

In Silico Study of Seven Novel Peronemin Compounds from Sungkai Leaves (*Peronema canescens* Jack) as Anti-Inflammatory Linkage Immunomodulatory

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Abstract: Sungkai (*Peronema canescens*) is a raw material used in traditional medicine to reduce fever (antipyretic) and can also be used as a treatment for malaria. The secondary metabolite content in sungkai leaves is in the form of alkaloids, flavonoids, triterpenoids, steroids, phenolics, and saponins. From the acetone extract of sungkai leaves, the compounds peronemin (A2, A3, B1, B2, B3, C1 and D1), sitosterol, isopropanol, phytol, diterpenoids and flavonoids are produced. Molecular docking of seven peronemin compounds was carried out using PyRx based on AutoDockTools using IFN- γ and IL-10 receptors, which can modulate the immune system and inflammatory reactions. The molecular docking process obtained the best results for the peronemin C1 compound against the IFN- γ receptor with a binding energy value of -3.65. Tethering with IL-10 obtained the best bond energy value for compound A3, -2.37.

Keywords: anti-inflammatory; immunomodulatory; molecular docking; peronemin; IL-10; IFN- γ .

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1. Introduction

Nowadays, the need for and use of traditional medicine is growing [1]. The use of plant-based medicines, herbs and secondary metabolite compounds, phytonutrients, or nutraceuticals continues to proliferate worldwide, and many people are now using these products to overcome various health challenges in various national health service settings [2]. Indonesia is a country that has abundant natural resources, where around 40,000 plant species have potential as medicine; of the majority of natural biological resources in Indonesia, only around 2.5% have been explored and utilized as medicine or alternative healing [3]. One of the plants that can be used as alternative medicine in Indonesia is the sungkai (*Peronema canescens*) plant.

Sungkai (*Peronema canescens*) belongs to the Verbenaceae family *P. canescens*. The young leaves of *P. canescens* are raw materials used in traditional medicine to reduce fever (antipyretic). The community can also use them as a treatment for malaria, according to various studies that have been conducted [4,5]. The secondary metabolite in sungkai leaves can help the body's homeostatic function and is an anticancer. Sungkai leaves also contain furano diterpenoid compounds of the type klero and (clerodane). Alkaloids, flavonoids, triterpenoids,

steroids, phenolics, and saponins [4,6]. From the acetone extract of sungkai leaves, the compounds peronemin (A2, A3, B1, B2, B3, C1, and D1), sitosterol, isopropanol, phytol, diterpenoids and flavonoids are produced [7]. The peronemin compound and its derivatives in sungkai leaves can improve the immune system by increasing the number of white blood cells (leukocytes) in the body. Leukocytes are cells that form blood components, which help increase white blood cell levels and protect the body from various diseases [8]. Infection can be inhibited through compounds that can modulate inflammation as well as improve the immune system by selecting the right receptors paired with ligands (active compounds) to obtain optimal results [9].

Traditional drug discovery and development requires a long process and costs quite a lot of money [3]. Traditional drug development takes an average of 10-15 years before it is ready to enter the marketing process. One of the efforts made to reduce time and costs in developing a drug compound is using the computer-aided drug discovery (CADD) approach. CADD has an important role in innovation in low-cost drug discovery, reducing the use of animals in pharmacological tests and helping design new drug candidates. One of the methods used is an in-silico test, which can identify drug targets by computationally predicting target proteins [10]. CADD approaches are generally divided into two types, namely structure-based drug design (SBDD) and ligand-based drug design (LBDD) [11].

A computational method, namely molecular docking, is used to predict the bond of a macromolecule (receptor) with a small molecule in the form of a ligand. This molecular docking aims to determine the conformations and binding free energies involved in the interaction between the macromolecule (receptor) and the ligand [12]. This docking simulation helps in studying drug or ligand or receptor/protein interactions to obtain good geometry of the ligand-receptor complex [13]. This research was carried out to simulate the interaction of ligands (peronemin compounds) with macromolecules (IL-10 and IFN- γ).

