Formosa Journal of Multidisciplinary Research (FJMR)
Vol. 4 No. 10, 2025: 5025-5044

Molecular Dynamics Simulation of Ecdysone from *Sida rhombifolia* Against PBP2a Protein of MRSA as a Potential New Antibacterial Candidate

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A R T I C L E I N F O

Keywords: Molecular Dynamics, Ecdysone, Sida Rhombifolia

Received : 16, September
Revised : 30, September
Accepted: 25, October

A B S T R A C T

This study aims to evaluate the predicted binding stability of the compound ecdysone in comparison with the native ligand, in order to determine its potential as an effective and stable antibacterial agent through molecular docking and molecular dynamics (MD) simulations. An exploratory computational approach was employed to assess the antibacterial activity, interaction patterns, and stability of compounds derived from *Sida rhombifolia*. A total of 15 bioactive compounds were docked against the molecular target PBP2a (PDB ID: 4JCN) using AutoDockTools and PyMol, followed by an analysis of amino acid residue similarity between each test ligand and the native ligand. The compound exhibiting the best interaction profile was further analyzed using MD simulation to evaluate its binding stability and pharmacokinetic properties. Validation parameters, including Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF), were calculated based on *in silico* predictions. MD simulations were conducted using YASARA Dynamics. The molecular docking results indicated that ecdysone demonstrated the highest binding affinity and interaction pattern comparable to that of the native ligand. The MD simulation further confirmed that ecdysone maintained stable binding throughout the simulation period, as reflected by RMSD and RMSF profiles of the MRSA target complex.

DOI: https://doi.org/10.55927/fjmr.v4i10.555
ISSN-E: 2829-8896
https://npaformosapublisher.org/index.php/fjmr

INTRODUCTION

Antibiotic resistance has emerged as a critical global health crisis, primarily driven by the rise of *Methicillin-Resistant Staphylococcus aureus* (MRSA). MRSA exhibits remarkable resilience against β-lactam antibiotics, including penicillins and cephalosporins, resulting in significant morbidity and mortality worldwide. The World Health Organization (WHO) has designated MRSA as a high-priority pathogen requiring the urgent discovery of new antibacterial agents (*World Health Organization, 2023*). This alarming scenario has prompted intensive research efforts to identify both synthetic and natural compounds capable of inhibiting bacterial targets involved in resistance mechanisms.

The key determinant of MRSA resistance lies in the *Penicillin-Binding Protein 2a* (PBP2a), a modified transpeptidase enzyme exhibiting low affinity toward β-lactam antibiotics. This unique adaptation allows MRSA to maintain cell wall biosynthesis even under antibiotic pressure (*Hartman & Tomasz, 2019*). The distinctive architecture of PBP2a's active domain restricts many β-lactam drugs from effective binding, rendering them therapeutically ineffective. Therefore, PBP2a has become a rational molecular target in the development of novel antibacterials aimed at counteracting β-lactam resistance (Fishovitz *et al.,* 2020).

In recent years, natural products have gained substantial attention as promising reservoirs for novel antibacterial agents. *Sida rhombifolia* L. (commonly known as Sidaguri) is one such plant, traditionally used in ethnomedicine and reported to contain diverse bioactive secondary metabolites, including alkaloids, flavonoids, saponins, and phytosterols (*Patel et al., 2021*). These phytoconstituents have demonstrated broad pharmacological activities, such as anti-inflammatory, antimicrobial, and immunomodulatory effects. This pharmacological diversity makes *Sida rhombifolia* an attractive candidate for further bioinformatic and molecular screening studies targeting resistant bacterial enzymes.

Among the various bioactive molecules isolated from *Sida rhombifolia*, ecdysone, a steroidal compound known as an insect molting hormone, has gained interest for its potential pharmacological applications. Beyond its physiological role in arthropods, ecdysone has exhibited antioxidant, antimicrobial, and immunomodulatory activities in mammalian systems (Kumar & Singh, 2022). Structurally, ecdysone's multiple hydroxyl groups and steroidal ring framework suggest its potential to form stable hydrogen bonds and hydrophobic interactions within the active site of bacterial enzymes such as PBP2a, making it a plausible candidate for molecular docking and simulation studies.

Advances in computational chemistry have revolutionized drug discovery, particularly through *in silico* techniques such as molecular docking and molecular dynamics (MD) simulations. These approaches allow for the rapid evaluation of ligand–protein interactions at the atomic level, reducing the cost and time associated with early-stage drug screening (*Lionta et al., 2014*). Through docking studies, potential binding affinities and interaction residues can be predicted, while MD simulations provide dynamic insights into the stability and

conformational changes of the ligand–protein complex under physiological conditions.

Recent *in silico* investigations on steroidal compounds have revealed that their rigid cyclic structures and high polarity facilitate strong interactions with resistant bacterial enzymes such as PBP2a (*Singh et al., 2023*). Docking ecdysone with PBP2a is expected to reveal favorable binding energies and key interactions within the enzyme's transpeptidase domain. Such interactions could potentially inhibit the catalytic function of PBP2a, thereby restoring bacterial susceptibility to β-lactam antibiotics.

While docking studies provide static binding predictions, they fail to account for the dynamic behavior of biomolecules in a solvated environment. Molecular dynamics (MD) simulations are therefore crucial for validating the stability and conformational flexibility of the ligand–protein complex over time. Key parameters such as *Root Mean Square Deviation* (RMSD), *Root Mean Square Fluctuation* (RMSF), and *Radius of Gyration* (Rg) can be analyzed to assess the structural integrity and binding stability of the ecdysone–PBP2a complex under physiological simulation (Durrant & McCammon, 2011).

This study aims to elucidate the molecular mechanism and dynamic stability of ecdysone from *Sida rhombifolia* in complex with the PBP2a protein of MRSA through molecular docking and MD simulations. The findings are expected to provide a theoretical foundation for developing ecdysone or its derivatives as novel antibacterial candidates targeting resistant strains of *S. aureus*. Integrating computational pharmacology with natural product research not only supports the sustainable use of local biodiversity but also contributes to the ongoing global efforts to combat antibiotic resistance through innovative drug discovery strategies.

