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Submission date: 25-May-2026 03:50PM (UTC+0530)
Submission ID: 2969053252
File name: AI_PLAG_CHECK.docx (5.4M)
Word count: 5810
Character count: 34981

ABSTRACT

Antibiotic infections are majorly caused by the development of resistance against methicillin in *Staphylococcus aureus.* Methicillin-resistant *Staphylococcus aureus,* or MRSA, shows the presence of Penicillin-Binding Protein 2a or PBP2a. This protein is responsible for the reduced effectiveness of β-lactam antibiotics against *S aureus.* In this study, we aim to explore the binding ability of Gedunin, a limonoid that is obtained from *Azadirachta indica* or neem, against PBP2a using molecular docking analysis studies.

Docking was performed AutoDock Vina against PBP2a receptor at its active and allosteric sites for Gedunin ligand. Re-docking of the co-crystallised ligand was performed which resulted in a RMSD of 0.537 Å. This confirmed the accuracy of the docking protocol used in the study. Further analysis of Gedunin showed favourable affinity towards the active and the allosteric site of the protein. The predicted binding to the active site (−6.935 kcal/mol) was much more than that of the allosteric site (−6.912 kcal/mol).

Further, Gedunin showed the presence of the formation of hydrogen bonds, the hydrophobic interactions, π-stacking bonds, and salt bridges along with key amino acid residues for the binding cavity at both sites. To validate the study, a comparative analysis of Gedunin was performed with oxacillin and ceftaroline to ensure that Gedunin can interact with functional regions of PBP2a.

Additionally, ADMET analysis and AMES analysis were done using SwissADME and pkCSM, respectively. The results obtained supported the use of Gedunin as a potential inhibitor of PBP2a. Overall, the results indicate the use of Gedunin in developing agents that target PBP2a and after experimental studies validate these computational findings, it can serve as an important naturally obtained product for targeting methicillin-resistant *S aureus*.

Keywords: Gedunin; PBP2a; MRSA; molecular docking; antimicrobial resistance; AutoDock Vina; natural products; beta-lactam resistance

CHAPTER 1

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the major health concerns across the globe (Halder et al., 2022). AMR has been listed among the top ten global risks to human health by the World Health Organisation (WHO, 2021). Some of the revolutionary analyses on epidemiological levels have quantified death rates caused by drug-resistant bacterial infections to approximately 1.27 million deaths globally in 2019, and this figure has increased in 2026 (Jiao et al., 2023). These studies have also reported that these drug-resistant infections contribute to approximately 4.95 million deaths when an attributable mortality rate is taken into consideration across various associated conditions during infections (Murray et al., 2022). It is believed that without any intervention and development of methods that help contain this problem of AMR, the estimated figures of deaths by 2050 would escalate to approximately 10 million deaths per annum. This would surpass cancer as the cause of most deaths worldwide (O'Neill, 2016).

Among the resistant bacteria, Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a critical priority pathogen (Jiao et al., 2023). MRSA has been reported to be responsible for a broad and clinically serious category of diseases, like pneumonia, bacteraemia, infective endocarditis, and osteoarticular infections (Tong et al., 2015). The figures of estimated deaths caused by MRSA in only the United States and the European Union is approximately 1,00,000 annually (European Centre for Disease Prevention and Control, 2023). This has led to the need for some novel compounds to help overcome the problem of AMR.

1.1 Molecular Basis of MRSA Resistance

The molecular mechanism behind the generation of MRSA resistance to β-lactam antibiotics is the acquisition and the expression of the *mecA* gene. This gene is located on the staphylococcal cassette chromosome *mec* or the SCC*mec* which is a mobile genetic element (Elabed et al., 2025). The *mecA* gene codes for the Penicillin-Binding Protein 2a (PBP2a), a high-molecular-weight transpeptidase of 78 kDa. PBP2a participates in the final cross-linking step of the peptidoglycan biosynthesis (Llarrull et al., 2009). Unlike native Staphylococcal PBPs, which are acylated and inactivated by β-lactam antibiotics with high efficiency, PBP2a maintains a constitutively closed active site conformation characterised by an intrinsically low acylation rate constant ($k2/Ks$) for all β-lactam compounds currently in clinical use. This property effectively renders the entire β-lactam class that includes penicillin, cephalosporins, carbapenems, and monobactams, which are clinically ineffective against MRSA strains harbouring this determinant (Lim & Strynadka, 2002).

Crystallographic and biochemical studies have further established that PBP2a possesses a distal allosteric binding site which is located approximately 60 Å away from the transpeptidase active site center. In case fragments of the peptidoglycan cell-wall or certain small molecules occupy the allosteric pocket of the protein, there is a cascade of conformational changes that occur, which ultimately open the active site and allow for necessary interactions (Otero et al., 2013; Fishovitz et al., 2014). This mechanism of active site opening due to changes in the allosteric site provides a secondary target site that can be targeted even when the active site is not directly

involved. The secondary site modulations disrupt PBP2a function and have been exploited for decades for the discovery of drugs and for therapeutic advancements.

1.2 Limitations of Current Therapeutic Options

The traditional therapy system against MRSA infections is very limited and lacks advancements. For decades, the treatment for MRSA infections was largely dependent on a glycopeptide antibiotic called as Vancomycin (van Hal & Fowler, 2013). However, this drug is associated with high levels of nephrotoxicity and also has additional requirements for administration and monitoring. Overuse of the drug for decades led to the development of vancomycin-resistant (VRSA) and vancomycin-intermediate *S. aureus* (VISA) strains (van Hal & Fowler, 2013).