2. Materials and Methods

2.1. Materials and instrumentation.

The main ingredient in this research is the 3D form of the Peronemin compound, which is saved in PDB format. as well as the receptor structures (target proteins) of IL-10 (1LQS) and IFN- γ (3BES), which are stored in PDB form on the web server of each database. The tools used in this research are hardware, namely Lenovo PC IdeaCentre AIO 5i 24IAH7 F0GR006RID Storm Gray (Intel Core i7 12700H, Win11 Home, 16GB DDR4, Intel ARC A370M 4GB GDDR6) and software PyRx, ChemDraw Ultra version 22.0, Chem 3D version 22.0, AutoDockTools, Discovery Studio Visualizer 2021 and UCSF Chimera. The webserver used is RCSB (Research Collaboratory for Structural Bioinformatics).

2.2. Ligand structure preparation.

Ligand preparation was carried out by converting the 2-dimensional (2D) molecular structures of seven Peronemin compounds, which were drawn using ChemDraw Ultra version 22.0, and then converted them into 3-dimensional (3D) structural models using the Chem3D version 22.0 application, which were saved in PDB file format. Then, hydrogen ions are added to the ligand using Discovery Studio 2021 software and saved in PDF file format. Next, the ligand is optimized using the AutoDockTools program, and then the number of torsion bonds in the ligand is adjusted and saved in the PDBQT file format.

2.3. Macromolecular preparation.

The three-dimensional macromolecules IL-10 and IFN- γ were downloaded from the Protein Data Bank data site <https://www.rcsb.org>. The PDB codes used are 1LQS (IL-10) and 3BES (IFN- γ). Macromolecules are separated from solvents and native ligands or non-standard residues using the UCSF Chimera application. Native ligands and unnecessary residues are removed by clicking the select feature, then clicking residues and selecting all non-standard, then selecting the actions feature, clicking atoms/bonds, and then clicking delete. Macromolecular (receptor) files are saved in PDB format. Next, the macromolecules were optimized using AutoDockTools by adding hydrogen ions and Kollman charges and saved in PDBQT file format.

2.4. Validation of molecular docking parameters.

Validation of the molecular docking method was carried out using AutoDock Tools software. This is done by re-docking the natural ligands of each macromolecule (receptor). The parameter used is Root Mean Square Deviation (RMSD). The results obtained in this process are the grid box parameters and RMSD values. The docking method is said to be valid if it has an RMSD value $< 3 \text{ \AA}$; this value indicates the protocol is accepted and docking can be carried out.

2.5. Molecular docking.

The molecular docking process is carried out using PyRx software based on AutoDock Tools. The macromolecule (receptor) and ligand structures that have been optimized separately are stored in one folder. According to validation results, the molecular docking process uses a grid box and energy minimization parameters. Grid box parameter settings are carried out using grid box coordinates, which are determined based on the ligand coordinates of the receptor used in the docking validation process. Next, docking is carried out using PyRx software with the AutoDock wizard feature. The docking data displayed is in the form of binding affinity values and amino acid residue interactions. Docking results are saved in PDB format.

2.6. Visualization and analysis of docking results.

The visualization process is carried out to see the interactions that occur in the docking results between the receptor and the ligand. Visualization of docking results was carried out using Discovery Studio Visualizer 2021 software.

2.7. Pharmacokinetic and toxicity prediction.

Pharmacokinetic and toxicity predictions were carried out using methods referring to the report by Terefa et al. [14]. Pharmacokinetic predictions are made for the test compound by copying the SMILES code on the test compound and then pasting it into the SWISSADME database (<http://www.swissadme.ch/>). Next, the SMILES code of the test compound is accessed via the admetSAR database (<http://lmmd.ecust.edu.cn/admetsar2/>) for toxicity prediction analysis tests.

3. Results and Discussion

3.1. Ligan structure and preparation.

Ligands are molecules or active compounds that bind to receptors to trigger a biomolecular response. The ligands used in the test are peronemin compounds A2, A3, B1, B2, B3, C1, and D1 (Figure 1). The 2D peronemin structure that was drawn using ChemDraw Ultra version 22.0 was then converted into 3D using Chem 3D version 22.0, which was saved in PDB format. Next, hydrogen ions were added using Discovery Studio 2021 software saved in PDF format, and optimization was carried out using the AutoDock Tools program by calculating the number of torsion bonds. All optimized ligands are saved in the PDBQT file format. Ligand preparation aims to determine the energy interaction between the ligand and the receptor. This is done to create ligand flexibility by increasing the number of bonds that can be rotated by the ligand [15].

