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Molecular Docking Analysis of Compounds in *Coleus blumei* Leaves as Potential Antibiotics Against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT: *This study investigated the potential of phytochemical compounds from Coleus blumei leaves as anti-Methicillin-Resistant Staphylococcus aureus (MRSA) candidates through a validated molecular docking and absorption-distribution-metabolism-excretion-toxicology (ADMET)-based screening approach. The ligand structures were energy-minimized using MMFF94 followed by PM7 to obtain stable conformers prior to docking. Protein targets consisted of PBP2a and the extracellular antibiotic-sensing domain of MecR1 (benzylpenicilloyl- and oxacilloyl-acylated forms), both of which play central roles in the mecA-mediated resistance pathway of MRSA. Docking simulations were performed using AutoDock Vina v1.2.7 and validated through redocking of co-crystallized ligands, yielding RMSD values <2.0 Å. Among the eight tested compounds, Hexahydro-3H-1[2'-trifluoromethyl]-6'[4''-trifluoromethylphenyl]- exhibited the strongest binding affinities, surpassing reference β-lactam antibiotics and demonstrating key interactions with residues involved in allosteric activation of PBP2a and ligand sensing in MecR1. ADMET predictions further supported its drug-likeness and safety profile, although experimental confirmation remains necessary. These findings highlight this compound as a promising anti-MRSA lead candidate and underscore the need for subsequent in vitro and in vivo evaluations to substantiate its mechanistic and therapeutic potential.*

Keywords: *ADMET; Coleus blumei; MecR1; molecular docking; MRSA; PBP2a*

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1. Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the primary pathogens of concern in nosocomial infections in hospitals. The disease spreads rapidly among patients, resulting in improper handling and treatment due to the lack of effective therapies. MRSA is capable of resisting conventional antibiotics due to its evolutionary adaptation and expression of penicillin-binding protein (PBP2a), which has a very low affinity for β-lactam antibiotics[1]. Nosocomial infections occur due to various medical interventions such as the use of medical devices, gradual antibiotic administration, surgeries, and post-operative procedures[2]. This infection continues to increase and spread worldwide, adding urgency to finding effective therapeutic solutions.

MRSA exhibits high resistance to antibiotics due to the mecA gene encoding PBP2a. This resistance allows the continued biosynthesis of peptidoglycan even in the presence of β-lactam antibiotics, enabling the bacteria to survive. This condition underscores the need for research to discover effective drugs, including the exploration of natural compounds in plants that have potential as antibiotics [3].

The selection of PBP2a and MecR1 as docking targets is based on their central and complementary roles in mediating methicillin resistance in *Staphylococcus aureus*. PBP2a, encoded by the mecA gene, possesses a uniquely low affinity for β-lactam antibiotics and is responsible for maintaining transpeptidase activity even in the presence of inhibitory drug concentrations, thereby enabling continued cell-wall biosynthesis under antibiotic pressure [1,3,4]. This makes PBP2a the defining molecular hallmark of MRSA and a critical target for structure-based inhibitor design. In parallel, MecR1 functions as a membrane-bound sensor–transducer that detects β-lactam antibiotics and subsequently activates the expression of mecA through regulated proteolysis of its cognate repressor MecI. The extracellular sensor domain of MecR1 initiates this signaling cascade upon antibiotic binding, ultimately triggering PBP2a overexpression [1,4,5]. Targeting MecR1 therefore offers an upstream strategy to prevent mecA induction entirely. Combining both targets, one functioning as the primary resistance effector (PBP2a) and the other as the regulatory activator (MecR1), provides a comprehensive approach for inhibiting MRSA resistance mechanisms at both effector and regulatory levels, thereby increasing the likelihood of identifying compounds capable of restoring β-lactam susceptibility.

Previous research has extensively explored various plants as sources of antibacterial compounds, but studies on the effectiveness of *Coleus blumei* leaves against MRSA remain limited. Some studies have shown the potential of compounds in plants as antibacterial agents, but in-depth research focusing on the molecular interaction mechanisms of compounds from *Coleus blumei* leaves against MRSA protein targets is still scarce [6]. This indicates a research gap that needs to be addressed through more comprehensive and evidence-based studies.

