Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 15, Issue 3, 2025, 36
https://doi.org/10.33263/BRIAC153.036

Bioactive Compound of Sungkai Leaves (*Peronema canescens* Jack) As Natural Inhibitor of Beta-Lactamase: *In Silico* Study

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Received: 9.03.2025; Accepted: 11.04.2025; Published: 8.06.2025

Abstract: Bacterial diseases have become a difficult challenge to the world's health, capable of causing a wide variety of infections that can lead to severe health complications and even death. The main method used to treat bacterial infections is generally antibiotics. Still, many bacteria have developed mechanisms to counteract the effects of existing antibiotics, making effective treatment of bacterial infections increasingly difficult. One common resistance is resistance to commercial beta-lactam. To counter this challenge, the development of alternative treatments for bacterial infections has begun to emerge, especially through approaches to plant secondary metabolites. This natural compound has shown promising antibacterial properties. The study is supplemented by considering the report of some additional properties of potential application in structure-activity relationships (SAR) research for the development of therapeutic drugs and the bioactivity radars related to the drug-like behavior of the studied compounds. Based on the results of the Sungkai (*Peronema canescens* Jack) bioavailability and toxicity test followed by molecular docking against beta-lactamase, the test ligand SMR000036195 is considered safe and had binding energy above avibactam (8.91 kcal/mol vs. 7.03 kcal/mol). It can be concluded that SMR000036195 can be an alternative inhibitor of beta-lactamase, replacing avibactam as a commercial inhibitor.

Keywords: beta-lactamase; ınhibitor; molecular docking; Sungkai; YASARA.

1. Introduction

Bacterial diseases have become a formidable challenge to the world's health, capable of causing a wide range of infections that can lead to severe health complications and even death [1]. Common bacterial infections include pneumonia caused by Streptococcus pneumoniae and urinary tract infections caused by *Escherichia coli*. The growing problem of antibiotic resistance among bacterial pathogens poses a major and increasing threat to public health worldwide. This resistance crisis is caused by various factors, one of which is the misuse of antibiotics in both the health and agricultural sectors, which makes many bacterial infections increasingly difficult to deal with effectively [2].

The main method used to treat bacterial infections is generally with antibiotics, which are designed to inhibit the growth or kill bacteria. However, the development and production of new antibiotics have experienced significant obstacles, such as high development costs, long trial processes, and antibiotic resistance that is beginning to occur [3]. Many bacteria have developed mechanisms to counteract the effects of existing antibiotics, making effective treatment of bacterial infections increasingly difficult. One worrying trend is the increasing prevalence of antibiotic-resistant bacterial strains, such as multi-spectral beta-lactamase-producing Gram-negative bacteria, which are becoming more common in the health and community sectors [4].

One of the commonly used antibiotics is beta-lactam, which works by inhibiting the synthesis of bacterial cell walls. These antibiotics attach to *penicillin-binding proteins* (PBPs), disrupting the cross-linking in the peptidoglycan layer, which is important for maintaining the stability of bacterial cell structures. This causes the cells to become lysed and the bacteria to die [5]. Beta-lactamase refers to a group of enzymes produced by certain bacteria that cause resistance to beta-lactam antibiotics, such as penicillin, cephalosporin, and carbapenem. These enzymes function by hydrolyzing the beta-lactam ring, one of the important structural components of these antibiotics, thereby causing these antibiotics to not work optimally against bacterial infections. The presence of beta-lactamase is one of the factors in the growth of antibiotic resistance [5].

To fight this challenge, the development of alternative treatments for bacterial infections is beginning to emerge, particularly through approaches targeting plant secondary metabolites. These natural compounds, produced by plants as part of their defense mechanism, have shown promising antibacterial properties. Research on this compound has shown potential as an alternative or adjunct to conventional antibiotics, paving the way for new treatments in an era where antibiotic resistance is a major concern [6,7]. This research is complemented by considering reports on several additional properties of potential applications in structure-activity relations (SAR) studies for therapeutic drug development, as well as with a bioactivity radar related to the drug-like behavior of the studied compounds, predicted biochemical targets and values associated with pharmacokinetics and ADMET properties through standard chemoinformatics procedures [8–14]. The current research represents studies on the properties of some families of therapeutic compounds of sungkai origin.