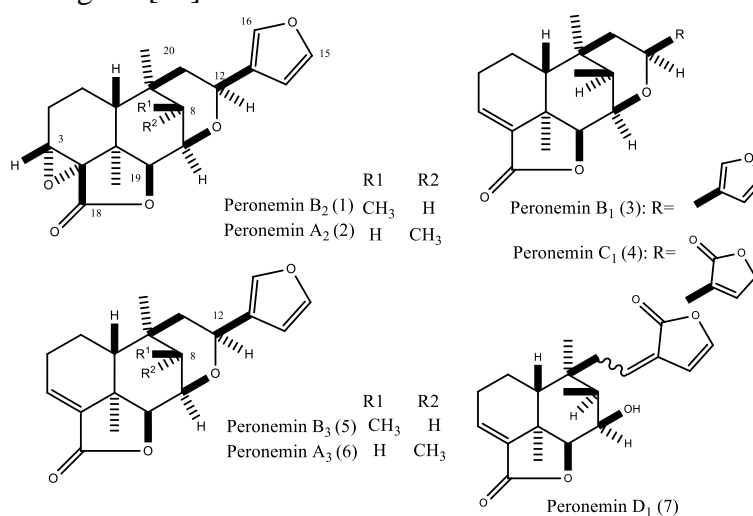


Figure 1. Structure of seven peronemin compounds.

3.2. Characterization of test ligands.

The initial screening in molecular docking, namely in the ligand selection process, must follow Lipinski's rules. A test compound (ligand) is considered to have the potential to enter cell membranes and be absorbed by the body if it meets Lipinski's rules with the following criteria: (1) molecular weight < 500 grams/mol, (2) number of hydrogen proton donor groups < 5, (3) number of hydrogen bond proton acceptor groups < 10, (4) value of the logarithm of the partition coefficient in water and 1-octanol < 5 [16].

By analyzing the physicochemical characteristics of the ligand according to Lipinski's rules, it is possible to determine a substance's hydrophobic/hydrophilic character to diffuse passively through the cell membrane. The log P value represents the solubility coefficient in fat/water, which has a range of -0.4 – 5. Molecular weights of more than 500 Da cannot diffuse across the cell membrane. The greater the log P value, the more hydrophobic the molecule is. Molecules that are too hydrophobic tend to have a high level of toxicity because they are retained longer in the lipid bilayer and are distributed more widely in the body, thereby reducing the selectivity of binding to the target enzyme. In addition, a log P value that is too negative is also unfavorable because the molecules cannot penetrate the lipid bilayer membrane. The number of hydrogen bond donors and acceptors shows that the energy required to continue the absorption process increases as the hydrogen bond capacity increases. Lipinski's

rule generally describes the solubility of certain compounds in penetrating cell membranes by passive diffusion [17]. The structure of the seven peronemin compounds that have been screened, as shown in Table 1, fulfills Lipinski's law so that it has the potential for high bioavailability in the body.

Table 1. Characterization of test ligands.

Characteristics	A2	A3	B1	B2	B3	C1	D1
Koefisien Log P	3.2449	4.0337	4.0337	3.2449	4.0337	2.5512	2.4923
Berat Molekul (BM)	344.40	328.40	328.40	344.40	328.40	344.40	344.40
HBA	5	4	4	5	4	5	5
HBD	0	0	0	0	0	0	1

3.3. Macromolecular preparation.

Macromolecules (receptors) are sites where drug molecules can interact to form a reversible complex that will ultimately cause a response. In general, macromolecules (receptors) are protein molecules/polymer structures that specifically recognize and can bind molecules (ligands). Macromolecular preparation was carried out by downloading the receptors in the <https://www.rcsb.org> database with the receptor codes 1LQS (IL-10) and 3BES (IFN- γ). The 3D structure of IFN- γ (3BES) was obtained from the PDB data bank, which was the result of crystallization using x-ray diffraction with a resolution of 2.20 Å and the 3D structure of IL-10 (1LQS) with a resolution of 2.70 Å. The smaller resolution of the protein structure obtained from the results of X-ray crystallography shows that the structure is close to the structure that exists in cells. Apart from that, the receptor must come from the Homo sapiens species so that the results of the docking are close to actual conditions [18].