Coleus blumei leaves originate from Southeast Asia and have spread to various parts of the world, including Indonesia. This plant is known to contain bioactive compounds that have potential therapeutic properties [7-9]. Given the high level of resistance of MRSA to conventional antibiotics, research on the potential of compounds from *Coleus blumei* leaves as alternative antibiotics is crucial. In addition, the in silico approach, particularly molecular docking, provides advantages in efficiently predicting and visualizing compound interactions with protein targets, thus reducing the need for animal testing [10-13].

Research on the use of in silico methods in molecular interaction analysis has shown promising results in drug discovery. This method allows for the identification and evaluation of interaction mechanisms between ligands and receptors, facilitating the discovery of drugs more quickly and efficiently [14]. Several studies have utilized molecular docking to investigate interactions between small molecules and target proteins, in-

cluding in the context of discovering antibacterial drugs [14-16].

In this study, field-based evidence indicates that MRSA infections remain a significant issue in hospitals, particularly in developing countries where infection control is often suboptimal. Epidemiological data shows an increasing incidence of MRSA across various healthcare facilities, emphasizing the urgent need for effective therapies [2,9,17,18]. Furthermore, clinical reports and laboratory surveys indicate that MRSA bacteria continue to develop new resistance mechanisms [1], reinforcing novel approaches in antibiotic discovery.

This study aimed to explore the potential of compounds found in *Coleus blumei* leaves as antibiotics against MRSA using a molecular docking approach. By employing in silico methods, this research predicted the interactions and affinities of *Coleus blumei* leaf compounds with MRSA target proteins, specifically the benzylpenicilloyl-acylated mecR1 extracellular antibiotic-sensor domain, mutant PBP2a, and oxacilloyl-acylated mecR1 extracellular antibiotic-sensor domain.The study intends to fill existing research gaps, provide strong scientific evidence regarding the potential of *Coleus blumei* leaves as a source of new antibiotics, and lay the groundwork for further research and development of effective therapies against MRSA infections.

2. Materials and methods

The study employs various computational tools and chemical databases to support the molecular docking analysis. The active compounds of *Coleus blumei* were identified through a systematic search of phytochemical and cheminformatics databases. The retrieval process used targeted keywords such as "*Coleus blumei* leaf compounds," "*Coleus blumei* phytoconstituents," "*Coleus blumei* secondary metabolites," and "*Coleus blumei* GC–MS profile." Searches were conducted across multiple databases, including PubChem, ChEBI, NPASS (Natural Products Atlas), and previously published phytochemical profiling studies. Each compound was confirmed by cross-checking structural identity, IUPAC nomenclature, and molecular formula. For compounds unavailable in 3D formats, the structures were drawn manually using ChemDraw and subsequently optimized prior to docking. This approach ensures that the selection of *Coleus blumei* constituents is traceable, reproducible, and based on verifiable digital chemical repositories. The three-dimensional (3D) structures of the target proteins PBP2a and mecR1, associated with methicillin-resistant *Staphylococcus aureus* (MRSA), were retrieved from the Protein Data Bank (PDB). Molecular docking simulations were performed using AutoDock Vina version 1.2.7, the latest stable release provided by the Scripps Research Molecular Modeling Laboratory, which incorporates improved scoring functions, enhanced local search algorithms, and expanded support for modern ligand file formats. This version was selected to ensure high accuracy and reproducibility of docking results in accordance with current computational chemistry standards. All docking runs were executed using the default Vina scoring function with an exhaustiveness level adjusted to optimize binding pose prediction. The selection of this updated version aligns with recommended practices for contemporary structure-based drug discovery workflows.

The active compounds analyzed in this study were derived from *Coleus blumei*, a medicinal plant known for its bioactive secondary metabolites. The phytochemical constituents of *Coleus blumei* were obtained from literature studies and phytochemical databases, ensuring the selection of well-characterized compounds with reported biological activity. The structural integrity of these compounds was confirmed using cheminformatics databases before being subjected to molecular docking analysis.

2.1. Method development and validation

2.1.1. Molecular docking simulation

The molecular docking workflow began with the preparation of ligand (active compound) and protein structures. The 3D structures of the active compounds were either downloaded from PubChem or manually drawn and optimized using ChemDraw. Prior to molecular docking, all ligand structures obtained from PubChem or manually constructed in ChemDraw were subjected to computational geometry optimization to ensure that each compound adopted its lowest-energy conformation. The optimization was performed using a two-stage protocol. First, a molecular mechanics (MMFF94) force field–based minimization was applied to remove steric clashes and stabilize the initial geometry. Subsequently, the structures were refined using a semi-empirical quantum chemical method (PM7) to obtain a more accurate representation of electronic distribution and bond orientation relevant to ligand–protein interactions. Only the fully energy-minimized ligand conformers were used in the docking simulations, ensuring reliability and reproducibility in predicting binding affinities.