A similar case is that of Ceftaroline, which is a fifth-generation cephalosporin. It is the only β-lactam that is approved for the treatment of MRSA, for it has exceptionally high affinity for both the active and allosteric sites of PBP2a (Long et al., 2014). Unfortunately, the overexploited use of ceftaroline has also been associated with documented evidence of resistance in the case of MRSA that arises due to point mutations in the mecA gene (Long et al., 2014). This increased resistance is the reason why there is an urgent need for the development of novel compounds that perform similar functions.

1.3 Natural Products as Antibacterial Lead Compounds

Natural products have historically accounted for the majority of clinically approved antibiotics, including penicillins, cephalosporins, aminoglycosides, macrolides, tetracyclines, and glycopeptides, among others. A comprehensive analysis demonstrated that, of new antibacterial agents approved between 1981 and 2019, approximately 70% were derived from or directly inspired by natural product scaffolds (Newman & Cragg, 2020). Plants, fungi, and marine organisms remain prolific sources of structurally diverse bioactive secondary metabolites that offer advantages over purely synthetic compounds in terms of molecular complexity, stereochemical richness, and evolutionary pre-optimisation for biological target interaction.

(4α,7β,14β)-7-acetoxy-1,14-epoxy-12β-hydroxy-4,8-dimethyl-3-oxo-A-homoandrostane-17-yl carboxylate or Gedunin (PubChem CID: 122767) is a tetranortriterpenoid limonoid isolated primarily from *Azadirachta indica* (neem) and species within the family Meliaceae, including *Entandrophragma* spp. The ancient knowledge system in India has mentioned Gedunin to possess antibacterial, antifungal, anti-inflammatory and even anti-cancer properties (Alzohairy, 2016; Yadav et al., 2021). Specifically for cases of *S. aureus,* a MIC assay showed Gedunin as an inhibitor for heat shock protein 90 (Rao et al., 2019). However, the potential of Gedunin as an inhibitor for PBP2a in MRSA has not been studied extensively and thus needs more theoretical and computational work.

1.4 Molecular Docking as a Drug Discovery Tool

To predict the binding affinity of Gedunin with PBP2a, a well-established and extensively used technique called Molecular Docking was employed. It helps us predict binding affinity, top poses of binding, and the binding orientation within the protein binding site (Meng et al., 2011). AutoDock Vina is a benchmarked docking platform that employs a local search algorithm, which provides a hybrid scoring function and efficient gradient-based methods to achieve favourable accuracy and computational speed (Trott & Olson, 2010). It has been extensively

used for applications in drug discovery and has been extensively documented in studies related to antimicrobial resistance, especially where the studies are related to targeting β-lactam resistance mechanisms in MRSA.

1.5 Objectives

- To evaluate the binding affinity of Gedunin against both the active site present on residue Ser403 and the allosteric site present on residue Trp374 of the PBP2a protein from MRSA using AutoDock Vina molecular docking.
- To characterise the key protein-ligand interactions at the atomic level using the Protein-Ligand Interaction Profiler (PLIP), including hydrogen bonds, hydrophobic contacts, π-stacking interactions, and salt bridges.
- To compare Gedunin PBP2a-targeting compounds like oxacillin and ceftaroline under identical docking conditions and to then predict their binding affinity and their interaction profile.
- To validate the molecular docking protocol through re-docking of the co-crystallised ligand and RMSD analysis against the crystallographic binding mode.
- To assess the AMES toxicity profile and ADMET profile of Gedunin using SwissADME and pkCSM in comparison with the reference compounds like oxacillin and ceftaroline.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 Biology and Clinical Significance of *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive bacterium that grows in colonies that look like a cluster of grapes. It is a non-motile and non-spore-forming bacterium. It is essentially a facultative anaerobe and can grow across various ranges of temperature and pH. It is these properties that help and enhance the chances of survival of *S. aureus* across various conditions (Tong et al., 2015). As a member of the human skin and nasal microbiota, *S. aureus* asymptomatically colonises approximately 30% of the general population; however, in immunocompromised individuals or following breaches in skin or mucosal integrity, it represents a major opportunistic pathogen of extraordinary virulence.

The pathogenicity of *S. aureus* is attributable to an extensive array of virulence factors, including surface adhesins (fibronectin-binding proteins, clumping factors), secreted toxins (alpha-toxin, Panton-Valentine leukocidin, exfoliative toxins, toxic shock syndrome toxin-1), immune evasion proteins (protein A, CHIPS, SCIN), and enzymes (coagulase, staphylokinase, nuclease). The interplay of these factors enables *S. aureus* to adhere to host tissues, evade phagocytic killing, disseminate haematogenously, and establish persistent biofilm-associated infections on implanted medical devices and damaged heart valves.

2.2 Emergence and Epidemiology of MRSA

In 1961, the United Kingdom was the first place where a case of MRSA was first identified. This discovery happened shortly after methicillin was invented as a stable β-lactamase penicillin so as to reduce the already arising cases of resistance in staphylococci that produced penicillinase (Jevons, 1961). Since the initial identification, MRSA has developed lineages into two broad categories of healthcare-associated (HA-MRSA) and community-associated (CA-MRSA) lines. These lines differ in their basic properties, such as different molecular bases and epidemiology.

MRSA has been reported to be responsible for a broad and clinically serious category of diseases, like infective endocarditis, osteoarticular infections, bacteraemia, and pneumonia (Tong et al., 2015). They cause 12% of all bloodstream-related infections across Europe. Figures of estimated deaths caused by MRSA in only the United States and the European Union are approximately 1,00,000 annually (European Centre for Disease Prevention and Control, 2023).