2. Materials and Methods

2.1. Materials.

This research was designed using a computer device with a Windows 11 Professional 64-bit operating system, an x64-based processor, and an AMD Ryzen 5 5600H @3.30GHz-4.20GHz processor specification. The software used was Yet Another Scientific Artificial Reality Application (YASARA) structure, BIOVIA Discovery Studio 2024, and ChimeraX. All test ligand materials were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) and receptors from RCSB PDB (https://www.rcsb.org).

2.2. Materials.

2.2.1. Protein functional sites.

Research on the functional sites of the identified protein was done using CD-search on CDD webserver11 (ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [12] and Computed Atlas of Surface Topography of the universe of protein Folds (CASTpFold) webserver https://cfold.bme.uic.edu/castpfold/ [14].

2.2.2. Pharmacokinetic test.

The molecular structures of the bioactive compound from *Peronema canescens* Jack were obtained from PubChem. One of the most crucial pieces of information to gather before beginning research on the identification and creation of novel therapeutic therapies is pharmacokinetics. Typically, this is accomplished using separate indicators known as ADMET (absorption, distribution, metabolism, excretion, and toxicity) factors. Using online SwissADME software, various ADME parameters were computed for this study (swissadme.ch) [11,15]. By using pkCSM (biosig.lab.uq.edu.au/pkcsm/prediction) and Deep-PK (biosig.lab.uq.edu.au/deeppk/), additional information related to the ADMET properties and the pharmacokinetics parameters were identified [13,15].

2.2.3. Toxicity prediction.

The compounds' toxicity prediction was performed to ensure these drugs were safe when used for humans. The analysis was performed using ProTox-III (tox.charite.de/protox3), a virtual lab for the prediction of toxicities of small molecules. The drugs were uploaded to the server, which yielded results showing the toxicity prediction in comparison to the already reported drugs, such as Aspirin and Digoxin [10].

2.2.4. Ligands and receptor preparation.

The test ligands were obtained from the PubChem database. The 8DE1 protein was chosen as the target receptor and imported into the Yasara Structure software. The protein structure was prepared by adding a hydrogen atom, removing the water molecule, and deleting unused ligands. The test compounds were then optimized by minimizing their binding energy using the "Energy minimization" experiment in the YASARA Structure program. Both receptor and ligand were saved in *.PDB file format [16].

2.2.5. Molecular docking between ligands and receptors.

The receptor was docked with ligands using YASARA. Redocking was performed to determine the most suitable Grid box size. This process was then continued for screening. YASARA structure with the dock_run.mcr.macro script was executed with 100 runs, and the Amber14 force field was used for validation [17]. The dock_runscreening macro file, written by Elmar Krieger for the YASARA structure, is used to attach an unlimited number of ligands to the target receptor using the VINA or AutoDock methods. The resulting binding energy values are then sorted accordingly (yasara.org/dock_runscreening.mcr). Screening uses the YASARA structure with the macrodock_runscreening.mcr set in the VINA method, runs=100, and Amber14 [16].

2.2.6. Data analysis.

BIOVIA Discovery Studio 2024 and ChimeraX version 1.8 software were used to analyze the screening data and visualize it in two and three dimensions. Using the YASARA structure, the (.yob) files or objects containing complexes are transformed into (.pdb) format for simpler 2D and 3D visualization with BIOVIA Discovery Studio and ChimeraX [17].