The protein structures used as docking targets consisted of Penicillin-Binding Protein 2a (PBP2a) and the extracellular antibiotic-sensor domain of MecR1, both of which play central roles in methicillin resistance in *Staphylococcus aureus*. The crystallographic structure of PBP2a was obtained from PDB ID 1VQQ, originating from *S. aureus* strain COL, a widely studied MRSA reference strain. The MecR1 extracellular sensor domain structures were retrieved from PDB IDs 2IWC and 2IWD, derived from *S. aureus* strain N315 [3,19]. Each protein structure was evaluated for missing residues, sequence gaps, and inconsistent geometry using PDBFixer, followed by local structural relaxation based on the OpenMM molecular mechanics framework [20]. Subsequent optimization steps, including removal of crystallographic water molecules, addition of hydrogen atoms, and protonation-state refinement, were performed using UCSF Chimera to ensure structural readiness for docking simulations.

AutoDock Vina was used for molecular docking, where grid boxes were set to encompass the active site of the target proteins. Default docking parameters were adjusted to ensure reliable binding affinity predictions. The docking scores (binding energies) were recorded, and the best docking poses were selected for further analysis.

2.1.2. Validation of docking results

To ensure the reliability of the docking protocol, a validation step was performed using a redocking approach in which the co-crystallized ligands present in the original PDB structures were extracted and re-docked into their respective binding sites. For PBP2a (PDB ID: 1VQQ), the native β-lactam–derived ligand was re-docked, yielding a root-mean-square deviation (RMSD) value of <2.0 Å between the docked pose and the experimentally observed crystallographic conformation, indicating high accuracy in pose prediction. A similar validation procedure was conducted for the MecR1 extracellular sensor domain using the ligands from PDB IDs 2IWC and 2IWD, which also resulted in RMSD values within acceptable thresholds for validated docking workflows. Additionally, reference antibiotics such as penicillin and oxacillin were docked as positive controls to verify appropriate ranking of binding affinities relative to the test compounds. These validation outcomes collectively confirm that the docking protocol used in this study is robust, reproducible, and suitable for predicting ligand–protein interactions with acceptable reliability.

2.1.3. ADMET analysis

To further characterize the pharmacokinetic and toxicity profiles of the active compounds from *Coleus blumei*, an in silico ADMET analysis was performed. The evaluation included absorption parameters (intestinal permeability, human oral bioavailability, and Caco-2 permeability), distribution properties (plasma protein binding and predicted blood–brain barrier penetration),

metabolism profiling based on cytochrome P450 isoform interactions, excretion predictions (total clearance and renal elimination potential), and toxicity assessment (hepatotoxicity, AMES mutagenicity, and LD_{50} estimation). The ADMET predictions were generated using freely accessible web-based platforms such as SwissADME, pkCSM, and admetSAR, which are widely utilized in early-phase drug discovery. Only compounds that fulfilled favorable ADMET characteristics, including non-mutagenicity, non-hepatotoxicity, acceptable oral absorption, and absence of major CYP inhibition, were selected for subsequent docking analysis.

2.2. Data analysis

The docking results were analyzed by evaluating key interaction parameters, including binding energy, hydrogen bonding, hydrophobic interactions, and other molecular interactions. PyMOL and Discovery Studio were used to visualize binding orientations and identify crucial ligand-protein interactions. The binding energy values of each compound were compared, and those with the highest affinities were identified as potential candidates for MRSA inhibition.

3. Results and discussion

The evaluation of compounds from *Coleus blumei* leaves as antibiotics against MRSA was conducted using the molecular docking method. The results provide deep insights into the interaction between active compounds from *Coleus blumei* leaves and MRSA target proteins.

