results of the Total Clearance test show that the glabrol compound has the highest Total Clearance, with a value of 0.691, while Kaempferitrin has the lowest Total Clearance, with a value of -0.102. Total Clearance is connected to bioavailability, which is a mix of metabolism in the liver and bile, and chemicals with the highest Total Clearance can be eliminated quickly (Yeni & Rachmania, 2022). The AMES Toxicity test is used to determine a compound's toxicity, which is a method for analyzing a compound's mutagenesis potential using bacteria (Halder *et al*., 2022). AMES Toxicity test results, all compounds are not hepatotoxic with an LD_{50} between 2,273-2,587 mg/kg and are not carcinogenic. Based on this, all test compounds are predicted to be safe and non-toxic (Ferrari & Mario, 2022).

Table 6. Pharmacokinetic profile analysis results (ADME and Toxicity)

Pharmacokinetic Profile	Parameter	Compounds				
		Eriocitrin	**Glabrol**	**Kaempferitrin**	**Linarin**	**Narirutin**
Absorption	CaCO2	-1.101	1.16	0.225	0.357	0.521
	HIA	26.972	90.398	35.385	36.679	36.625
	Skin Permeability	-2.735	-2.795	-2.735	-2.735	-2.735
Distribution	VDss	1.529	-0.055	1.487	0.919	1.295
	BBB	-1.831	-0.161	-1.823	-1.636	-1.594
	SSP	-4.859	-1.635	-4.673	-4.674	-4.708
Metabolism	CYP 2D6 *substrate*	No	No	No	No	No
	CYP 2D6 *inhibitor*	No	No	No	No	No
	CYP 3A4 *substrate*	No	Yes	No	No	No
	CYP 3A4 *inhibitor*	No	Yes	No	No	No
	CYP 2C9 *inhibitor*	No	Yes	No	No	No
	CYP 2C19 *inhibitor*	No	Yes	No	No	No
Excretion	*Total Clearance*	0.115	0.691	-0.102	0.103	0.307
Toxicity	LD50	2.514	2.273	2.587	2.521	2.519
	AMES Toxicity	No	No	No	No	No
	Hepatoksisitas	No	No	No	No	No

4. CONCLUSION

The flavonoid chemicals eriocitrin, glabrol, kaempferitrin, linarin, and narirutin have been identified with anticancer properties based on molecular docking and pharmacokinetic studies. The kaempferitrin outperforms the other compounds when the interaction stability outcomes of the docking molecules are assessed using 100 ns molecular dynamics simulations. Kaempferitrin has the lowest RMSD and RMSF values when compared to other drugs. As a result, more research on kaempferitrin compounds can be done in order to discover and develop novel molecules as prospective therapeutic options for breast cancer treatment.

ACKNOWLEDGEMENT

The authors thank the Universitas Bakti Tunas Husada and Universitas Perjuangan Tasikmalaya for the facilities in research.

REFERENCES

Alnajjar, R., Mostafa, A., Kandeil, A., Al-Karmalawy, A. A. (2020). Molecular docking, molecular dynamics, and in vitro studies reveal the potential of angiotensin II receptor blockers to inhibit the COVID-19 main protease. *Heliyon*, *6*(12), e05641. https://doi.org/10.1016/j.heliyon.2020.e05641

Aziz, F. K., Nukitasari, C., Oktavianingrum, F. A., Aryati, L. W., Santoso, B. (2016). Hasil In Silico Senyawa Z12501572, Z00321025, SCB5631028 dan SCB13970547 dibandingkan Turunan Zerumbon terhadap Human Liver Glycogen Phosphorylase (1l5Q) sebagai Antidiabetes. *Jurnal Kimia VALENSI*, *2*(2), 120–124. https://doi.org/10.15408/jkv.v2i2.4170

Baksi, R., Singh, D. P., Borse, S. P., Rana, R., Sharma, V., Nivsarkar, M. (2018). In vitro and in vivo anticancer efficacy potential of Quercetin loaded polymeric nanoparticles. *Biomedicine and Pharmacotherapy*, *106*(July), 1513–1526. https://doi.org/10.1016/j.biopha.2018.07.106

Binotto, M., Reinert, T., Werutsky, G., Zaffaroni, F., Schwartsmann, G. (2020). Health-related quality of life before and during chemotherapy in patients with early-stage breast cancer. *Ecancermedicalscience*, *14*, 1–11. https://doi.org/10.3332/ECANCER.2020.1007

Chen, X., Li, H., Tian, L., Li, Q., Luo, J., Zhang, Y. (2020). Analysis of the Physicochemical Properties of Acaricides Based on Lipinski's Rule of Five. *Journal of Computational Biology*, *27*(9), 1397–1406. https://doi.org/10.1089/cmb.2019.0323

Chow, E., Rendleman, C. A., Bowers, K. J., Dror, R. O., H, D., Gullingsrud, J., Sacerdoti, F. D., Shaw, D. E. (2008). Desmond Performance on a Cluster of Multicore Processors. *Simulation*, *July*, 1–14.