LITERATURE REVIEW

Resistant Staphylococcus aureus (MRSA)

Methicillin-Resistant Staphylococcus aureus

(MRSA) is a strain of *S. aureus* that has developed resistance to the antibiotic methicillin. In contrast, strains that remain sensitive to methicillin are referred to as Methicillin-Sensitive *Staphylococcus aureus* (MSSA). MRSA is also resistant to other classes of antibiotics, including β-lactams, macrolides, tetracyclines, chloramphenicol, and quinolones. MRSA infections are opportunistic in nature, similar to those caused by *S. aureus*. This bacterium is a Gram-positive coccus that typically appears in clusters resembling grape-like structures. It is non-spore-forming and non-motile. *S. aureus* belongs to the *Staphylococcus* genus and is characterized by its ability to produce catalase and yield a positive result in the coagulase test (Nandhini et al., 2022).

Molecular Target

PBP2a

Penicillin-Binding Protein 2a (PBP2a) is an enzyme that plays a crucial role in conferring resistance to β-lactam antibiotics, including methicillin, in MRSA strains of *Staphylococcus aureus*. PBP2a is responsible for catalyzing the production of peptidoglycan, an essential component of the bacterial cell wall

This enzyme exhibits a significantly lower binding affinity toward β-lactam antibiotics compared to other PBPs (Penicillin-Binding Proteins). As a result, MRSA is able to continuously catalyze cell wall synthesis even in the presence of β-lactam-derived antibiotics such as methicillin, oxacillin, nafcillin, and cephalosporins. Under normal physiological conditions, PBP2a functions in peptidoglycan biosynthesis by catalyzing the cross-linking of peptide chains, which provides the structural strength and stability necessary for maintaining the integrity of the bacterial cell wall.

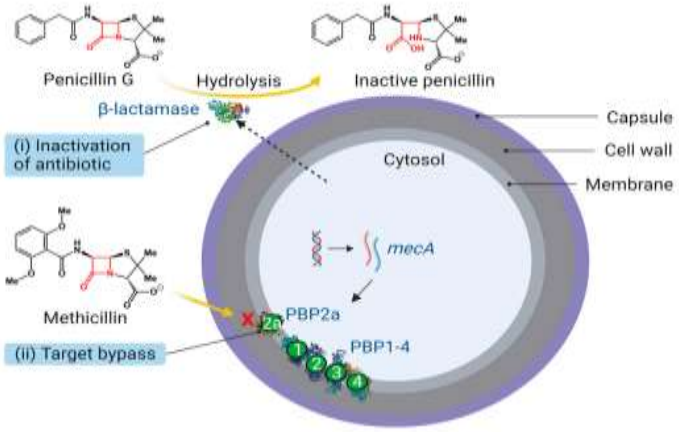


Figure 1. Molecular mechanism of β-lactam resistance in *Staphylococcus aureus* (H. L. and J.-S. Kim, 2023).

Molecular Docking

Molecular docking is an *in silico* technique used to predict the orientation and positioning of small molecules or ligands within the active site of their target protein (receptor). This method is primarily employed to accurately estimate the most favorable binding mode and the bio-affinity between a ligand and its receptor. The molecular docking methodology generally comprises three interrelated objectives: predicting the binding pose, evaluating the binding affinity, and performing virtual screening. In molecular docking, the essential components include a search algorithm and a scoring function, which are used to generate and analyze various ligand conformations (Surabhi & Singh, 2018).

Molecular Dynamics (MD)

Molecular Dynamics (MD) simulation predicts how each atom in a protein or other molecular system moves over time, based on fundamental physical principles governing atomic interactions. This simulation technique is widely used to study the motion of complex macromolecular systems, including biologically significant biomolecules (Karplus & Petsko, 1990). MD is a computational method that simulates molecular behavior within a specific timescale to observe the biological functions of proteins or nucleic acids that are often difficult to examine experimentally. MD simulations provide both static and dynamic information at the atomic level—such as atomic positions and velocities—which can be further analyzed to derive macroscopic properties including pressure, temperature, and other thermodynamic parameters.

Essentially, MD involves examining the time-dependent behavior of molecules using classical mechanics principles, which are conceptually similar to those applied in molecular mechanics calculations (Bakal et al., 2022).

METHODOL.OGY

This study is an exploratory investigation aimed at evaluating the activity, interaction pattern, and stability of bioactive compounds derived from *Sida rhombifolia* (Sidaguri) as potential anti-MRSA drug candidates through molecular docking and molecular dynamics simulations.

Materials and Equipment

1. Materials

The three-dimensional (3D) structure of the test ligand was constructed based on its SMILES code obtained from the PubChem database. The 3D structure was generated and optimized using ChemDraw and MarvinSketch software.

The three-dimensional (3D) structure of the target macromolecule, namely Penicillin-Binding Protein 2a (PBP2a) with PDB ID 4CJN, was retrieved from the Protein Data Bank (RCSB PDB) for further computational analysis.

2. Equipment

1.1 Hardware

All computational analyses were performed using an ASUS ROG S13 X330FA_S3330FA laptop operating on Windows 11 Pro 64-bit (Build 22621), equipped with an Intel® Core™ i7-8145U CPU @ 2.10 GHz processor, 8 GB RAM, and DirectX 12 graphics environment.

1.2 Software and Online Resources

The software and online platforms utilized in this study included: AutoDock 4.0, AutoDockTools, MarvinSketch, PyMOL, LigPlot+, BIOVIA Discovery Studio, Notepad++, SwissADME (*http://www.swissadme.ch/*), and YASARA Dynamics.

1. Ligand Screening

The ligand screening process began with the identification of potential compounds that could serve as therapeutic candidates against *Methicillin-Resistant Staphylococcus aureus* (MRSA). The Dr. Duke's Phytochemical Database was utilized as one of the web-based resources to identify bioactive compounds potentially active against MRSA targets. Subsequently, chemical constituents of *Sida rhombifolia* were retrieved from the KNApSAcK phytochemical database, tabulated, and evaluated based on Lipinski's Rule of Five to determine their suitability as orally active drug candidates.