To show that the complex between IFN- γ (3BES) and IL-10 (1LQS) has good quality is to depict it in a Ramachandran plot (Figure 2). Ramachandran plots are used to obtain information about the quality of proteins that will be used in the molecular docking process. The method is to look at the non-glycine residue plot in the prohibited dihedral corner area (disallowed region). Glycine is an amino acid with no side chain, so the angles Φ and ψ can be in the four quadrants of the Ramachandran plot [19]. The results of the Ramachandran plot visualization for each target receptor can be used because they all meet the rules of the Ramachandran plot analysis parameters, namely 0.0% for IFN- γ and IL-10.

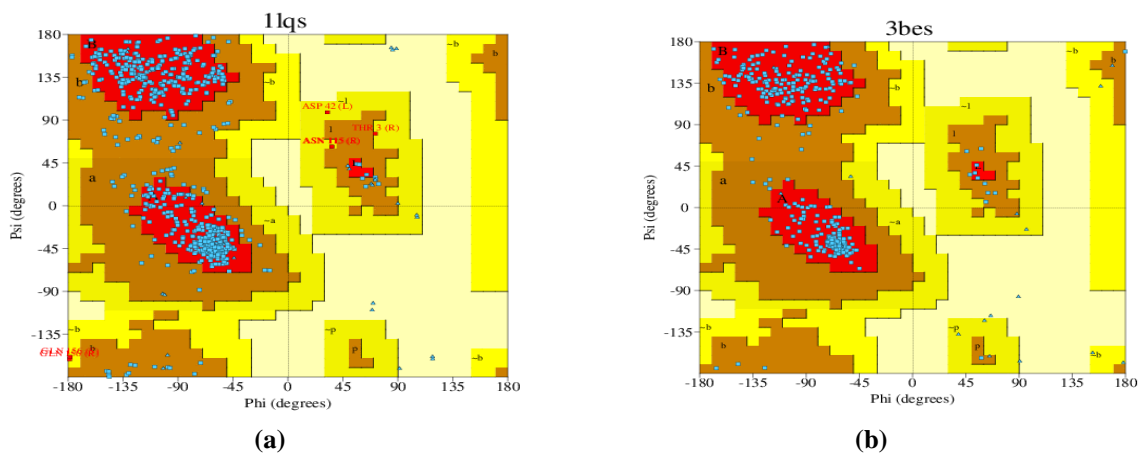


Figure 2. Plot ramachandran protein (a) IL-10; (b) IFN- γ .

The procedure is to look at the plot of non-glycine residues found in dihedral corner regions that are disallowed (disallowed region). A protein structure can be good if the number of residual plots in the disallowed region is less than 0.8% [20]. Next, macromolecular

preparation is carried out by separating the solvent and the original ligand or non-standard residue. According to Dewi et al. [21], macromolecular preparation is carried out with the aim of obtaining a target protein structure without a native ligand so that space (pocket cavity) is available for the docking process of the test compound, as well as obtaining a native ligand structure for the method validation process. Macromolecular preparation is carried out by removing water molecules (H₂O) from each target protein whose original ligand has been removed, leaving the protein's amino acids. As well as the addition of hydrogen ions and Kollman calculations.

3.4. Molecular docking validation.

Validation of the molecular docking method was carried out by docking the original ligand back to the target protein that had been removed using the AutoDock Tools program. The docking validation parameter used is the RMSD (Root Mean Square Distance) value. RMSD is the value used to determine if the binding mode prediction is successful and is important for validating the docking program. The RMSD value is said to be good if $< 3 \text{ \AA}$ (Table 2). The greater the deviation, the greater the error in predicting ligand-protein interactions. This validation process aims to see deviations between the native ligand conformation before and after docking again [22].