3. Results and Discussion

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, and the conclusions that can be drawn. The test ligands used in this research are 42 active compound ligands of *Peronema canescens* Jack and one comparison ligand, Avibactam [18]. The first physicochemical test that was performed was the Lipinski Rules of Five. Four ligands were eliminated from the 43 that were tested. ADMET parameters, which include intestinal absorption, AMES toxicity, hERG blockers, carcinogenesis, and LD50, were applied to test the remaining 39 ligands. Thirteen of the 39 evaluated ligands were eliminated, while the remaining ligands proceeded to the next evaluation phase. Five compounds with the best Gibbs free energy values were selected through a virtual screening of 26 test ligands. After docking the five ligands with the receptor, the results were compared with the docking result for Avibactam. Based on the interaction that formed between the ligand and receptor, the best ligands were then visualized and compared with Avibactam.

3.1. Pharmacokinetic test.

The Lipinski rules of 5 (Table 1) are pharmacokinetic criteria when studying substances resembling important drugs. Molecular weight, log p, rotatable bonds, donors and acceptors, and surface area are the criteria of the Lipinski Rules of 5. If a compound fails to meet one or more of these criteria, it is predicted to have a bioavailability problem. Utilizing established criteria, Lipinski's rule explains each compound's physicochemical characteristics [13]. Out of 43 ligands tested, 4 ligands were eliminated. The bioavailability radars of the top 5 ligands that passed the bioavailability test and Avibactam is shown in Figure 1.

Table 1. Results of Lipinski's rules of 5 bioavailability test.

Ligand	Lipinski's Rules of 5					
	MW	**Log P**	**RB**	**H_A**	**H_D**	**TPSA**
Acceptable Value	≤ 500	≤ 5	≤ 10	≤ 10	≤ 5	≤ 140
Prednisone	358.43	1.77	2.00	5.00	2.00	91.67
SMR000036195	382.43	3.11	6.00	7.00	0.00	107.20
DTXSID50454478	398.52	7.76	1.00	0.00	0.00	28.24
MLS001002312	398.50	1.62	9.00	6.00	2.00	156.89
SCHEMBL13963031	224.13	-2.99	3.00	8.00	2.00	145.06
Avibactam	265.24	-1.21	3.00	6.00	2.00	138.62

The red value indicates the failure to fulfill Lipinski Ro5 test parameters.

The remaining 39 ligands were tested using ADMET parameters, such as Intestinal Absorption, AMES Toxicity, hERG Blockers, Carcinogenesis, and LD50 (Table 2). Out of 39 ligands tested, 13 ligands were eliminated, and the rest advanced to the next test stage. Virtual screening showed that between 26 test ligands, five compounds showed up with the best Gibbs free energy values. The five ligands were then docked with the receptor, and the results were compared to the Avibactam docking result.