These figures underscore the continuing clinical significance of MRSA as a target for novel therapeutic intervention.

2.3 Structural Biology and Function of PBP2a

Penicillin-Binding Proteins are membrane-associated enzymes that catalyse the final steps in peptidoglycan biosynthesis: trans glycosylation, which elongates glycan chains, and transpeptidation, which cross-links adjacent peptide stems. The transpeptidase activity is the primary target of β-lactam antibiotics, which act as structural analogues of the D-Ala-D-Ala terminus of the stem peptide substrate. β-Lactams form a stable covalent acyl-enzyme

intermediate with the catalytic serine residue of the active site, irreversibly inactivating the enzyme and leading to peptidoglycan defects that ultimately cause cell lysis.

PBP2a, encoded by the mecA gene with an open reading frame of 2,052 base pairs encoding a 668 amino acid protein, is a class B high-molecular-weight PBP with a modular architecture comprising an N-terminal transmembrane anchor, a non-penicillin-binding (NPB) domain of unknown function, and a C-terminal transpeptidase (TP) domain. The TP domain carries the three conserved PBP motifs characteristic of class B PBPs: SXXK (containing the catalytic Ser403), SXN, and KTG.

Crystallographic analysis has established that the PBP2a active site maintains a closed conformation through a distinctive β5-α11 loop configuration that sterically occludes access of β-lactam molecules to Ser403, explaining the kinetically impaired acylation of PBP2a by the entire β-lactam class (Lim & Strynadka, 2002).

The discovery of the allosteric regulatory site in PBP2a, reported by Otero et al. (2013), represented a landmark advance in understanding PBP2a function and in drug design strategy. The allosteric site is located on the non-penicillin-binding domain, approximately 60 Å from the transpeptidase active site, and is characterised by a predominantly hydrophobic cavity bounded by residues including Trp374, Tyr446, Met476, Leu421, and Ile434.

The binding of peptidoglycan cell-wall fragments or synthetic allosteric ligands to this site induces a cascade of conformational rearrangements transmitted through an α-helical linker, resulting in transient opening of the active site and enabling the brief transpeptidase activity necessary for cell-wall homeostasis. This mechanism of active site opening due to changes in the allosteric site provides a secondary target site that can be targeted even when the active site is not directly involved. The secondary site modulations disrupt PBP2a function, resulting in competitive active site inhibition have been exploited for decades for the discovery of drugs and for therapeutic advancements.

2.4 Current Therapeutic Landscape for MRSA Infections

The traditional therapy system against MRSA infections is very limited and lacks advancements. For decades, the treatment for MRSA infections was largely dependent on a glycopeptide antibiotic called as Vancomycin (van Hal & Fowler, 2013). It inhibits the cell-wall biosynthesis and thus was used as the primary line for treatment. However, this drug is associated with high levels of nephrotoxicity and also has additional requirements for administration and monitoring. Overuse of the drug for decades led to the development of vancomycin-resistant (VRSA) and vancomycin-intermediate *S. aureus* (VISA) strains (van Hal & Fowler, 2013).

Ceftaroline, the only β-lactam with regulatory approval for MRSA (approved by the FDA in 2010 and EMA in 2012), is a fifth-generation cephalosporin with unusually high affinity for PBP2a, attributable to its conformationally flexible side chain that stabilises the open-active-site conformation of the enzyme. However, ceftaroline-resistant MRSA clinical isolates have been described, arising through diverse mecA mutations including T600A, E239K, and Y446F substitutions in the PBP2a transpeptidase domain (Long et al., 2014), highlighting the relentless evolutionary pressure exerted by antibiotic use.

Linezolid, a protein synthesis inhibitor targeting the 23S ribosomal RNA, is effective but carries risks of haematopoietic toxicity with prolonged use.

2.5 Natural Products as Sources of Anti-MRSA Leads

The natural kingdom continues to provide structurally diverse scaffolds with demonstrated or predicted anti-MRSA activity. Compounds from multiple structural classes have been investigated, including flavonoids (e.g., quercetin, epigallocatechin gallate), terpenoids (e.g., carvacrol, thymol, ursolic acid), alkaloids (e.g., berberine), and limonoids. Among limonoids, gedunin and its congeners have attracted particular interest owing to their multi-target biological activities and their accessibility from *Azadirachta indica*, a plant with extensive ethnopharmacological use across South and Southeast Asia (Alzohairy, 2016).

Gedunin was first isolated and characterised structurally in 1960 (Taylor, 1960). The structural core of Gedunin comprises of a tetranortriterpenoid skeleton core. There is a 4,4,8-trimethyl ring system in the structural configuration. This ring is tetracyclic and has a δ-lactone present on the ring D. Additionally, gedunin also comprises an acetoxy group at the C-7 position. This group is necessary for providing considerable structural rigidity and strategic hydrogen bond acceptor and hydrogen bond donor groups (Taylor, 1960).

Investigations that have been carried out earlier have reported that Gedunin possesses inhibitory activities against *S. aureus*. These inhibitory activities are also reported against the MRSA strains using various experiments (Rao et al., 2019). Use of the disc diffusion method and the broth microdilution assay has shown MIC values ranging from 32 to 128 microgram per millilitre. The variation between 32 and 128 µg/mL is seen, depending largely on the strain used and the conditions of the assay (Rao et al., 2019).

Computational investigations have previously examined Gedunin as an HSP90 inhibitor and have demonstrated its potential to disrupt virulence-associated chaperone activity; however, its direct interaction with PBP2a as an antibacterial mechanism has not been reported in the peer-reviewed literature prior to the present study.