Dermawan, D., Sumirtanurdin, R., Dewantisari, D. (2019). Molecular Dynamics Simulation Estrogen Receptor Alpha againts Andrographolide as Anti Breast Cancer. *Indonesian Journal of Pharmaceutical Science and Technology*, *6*(2), 65. https://doi.org/10.24198/ijpst.v6i2.18168

Villa, F. X. D, Durán-Iturbide, N. A., Ávila-Zárraga, J. G. (2021). Synthesis, molecular docking, and in silico ADME/Tox profiling studies of new 1-aryl-5-(3-azidopropyl)indol-4-ones: Potential inhibitors of SARS CoV-2 main protease. *Bioorganic Chemistry*, *106*(October). https://doi.org/10.1016/j.bioorg.2020.104497

Eberhardt, J., Santos-Martins, D., Tillack, A. F., Forli, S. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *Journal of Chemical Information and Modeling*, *61*(8), 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203

Elengoe, A., Kumar, Selvam, S., Selvarajah, M., Manikam, H., Vijaya, R. (2022). Sequence analysis and structure prediction of malaysia sars-cov-2 strain's structural and accessory proteins. In *Biointerface Research in Applied Chemistry*, 12(3). https://doi.org/10.33263/BRIAC123.32593304

Ezzati, M., Yousefi, B., Velaei, K., Safa, A. (2020). A review on anti-cancer properties of Quercetin in breast cancer. *Life Sciences*, *248*(September 2019). https://doi.org/10.1016/j.lfs.2020.117463

Ferrari, I. V., Mario, M. D. (2022). Comparison and Prediction of toxicity parameters of principal Aflatoxins , antimicrobial compounds and Antifungal Drugs by pKCSM server. *International Journal of Scientific Research in Biological Sciences*, *9*(1), 48–52.

Frimayanti, N., Djohari, M., Khusnah, A. N. (2021). Molekular Docking Senyawa Analog Kalkon sebagai Inhibitor untuk Sel Kanker Paru-Paru A549. *Jurnal Ilmu Kefarmasian Indonesia*, *19*(1), 87. https://doi.org/10.35814/jifi.v19i1.765

Globocan. (2020). Cancer Incident in Indonesia. *International Agency for Research on Cancer*, *858*, 1–2. https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf

Gyebi, G. A., Ogunro, O. B., Adegunloye, A. P., Ogunyemi, O. M., Afolabi, S. O. (2021). Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): an in silico screening of alkaloids and terpenoids from African medicinal plants. *Journal of Biomolecular Structure and Dynamics*, *39*(9), 3396–3408. https://doi.org/10.1080/07391102.2020.1764868

Halder, S. K., Ahmad, I., Shathi, J. F., Mim, M. M., Hassan, M. R., Jewel, M. J. I., Dey, P., Islam, M. S., Patel, H., Morshed, M. R., Shakil, M. S., Hossen, M. S. (2022). A Comprehensive Study to Unleash the Putative Inhibitors of Serotype2 of Dengue Virus: Insights from an In Silico Structure-Based Drug Discovery. *Molecular Biotechnology*, *0123456789*. https://doi.org/10.1007/s12033-022-00582-1

Hanif, A. U., Lukis, P. A., Fadlan, A. (2021). Pengaruh Minimisasi Energi MMFF94 dengan MarvinSketch dan Open Babel PyRx pada Penambatan Molekular Turunan Oksindola Tersubstitusi. *ALCHEMY : JOURNAL OF CHEMISTRY*.

Hartini, S., Winarsih, B. D., Galih, E., Nugroho, Z., Studi, P., Ners, P. (2020). *Peningkatan Pengetahuan Perawat Untuk Perawatan Anak Penderita Kanker*. *3*(2), 141–149.

Ivanović, V., Rančić, M., Arsić, B., Pavlović, A. (2020). Lipinski's rule of five, famous extensions and famous exceptions. *Popular Scientific Article*, *3*(1), 171–177.

