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Exploring Oxygenated Sesquiterpenes from Merauke Agarwood as PBP1a and PBP3 Inhibitors in *Acinetobacter baumanii:* An *In-Silico* Approach

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Abstract: *Acinetobacter baumannii* is a multidrug-resistant pathogen causing severe nosocomial infections, primarily associated with alterations in penicillin-binding proteins (PBPs) that reduce the effectiveness of β- lactam antibiotics. Despite increasing interest in plant-derived bioactive compounds, the potential of oxygenated sesquiterpenes from *Aquilaria malaccensis* as inhibitors of PBPs remains underexplored, indicating a clear research gap. This study aimed to evaluate the antibacterial potential of three oxygenated sesquiterpenes—Agaruspirol, Eudesmol, and Sinenofuranol—against PBP1a (3UE3) and PBP3 (3UDF). A systematic literature review (SLR) integrated with an in silico approach was conducted, including ligand retrieval from PubChem, ADMET prediction using ADMETlab 3.0, molecular docking using CB-Dock2, interaction analysis with UCSF ChimeraX and PLIP, and antibacterial activity prediction using PASS Online. The results showed that all compounds satisfied Lipinski's rule of five and exhibited favorable ADMET profiles. Molecular docking demonstrated stable interactions with both PBPs, dominated by hydrogen bonding and hydrophobic interactions. Agaruspirol exhibited the strongest binding affinity toward PBP3 (–6.9 kcal/mol) and PBP1a (–6.5 kcal/mol), along with the highest predicted antibacterial activity (Pa = 0.364; Pi = 0.039). These findings suggest that oxygenated sesquiterpenes, particularly agaruspirol, have potential as lead compounds for the development of antibacterial agents targeting PBP-mediated resistance in *A. baumannii*, and provide a scientific basis for further in vitro and in vivo validation.

Keywords: *Acinetobacter baumanii*; Agarwood Merauke; Molecular docking oxygenated sesquiterpenes; PBP

Introduction

Acinetobacter baumannii is a Gram-negative, strictly aerobic, non-motile coccobacillus belonging to the family Moraxellace (Harding et al., 2018). Morphologically, it appears as a short, pleomorphic rod that forms smooth, opaque colonies on standard nutrient media (Shamsizadeh et al., 2017). The bacterium is oxidase-negative, catalase-positive, and remarkably resistant to desiccation, allowing it to persist for long periods on hospital surfaces and medical equipment (Tsioutis et al., 2016). This environmental resilience contributes to its role as an opportunistic pathogen in healthcare settings. It is frequently associated with infections of the respiratory tract, bloodstream, urinary tract, and wounds, especially among immunocompromised or critically ill patients (Jang et al., 2024).

Globally, *A. baumannii* is recognized by the World Health Organization (WHO) as part of the ESKAPE group—bacterial pathogens responsible for the majority of hospital-acquired infections and noted for their extensive multidrug resistance (Shamsizadeh et al., 2017). Recent epidemiological studies report that *A. baumannii* causes approximately 20 percent of ventilator-associated pneumonia and up to 15 percent of

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bloodstream infections in intensive care units (Tsioutis et al., 2016). The prevalence of multidrug-resistant (MDR) and carbapenem-resistant *A. baumannii* (CRAB) has increased dramatically, exceeding 60 percent in Southeast Asia and reaching as high as 70 to 90 percent resistance to imipenem and meropenem in several Indonesian hospitals (Suwantarat & Carroll, 2016), including those in Java and Papua. These findings underscore the urgent need for new antibacterial agents targeting essential bacterial pathways.

Penicillin-binding proteins (PBPs) play crucial roles in bacterial cell wall biosynthesis and are major targets of β-lactam antibiotics (Jang et al., 2024). Among them, PBP1a (PDB ID: 3UE3) and PBP3 (PDB ID: 3UDF) have distinct yet complementary functions. PBP1a acts as a bifunctional transglycosylase and transpeptidase involved in cell wall elongation, while PBP3 mediates the formation of septal peptidoglycan during bacterial cell division (Solanki et al., 2023). Inhibition of these enzymes compromises cell wall integrity, leading to bacterial lysis(Boll et al., 2016). Simultaneous inhibition of both PBP1a and PBP3 has been suggested to enhance antibacterial activity and delay the emergence of resistance, making these enzymes attractive dual targets for the development of new antibacterial agents against *A. baumannii* (Sauvage & Terrak, 2016). Several in silico and experimental studies have explored β-lactam antibiotics and synthetic inhibitors targeting PBPs; however, investigations focusing on plant-derived compounds as dual inhibitors of PBP1a and PBP3 in A. baumannii remain limited, particularly those derived from Indonesian biodiversity.