The process of docking macromolecules with ligands utilized AutoDock Vina within the PyRx software. At this stage, both the proteins and ligands had been optimized, with proteins optimized using Discovery Studio and ligands using Open Babel within the PyRx application. Subsequently, docking was performed by inputting proteins and ligands into the Vina Wizard, followed by forward and global optimum search using local search algorithms. The grid box volumes used for the proteins with the protein codes 1VQQ were (x= 1.64, y=36.8, z=47.9), for 2IWC are (x= 27.9439, y=6.38, z=87.19), and for 2IWD are (x=28.07, y= 6.12, z= 85.9).

Based on the entire docking process conducted, the results from Table 1 indicate that out of the eight test compounds docked with the three target proteins, one compound shows potential as an antibiotic against MRSA. Hexahydro-3H-1[2'-trifluoromethyl]-6'[4''-trifluoromethylphenyl]- demonstrated the lowest binding free energy among all tested compounds, indicating a stronger predicted affinity and a more stable ligand–protein interaction compared with the other phytoconstituents [21,22].

To provide biological context for the binding affinity results, the docking scores of the *Coleus blumei* compounds were compared to clinically relevant reference ligands known to interact with MRSA resistance proteins. For PBP2a, the native β-lactam–derived ligand in PDB 1VQQ and reference antibiotics such as penicillin and oxacillin typically exhibit binding energies ranging from –6.0 to –8.5 kcal/mol in validated docking simulations [3,4]. The test compound Hexahydro-3H-1[2'-trifluoromethyl]-6'[4''-trifluoromethylphenyl]- displayed a binding affinity of –9.0 kcal/mol, which is stronger than the values commonly achieved by standard β-lactams, suggesting that this compound may bind more favorably to the PBP2a allosteric site.

For the MecR1 sensor domain, penicillin and oxacillin showed docking scores around –8.0 kcal/mol, consistent with their roles as induction ligands in the mecA regulatory pathway. The comparable or slightly superior affinity of the test compound (–8.3 and –8.1 kcal/mol for the benzylpenicilloyl- and oxacilloyl-acylated MecR1 structures, respectively) indicates that it may competitively interfere with β-lactam sensing, thereby preventing MecR1 activation and subsequent mecA transcription. These results suggest that the compounds binding affinity values fall within biologically meaningful thresholds for

Table 1. Binding affinity of *Coleus blumei* leaf compounds with target proteins

Macromolecules	Ligand of *Coleus blumei* leaf compounds	Binding affinity (kcal/mol))
Protein mutant penicillin-binding Protein 2a	***Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]-***	**-9.0**
	2-methyl thiophene	-3.5
	Z)-3-heptadecen-5-yne	-4.1
	Octadecane	-5.0
	Dotriacontan	-5.2
	Hexadecahydro-pyrene	**-7.4**
	Aristolone	-6.8
	Triacontane	-4.8
Benzylpenicilloyl-acylated mecR1 extracelluler antibiotic-sensor domain	*Pnm C_{16} H_{20} N_2 O_4 S (Penicillin)*	-8.0
	Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]-	**-8.3**
	2-methyl thiophene	-3.1
	Z)-3-heptadecen-5-yne	-4.1
	Octadecane	-3.8
	Dotriacontan	-4.0
	Hexadecahydro-pyrene	-5.9
	Aristolone	-6.1
	Triacontane	-4.2
Oxacilloyl-acylated mecR1 extracellular antibiotic-sensor	***2r,4s)-5,5-Dimethyl-2-[(1r)-1-{[(5-Methyl-3-Phenyl-1,2-Oxazol-4-Yl)Carbonyl]Amino}-2-Oxoethyl]-1,3-Thiazolidine-4-Carb Oxylic Acid***	-8.0
	Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]-	**-8.1**
	2-methyl thiophene	-2.9
	Z)-3-heptadecen-5-yne	-3.6
	Octadecane	-3.8
	Dotriacontan	-3.8
	Hexadecahydro-pyrene	-6.3
	Aristolone	-6.0
	Triacontane	-3.8

ligand–protein interactions in MRSA resistance mechanisms, supporting its potential as an antibacterial candidate worthy of further investigation.