2.6 Computational Methods in Antibacterial Drug Discovery

The integration of computational tools in the drug discovery pipeline has transformed the efficiency of lead identification and optimisation. Structure-based virtual identification, of which MD is one of the most widely implemented method, enables the rapid evaluation of enormous compound libraries with the validated protein targets, generating ranked interaction predictions that guide experimental prioritisation. The AutoDock Vina scoring function approximates the free energy of binding through a linear combination of steric, hydrogen bonding, and torsional entropy terms, and has been validated against datasets of hundreds of protein-ligand co-crystal structures, consistently achieving cross-docking RMSD values below 2.0 Å for the majority of test cases (Trott & Olson, 2010).

Pharmacokinetic and toxicological prediction using platforms such as SwissADME and pkCSM has similarly become an integral component of early drug discovery. These tools deploy quantitative structure-property relationship (QSPR) models to help predict various factors that determine the use of the said compound as a drug. They use very large, experimental pharmacokinetic databases to help predict ADMET parameters. They are capable of doing this from molecular structure alone, hence allowing room for rapid identification of

risk associated with the compound and at a very low cost to help strengthen the experimental basis of a study (Daina et al., 2017; Pires et al., 2015).

A lot of natural product-based drug discovery studies aimed at targeting the bacterial resistance mechanisms use integration of docking and ADMET prediction to validate the integrated computational approach. This integrated approach also strengthens this study.

CHAPTER 3

MATERIALS AND METHODS

3.1 Protein Structure Retrieval and Preparation

Three-dimensional (3D) crystal structure of PBP2a from MRSA was retrieved from the RCSB Protein Data Bank (rcsb.org) in PDB format. Two structures were employed: PDB ID 1VQQ (apo form, resolution 1.80 Å) for primary docking experiments, and PDB ID 4CJN (co-crystallised form, resolution 2.20 Å) for re-docking validation (Berman et al., 2000). Protein preparation was performed in AutoDockTools (ADT) version 1.5.7 (Scripps Research Institute). Crystallographic water molecules and non-essential HETATM records were removed. Polar hydrogen atoms were added to the protein which were followed by the addition of Gasteiger partial charges. The protein coordinates that were prepared at the end of this step were then stored as PDBQT format files to attain maximum compatibility with AutoDock Vina.

3.2 Binding Site Identification

Druggable binding pockets on the PBP2a structure were identified using two independent computational tools: CASTp 3.0 (Computed Atlas of Surface Topography of Proteins) and DoGSiteScorer (Tian et al., 2018; Volkamer et al., 2012). Based on consensus pocket prediction and corroboration with the published structural literature, two sites were selected for docking. The transpeptidase active site, centred on the catalytic residue Ser403, with key surrounding residues Lys406 and Thr600. The allosteric site near residue Trp374, whose occupancy is associated with conformational opening of the active site (Fishovitz et al., 2014). Grid boxes of 25 × 25 × 25 Å were defined for each site using the centre-of-mass coordinates of the respective pocket residues.

3.3 Ligand Preparation

The three-dimensional structure of Gedunin (molecular formula $C_{28}H_{34}O_7$; molecular weight (MW): 482.55 gram per mol was retrieved from the PubChem Compound Database in SDF format (Kim et al., 2021). The PubChem CID for Gedunin was 122767. Structural conversion and energy minimisation were performed using Open Babel version 3.1.1 with the MMFF94 force field (O'Boyle et al., 2011). Rotatable bonds were assigned automatically in AutoDockTools, and Gasteiger charges were computed. The prepared ligand was saved in PDBQT format. Reference compounds that were used included oxacillin and ceftaroline. The PubChem CID for oxacillin 6196 and for ceftaroline is 16007562. These were also prepared using a similar workflow for the comparative docking studies.

3.4 Molecular Docking Studies

AutoDock Vina is a powerful tool to perform MD studies. We used the version 1.1.2 to perform all docking studies (Trott & Olson, 2010). The exhaustive parameter for docking was set at 16, which generated a total of ten docking poses for each compound-site combination. All the compounds that are Gedunin, oxacillin, and ceftaroline were docked with both the active and the allosteric site of the PBP2a protein. We had ensured that all the conditions for docking were kept the same so as to obtain fair results. Comparative analysis of the binding

affinity was done using a graph. It was observed that the more negative the value obtained, the stronger the binding. Further analysis was performed using the most negative value obtained.

3.5 Re-Docking Validation

To validate the docking protocol, the co-crystallised ligand was extracted from PDB structure 4CJN and re-docked into the prepared apo-form receptor under identical conditions. The RMSD value or the root mean square deviation obtained for the original crystallographic ligand and the outcome after docking was calculated using the PyMOL version 2.5 (Schrödinger LLC). An RMSD threshold of 2.0 Å was applied as the criterion for protocol validity, consistent with established standards in the field (Warren et al., 2006).

3.6 Protein-Ligand Interaction Analysis

Protein-ligand interactions for the best-ranked Gedunin pose were characterised by employing use of a a Protein-Ligand Interaction Profiler webserver, which identifies and quantifies hydrogen bonds, hydrophobic contacts, pi-stacking interactions, pi-cation interactions, and salt bridges with precise bond geometry parameters (Salentin et al., 2015). The 2D diagrams for the schematic interaction were generated using LigPlot+ version 2.2 (EMBL-EBI) (Laskowski & Swindells, 2011). 3D visualisations of binding poses and protein surface representations were rendered in PyMOL version 2.5.