In molecular docking validation, a grid box is arranged to provide space for the native ligand to form a conformation when docked with the target protein. The grid box is where the ligand interacts with amino acid residues on the target protein. The grid box is determined to find the coordinates in the binding site area of a protein. From the results of the redocking process on native ligands on the IFN- γ and IL-10 receptors, the RMSD value is below 3 \AA , so the method used is valid. The acceptable RMSD value limit is $< 3 \text{ \AA}$ [23].

Table 2. Molecular docking validation.

Native ligand	Binding free energy (kcal mol ⁻¹)	Inhibition constant (μM)	RMSD	Amino acid residue
IFN- γ (3BES)	-1.44	87.78	2.87	ILE 160, TRP 201, GLU 156, ASN 149, PRO 158, GLU 157
IL-10 (1LQS)	-1.39	95.87	2.60	ASP 133, GLU 129, ASN 134

3.5. Molecular docking analysis.

The goal of drug discovery is to identify, optimize, and clinically validate compounds that target and modulate the function of target proteins involved in the occurrence of disease. One bioinformatics analysis method that can be used is molecular docking. This method will predict which macromolecules can be used as targets by comparing the structural similarity of the test compound (ligand) with a collection of compounds that are known to be targets in one or more databases. Docking analysis allows the initial identification of target molecules that have the potential to be immunomodulatory and anti-inflammatory from natural compounds. Docking analysis also makes it possible to find out the binding model that occurs so that it can be used to confirm its function as an immunomodulator and anti-inflammatory [22]. The molecular docking used in this research was through the PyRx program with the AutoDock wizard feature. The results of molecular docking can be visualized using Discovery Studio 2021 software in 2D and 3D (Figure 3).

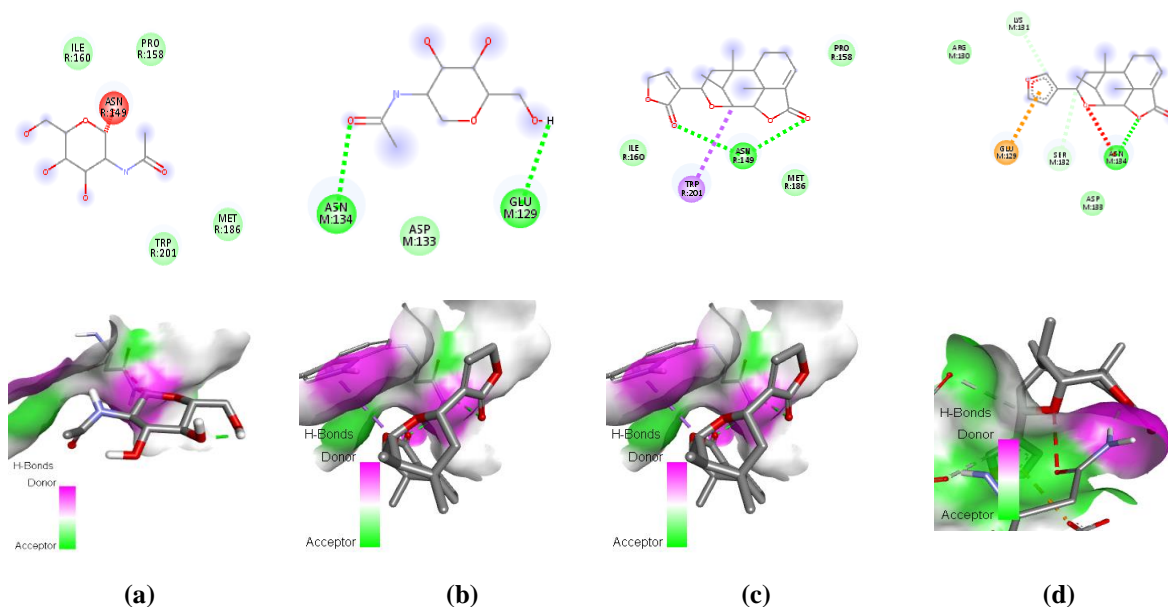


Figure 3. 2D and 3D Visualization of Native Ligands and Compounds Resulting from Molecular Docking; (a) Native Ligand IFN- γ ; (b) Native ligand IL-10; (c) Peronemin Compound C1 (IFN- γ); (d) Peronemin Compound A3 (IL-10).