The visualization of the interaction between the ligand Hexahydro-3H-1[2'-trifluoromethyl]-6'[4"-trifluoromethylphenyl]- and the three MRSA target proteins is depicted in Figures 1, 2, and 3. The visualization of ligand-protein interactions provides a graphical representation illustrating how a small molecule (ligand) interacts with a protein at the molecular level. These visualizations demonstrate how the active compound Hexahydro-3H-1[2'-trifluoromethyl]-6'[4"-trifluoromethylphenyl]- interacts with the MRSA target proteins, specifically PBP2a or MecR1. Such visualizations clarify the binding mechanisms and the potential effects of the compound in inhibiting the function of the target proteins, which ultimately may address antibiotic resistance me-

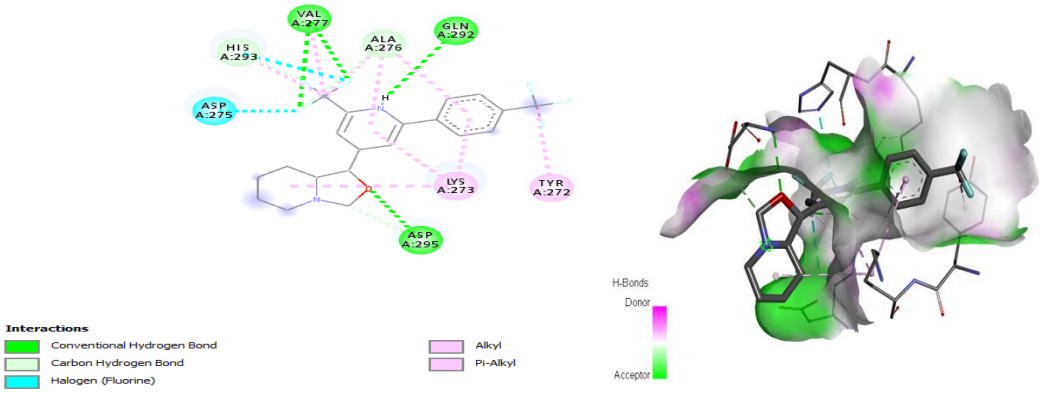

Figure 1. 2D and 3D visualization of the interaction of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- with the mutant penicillin-binding protein 2a (PBP2a or PBP2')

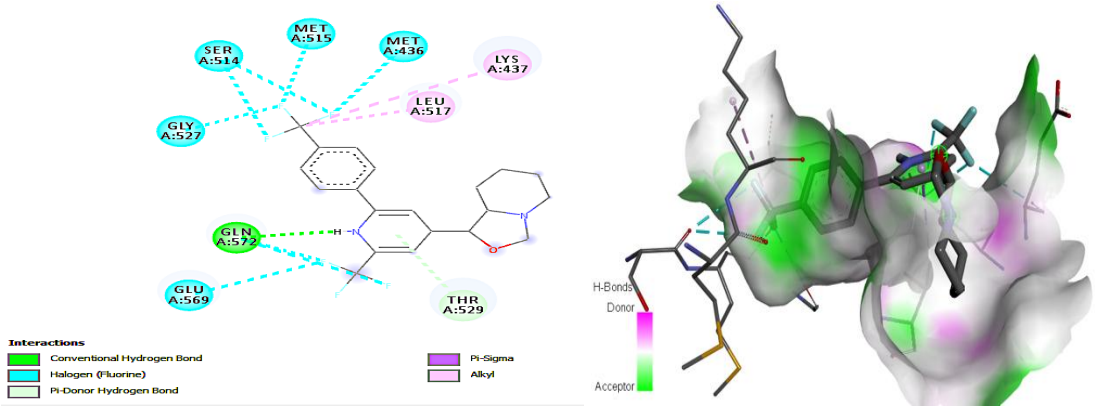

Figure 2. 2D and 3D visualization of the interactions of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- with Benzylpenicilloyl-acylated mecR1 extracellular antibiotic-sensor domain