Furthermore, pathway screening was conducted using the KEGG Pathway database (https://www.genome.jp/kegg/pathway.html) with the MRSA-specific keyword *map01501*, to confirm the involvement of relevant signaling pathways consistent with *in silico* predictive analysis (Fernández & Hancock, 2012).

The drug-likeness evaluation of *Sida rhombifolia* phytoconstituents was then carried out using the SwissADME web tool. The canonical SMILES of each

compound was obtained from PubChem and entered into the SwissADME platform (http://www.swissadme.ch/). The procedure involved copying and pasting the canonical SMILES into the input box labeled "*Enter a list of SMILES here*", followed by selecting "*Run!*" to generate the ADME (Absorption, Distribution, Metabolism, and Excretion) and drug-likeness profiles. The resulting pharmacokinetic and physicochemical properties were analyzed to determine whether each compound met the criteria for oral drug candidates according to Lipinski's parameters (Daina et al., 2017).

2. Ligand Optimization and Preparation

The two-dimensional (2D) structure of the test ligand was first constructed prior to generating its three-dimensional (3D) conformation. The 2D structure was built using MarvinSketch software in *.mol* format by inserting the canonical SMILES string obtained from the PubChem webserver. The 3D visualization of the ligand structure was then displayed using BIOVIA Discovery Studio (DS). Subsequently, structural optimization was carried out in MarvinSketch to generate an energetically minimized 3D structure of the ligand.

Protonation of the ligand was adjusted to a physiological pH of 7.4, corresponding to human blood conditions, to ensure appropriate ionization states. The structure was then optimized to achieve the most stable and favorable conformation and saved in the *.mol2* file format (Dwi Agistia *et al.,* 2013).

Following optimization, the ligand was imported into YASARA View to remove any water molecules that could interfere with docking calculations, thereby improving computational efficiency. Hydrogen atoms were added to complete valence structures and stabilize molecular geometry. After the preparation process was finalized, the optimized ligand structure was saved again in the *mol2* format for subsequent docking analysis (Dwi Agistia *et al.,* 2013).

3. Macromolecule Retrieval

The target macromolecule was retrieved from the RCSB Protein Data Bank (PDB) with the PDB ID 4CJN, corresponding to Penicillin-Binding Protein 2a (PBP2a), in *.pdb* format.

4. Macromolecule Preparation

The preparation of the macromolecule involved separating the native ligand from the protein structure using YASARA View. Several macromolecules that constituted the target protein were isolated into a single sequence by opening the downloaded *.pdb* file. In the docking protocol, unnecessary parts of the complex were removed to retain only the protein and its native ligand. The macromolecule was optimized using YASARA View by adding hydrogen atoms, and the optimized structure was saved in *yob* format. The prepared protein target was then saved as protein mol2 (Tegar & Purnomo, 2013).

The native ligand was extracted from the macromolecular complex and optimized separately in YASARA by adding hydrogen atoms, then saved as ref_ligand.mol2 (*Tegar & Purnomo, 2013*). Ligand preparation followed the docking protocol, combining multiple conformations using MarvinSketch. The

ref_ligand.mol2 file was opened in MarvinSketch, and protonation was checked at physiological pH (7.4) via the *Tools → Protonate* menu, then saved as ligand_2D.mrv. Conformational search was performed through *Tools → Conformation → Conformers*, and the resulting conformers were saved as ligand.mol2 (Tegar & Purnomo, 2013).

5. Validation of Molecular Docking Method

Docking validation was conducted by comparing the conformation of the native ligand from the experimental crystallographic structure with the conformation obtained through redocking using AutoDockTools (ADT). Default settings were used for grid box dimensions (x, y, z), grid center (x, y, z), and spacing. The validation result was assessed using the Root Mean Square Deviation (RMSD) value. The docking method was considered valid if RMSD ≤ 2 Å. If RMSD exceeded 2 Å, grid box parameters were adjusted manually until a valid RMSD (≤ 2 Å) was achieved (Mukherjee *et al., 2010*).

6. Molecular Docking Procedure

Molecular docking simulations were performed using AutoDock 4.0 (AD4.0) and AutoDock Tools (ADT). The optimized protein and ligand structures were stored in the same directory. Prior to docking, a Grid Parameter File (GPF) was prepared as follows:

a) Select *Grid → Macromolecule → Open*, and load the protein (*.pdbqt*).
b) Select *Grid → Set Map Types → Open*, and load the ligand (*.pdbqt*).
c) Define the binding site region using *Grid → Grid Box → Center on Ligand* (for validation).
d) Adjust the grid dimensions and spacing according to the validated grid parameters.
e) Save the GPF file (*File → Output → Save GPF*).

The AutoGrid process was then executed (*Run → Run AutoGrid*), specifying "autogrid4.exe" as the executable and the GPF file as the parameter input. After completion, docking parameters were configured by selecting *Docking → Macromolecule → Set Rigid Filename* (protein file) and *Docking → Ligand → Choose Ligand → Accept*. Docking runs were set using the Lamarckian Genetic Algorithm (LGA) with 100 GA runs and a population size of 150.

Docking parameters were saved as a Docking Parameter File (DPF) (*Docking → Output → Lamarckian GA*). The AutoDock process was executed (*Run → Run AutoDock*), specifying "autodock4.exe" as the executable and the DPF file as input. Docking results were analyzed and visualized using BIOVIA Discovery Studio and PyMOL to identify binding interactions and amino acid residues involved in ligand-protein interactions (Huey *et al.,* 2012*;* Rizv*i et al.,* 2013*;* Dwi Agistia *et al.,* 2013).

7. Molecular Dynamics (MD) Simulation

The best-performing ligand for PBP2a, based on the lowest binding free energy and key amino acid interactions similar to the native ligand, was selected for molecular dynamics (MD) simulations using YASARA Dynamics. If a

compound exhibited favorable binding energy but did not interact with residues consistent with the native ligand, it was excluded from MD analysis.

Complexes of both the native ligand–protein and test ligand–protein were placed in the same working directory. The simulation script *md_run.mcr* was configured with 0.9% NaCl, pH 7.4, temperature 298 K, a simulation time of 20 ns, and the AMBER force field parameters (Karplus & Petsko, 1990; Bakal *et al., 2022*).