The visualization process is carried out by taking data from docking results or scoring values. Molecules with the lowest scoring value show affinity and good stability and can be visualized with software. The smaller the results from the docking process, the more stable the protein-ligand complex will be, so the compound will be more patent [24]. The docking results can be visualized with the help of Discovery Studio Visualizer 2021 software. The purpose of visualization is to see how the interaction between the ligand and the amino acid residues on the receptor. Visualization of the interaction of the ligand with the receptor shows the amino acid residues of the receptor that play an important role in the binding site area. In the molecular docking test with ligands in the form of seven peronemin compounds with IL-10 and IFN- γ test receptors, which are thought to be able to modulate the immune system and inflammatory reactions. The test compound has good potential as an immunomodulator and anti-inflammatory, as can be seen from the comparison of the ligand used as a control. The ligand used is the native ligand on the test receptor. A test compound that has a lower affinity value is predicted to be able to bind more stably when compared with the comparison compound. Amino acid residue interactions can also determine whether a compound has the same biological activity as a comparison or native ligand [25]. The docking results of seven peronemin compounds, namely A2, A3, B1, B2, B3, C1, and D1 can be seen from Table 3.ble 3.

Table 3. Results of docking of the test ligand with the IFN- γ receptor.

Test ligand	Binding free energy (kcal mol ⁻¹)	Inhibition constant (μ M)	Hydrogen bond	Hydrogen bond distance (Å)	Amino acid residue*
Peronemin A2	-2.68	10.80	ASN 149	2.10547	ILE 160, TRP 201, ASN 149, MET 186, PRO 158
Peronemin A3	-3.40	3.21	ASN 149	2.07832	ILE 160, TRP 201, MET 186, ASN 149, PRO 158
Peronemin B1	-3.40	3.23	ASN 149	2.11879	ILE 160, TRP 201, MET 186, ASN 149, PRO 158
Peronemin B2	-3.37	3.40	ASN 149	2.12783	ILE 160, TRP 201, MET 186, ASN 149, PRO 158
Peronemin B3	-3.39	3.27	ASN 149	2.10443	ILE 160, TRP 201, MET 186, ASN 149, PRO 158

Test ligand	Binding free energy (kcal mol ⁻¹)	Inhibition constant (μM)	Hydrogen bond	Hydrogen bond distance (Å)	Amino acid residue*
Peronemin C1	-3.65	2.11	ASN 149	2.07481 2.18765	ILE 160, TRP 201 , MET 186, ASN 149, PRO 158
Peronemin D1	-3.01	6.24	ASN 149	2.34191	ILE 160 , TRP 201, MET 186, ASN 149, PRO 158 , GLU 157

Amino acids in bold are hydrophobic interactions.

Molecular docking was carried out using PyRx based on the Autodock Tools wizard, which had grid box sizes and coordinates obtained during the validation process. The results obtained from the docking process are in the form of bond energy and type of interaction (hydrogen bonds). The calculation (scoring function) of the ligand conformation formed in a macromolecule under equilibrium conditions is known as the bond energy. The binding free energy will reach balance if it is negative (Table 4). The binding free energy will also be directly proportional to the inhibition constant. The greater the negative value of a compound's ΔG, the more spontaneous its ability to interact with the target receptor. The inhibition constant can be declared strong if it has a value of ≤100 μM and conversely weak if it is ≤100 Mm [26]. A low binding value indicates that the ligand-protein complex formed is stable. Based on this, the conformation of the test compound, which has the lowest binding energy and interacts with the amino acid residues at the binding site, is selected [19]. From the results obtained from docking the seven peronemin compounds with the IFN-γ receptor, the best scoring results were obtained for the peronemin compound C1 with a free binding energy value of -3.65 with an inhibition constant value of 2.11 μM.

Table 4. Results of docking of the test ligand with the IL-10 receptor.