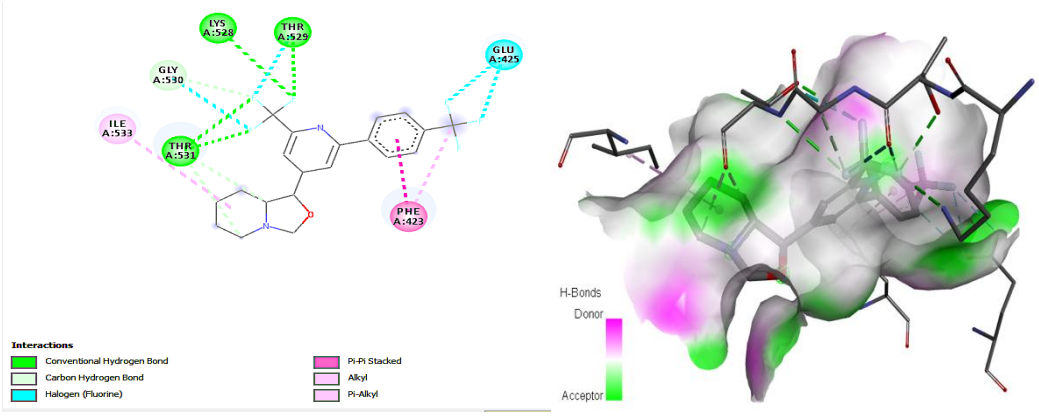

Figure 3. 2D and 3D visualization of the interactions of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- with Oxacilloyl-acylated mecR1 extracellular antibiotic-sensor

diated by MRSA. These visualizations were generated using software such as PyMOL and Discovery Studio, capable of modelling and predicting molecular interactions based on structural data.

Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- binds to the mutant penicillin-binding protein 2a (PBP2a or PBP2) with hydrogen bonding occurring between the ligand and receptor macromolecule residues (Figure 1). Specifically, conventional hydrogen bonds form

with VAL A:277 (Valine) and ASP A:295 (Aspartic Acid), as well as carbon-hydrogen bonds with ALA A:276 (Alanine) and HIS A:293 (Histidine). Hydrophobic bonds observed in the figure involve LYS A:273 (Lysine) and ALA A:276 (Alanine), facilitating hydrophobic interactions. Additionally, hydrogen bonds are evident where GLN A:292 (Glutamine) and ASP A:295 (Aspartic Acid) act as donors, while HIS A:293 (Histidine), ASP A:275 (Aspartic Acid), and ALA A:276 (Alanine) serve as halogen acceptors.

The docking and interaction results of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- with the Benzylpenicilloyl-acylated mecR1 extracellular antibiotic-sensor domain mecR1 indicate hydrogen bonding between the ligand and the receptor macromolecule residues (Figure 2). Specifically, hydrogen bonding occurs with GLN A:572 (Glutamine), involving conventional hydrogen bonds, and THR A:529. Additionally, hydrophobic interactions are observed with LYS A:437 (Lysine) and LEU A:517 (Leucine). The figure also illustrates halogen atoms (Fluorine) at amino acid residues MET A:436 (Methionine), SER A:514 (Serine), MET A:515 (Methionine), GLY A:527 (Glycine), GLU A:569 (Glutamic Acid), GLN A:572 (Glutamine), and THR A:529 (Threonine).

The docking and interaction results of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- with the Oxacilloyl-acylated MecR1 extracellular antibiotic-sensor show hydrogen bonding (Figure 3). Specifically, hydrogen bonds occur with LYS A:528 (Lysine), THR A:529 (Threonine), and THR A:531 (Threonine), involving conventional hydrogen bonds, and GLY A:530 (Glycine) with carbon-hydrogen bonds. Hydrophobic interactions in the figure involve PHE A:432 and ILE A:535. GLU A:425 and GLY A:530 act as halogen acceptors.

From the eight test ligands, Hexahydro-3H-1[2'-trifluoromethyl]-6'[4''-trifluoromethylphenyl]- showed favorable physicochemical characteristics that align with the general principles of drug-likeness, including an acceptable molecular weight and appropriate numbers of hydrogen bond donors and acceptors. These basic parameters suggest that the compound possesses preliminary suitability for further consideration in early drug discovery phases.

Although the Lipinski rule-of-five provides useful preliminary guidance for assessing the drug-likeness and absorption potential of small molecules, its application must be interpreted with caution, particularly for antibiotic candidates. Lipinski parameters do not predict biological activity, target engagement, metabolic stability, or suitability for non-oral routes, and the rule itself is known to be less applicable to antimicrobial agents, which often violate one or more of its criteria while remaining clinically effective. Therefore, the favorable Lipinski profile observed for Hexahydro-3H-1[2'-trifluoromethyl]-6'[4''-trifluoromethylphenyl]- should be considered only as preliminary support for its potential developability, not as confirmation of pharmacological efficacy. Experimental validations including in vitro antibacterial assays, membrane permeability studies, cytotoxicity testing, and in vivo pharmacokinetic profiling were required to determine whether the compound can achieve biologically relevant concentrations at the site of infection and exert meaningful inhibition of MRSA. Incorporating these considerations provides a more balanced interpretation of Lipinski's rule in the context of early-phase antibiotic discovery.