The simulation was initiated by selecting *Options → Macro & Movie → Set Target* (selecting the target *.pdb* file), followed by *Options → Macro & Movie → Play Macro → md_run.mcr*. Upon completion, analysis was performed using *md_analyze.mcr* to calculate the RMSD (Root Mean Square Deviation) and RMSF (Root Mean Square Fluctuation) values. If the system exhibited instability, the second-best ligand from docking results was subjected to MD simulation (Land & Humble, *2018*).

Data Analysis

1. Validation

Validation was performed to confirm the 3D conformational alignment of the native ligand within the receptor's binding pocket. According to *Listyani et al. (2019)*, this step ensures structural consistency between the native ligand before and after docking. The docking method was considered valid when the RMSD value was less than 2 Å (Sari *et al., 2024*).

2. Binding Energy

The binding energy values of the receptor–ligand complexes obtained from the docking simulations were tabulated and compared with those of the receptor–native ligand complex. A lower binding energy indicates a stronger interaction between the ligand and receptor. Consequently, the smaller the binding energy, the greater the predicted stability and affinity of the ligand–receptor complex.

3. Receptor–Ligand Interactions

The receptor–ligand interaction data were visualized using BIOVIA Discovery Studio to examine the binding interactions between the test ligands and the amino acid residues within the active site of the target protein. The type and nature of the interactions, such as hydrogen bonding, hydrophobic contacts, and electrostatic interactions, were analyzed in detail.

The effectiveness of a ligand in binding to its target can be inferred from the similarity of its interacting amino acid residues to those of the native ligand. The higher the similarity in residue interactions, the more favorable the binding affinity of the ligand toward the target protein. Amino acid residues within the binding pocket play a critical role in stabilizing the ligand through non-covalent interactions (*Sari et al., 2024*).

4. Stability of Receptor–Ligand Complexes

The stability of the receptor–ligand complexes was assessed by comparing the native ligand–receptor complex with the test ligand–receptor complex. The results of this comparison were evaluated based on the Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) values obtained from molecular dynamics simulations. These parameters reflect the

conformational stability and flexibility of the ligand–receptor complex over the course of the simulation

RESEARCH RESULT AND DISCUSSION

Preparation of Test Compounds

1. Screening of Test Compounds

The success or failure of the molecular docking process is influenced by the physicochemical properties of the compounds, as described by Lipinski's Rule of Five (*Patrick, 2013*). This rule serves as a fundamental guideline for evaluating the oral bioavailability of small-molecule drug candidates. According to Lipinski's criteria, a compound is more likely to exhibit favorable pharmacokinetic properties if it meets the following conditions: a molecular weight ≤ 500 g/mol, a partition coefficient (MLogP) ≤ 4.15, no more than 10 hydrogen bond acceptors, and no more than 5 hydrogen bond donors.

Table 1. Results of Lipinski's Rule of Five Screening

Compounds	Filter Lipinski				Ket
	BM ≤ 500	MLOGP ≤ 4,15	N or O ≤ 10	NH or OH ≤ 5	
2D-Hydroxyecdysone	✓	✓	✓	□	*Yes*
Acacetin	✓	✓	✓	✓	*Yes*
Ecdysone	✓	✓	✓	✓	*Yes*
Peganine	✓	✓	✓	✓	*Yes*
Vasicinol	✓	✓	✓	✓	*Yes*
Vasicinone	✓	✓	✓	✓	*Yes*
Kaempferol	✓	✓	✓	✓	*Yes*
Pterosterone-3-O-β-D-Glucopyranoside	✓	✓	✓	□	*Yes*
Sanguinine	✓	✓	✓	✓	*Yes*
Quercetin	✓	✓	✓	✓	*Yes*
24-Methylenecholesterol	✓	✓	✓	✓	*Yes*
Scopoletin	✓	✓	✓	✓	*Yes*
Quindolinone	✓	✓	✓	✓	*Yes*
Stigmasterol	✓	✓	✓	✓	*Yes*
Campesterol	✓	✓	✓	✓	*Yes*

Explanation:
Yes = Meets the criteria (violates one rule)
No = Does not meet the criteria (violates more than one rule)
✓ = Complies with Lipinski's Rule
O = Does not comply with Lipinski's Rule

Based on Table 1, fifteen compounds tested for drug-likeness were predicted to have potential as orally active drug candidates. Lipinski's Rule of Five suggests that a compound is considered suitable for oral administration if it does not violate more than one of the established criteria. This rule is commonly used to assess drug-likeness because oral drug administration remains one of the most common and convenient methods in clinical practice. Oral drugs offer several practical advantages, including ease of use by patients without the need for specialized medical assistance.

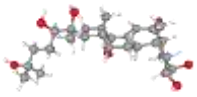
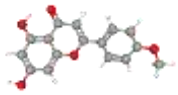
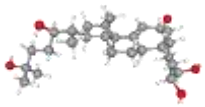
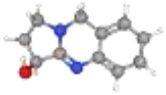
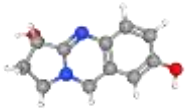
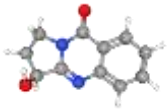
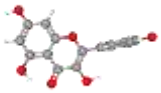
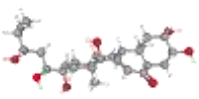
Natural compounds often present unique structural characteristics that differentiate them from synthetic drugs traditionally used in pharmaceutical development. These bioactive natural products, which are frequently employed in traditional or alternative medicine, may have higher molecular weights,

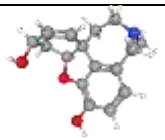
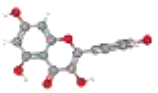
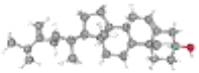
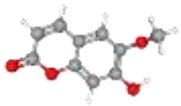
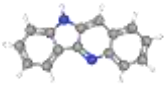
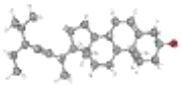
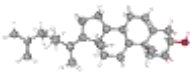
increased log P values, or other variations that deviate from Lipinski's parameters. As such, not all natural or complex molecules can be strictly evaluated using this rule-based criterion, highlighting the need for flexibility when assessing drug-likeness for these compounds.