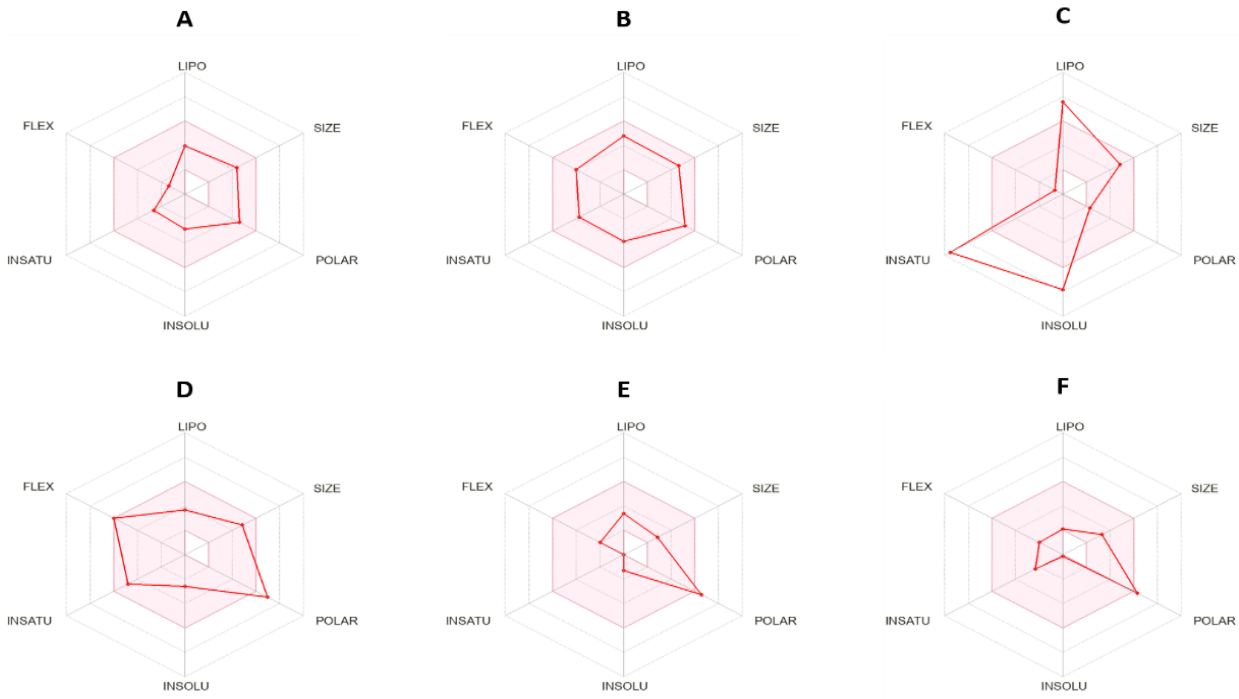

Figure 1. The bioavailability radars of Beta-Lactamase test ligands **(A)** Prednisone; **(B)** SMR000036195; **(C)** DTXSID50454478; **(D)** MLS001002312; **(E)** SCHEMBL13963031; **(F)** Avibactam.

Table 2 describes the result of several criteria selected from ADMET, consisting of Intestinal Absorption (>30% to be in the safe category), AMES Toxicity ("safe" result to enter the safe category), Human Ether-A-Go-Go Related Gene (hERG) blocker ("safe" result to enter the safe category) carcinogenesis (“Safe” result to enter the safe category), and LD50 (>300mg/KgBW/day to enter the safe category) [10,13].

Table 2. Results of ADMET test.

Ligand	ADMET				
	Intestinal absorption	**AMES toxicity**	**hERG blockers**	**Hepatotoxicity**	**LD50**
Acceptable Value	>0.3	Safe	Safe	Safe	≥300mg/kg
Prednisone	1.00	Toxic	Safe	Toxic	1680mg/kg, Class 4
SMR000036195	0.98	Safe	Safe	Safe	2730mg/kg, Class 5
DTXSID50454478	1.00	Toxic	Safe	Toxic	1000mg/kg, Class 4
MLS001002312	0.91	Safe	Safe	Safe	1000mg/kg, Class 4
SCHEMBL13963031	0.93	Toxic	Safe	Toxic	100mg/kg, Class 3
Avibactam	0.39	Safe	Safe	Safe	1500mg/kg, Class 4

The red value indicates failure to fulfill one/more of the ADMET test parameters.

Lipinski's criteria can be used for pharmacokinetic screening. These say that the molecules that are most "drug-like" should have a log P value of ≤ 5, a molecular weight of ≤ 500, no more than 10 hydrogen bond acceptors, no more than 5 hydrogen bond donors, and a topological polar surface area (TPSA) of ≤ 140 Å^2. Molecules not meeting one or more of these criteria may face bioavailability issues. [19]. This rule is called the rule of 5. Having no more than 10 rotatable bonds and a maximum of 15 hydrogen bond donors and acceptors combined are the fifth and sixth requirements. In the human body, drug absorption starts at the intestinal epithelium, moves through the bloodstream, and ends at the drug's site of action. Compounds weighing more than 500 Daltons have been shown in earlier research to have less-than-ideal absorption properties [19]. According to Table 1, all tested ligands met these criteria successfully.