Test ligands	Binding free energy (kcal mol ⁻¹)	Inhibition constant (μM)	Hydrogen bond	Hydrogen bond distance (Å)	Amino acid residue
Peronemin A2	-1.79	49.09	ASN 134 SER 132 LYS 131	1.72578 3.57106 2.83203	ARG 130, LYS 131, SER 132, GLU 129, ASP 133, ASN 134
Peronemin A3	-2.37	18.19	ASN 134 SER 132 LYS 131	1.78159 3.57029 2.81347	ARG 130, LYS 131, SER 132, GLU 129, ASP 133, ASN 134
Peronemin B1	-2.32	19.90	ASN 134 SER 132 LYS 131	1.79755 3.62124 2.78539	LYS 131, SER 132, GLU 129, ASP 133, ASN 134
Peronemin B2	-2.32	19.95	ASN 134 SER 132 LYS 131	1.78967 3.60277 2.79127	LYS 131, SER 132, GLU 129, ASP 133, ASN 134
Peronemin B3	-2.32	20.01	ASN 134 SER 132 LYS 131	1.80369 3.61207 2.77494	LYS 131, SER 132, GLU 129, ASP 133, ASN 134
Peronemin C1	2.31	20.17	ASN 134 SER 132	2.09073 3.50206	LYS 131, SER 132, GLU 129, ASP 133, ASN 134
Peronemin D1	-2.07	30.16	ASN 134	1.83912	LYS 131, SER 132, GLU 129, ASP 133, ASN 134

The results of docking seven peronemin compounds with the IL-10 receptor (1LQS) can be seen in Table 4, where the results obtained show that the peronemin compound A3 has the best value of the seven compounds tested. Compound A3 has the highest bond energy, namely -2.37, with an inhibition constant value of 18.19 μM, and has three hydrogen bonds: ASN 134, SER 132, and LYS 131. Judging from Tables 3 and 4, the molecular docking with

the IFN- γ receptor has hydrogen bonds in ASN 149, which has a hydrophobic bond to TRP 201 in the best molecular docking results in compound C1. Hydrogen bonds are used as parameters that characterize pharmacological interactions in the mechanism between drug and receptor. Hydrogen bonds are the central bonds that can maintain protein stability, so hydrogen bonds formed in molecular dynamics simulations indicate the affinity between the compound and the target receptor. Meanwhile, hydrophobic interactions can increase protein stability by changing the nature of amino acids that have hydrophilic properties in a hydrophobic environment and can also determine amino acid residues that significantly contribute to maintaining protein stability. The results of the native ligand test with the IFN- γ and IL-10 receptors have similar amino acid residues. The similarity of these amino acid residues can prove that the test ligand has capabilities like the native ligand [27].

The molecular docking of seven peronemin compounds A2, A3, B1, B2, B3, C1, and D1 by the IFN- γ and IL-10 receptors modulates the immune system and inflammatory reactions. Interferon-gamma (IFN- γ) plays a vital role in inducing and modulating a range of immune responses. IFN- γ plays a substantial role in the formation of cellular immunity. A critical role of IFN- γ is to regulate immunity and bridge innate and specific immune response pathways. Interleukin 10 (IL-10) has strong anti-inflammatory properties, which play a central role in limiting the immune response to pathogens, thus preventing damage and maintaining normal tissue homeostasis [28].

3.6. Prediction of pharmacokinetic properties.

Pharmacokinetics is the process experienced by a drug when it enters the human body. It is included in the pharmacokinetic process, including absorption, distribution, metabolism, and excretion. Differences in physicochemical properties affect the absorption, distribution, efficacy, metabolism, and excretion of the components of these compounds in the body; apart from that, these differences are also correlated with interactions between the components of these compounds. This is related to the compound's high hydrophobicity, molecular weight, structural flexibility (number of bonds that can be rotated), and low target selectivity. From pharmacokinetic prediction tests on seven peronemin compounds, the following data were obtained:

Table 5. Pharmacokinetic parameter values of test compounds.