3.7 ADMET Analysis

Drug-likeness and pharmacokinetic profiling of Gedunin and reference compounds were evaluated using two established web-based platforms. SwissADME was used to predict physicochemical properties, lipophilicity, water solubility, and adherence with Lipinski's Rule of Five (Daina et al., 2017). The ADMET parameters, along with the intestinal permeability (Caco-2), the blood-brain barrier (BBB) penetration ability, the CYP450 isoform inhibition action, the hERG cardiotoxicity, and the AMES mutagenicity, were assessed using pkCSM (Pires et al., 2015).

CHAPTER 4

RESULTS

1. Re-Docking Validation

To confirm the reliability of the docking protocol before the primary experiments, QNZ (co-crystallised ligand) extracted from PDB structure 4CJN was re-docked into the prepared PBP2a receptor. The pose after re-docking achieved an RMSD value of 0.537 Å with respect to the original crystallographic binding mode, which is below the 2.0 Å threshold accepted as indicative of a valid docking protocol. The superimposition of the re-docked and crystal poses demonstrated high positional concordance with retention of critical interactions at the active site (Figure 1).

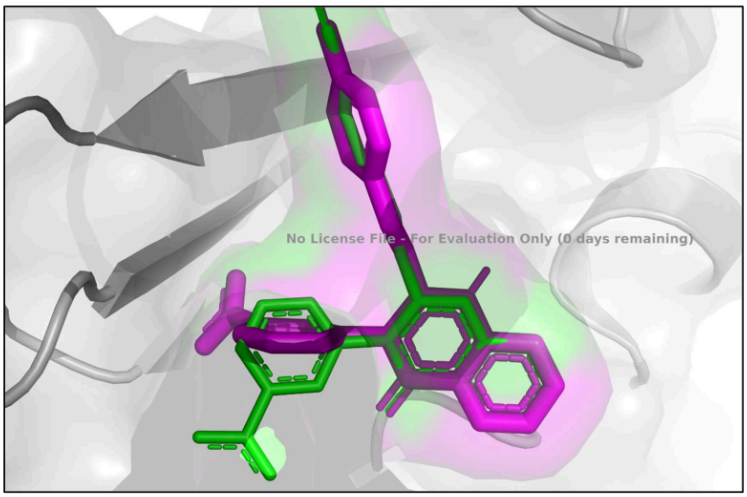

Figure 1. Re-docking validation of the molecular docking protocol. The co-crystallised ligand extracted from PDB 4CJN (green sticks) is superimposed with the top-ranked re-docked pose (purple sticks) within the active site of PBP2a protein (grey cartoon).

2. Binding Affinity of Gedunin Against PBP2a

Gedunin demonstrated a favourable predicted binding affinity against both the active centre and the allosteric centre of PBP2a. At the active centre, which was centred on Ser403, the best obtained docking pose yielded an affinity of binding at -6.935 kilo calorie per mol, with a mean affinity of -6.935 kcal/mol across ten poses, indicating consistent convergence. The allosteric site is centred on the residue Trp374. At this allosteric site, a binding affinity of -6.912 kcal/mol was achieved by Gedunin with a mean affinity of -6.656 kcal/mol across ten poses. Comparative analysis shows that the binding affinity of gedunin is stronger at the active site than at the allosteric site. This inference results in understanding that Gedunin can interact

preferentially with the active centres of PBP2a and maintain protein function using the allosteric centre.

Table 1. Predicted binding affinities (AutoDock Vina) of Gedunin, oxacillin, and ceftaroline against the active site and allosteric site of PBP2a.

Compound	**Binding Site**	**Best Affinity (kcal/mol)**	**Mean Affinity (kcal/mol)**
Gedunin	Active site (Ser403)	-6.935	-6.607
Gedunin	Allosteric site (Trp374)	-6.912	-6.656
Oxacillin	Active site	-7.455	-6.618
Oxacillin	Allosteric site	-7.377	-6.773
Ceftaroline	Active site	-7.48	-6.777
Ceftaroline	Allosteric site	-8.652	-7.526

3. Comparative Docking with Reference Compounds

The binding affinity of Gedunin and other compounds, including oxacillin and ceftaroline, which are potential binders to PBP2a after docking under a set of identical conditions, is all summarised in Table 1.

Gedunin demonstrated slightly weaker docking affinity than oxacillin at PBP2a's active and allosteric site. The comparable interaction profile observed for gedunin may still indicate its ability to associate with functionally relevant regions of PBP2a, given the known reduced effectiveness of oxacillin against the protein's conformationally restricted active site (Figure 2).

In comparison with ceftaroline, Gedunin exhibited lower binding affinity at both binding pockets, particularly at the allosteric site. Nevertheless, the ability of gedunin to dock within the allosteric region suggests a possible interaction with regulatory residues of PBP2a. This, however remains a subject of further experimental validation.