Among the compounds analyzed, 15 fulfilled Lipinski's requirements, but two compounds—2D-Hydroxyecdysone and Pterosterone-3-O-β-D-Glucopyranoside—exceeded the acceptable limits for hydrogen bond donors or acceptors, indicating higher molecular polarity and potentially reduced membrane permeability. However, both compounds were still considered to possess acceptable drug-likeness, as they violated only one of the five rules. According to Doak et al. (2014), compounds with high molecular weight and multiple hydroxyl groups may still exhibit significant biological activity, especially if they interact with specific membrane-bound targets or employ active transport mechanisms. This suggests that their activity is not solely dependent on passive diffusion, allowing for their potential as oral drug candidates.

2. *Retrieval and Preparation of Test Compounds*

Table 2. Results of 3D Structure Retrieval of Test Compounds

Compounds	SMILES	Structure 3D
2D-Hydroxyecdysone	C[C@]12CC[C@@H](C[C@H]1C(=O)C=C3[C@@H]2CC[C@]4([C@]3(CC[C@@H]4[C@](C)([C@@H](CCC(C)(C)O)OP(=O)(O)O)O)O)C)O	
Acacetin	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O	
Ecdysone	C[C@@H]([C@H]1CC[C@@]2([C@@]1(CC[C@H]3C2=CC(=O)[C@H]4[C@@]3(C[C@@H]([C@@H](C4)O)O)C)C)O)[C@@H](CCC(C)(C)O)O	
Peganine	C1CN2CC3=CC=CC=C3N=C2C1O	
Vasicinol	C1CN2CC3=C(C=CC(=C3)O)N=C2[C@H]1O	
Vasicinone	C1CN2C(=NC3=CC=CC=C3C2=O)[C@H]1O	
Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	
Pterosterone-3-O-β-D-Glucopyranoside	CC(C)[C@H](C[C@H]([C@@](C)([C@H]1CC[C@@]2([C@@]1(CC[C@H]3C2=CC(=O)[C@H]4[C@@]3(C[C@@H]([C@@H](C4)O)O)C)C)O)O)O)O	

Compounds	SMILES	Structure 3D
Sanguinine	CN1CC[C@@]23C=C[C@@H](C[C@@H]2OC4=C(C=CC(=C34)C1)O)O	
Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	
24-Methylenecholesterol	C[C@H](CCC(=C)C(C)C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C	
Scopoletin	COC1=C(C=C2C(=C1)C=CC(=O)O2)O	
Quindolinone	C1=CC=C2C(=C1)C3=NC4=CC=CC(=O)C4=CC3=N2	
Stigmasterol	CC[C@H](/C=C/[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C)C(C)C	
Campesterol	C[C@H](CC[C@@H](C)C(C)C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C	

Based on Table 2, the optimized three-dimensional (3D) structures of the analyzed compounds display conformational characteristics typical of bioactive molecules reported in the literature. The structures of 2D-Hydroxyecdysone and Ecdysone exhibit a steroidal backbone with trans configurations between the A/B and B/C rings, alongside hydroxyl groups positioned at strategic sites (such as C2, C3, and C14) that facilitate hydrogen bond formation. These findings align with those of Lafont and Dinan (2003), who emphasized the importance of the spatial orientation of hydroxyl groups in ecdysteroids for optimal receptor recognition in insects.

Acacetin, a flavonoid compound, features a conjugated aromatic ring system with hydroxyl substitutions at the C5 and C7 positions. This structural configuration supports both π–π stacking and hydrogen bonding interactions, which are consistent with Zhang et al. (2017), who highlighted the significance of these features in antioxidant and enzyme inhibitory activities. Similarly, the alkaloids Peganine and Vasicinone, members of the quinolizidine class, exhibit rigid 3D frameworks characterized by saturated ring systems and a single chiral center. This contributes to partial conformational flexibility during target binding, supporting the findings of Ali et al. (2019), who demonstrated that such conformations are critical for bronchodilatory activity.

Sterol compounds like Stigmasterol and Campesterol exhibit a tetracyclic scaffold with a rigid isoprenoid side chain, in line with crystallographic data

reported by Nes et al. (2008). This structural rigidity is necessary for specific interactions with cholesterol biosynthetic enzymes. Overall, the optimized 3D structures of these phytochemical compounds are consistent with previous crystallographic or simulation data, reinforcing their potential biological activity and suitability for molecular docking and dynamics simulations.

Target Protein Preparation

1. Screening and Retrieval of Target Protein

The target protein used in this study was identified based on the NetPharm screening results of compounds from *Sida rhombifolia* against methicillin-resistant *Staphylococcus aureus* (MRSA) proteins. A higher prediction score indicated a stronger likelihood of interaction between the compound and the target protein, supported by prior literature data. The selected protein was then retrieved from the Protein Data Bank (PDB), an online repository that archives experimentally determined three-dimensional (3D) structural data of biomacromolecules, to facilitate the identification and selection of target proteins for molecular modeling studies. The selection criteria for the target protein included structures resolved using X-ray diffraction methods with a resolution of less than 2.5 Å, the presence of a co-crystallized ligand (serving as a positive control), and validation through experimental evidence demonstrating structural accuracy. The MRSA target protein selected and downloaded from the PDB for this study is presented in Table 3.

Table 3. Results of MRSA Target Protein Retrieval

Target Proteins	PDB	3D Structure
Pbp2a	4CJN	

The target protein PBP2a met all the required specifications for molecular target selection. The selection process was conducted based on several parameters, including the predicted interaction between the compound and the MRSA target protein obtained from the STRING web server, the interaction score of the compound–target association, and the structural specifications of the protein retrieved from the Protein Data Bank (PDB). The selected target protein was subsequently downloaded from the PDB web server, as shown in Table 3 (Burley et *al.*, 2025).

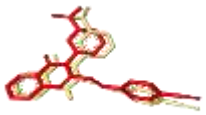
2. Target Protein Preparation

The target protein must undergo a preparation process prior to molecular docking and molecular dynamics simulations. This preparation involves separating the native ligand from the target protein so that it can be replaced with the test compound for subsequent docking and dynamic analysis. The protein preparation process can be performed using BIOVIA Discovery Studio, PyMOL, and the SwissDock web server.