Test compound	Molecular weight	HBA	HBD	MLogP	Lipinski's violations	TPSA	WLogP	BBM permeation
Peronemin A2	344.40	5	0	1.93	0	61.20	2.92	Yes
Peronemin A3	328.40	4	0	2.67	0	48.67	3.71	Yes
Peronemin B1	328.40	4	0	2.67	0	48.67	3.71	Yes
Peronemin B2	344.40	5	0	1.93	0	61.20	2.92	Yes
Peronemin B3	328.40	4	0	2.67	0	48.67	3.71	Yes
Peronemin C1	344.40	5	0	2.70	0	61.83	2.55	Yes
Peronemin D1	344.40	5	1	2.61	0	72.83	2.66	Yes

Physicochemical parameters can be determined using Lipinski's Rules of Five regarding permeability for passive diffusion, including lipophilicity (C Log P) < 5, number of hydrogen bond donors < 5, number of hydrogen bond acceptors < 10, and molecular weight (BM) < 500 g/mol [12]. Test compounds (ligands) with a BM < 500 g/mol will find it challenging to penetrate skin or digestive tract membranes. MLogP is a calculation to estimate LogP values and is also used to search for drug candidates. The MLogP value is directly proportional to the molecular weight; when the MLogP value is superior, the molecular weight

will also be superior. The greater the MLog P value, the more hydrophobic the molecule is, so the test compound has high toxicity because it will be retained longer in the lipid bilayer, and its distribution will be more comprehensive in the body so that the selectivity of binding to the target enzyme is reduced. A Log P value that is too negative is also not good because molecules cannot pass through the lipid bilayer membrane [29]. From the data obtained in the pharmacokinetic test, all test compounds were by Lipinski's law (Table 6).

Table 6. Prediction results of the toxicity of seven peronemin compounds.

Test Compound	Toxicity				
	Inhibition hERG	Carcinogenicity		Acute oral toxicity	
	Probability Category	Class (Danger/Warning/Non-Required)	Category (Carcinogen/Not carcinogenic)	Probability	Category
Peronemin A2	0.839 (+)	Non-required (0.5873)	Not carcinogenic (0.9900)	0.3739	III
Peronemin A3	0.836 (+)	Non-required (0.4427)	Not carcinogenic (0.9900)	0.2719	I
Peronemin B1	0.836 (+)	Non-required (0.4427)	Not carcinogenic (0.9900)	0.2719	I
Peronemin B2	0.839 (+)	Non-required (0.5873)	Not carcinogenic (0.9900)	0.3739	III
Peronemin B3	0.836 (+)	Non-required (0.4427)	Not carcinogenic (0.9900)	0.2719	I
Peronemin C1	0.4188 (-)	Non-required (0.4574)	Not carcinogenic (0.9800)	0.3796	III

Peronemin D1	0 . 6 5 5 6	(+)	Danger (0.4498)	Not carcinogenic (0.9800)	0.6103	I
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Toxicity prediction is the key to finding various medicines that are helpful in assessing a compound's danger level to the environment and humans. Prediction of the toxicity of a compound is carried out with the admetSAR webserver using three control parameters: human ether, a go-go-related gene (herG), carcinogenicity, and acute toxicity. Based on the level of toxicity of a compound, which is expressed in median lethal dose (LD50) and reviewed based on criteria by the US Environmental Protection Agency (EPA), there are four categories: a) category I has a value of ≤ 50 mg/kg classified as very toxic and can fatal if swallowed; b) category II has a value of $50 < x \leq 500$ mg/kg which is classified as moderately toxic; c) category III has a value of $500 < x \leq 5000$ mg/kg which is classified as slightly toxic; and d) category IV has a value of > 5000 mg/kg classified as non-toxic [26]. A compound is said to pass the toxicity test if the human ether a go-go related gene (herG) parameter is a weak inhibitor, not carcinogenic, and the acute oral toxicity is in group III or IV [30]. From the results of the toxicity predictions of seven peronemin compounds, it was found that the compound that met the criteria for having the lowest level of toxicity was the peronemin compound C1.

4. Conclusions

Molecular docking of the seven peronemin compounds A2, A3, B1, B2, B3, C1, and D1 with the IFN- γ and IL-10 receptors produced the best docking results in compound C1 with the IFN- γ receptor, which had a binding free energy value of -3.65 and The inhibition constant value is 2.11 which has a hydrogen bond on ASN 149. Meanwhile, the best docking results are on the IL-10 receptor with a binding free energy value of -2.37 and an inhibition constant of 18.19 with three hydrogen bonds on ASN 134, SER 132, and LYS 131, peronemin compound A3.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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