The interactions between the test compound and the key amino acid residues of the MRSA proteins demonstrate mechanistic relevance that extends beyond simple ligand positioning. In PBP2a, interactions involving residues such as Val277, Asp295, and His293 are noteworthy because these residues lie adjacent to the allosteric communication pathway that regulates conformational changes leading to the opening of the PBP2a active site. Stabilization of these residues through hydrogen bonding and hydrophobic contacts may promote allosteric activation, thereby exposing the normally shielded catalytic Ser403

residue, a mechanism consistent with previous studies showing that allosteric ligands can sensitize PBP2a to β-lactam antibiotics [1]. This provides a plausible structural explanation for how the compound may counteract PBP2a-mediated resistance.

For the MecR1 extracellular sensor domain, interactions with residues such as Gln572, Thr529, Lys437, and Leu517 are functionally significant because these residues participate in the β-lactam recognition pathway that initiates the MecI degradation cascade. Binding at these positions may sterically or electrostatically interfere with antibiotic sensing, preventing conformational activation of MecR1 and thereby inhibiting downstream mecA transcription. This mechanism aligns with the established role of the MecR1 sensor loop in detecting β-lactam inducers [4,23].

Similarly, in the oxacilloyl-acylated MecR1 structure, interactions with Lys528, Thr531, Gly530, Phe432, and Ile535 occur within regions implicated in ligand-induced activation. Stabilizing or blocking these residues can disrupt signal transduction required for MecI cleavage, maintaining repression of mecA and ultimately preventing PBP2a overexpression. Collectively, these residue-level interactions highlight that the compound does not merely bind strongly, but binds in structurally strategic regions capable of modulating MRSA resistance pathways at both the regulatory and effector protein levels.

Based on the docking results between the eight test ligands and the target proteins Mutant Penicillin-binding protein 2a, Oxacilloyl-acylated mecR1 extracellular antibiotic-sensor, and Benzylpenicilloyl-acylated mecR1 extracellular antibiotic-sensor domain, it was found that the ligand with potential as a candidate antibiotic against Methicillin-Resistant *Staphylococcus aureus* (MRSA) was Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl].

The mechanism of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]-in addressing resistance can be understood from the molecular docking results of this ligand with the mecR1 protein. The mecR1 protein is a membrane sensor protein that detects the presence of β-lactams, acting as inducers for mecA gene activation, which encodes the synthesis of PBP2a. The activation of the mecA gene is suppressed by mecI as a repressor that binds to the promoter site and blocks mecA transcription [5,24].

Based on the visualization of docking results, it is known that the ligand Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- binds to the active site of the MecR1 protein. This binding leads to the inactivation of mecR1 through a competitive inhibition mechanism, allowing mecI to remain bound to the promoter and block the transcription of the MecA gene for PBP2a protein synthesis. The mechanism of MRSA resistance and the role of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- in overcoming resistance.

The analysis conducted indicates that Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- compound binds to the active site of the MecR1 protein. This binding results in the inactivation of MecR1 through a competitive inhibition mechanism, allowing MecI to remain bound to the promoter and block the transcription of the mecA gene for the synthesis of PBP2a protein. The mechanism of MRSA resistance and the role of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- in overcoming this resistance can be explained through several steps: β-lactam is detected by the penicillin-binding domain (PBD) on MecR1; the autolysis activity of the metalloprotein domain (MPD) on MecR1 is triggered and mediates the lysis of MecI; disruption of the cell wall fragment occurs due to β-lactam acting as a co-activator that binds to MecI and interferes with MecI binding to the operator, thereby promoting MecI proteolysis; degradation of MecI leads to transcription of mecA and expression of the PBP2a protein. When the Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- ligand binds to the active site of MecR1, the sensor activity of MecR1

is disrupted, preventing the lysis of MecI. As a result, MecI remains bound to the operator and blocks the transcription of mecA for the synthesis of PBP2a.