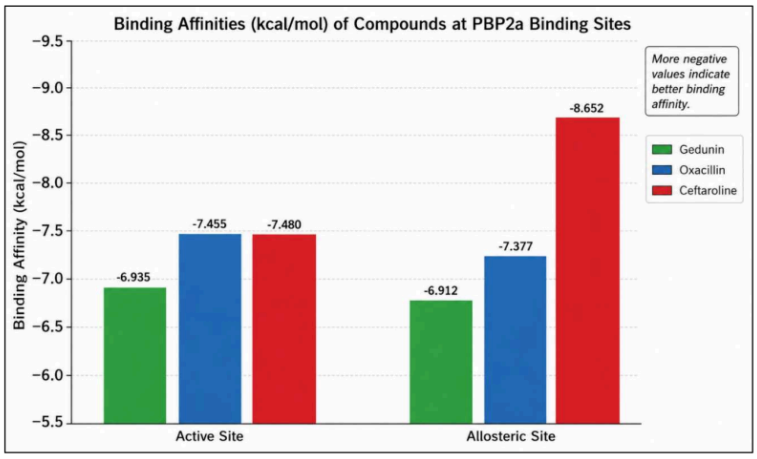

Figure 2. Comparative binding affinities of Gedunin, oxacillin, and ceftaroline against the PBP2a active site and allosteric site as predicted by AutoDock Vina. Values represent the best-ranked pose for each compound-site combination. The more negative the values obtained are, the stronger the predicted binding affinity of the compound is.

4. Analysis of the Protein-Ligand Interaction.

PLIP was used to perform an elaborative analysis for the top-ranked outcome obtained after docking Gedunin with the PBP2a active site. The analysis resulted in the revelation of 5 key interactions, which comprise hydrogen bonds (1), hydrophobic bonds (3) and π-stacking interactions (1) (Table 2). Residue ASN146B of PBP2a was associated with the formation of a hydrogen bond with Gedunin, about 2.27Å. Residues THR308A, ILE309A, and ASN146B were associated with the formation of the hydrophobic interactions, which are necessary for stabilisation of the ligand in the binding cavity. In addition, a π-stacking interaction with TRP205B further enhanced binding stability. These interactions are illustrated in the three-dimensional binding pose (Figure 3) and the two-dimensional schematic interaction map (Figure 4).

Table 2. Protein-ligand interactions between Gedunin and PBP2a active-site residues, as identified by PLIP. Bond distances are given in Angstroms (Å).

Residue	**Interaction Type**	**Bond Distance (Å)**	**Atom (Gedunin)**	**Atom (Residue)**
ASN146B	Hydrogen bond	2.91	O2	ND2
ASN146B	Hydrophobic contact	3.46	C22	Side-chain carbon atoms

THR308A	Hydrophobic contact	3.41	C14	Side-chain carbon atoms
ILE309A	Hydrophobic contact	3.69	C28	Side-chain carbon atoms
TRP205B	π-Stacking interaction	4.25	Aromatic ring atoms (23,24,25,26,33)	Indole ring of TRP205B

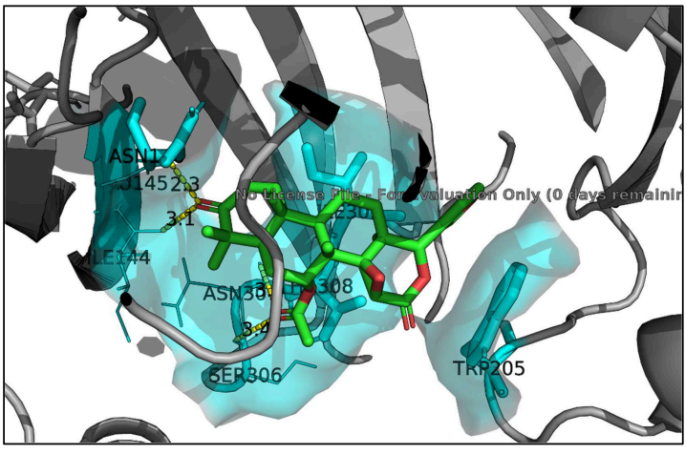

Figure 3. Three-dimensional binding pose of Gedunin within the active site of PBP2a. Gedunin is shown as green sticks within the cyan semi-transparent binding pocket surface, the backbone of the protein is represented by the grey cartoon. Key residues that show interactions, include ASN223, ILE144, ASN308, SER306, and TRP205 are labelled. Hydrogen bond interactions are depicted as yellow dashed lines with bond distances indicated in Å. The figure was rendered using PyMOL version 2.5.

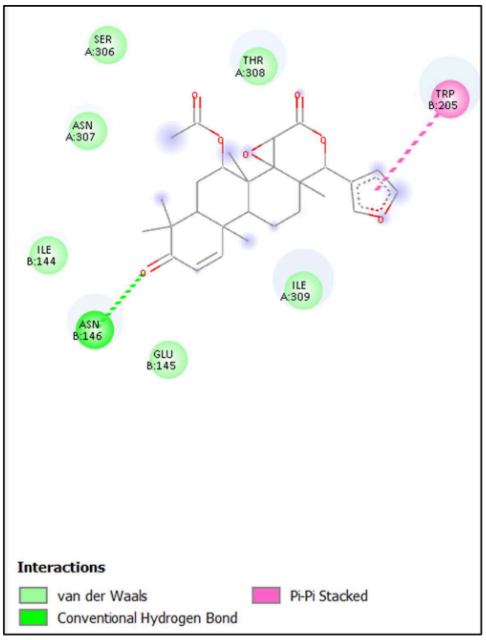

Figure 4. Two-dimensional schematic diagram showing the binding interactions between Gedunin and active-site residues of PBP2a, generated using Discovery Studio Visualizer. Conventional hydrogen bonds are shown using the dashed green lines, π–π stacking interactions as magenta dashed lines, and van der Waals contacts as light green circles.