The second criterion is Log P, which indicates that the acceptable limit for lipophilicity is Log P ≤ 5. Drug candidates that exceed this threshold often exhibit lower solubility in physiological solutions, hindering their ability to access the membrane surface. If a compound is excessively hydrophobic (Log P > 5), it tends to remain at the first membrane it encounters. In contrast, overly hydrophilic compounds may struggle to cross cell membranes to reach their intended site of action [19]. According to Table 1, DTXSID50454478 failed the Log P test. Rotatable bonds (Rot.Bond) are defined as any single non-ring bond connected to a non-terminal heavy atom (i.e., non-hydrogen atom). Amide C-N bonds are excluded from this definition due to their high rotational energy barrier. This straightforward topological parameter serves as an indicator of molecular flexibility and has been shown to be a reliable predictor of oral bioavailability for drugs [19]. Based on Table 1, all the test ligands passed this test.

The number of hydrogen bond donors and acceptors also significantly affects a molecule's physicochemical properties, including solubility, absorption, and distribution, directly influencing drug efficacy. For optimal absorption and permeability, it is recommended that the number of hydrogen bond donors be less than 5 and the number of acceptors be less than 10. If a compound does not adhere to this guideline, it may be too polar to pass through cell membranes [19] effectively. According to Table 1, all ligands passed the hydrogen donor and acceptor test. The topological polar surface area (TPSA) of drug molecules is known to directly affect their absorption through biological membranes, such as those in $CaCO^{-2}$ cells (large intestine carcinoma) and brain and nerve cells within the central nervous system. Research indicates that drugs with a dynamic TPSA of less than 60 $Å^2$ are fully absorbed, while those with a TPSA greater than 140 $Å^2$ experience limited permeability [20]. Based on Table 1, MLS001002312 and SCHEMBL13963031 failed the TPSA test.

A crucial factor when assessing the pharmacokinetic and pharmacodynamic characteristics of pharmacological molecules is ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity). Optimizing new drug possibilities requires a computation of ADMET characteristics. Both attractive ADMET properties and effective therapeutic activity are necessary for successful drug development. A key component of drug development is comprehending how drugs are absorbed, especially when taken orally, and how they are absorbed in the digestive system. Neurotoxicity and nephrotoxicity may result from poor absorption, which may adversely impact distribution and metabolism. Therefore, the goal of this study is to clarify how drug molecules behave in an organism, which analyzes ADMET features, an essential part of computational drug design [15].

A compound can reach a tissue if it is taken into the bloodstream. Usually, a drug is administered through mucous surfaces such as the digestive tract before it is taken up by the target cells. Factors like poor compound solubility, intestinal transit time, gastric emptying time, inability to permeate the intestinal wall, and chemical instability in the stomach are responsible for reducing the extent of drug absorption after oral administration. Drugs with poor absorption are less desirable for oral administration, such as by inhalation or intravenously [13,15]. A molecule with an Intestinal Absorption of less than 30% is considered poorly absorbed. Based on Table 2, all tested ligands met these criteria successfully.

AMES toxicity testing is a commonly used method for evaluating the mutagenic potential of a drug by utilizing bacterial assays. A positive result from this test suggests that the compound being studied may be mutagenic and could act as a carcinogen [9,10,13]. Based on Table 2, prednisone, DTXSID50454478, and SCHEMBL13963031 failed this test. The

criteria for hERG inhibitors indicate that the blockage of potassium channels encoded by the hERG (human ether-a-go-go gene) is a key factor in developing QT syndrome, which can lead to serious ventricular arrhythmias in the heart. [13]. Based on Table 2, all tested ligands met these criteria successfully. Drug-induced liver injury is a significant safety issue in drug development and a major reason for the failure of drugs in the market. A compound is classified as hepatotoxic if it is linked to at least one pathological or physiological liver event that is closely associated with impaired normal liver function, based on Table 2, prednisone, DTXSID50454478, and SCHEMBL13963031 failed the test.

Potential toxicity is very important for potential compounds. Lethal dosage value (LD50) is a standard measure of acute toxicity to assess toxicity relative to other molecules. LD50 is the amount of compound administered directly capable of causing the death of 50% of test animals. In ProTox-III, there are 6, which is fatal. On the other hand, class 6 shows classes for toxicity (1 to 6) in which class 1 has LD50≤5, which is fatal if consumed up to LD50>5000, which means the compound is non-toxic [9,10]. Based on Table 2, all the test ligands passed this test.