Validation of the Molecular Docking Method

Root Mean Square Deviation (RMSD) is a crucial parameter for validating the accuracy and reliability of the molecular docking method, as it reflects the deviation in the protein–ligand complex conformation between the crystal structure before docking and the redocked structure (Mukherjee et al., 2010; Sándor et al., 2010; de Oliveira et al., 2022; Venkatesh, 2022). The docking protocol is considered valid if the RMSD value is less than 2 Å (Indah et al., 2020). RMSD values were obtained from the validation process conducted using BIOVIA Discovery Studio, by comparing the native ligand's conformation before and after docking. Based on the validation results, all target proteins exhibited RMSD values below 2 Å, confirming the validity of the docking method used in this study. The RMSD values of the validated target proteins are presented in Table 4.

Table 4. RMSD Values of Validated Target Proteins

Protein Target	RMSD	Overlay 3D
PBP2a	0,954 Å	

Based on the data presented in Table 5, the RMSD (Root Mean Square Deviation) value was used to evaluate the conformational stability of the protein during molecular dynamics simulations. In this study, the RMSD value of the target protein indicated a relatively stable structural conformation throughout the 100 ns simulation period. The PBP2a protein exhibited an average RMSD of 0.954 Å, suggesting that it remained within a stable range, as RMSD values below 3 Å are generally considered indicative of good conformational stability during simulation (Duan *et al*., 2020).

When compared with the previous study conducted by Khan *et al*. (2022), RMSD values within the range of <3.0 Å are often classified as indicators of protein–ligand system stability. In that study, proteins exhibiting RMSD values between 2.0 Å and 2.8 Å were considered to have good complex affinity and structural stability throughout the simulation period. Similarly, Sharma *et al*. (2021) reported that consistent RMSD values below 3.0 Å suggest that the protein structure remains within its native conformational range without exhibiting extreme fluctuations.

A relatively constant RMSD value after the equilibration phase further indicates that the protein system achieved structural stability and did not experience major fluctuations that could suggest structural distortion or irregularity. Moreover, the absence of significant RMSD spikes implies that the interactions between the protein and ligand remained stable throughout the simulation (Prabhakar *et al*., 2021; Hollingsworth & Dror, 2018).

Considering these findings, it can be concluded that all target proteins in this study maintained good structural stability during molecular dynamics simulations. This reinforces the validity of the protein–ligand interaction models used and provides a strong foundation for further analyses, such as binding

energy calculations and ligand affinity predictions (Hospital *et al*., 2015; Durrant & McCammon, 2011).

Analysis of Molecular Docking Results

In this study, fifteen compounds derived from *Sidaguri* (*Sida rhombifolia*) were docked against the selected target proteins using the molecular docking software AutoDockTools. Molecular docking aims to predict the binding mode and affinity of a small molecule toward the active site of a specific target protein. The outcome of docking is expressed as the binding free energy, which represents the amount of energy required for the ligand to interact with and bind to the receptor (Guedes et al., 2013).

The evaluation of the test ligands in molecular docking involves assessing several key parameters, including binding free energy and the similarity of amino acid residues involved in the interactions with those of the native ligand. These factors play an essential role in selecting ligands with high potential to bind specifically and effectively to the target protein. Binding energy is a critical factor in determining the stability of the receptor–ligand complex (de Oliveira et al., 2022). Lower binding free energy values generally indicate stronger and more stable interactions between the target protein and ligand (Kurczab, 2017). Moreover, the similarity of interacting amino acid residues between the test and native ligands may enhance the likelihood of forming a favorable and stable binding complex, as ligand–protein binding is largely governed by specific interactions within the active site (Wu & Huang, 2023).

PBP2a

In the molecular docking analysis of the PBP2a protein, out of the fifteen screened compounds, only five were selected for further discussion based on their highest binding affinity values. This selection was guided by the binding energy, which serves as a crucial indicator in docking studies to describe the strength of the interaction between a ligand and its target protein. A more negative binding energy value signifies a stronger binding affinity, reflecting a higher potential inhibitory effect on the biological activity of the target (Morris & Lim-Wilby, 2008).

The five selected compounds exhibited lower binding energy values than the others, suggesting that they possess the strongest potential to inhibit the PBP2a enzyme. This is significant because PBP2a is an enzyme responsible for β-lactam antibiotic resistance in *Staphylococcus aureus*; therefore, inhibition of this enzyme represents a key strategy in the development of novel antimicrobial agents (Fuda et al., 2005).

A previous study by Mohamed et al. (2020) reported that compounds with binding energies below −7.0 kcal/mol against PBP2a were considered strong candidates for overcoming methicillin-resistant *Staphylococcus aureus* (MRSA) resistance, which aligns with the findings and approach used in this study. Similarly, Shamsi et al. (2021) applied a comparable selection strategy, identifying potential PBP2a inhibitors based on their binding affinities, and demonstrated that only ligands with binding energies below a certain threshold exhibited stable and pharmacologically effective interactions.

In this study, PBP2a (PDB ID: 4CJN) showed favorable binding interactions with ecdysone, 24-methylenecholesterol, quercetin, sanguinine, and stigmasterol. The native ligand, serving as the positive control, was identified as *(E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl) benzoic acid*. The binding energy values for PBP2a are summarized in Table 5.