Kikuchi, H., Yuan, B., Hu, X., Okazaki, M. (2019). Chemopreventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. *American Journal of Cancer Research*, *9*(8), 1517–1535. http://www.ncbi.nlm.nih.gov/pubmed/31497340%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6726994

Kumar, S., Sharma, P. P., Shankar, U., Kumar, D., Joshi, S. K., Pena, L., Durvasula, R., Kumar, A., Kempaiah, P., Poonam, Rathi, B. (2020). Discovery of New Hydroxyethylamine Analogs against 3CLproProtein Target of SARS-CoV-2: Molecular Docking, Molecular Dynamics Simulation, and Structure-Activity Relationship Studies. *Journal of Chemical Information and Modeling*, *60*(12), 5754–5770. https://doi.org/10.1021/acs.jcim.0c00326

Leung, S. H., Bodkin, M. J., von Delft, F., Brennan, P. E., Morris, G. M. (2019). SuCOS is better than RMSD for evaluating fragment elaboration and docking poses. *ChemRxiv*, 1–47.

Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Mardianingrum, R., Ruswanto, R., Agustien, G. S., Nuraisah, A. (2020). The Active Compound of Bangle Essential Oil as Cyclooxygenase-2 (Cox-2) Inhibitor: In Silico Approach. *Jurnal Kimia Valensi*, *6*(2), 156–168. https://doi.org/10.15408/jkv.v6i2.16943

Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., Jemal, A., Kramer, J. L., Siegel, R. L. (2019). Cancer treatment and survivorship statistics, 2019. *CA: A Cancer Journal for Clinicians*, *69*(5), 363–385. https://doi.org/10.3322/caac.21565

Mvondo, J. G. M., Matondo, A., Mawete, D. T., Bambi, S.-M. N., Mbala, B. M., Lohohola, P. O. (2021). In Silico ADME/T Properties of Quinine Derivatives using SwissADME and pkCSM Webservers. *International Journal of TROPICAL DISEASE & Health*, *42*(11), 1–12. https://doi.org/10.9734/ijtdh/2021/v42i1130492

Pires, D. E. V., Blundell, T. L., Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, *58*(9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104

Pratama, P. R., Isman, F., Fadlan, A. (2022). Penyelidikan Aktivitas Antikanker Payudara Oleh Minyak Atsiri Bunga Michelia Alba Secara in Silico. *Jurnal Ilmu Kimia Dan Terapan*, *9*(1).

Protein Data Bank. (n.d.). *Enabling Breakthroughs in Scientific and Biomedical Research and Education*. https://www.rcsb.org/

Puspita, P. J., Ambarsari, L., Adiva, A., Sumaryada, T. I. (2021). In Silico Analysis of Glucose Oxidase H516r and H516d Mutations for an Enzymatic Fuel Cell. *Jurnal Kimia Valensi*, *7*(2), 83–93. https://doi.org/10.15408/jkv.v7i2.20733

Putra, P. P., Fauzana, A., Lucida, H. (2020). Analisis Sifat Fisika-Kimia, Potensi Target dan Toksikologi Senyawa Isolat Murni dari Bahan Alam dengan Metode In Silico. *Indonesian Journal of Pharmaceutical Science and Technology Journal Homepage*, *7*(3), 107–117. http://jurnal.unpad.ac.id/ijpst/UNPAD

Qasaymeh, R. M., Rotondo, D., Oosthuizen, C. B., Lall, N., Seidel, V. (2019). Predictive binding affinity of plant-derived natural products towards the protein kinase g enzyme of mycobacterium tuberculosis (Mtpkng). *Plants*, *8*(11), 1–14. https://doi.org/10.3390/plants8110477

Rachmania, R. A., Hariyanti, H., Zikriah, R., Sultan, A. (2018). Studi In Silico Senyawa Alkaloid Herba Bakung Putih (Crinum Asiaticum L.) pada Penghambatan Enzim Siklooksigenase (COX). *Jurnal Kimia VALENSI*, *4*(2), 124–136. https://doi.org/10.15408/jkv.v4i2.7686

Ramadhan, D. S. F., Fakih, T. M., Arfan, A. (2020). Activity Prediction of Bioactive Compounds Contained in Etlingera elatior Against the SARS-CoV-2 Main Protease: An In Silico Approach. *Borneo Journal of Pharmacy*, *3*(4), 235–242. https://doi.org/10.33084/bjop.v3i4.1634

Rathod, S., Shinde, K., Porlekar, J., Choudhari, P., Dhavale, R., Mahuli, D., Tamboli, Y., Bhatia, M., Haval, K. P., Al-Sehemi, A. G., Pannipara, M. (2022). Computational Exploration of Anti-cancer Potential of Flavonoids against Cyclin-Dependent Kinase 8: An In Silico Molecular Docking and Dynamic Approach. *ACS Omega*. https://doi.org/10.1021/acsomega.2c04837

Ruswanto, Mardianingrum, R., Siswandono, Kesuma, D. (2020). *Reverse Docking, Molecular Docking, Absorption, Distribution, and Toxicity Prediction of*

Artemisinin as an Anti-diabetic Candidate. *15*(2), 88–96.