On the other hand, protein PBP2a is an inducible protein that regulates resistance to methicillin. Based on the visualization results of Hexahydro-3H-1[2'trifluoromethyl]-6'[4"-trifluoromethylphenyl]- binding to PBP2a protein, it is known that the ligand does not bind to the active site of the PBP2a protein but rather to an allosteric site. According to previous research, the crystal structure of the PBP2a protein reveals a closed conformation of the active site due to transpeptidase activity. This structure does not provide access for β-lactam antibiotics to reach the catalytic site Ser403, thereby allowing cell wall synthesis to proceed [1,4]. When the ligand binds to the allosteric site, the conformation of the target protein changes, opening up the catalytic site residue Ser403, enabling antibiotics to reach the catalytic site and inhibit the synthesis of the *Staphylococcus aureus* cell wall [25].

Further evidence supports that the active compounds from *Coleus blumei* have potential as new therapeutic agents against MRSA. This study provides a strong foundation for further development and clinical applications. This potential is supported by previous research indicating that compounds with strong binding affinity to MRSA protein targets can be effective drug candidates [26,27].

Although these in silico results showed promising potential, further steps were necessary for experimental validation. In vitro and in vivo validation are crucial to confirm the biological activity of these compounds, evaluate efficacy, and assess safety in more complex biological systems. The molecular docking method used in this study, while effective, has limitations such as dependence on the quality of the protein and compound three-dimensional structures, as well as assumptions made in the docking model that may not fully reflect real biological condition [15,28].

This research also highlights the importance of a multi-target approach in addressing antibiotic resistance. Compounds that can target more than one resistance mechanism may provide a more effective solution and reduce the likelihood of further resistance development. This strategy aligns with recent trends in drug design that emphasize multi-targeted therapies for infectious diseases [11,29].

In a broader context, this study adds evidence supporting the exploration of natural compounds as a potential source for developing new drugs. Medicinal plants like *Coleus blumei* offer a variety of bioactive compounds that can be further explored for therapeutic applications. This trend also reflects the increasing interest in safer and sustainable natural-based treatments [30,31].

While the docking simulations provide a plausible structural rationale for how Hexahydro-3H-1[2'-trifluoromethyl]-6'[4''-trifluoromethylphenyl]-might modulate the MecR1–MecI–mecA regulatory pathway and interact with the allosteric site of PBP2a, these findings represent computational predictions that require empirical verification. The proposed mechanism namely, competitive interference with MecR1 sensor activation and potential allosteric modulation of PBP2a, should therefore be considered hypothetical until supported by experimental data. To substantiate this model, targeted in vitro assays such as β-lactam induction tests, MecR1–MecI proteolysis assays, and PBP2a allosteric activation studies would be necessary to determine whether the compound indeed disrupts regulatory signaling or enhances accessibility to the catalytic Ser403 residue. Furthermore, in vivo infection models, PBP2a expression profiling, and pharmacokinetic evaluations would be essential to confirm whether the compound can achieve effective concentrations at physiological sites of infection and exert measurable antibacterial effects. Integrating these experimental approaches is crucial for validating the mechanistic predictions derived from the in-silico results and determining the true therapeutic potential of the compound.

4. Conclusions

This study identified Hexahydro-3H-1[2′-trifluoromethyl]-6′[4′′-trifluoromethylphenyl]- as the most promising compound from *Coleus blumei* leaves based on its favorable binding affinities toward both the PBP2a and MecR1 proteins, which are central components of the MRSA resistance pathway. The compound demonstrated stronger predicted interactions than reference β-lactam antibiotics and exhibited binding patterns consistent with potential allosteric modulation of PBP2a as well as competitive interference with MecR1 sensor activation.

However, these results remain predictive and require comprehensive biological evaluation. Future research should include in vitro antibacterial assays, MecR1–MecI regulatory pathway studies, PBP2a allosteric activation tests, and cytotoxicity screening to determine whether the compound exhibits measurable biological activity. Additionally, in vivo pharmacokinetic and efficacy assessments, including infection-model experiments, are essential to evaluate its therapeutic feasibility. Key challenges include the extraction or synthesis of sufficient compound quantities, confirming its stability under physiological conditions, evaluating potential off-target effects, and ensuring adequate membrane permeability to reach intracellular or periplasmic targets in MRSA cells.

Overall, the present findings provide a structural and mechanistic foundation for the development of *Coleus blumei* derived molecules as potential anti-MRSA agents. The integration of computational predictions with targeted experimental validation will be critical to advancing this compound toward preclinical evaluation.

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