Table 5. Binding Energy Values of PBP2a

Compounds	Energy Binding (kkal/mol)	Amino Acids Involved in the Binding Interaction			
		Hydrogen	%	Non-Hydrogen	%
(E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl) benzoic acid (ligan alami)	-5,6	THR:165, ARG:241, LYS:148	100%	GLU:239, SER:240, HIS:293, VAL:277, SER:149, ARG:151, VAL:256, GLU:150, ASN:164	100%
24hydroxyecdysone	-9,4	THR:165, ARG:241, LYS:148	67%	SER:149, VAL:277, HIS:293, GLN:292, GLU:239, ASN:164, GLU:150, VAL:256, ARG:151	78%
Ecdysone	-9,7	THR:165, ARG:241,	67%	LYS:148, GLU:239, HOH:2026, ASN:164, GLU:150, ARG:151, HIS:293, HOH:2076, GLN:292, VAL:277, ILE:278, VAL:256, HOH:2060, SER:240	89%
Quercetin	-8,5	THR:165, ARG:241, LYS:148	67%	SER:149, VAL:277, HIS:293, GLU:239, ASN: 164, GLU:150, VAL:256, ARG:151	67%
Sanguinine	-8,3	THR:165, ARG:241, LYS:148	67%	SER:149, VAL:277, GLN:292, SER:240, GLU:239, ASN:164, GLU:150, ARG:151	67%
Stigmasterol	-8,3	THR:165, ARG:241, LYS:148	67%	SER:149, VAL:277, HIS:293, GLN:292, SER:240, GLU:239, ASN:164, GLU:150,	67%

Based on the molecular docking results presented in Table 5, the binding energy values, hydrogen bond interactions, and non-hydrogen bond interactions between the tested ligands and the target protein were determined. The compound 24-hydroxyecdysone formed both hydrogen and non-hydrogen interactions similar to those of the native ligand. It exhibited a favorable binding energy of −9.4 kcal/mol with the target protein PBP2a, which is lower (thus more favorable) than that of the native ligand (−5.6 kcal/mol). However, its amino acid interaction similarity was 67% for hydrogen bonds and 78% for non-hydrogen bonds compared to the native ligand.

Ecdysone also formed both hydrogen and non-hydrogen interactions similar to those of the native ligand. It showed a strong binding affinity toward PBP2a, with a binding energy of −9.7 kcal/mol, outperforming the native ligand (−5.6 kcal/mol). Despite this favorable binding energy, the amino acid interaction analysis revealed that the compound shared 67% of the hydrogen bonds and 89% of the non-hydrogen bonds with the native ligand, although no direct hydrogen bond interaction was observed in some binding regions.

Quercetin formed hydrogen and non-hydrogen interactions comparable to the native ligand, exhibiting a binding energy of −8.5 kcal/mol, which was

more favorable than the native ligand (−5.6 kcal/mol). However, quercetin shared only 67% hydrogen bond interactions and 67% non-hydrogen interactions with the native ligand. Similarly, sanguinine displayed both hydrogen and non-hydrogen interactions similar to those of the native ligand, with a binding energy of −8.3 kcal/mol, also better than that of the native ligand (−5.6 kcal/mol). Nevertheless, the amino acid interaction similarity was limited to 67% hydrogen bonds and 67% non-hydrogen bonds. Stigmasterol also exhibited comparable hydrogen and non-hydrogen interactions with the native ligand, showing a binding energy of −8.3 kcal/mol, which again was better than the native ligand (−5.6 kcal/mol). However, its amino acid interaction similarity was only 67% hydrogen bonds and 67% non-hydrogen bonds. Overall, ecdysone demonstrated the most favorable interaction profile with the target protein PBP2a, exhibiting the lowest binding energy and the highest similarity in both hydrogen and non-hydrogen interactions compared to the native ligand and the other compounds, making it the most promising candidate for potential inhibition of PBP2a (Alexander et al., 2020).

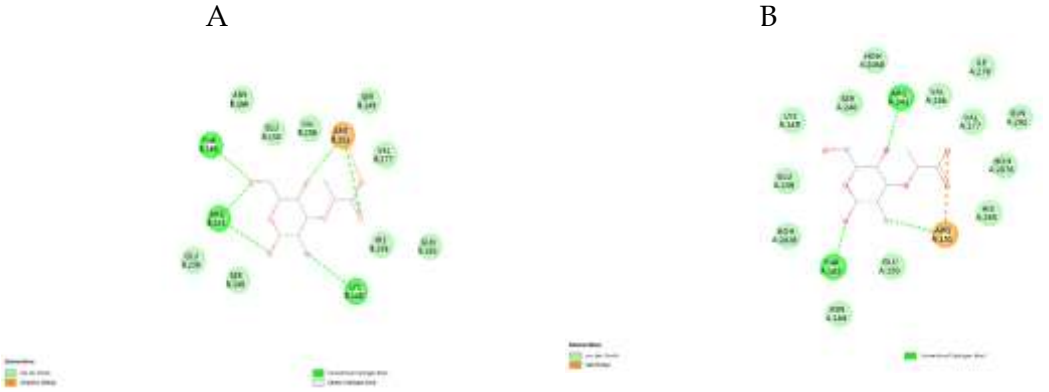


Figure 2. Amino Acid Interactions of the Native Ligand (A) and Ecdysone (B) with the Target Protein PBP2a

The amino acid interaction analysis of the native ligand (A) and ecdysone (B) with the target protein PBP2a revealed that both compounds shared common hydrogen bond interactions with the amino acid residues THR165 and ARG241, as well as common non-hydrogen interactions with residues LYS148, GLU239, ASN164, GLU150, ARG151, HIS293, VAL277, VAL256, and SER240. Both the native ligand and ecdysone also exhibited similar van der Waals interactions involving residues GLU239, SER240, HIS293, GLN292, VAL277, VAL256, GLU150, and ASN164 (Lee et al., 2018).

Biologically, PBP2a (Penicillin-Binding Protein 2a) is a key enzyme in the β-lactam resistance mechanism of *Staphylococcus aureus*, particularly in MRSA strains, as it can catalyze peptidoglycan synthesis even in the presence of β-lactam antibiotics, which inhibit other PBPs. Its low affinity for β-lactam antibiotics ensures that it remains functional in maintaining bacterial cell wall integrity (Fuda et al., 2005). In this study, the amino acid interaction analysis indicated that, in addition to ARG241, other key residues such as LYS316, ASN146, and GLY307 contribute to antibacterial activity. LYS316, involved directly in the transpeptidation process, is crucial for bacterial cell wall synthesis, and its interaction with the ligand may significantly disrupt the enzymatic

function of PBP2a. The fact that ecdysone interacts with these critical residues suggests its potential as a PBP2a inhibitor, potentially compromising MRSA's cell wall integrity (Shamsi et al., 2021).