3.2. Molecular docking between ligands and receptors.

The grid box size used for the screening is 4 Å because this size had an RMSD value of 0.45 Å with a binding energy of 7.03 kcal/mol when redocking was done. This research provides screening results in the form of binding energy, residual contact, and the types of interactions formed. The Beta-Lactamase (PDB: 8DE1) has one functional site predicted by the conserved domain databases (CDD) server. The active site was in residue SER 337 [12]. CASTpFOLD results showed that residue SER 70 and GLU 168 were the active sites, and LYS 234, SER 235, and GLY 256 were the binding sites for this protein [14,18]. Table 3 explains the ranking of the top five ligands based on their binding energy. A positive score indicates the best binding energy in the YASARA structure. Thus, a score with a more positive result indicates better binding of the ligand to the receptor [17]. Receptor interactions with Avibactam are shown in Figure 2, while receptor interactions with SMR000036195 are shown in Figure 3.

Table 3. Sungkai molecular docking results with Beta-Lactamase receptor.

Ligand	Effi (kcal/mol*atom)	Bind energy (kcal/mol)	Dissoc. constant (pM)
Prednisone	0.35	9.00	2.52E+05
SMR000036195	0.34	8.91	2.95E+05
DTXSID50454478	0.27	8.05	1.27E+06
MLS001002312	0.29	7.57	2.84E+06
SCHEMBL13963031	0.50	7.52	3.10E+06
Avibactam	0.41	7.03	7.01E+06

Description: The red ligand violates one/more of the pharmacokinetic test parameters.

Avibactam was used as a binding energy control so that the test ligand with binding energy above Avibactam can be considered a potential ligand to be developed. The ligand used as control, Avibactam (CID_ 9835049), has a binding energy of 7.03 kcal/mol [18]. The test ligands with binding energy above Avibactam are Prednisone (9.00 kcal/mol), SMR000036195 (8.91 kcal/mol), DTXSID50454478 (8.05 kcal/mol), MLS001002312 (7.57 kcal/mol), and SCHEMBL13963031 (7.52 kcal/mol). By sorting the best receptor-ligand complex, the YASARA structure identifies the best score. Therefore, a score with a more positive result indicates better binding of the ligand to the receptor [16].