PLIP was used to perform the analysis for the top-ranked outputs obtained after docking Gedunin with the allosteric site of PBP2a. Further, analysis revealed a total of 4 key interactions, which comprise hydrophobic bonds (3) and salt bridge interactions (1) (Table 3). Hydrophobic interactions were observed with LYS318A, ASP320A, and LYS322A, with interaction distances ranging from 3.57–3.93 Å, contributing to stabilisation of the ligand within the allosteric binding cavity. In addition, Gedunin formed a salt bridge interaction with LYS317A (5.29 Å), which may further enhance electrostatic stabilisation of the ligand–protein complex. These interactions are illustrated in the three-dimensional binding pose (Figure 5) and the two-dimensional schematic interaction map (Figure 6).

Table 3. Protein-ligand interactions between Gedunin and PBP2a allosteric-site residues, as identified by PLIP. Bond distances are given in Angstroms (Å).

Residue	Interaction Type	Bond Distance (Å)	Atom (Gedunin)	Atom (Residue)
LYS318A	Hydrophobic contact	3.69	Atom 24	Side-chain carbon atoms
ASP320A	Hydrophobic contact	3.93	Atom 8	Side-chain carbon atoms
LYS322A	Hydrophobic contact	3.47	Atom 22	Side-chain carbon atoms
LYS317A	Salt bridge	5.29	Carboxylate atoms (30,31)	NZ atom of Lys317

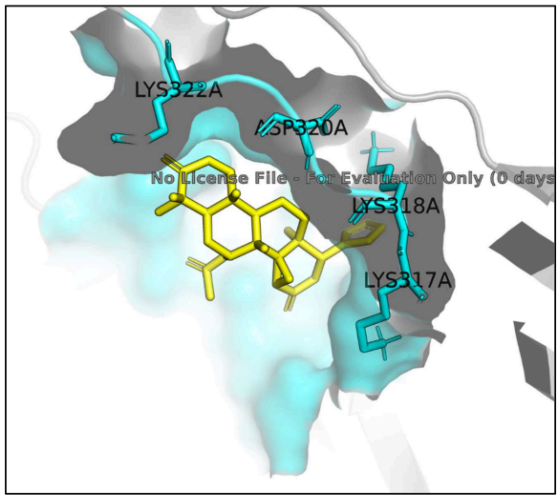

Figure 5. Three-dimensional binding pose of Gedunin within the allosteric site of PBP2a. Gedunin is shown as yellow sticks within the cyan semi-transparent binding pocket surface, while the protein backbone is represented as a grey cartoon. Key interacting residues including ASN223, ILE144, ASN308, SER306, and TRP205 are labelled. The figure was rendered using PyMOL version 2.5.

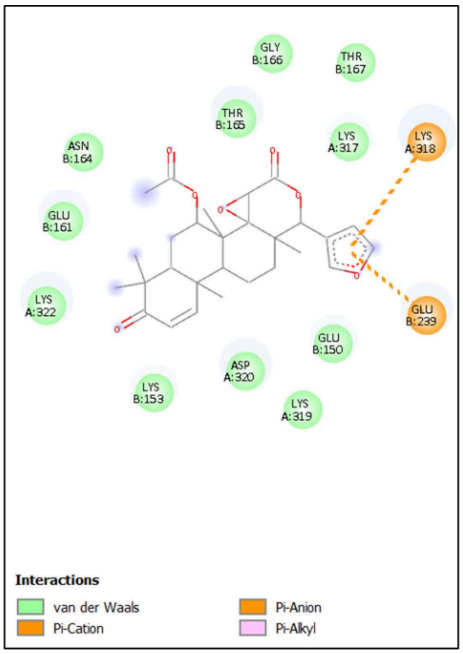

Figure 6. Two-dimensional schematic diagram showing the binding interactions between Gedunin and allosteric-site residues of PBP2a, generated using Discovery Studio Visualizer. π-anion and π -cation interactions appear orange and van der Waals contacts as light green circles.

5. ADMET Pharmacokinetic Profile

The physicochemical and pharmacokinetic properties of Gedunin, as predicted by SwissADME and pkCSM, are presented in Tables 3 and 4 alongside the reference compounds. Gedunin has a Mol. Weight of 482.55 grams per mol, which falls within the commonly cited drug-like range (< 500 g/mol) according to the Lipinski's Rule of Five. Gedunin exhibited no violations in the Lipinski Rule, indicating favourable likeness for the drug and suitability for oral administration. The observed GI absorption rate for gedunin was high. This is supported by acceptable levels of aqueous solubility and moderate lipophilicity of the molecule. The ability to penetrate the BBB was absent, thus ensuring very limited exposure to parts of the nervous system. The analysis also showed that Gedunin does not inhibit the key isoforms like CYP450, which ensures that there is no drug-drug interaction. Additionally, gedunin was seen to be a non-substrate for P-glycoproteins, which ensures high intracellular retention.

Concerns arose when three Brenk alerts were also observed with respect to functional groups, they rise problems regarding chemical reactivity or metabolic instability of the compound in the body. The bioavailability score of Gedunin was predicted to be 0.55, indicating moderate oral bioavailability potential.

Table 3. Comparative physicochemical properties of Gedunin, oxacillin, and ceftaroline as predicted by SwissADME.

Property	**Gedunin**	**Oxacillin**	**Ceftaroline**
M. W. (g/mol)	482.55	401.44	684.74
LogP	2.50	2.41	2.30
H-bond Donors	0	2	4
H-bond Acceptors	6	6	17
TPSA (Å²)	86.74	138.04	330
Rotatable Bonds	2	4	10
Absorption Rates in the Gastrointestinal Tract	High	High	Low
Blood Brain Barrier Permeability	No	No	No
Substrate (P-gp)	No	Yes	Yes
CYP3A4 Inhibition	No	No	No
CYP2D6 Inhibition	No	No	No
Lipinski Violations	0	0	3
Water Solubility	Soluble / Moderately soluble	Soluble	Highly soluble
Score for Bioavailability	0.55	0.55	0.17
Alerts of PAINS	0	0	0
Brenk Alerts	3	1	2
Lead-likeness	No	Yes	No
Synthetic Accessibility	4.39	4.01	6.12

ADMET profiling indicated that Gedunin possesses favourable pharmacokinetic and safety properties (Table 4). Gedunin was predicted to be non-mutagenic with low hERG toxicity risk, although moderate oral toxicity was observed. These findings overall support its potential as a promising therapeutic candidate (Figure 5).