Analysis of Molecular Dynamics Simulation Results
PBP2a (4CJN)

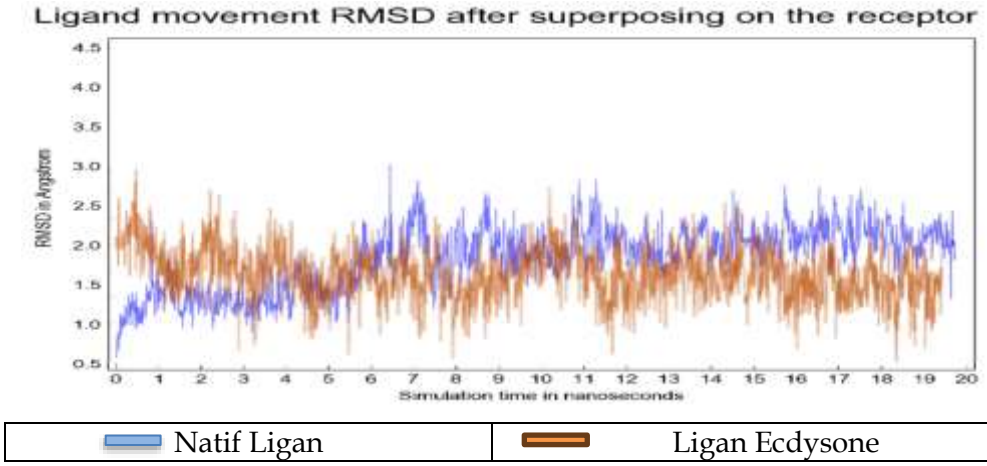


Figure 3. RMSD Plot of PBP2a

The RMSD values for both the ligand and the protein were calculated from the graph, with the initial values for the protein shown in Figure 3. The ecdysone compound bound to the target protein PBP2a initially exhibited an increase in RMSD from 2.0 to 2.5 Å, followed by a decrease at 0.2 ns from 2.5 to 2.3 Å. After 0.5 ns, the RMSD rose again from 2.3 to 3.0 Å. A reduction in RMSD occurred at 0.9 ns, reaching 2 Å, and during 8–20 ns, the RMSD remained stable with an average of 1.5 Å. For the native ligand of PBP2a, the simulation at 0.00 ns showed an increase in RMSD from 0.5 to 1.0 Å. Stability was observed between 1–4 ns with an average RMSD of 1.3 Å. An increase in RMSD occurred between 6.7–6.9 ns, reaching 3 Å, followed by a decrease to 1.7 Å between 6.9–7 ns. Afterward, the RMSD remained stable until the end of the simulation, with an average value of 2 Å.

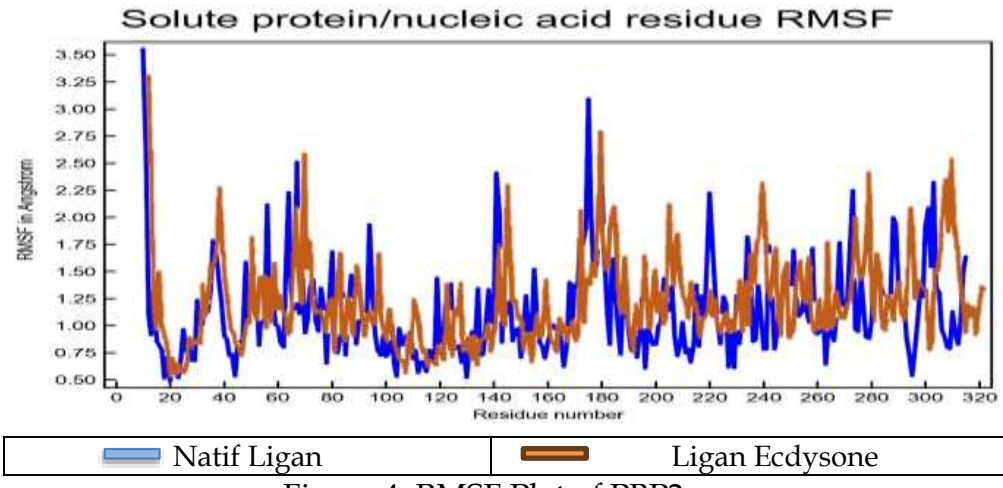


Figure 4. RMSF Plot of PBP2a

The RMSF values indicate low and stable fluctuations for most residues, remaining within the range of 0.75–1.50 Å throughout the simulation period. An

increase in the RMSF value was observed at residue 180, reaching up to 2.75 Å. The higher fluctuation suggests that this residue is more flexible and prone to structural changes, indicating that quercetin affects the protein's dynamics. At the end of the simulation, the RMSF value of the quercetin complex increased to 1.25 Å, while that of the native ligand reached 1.50 Å. Most residue values exhibited similar fluctuations to those of the native ligand of the 4CJN target protein. The low fluctuation results confirm the formation of a strong ligand–protein interaction. Ecdysone interacts with the same hydrogen-bonding amino acids as the native ligand, namely THR165 and ARG241, with bond distances below 2 Å, indicating structural stability (Rashid et al., 2022).

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of this study, it can be concluded that several bioactive compounds from *Sida rhombifolia* demonstrate strong potential as antibacterial agents against MRSA. Ten compounds—namely acacetin, ecdysone, peganine, kaempferol, sanguinine, quercetin, 24-methylenecholesterol, quindolinone, stigmasterol, and campesterol—were predicted to exhibit good binding affinity toward MRSA target proteins. Among these, ecdysone showed the most favorable interaction model and strong affinity with target proteins 4CJN. Moreover, molecular dynamics simulation results revealed that quercetin, ecdysone, and 24-methylenecholesterol maintained stable protein–ligand interactions throughout the simulation period, indicating consistent conformational stability. Furthermore, the pharmacokinetic prediction of ecdysone demonstrated a favorable ADMET profile, supporting its potential as a promising drug candidate for further development as an antibacterial agent targeting MRSA.

ADVANCED RESEARCH

The stability test can be extended up to 100 ns to further evaluate the stability of the compound–protein interactions. Further studies on the tested compounds, as well as the design of new derivatives, are recommended to optimize the chemical activity of *Sida rhombifolia* constituents as potential drug candidates.

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