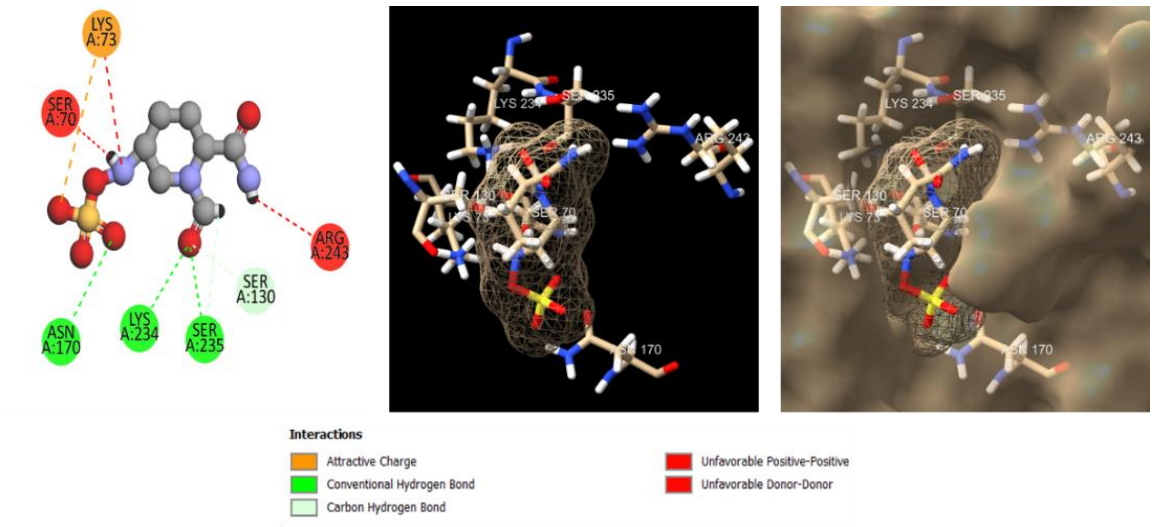

Figure 2. Avibactam interaction with beta-lactamase receptor visualized in 2D and 3D.

In addition, a small Kd value indicates a stronger ligand binding to the receptor [16,21]. The ligand efficiencies of SMR000036195 were 0.34, while Avibactam was 0.41, indicating that the binding energy contributed per atom of the compounds was close enough to the required energy to develop key contacts with the Beta-Lactamase target [16,22]. The range of ligand efficiency under Avibactam (based on binding energy) is 0.27 to 0.50. Based on the result from Lipinski Ro5, ADMET, followed by molecular docking with the Beta-Lactamase receptor, we chose SMR000036195 as the best test ligand to be used as a candidate alternative inhibitor.

Avibactam is the ligand that acts as an inhibitor standard for this study [18]. Avibactam forms two types of hydrogen bonds with Beta-Lactamase, such as conventional hydrogen bonds and carbon-hydrogen bonds. A conventional hydrogen bond was formed at the amino acid residues ASN 170, A LYS 234, and SER 235, with the interaction distance varying from 2,61 to 2,73 Å. While a carbon-hydrogen bond formed at the amino acid residue of SER 130, with an interaction distance of 3,02 Å.

SMR000036195 (Figure 3) formed both hydrogen bonds and hydrophobic bonds. Hydrogen bonds formed were divided into three types of hydrogen bonds: conventional hydrogen bonds, carbon-hydrogen bonds, and Pi-donor hydrogen bonds. A conventional hydrogen bond was formed at the amino acid residues ASN 132 and TYR 237 with the interaction distance of 2.44 and 2.29 Å, respectively. Carbon hydrogen bonds were formed at the amino acid residues of SER 130 and GLY 236 with the interaction distance of 2.72 and 2.28Å, respectively. A pi-donor hydrogen bond was formed at the amino acid residue of SER 70 with an interaction distance of 2.66 Å. Pi-Pi Stacked and pi-alkyl bonds were the two kinds of hydrophobic bonds that were identified. Pi-Pi Stacked bonds with interaction distances of 3.91 and 4.36 Å were formed at the amino acid residue of TYR 237. Pi-alkyl bonds with interaction distances ranging from 5.03 to 5.24 Å were formed at the amino residue of TYR 237.