Table 4. Comparative ADMET properties of Gedunin, oxacillin, and ceftaroline as predicted by pkCSM.

ADMET Parameter	**Gedunin**	**Oxacillin**	**Ceftaroline**
Caco-2 Permeability	High permeability predicted	Moderate permeability	Low permeability
BBB Penetration	No	No	No
CYP450 Inhibition	No inhibition of CYP3A4/CYP2D6	Minimal inhibition predicted	Minimal inhibition predicted
hERG Toxicity	Low risk	Low risk	Low risk
AMES Toxicity	Negative (non-mutagenic)	Negative	Negative
Oral LD50 (mg/kg)	Moderate toxicity	Low toxicity	Low toxicity

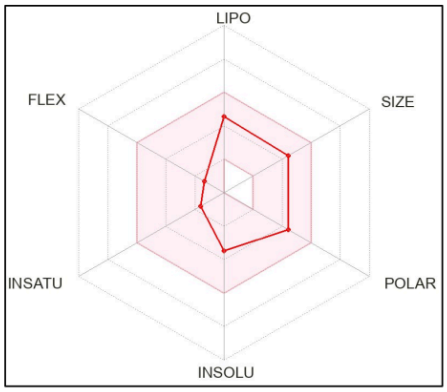

Figure 5. SwissADME bioavailability radar of Gedunin showing predicted drug-likeness properties including lipophilicity, size, polarity, flexibility, insolubility, and saturation. The pink region represents the optimal physicochemical space for oral bioavailability.

CHAPTER 5

DISCUSSION AND CONCLUSION

An increase in cases of antimicrobial resistance has been an emerging threat to human health. It is very important to find solutions that help overcome this problem. Ancient Indian knowledge systems have shown evidence of plants producing compounds that can be used as potential inhibitors of increased resistance in microorganisms. Our study explored the potential of Gedunin, a compound that is extracted from Azadirachta indica, for its potential in inhibiting resistance in MRSA. PBP2a in MRSA is responsible for conferring resistance against methicillin. Gedunin is explored as an inhibitor of PBP2a in this study.

We have used molecular docking studies to study interactions between the active and the allosteric sites of the PBP2a receptor. Analysis resulted in the establishment of the fact that Gedunin alters the normal functioning of the protein within the microorganism. Since PBP2a is responsible for the reduced efficiency of the β-lactam antibiotics in MRSA, the affinity of Gedunin for binding along this protein is important for the development of new therapeutic strategies against resistant bacterial infections.

Before the main docking experiments, a test docking protocol was validated through re-docking of the co-crystallised ligand. We kept the acceptable threshold limit for the RMSD value at 2.0 Å. After the re-docking was completed, the resulting RMSD value obtained was 0.537 Å, which was well within the limit. This value indicated that the procedure undertaken to perform molecular docking was accurate.

In the study, molecular docking analysis was performed, which showed that gedunin had favourable binding affinity for both the active and allosteric sites of the PBP2a protein. However, more prominent binding was seen at the active site. Although its docking scores were lower than those of oxacillin and ceftaroline, Gedunin was able to demonstrate meaningful interactions with residues located within functionally important regions of the protein. Protein–ligand interaction analysis has further supported the docking results.

Gedunin formed hydrogen bonds, hydrophobic interactions, π-stacking interactions, and salt bridge interactions with several amino acid residues within the binding pockets. These interactions with residues are important because they contribute to the stability of the ligand–protein complex. Residues THR308A, ILE309A, and ASN146B were associated with the formation of the hydrophobic interactions, which are necessary for stabilisation of the ligand in the binding cavity. π-stacking interaction with TRP205B further enhanced binding stability. Similarly, hydrophobic interactions observed with residue LYS318A, ASP320A, and LYS322A contribute to the stabilisation of the ligand within the allosteric binding cavity. In addition, Gedunin formed a salt bridge interaction with residue LYS317A, which enhances electrostatic stabilisation of the ligand–protein complex.

Our study also performs ADMET screening of Gedunin to ensure its use as a potential inhibitor. We observed that the compound fulfilled all necessary criteria, such as following Lipinski's rule of five and reported no violations. Compound also shows great solubility, high absorption rates in the GI track and low BBB permeability. It also shows no inhibitory action towards any of the cytochrome enzymes and has very low cardiotoxicity levels. The compound also does

not violate AMES test. However, three Brenk alerts were also observed with respect to functional groups, these rise problems regarding chemical reactivity or metabolic instability of the compound in the body.

Overall, the findings suggest that Gedunin possesses promising characteristics as a natural-product scaffold targeting PBP2a. However, this procedure has certain drawbacks, as it only takes the theoretical prediction into consideration for the ligand binding. It does not take into consideration the flexibility of the protein or the various conformational changes that occur under physiological conditions. In addition, the results obtained are computational predictions and therefore show a need for experimental confirmation, which can be obtained using in vitro antibacterial assays, MIC studies, enzyme inhibition experiments, and molecular dynamics simulations.

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