Ruswanto, Nofianti, T., Mardianingrum, R., Lestari, T., sepriliani, a. (2018). desain dan studi in silico senyawa turunan Kuwanon-H sebagai kandidat obat Anti-HIV. *Jurnal Kimia VALENSI*, *4*(1), 57–66. https://doi.org/10.15408/jkv.v4i1.6867

Ruswanto, R. (2015). Molecular Docking Empat Turunan Isonicotinohydrazide Pada Mycobacterium Tuberculosis Enoyl-Acyl Carrier Protein Reductase (InhA). *Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-Ilmu Keperawatan, Analis Kesehatan Dan Farmasi*, *13*(1), 135–141. https://doi.org/10.36465/jkbth.v13i1.25

Ruswanto, R., Garna, I. M., Tuslinah, L., Mardianingrum, R., Lestari, T., Nofianti, T. (2018). Kuersetin, Penghambat Uridin 5-Monofosfat Sintase Sebagai Kandidat Anti-kanker. *ALCHEMY Jurnal Penelitian Kimia*, *14*(2), 236. https://doi.org/10.20961/alchemy.14.2.14396.236-254

Ruswanto, R., Mardianingrum, R., Yanuar, A. (2022). Computational studies of thiourea derivatives as anticancer candidates through inhibition of Sirtuin-1 (SIRT1). *Jurnal Kimia Sains Dan Aplikasi*, *25*(3), 87–96. https://doi.org/10.14710/jksa.25.3.87-96

Ruswanto, R., Nofianti, T., Mardianingrum, R., Kesuma, D., Siswandono. (2022). Design, molecular docking, and molecular dynamics of thiourea-iron (III) metal complexes as NUDT5 inhibitors for breast cancer treatment. *Heliyon*, *8*(9). https://doi.org/10.1016/j.heliyon.2022.e10694

Salman, M. M., Al-Obaidi, Z., Kitchen, P., Loreto, A., Bill, R. M., wade-martins, r. (2021). advances in applying computer-aided drug design for neurodegenerative diseases mootaz. *International Journal of Molecular Sciences Review*, *42*(18), 3436–3446. https://doi.org/10.1021/jm990129n

Sari, I. W., Junaidin, Pratiwi, D. (2020). *Molecular Docking Study Flavonoid Compounds From Kumis Kucing (Orthosiphon stamineus B.) In a-Glukosidase Receptor as Antidiabetic Type 2*. *VII*(2), 54–60.

Selvakumar, P., Badgeley, A., Murphy, P., Anwar, H., Sharma, U., Lawrence, K., Lakshmikuttyamma, A. (2020). Flavonoids and other polyphenols act as epigenetic. *Nutrients*, *12*, 1–18. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.1002/cncr.30614

Sharma, V. R., Panwar, A., Sharma, A. K. (2020). Molecular dynamic simulation study on chromones and flavonoids for the in silico designing of a potential ligand inhibiting mTOR pathway in breast cancer. *Current Pharmacology Reports*, *6*(6), 373–379. https://doi.org/10.1007/s40495-020-00246-1

Surabhi, S., Singh, B. (2018). Computer aided drug design: an overview. *Journal of Drug Delivery and Therapeutics*, *8*(5), 504–509. https://doi.org/10.22270/jddt.v8i5.1894

Utami, W., Aziz, H. A., Fitriani, I. N., Zikri, A. T., Mayasri, A., Nasrudin, D. (2020). In silico anti-inflammatory activity evaluation of some bioactive compound from ficus religiosa through molecular docking approach. *Journal of Physics: Conference Series*, *1563*(1). https://doi.org/10.1088/1742-6596/1563/1/012024

Victory, A., Syahdi, R. R., & Yanuar, A. (2018). Virtual screening of Indonesian herbal database as murine double minute-2 (MDM2) inhibitor. *Pharmacognosy Journal*, *10*(6), 1184–1189. https://doi.org/10.5530/pj.2018.6.203

Weni, M., Safithri, M., & Seno, D. S. H. (2020). Molecular docking of active compounds Piper crocatum on the α-glucosidase enzyme as antidiabetic. *Indonesian Journal of Pharmaceutical Science and Technology*, *7*(2), 64. https://doi.org/10.24198/ijpst.v7i2.21120

Yeni, Y., Rachmania, R. A. (2022). The prediction of pharmacokinetic properties of compounds in Hemigraphis alternata (Burm.F.) T. ander leaves using pkCSM. *Indonesian Journal of Chemistry*, *22*(4), 1081–1089. https://doi.org/10.22146/ijc.73117