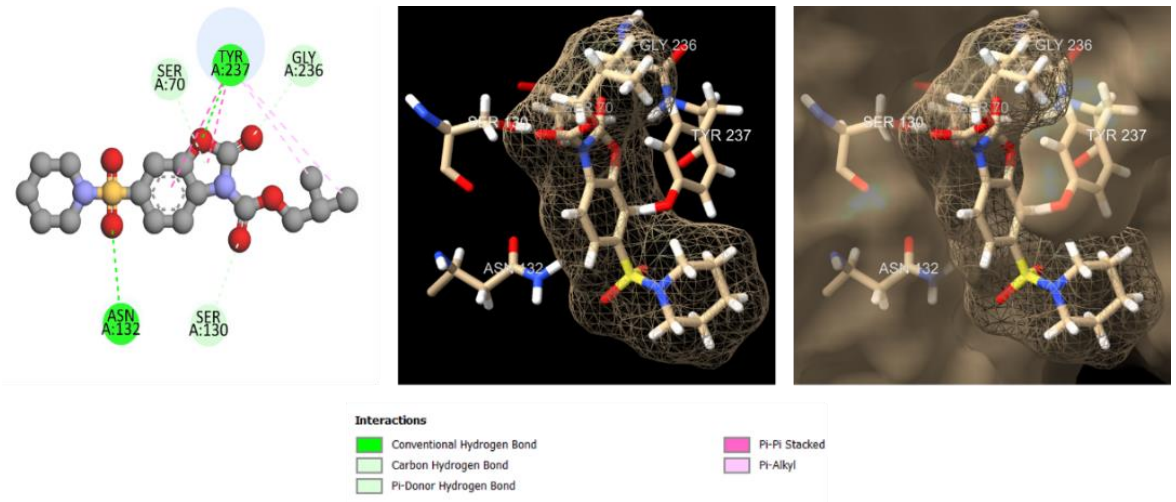

Figure 3. SMR000036195 interaction with beta-lactamase receptor visualized in 2D and 3D.

Table 4 shows the interaction between each test ligand, which was divided into two main categories: hydrogen bond and hydrophobic bond. The more hydrogen bonds the ligand, hypothetically, the easier and stronger a ligand binds to the active side of the receptor. A good hydrogen bond has a distance of less than 2.3 Å. Hydrophobic bonds are considered important due to their role in stabilizing the interaction between ligands and receptors [23,24]. Based on Table 4, nearly all formed interaction bonds occurred in the active and/or binding sites.

Table 4. Residue interaction analysis between test ligands and Beta-Lactamase receptor.

Ligand	Residues/Amino acids involved	
	Hydrophobic interaction	**Hydrogen bond**
Avibactam		A SER 130, A ASN 170, **A LYS 234**, **A SER 235**
Prednisone	A TYR 105, A VAL 216, A TYR 237	**A SER 70**, A SER 130 , A VAL 216
SMR000036195	A TYR 237	**A SER 70,** A SER 130, A ASN 132, **A GLY 236,** A TYR 237
DTXSID50454478	A PRO 167, A TYR 237	A SER 70,
MLS001002312	A TYR 105, A TYR 237	A ASN, A VAL 216, A TYR 237, A ARG 243
SCHEMBL13963031		A TYR 105, A ASN 170, **A GLY 236**, A TYR 237

Amino acids in bold indicate the presence of contact with the active site and/or binding site.

Table 4 shows the interaction between each test ligand and divided it into two main categories: hydrogen bond and hydrophobic bond. The more hydrogen bonds the ligand, hypothetically, the easier and stronger a ligand binds to the active side of the receptor). A good hydrogen bond has a distance of less than 2.3 Å. Hydrophobic bonds are considered important due to their role in stabilizing the interaction between ligands and receptors [23,24]. Based on **Table 4**, nearly all formed interaction bonds occurred in the active and/or binding sites.

4. Conclusions

Based on the results of the sungkai (*Peronema canescens Jack*) bioavailability and toxicity test followed by molecular docking against Beta-Lactamase, the test ligand SMR000036195 is considered safe and has binding energy above Avibactam (8.91 kcal/mol vs 7.03 kcal/mol). It can be concluded that SMR000036195 can be an alternative inhibitor of Beta-Lactamase, replacing Avibactam as a commercial inhibitor.

Author Contributions

Conceptualization, M.M.A.H. and R.H.B.S; methodology, M.M.A.H., D.A., and R.H.B.S.; software, M.M.A.H.; validation, D.A., and R.H.B.S.; formal analysis, M.M.A.H.; investigation, D.A., and R.H.B.S.; resources, M.M.A.H., D.A., and R.H.B.S.; data curation, M.M.A.H; writing—original draft preparation, M.M.A.H., D.A., and R.H.B.S.; writing—review and editing, M.M.A.H., D.A., and R.H.B.S.; visualization, M.M.A.H.; supervision, D.A., and R.H.B.S.; project administration, D.A., and R.H.B.S. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

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

Funding

This research received no external funding.

Acknowledgments

The author would like to thank all parties who have helped complete this research. All authors made equal contributions as the main contributors to this manuscript paper.

Conflicts of Interest

The authors declare no conflict of interest.

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