Natural products are a prolific source of structurally diverse compounds with potential pharmacological activities. Agarwood (*Aquilaria malaccensis*), locally known as gaharu, has long been valued for its medicinal, aromatic, and cultural uses throughout Southeast Asia(Gogoi et al., 2023). The species growing in Merauke, Papua, Indonesia, develops under unique ecological and climatic conditions, leading to a distinct phytochemical profile compared with agarwood from other regions (Gogoi et al., 2023). Phytochemical investigations of Merauke agarwood have identified several oxygenated sesquiterpenes, including agaruspirol, eudesmol, and sinenofuranol, which possess antimicrobial, anti-inflammatory, and antioxidant properties(Sen et al., 2017). However, their potential antibacterial activity against *A. baumannii*, particularly through inhibition of PBP1a and PBP3, has not been specifically investigated. Therefore, the novelty of this study lies in evaluating oxygenated sesquiterpenes from *A. malaccensis* (Merauke) as potential dual inhibitors targeting both PBP1a and PBP3 using an integrated in silico approach. This study is important to support the discovery of alternative antibacterial agents against multidrug-resistant *A. baumannii* and to explore the potential of Indonesian natural resources in drug development.

From a theoretical perspective, the escalating multidrug resistance of Acinetobacter baumannii can be explained through antimicrobial selective pressure, in which prolonged exposure to β-lactam antibiotics promotes genetic adaptations such as β-lactamase production, altered porin expression, and structural modification of penicillin-binding proteins (PBPs), ultimately reducing antibiotic binding affinity (Bodea et al., 2022). Inhibition of bacterial cell wall biosynthesis remains a validated therapeutic strategy because PBPs are essential enzymes absent in mammalian cells, ensuring selective toxicity (Veeraraghavan et al., 2025). Simultaneous targeting of elongation-associated PBP1a and septation-associated PBP3 is supported by multi-target drug design theory, which suggests that dual inhibition of complementary enzymatic pathways may enhance bactericidal efficacy and reduce the probability of resistance development (Benkerroum, 2010). Furthermore, natural product-based drug discovery highlights that plant secondary metabolites possess structurally diverse pharmacophores capable of interacting with essential bacterial enzymes (Ahmaed, 2017). Oxygenated sesquiterpenes from Aquilaria malaccensis grown in Merauke may exhibit distinct bioactivity due to ecological adaptation and unique phytochemical profiles shaped by local environmental conditions (Sen et al., 2017). Structure-based drug design using molecular docking provides a rational and cost-effective approach to predict ligand–protein interactions prior to experimental validation, thereby accelerating early-stage antibacterial screening (Yang et al., 2022)

Method

Hardware and Computational Environment

The workflow of the in-silico screening of Merauke Agarwood oil compunds against PBP1a dan PBP3 is shown Figure 1. All computational analyses were conducted on a MacBook Air 12-inch running macOS version 26.1 (Apple Inc., USA). All applications used were open-access web-based platforms; no stand-alone molecular-modeling software installation was required. Visualization of protein–ligand complexes was performed using UCSF ChimeraX version 1.6 (University of California, San Francisco; https://www.cgl.ucsf.edu/chimerax/)(Pettersen et al., 2021).

Ligand Retrieval and Preparation

Three oxygenated sesquiterpenes from *Aquilaria malaccensis* (Merauke origin)—agaruspirol, eudesmol, and sinenofuranol—were selected based on previous

phytochemical reports. The 3-dimensional ligand structures were downloaded in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Each ligand was checked for stereochemical correctness and converted automatically into PDBQT format during the docking process. Prior to docking, the molecular integrity and bond orders were verified to ensure compatibility with AutoDock Vina parameters.

Protein Retrieval and Preparation

The target proteins were the penicillin-binding proteins PBP1a (PDB ID: 3UE3) (Harding et al., 2018) and PBP3 (PDB ID: 3UDF) of *Acinetobacter baumannii*, obtained from the RCSB Protein Data Bank (https://www.rcsb.org). Each protein structure was examined for completeness and prepared by removing co-crystallized ligands, heteroatoms, and water molecules while maintaining the main catalytic residues. Energy minimization was handled internally by the docking server. The cleaned structures were used to evaluate potential ligand interactions at catalytically relevant binding sites.

ADMET and Drug-Likeness Prediction

Pharmacokinetic and toxicity properties were predicted using the ADMETlab 3.0 platform (https://admetmesh.scbdd.com) (Dong et al., 2018). The Simplified Molecular Input Line Entry System (SMILES) for each ligand was uploaded to obtain predicted absorption, distribution, metabolism, excretion, and toxicity parameters. Predicted descriptors included Lipinski's rule of five, topological polar surface area (TPSA), partition coefficient (logP), gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 inhibition, and hepatotoxicity. These data were compared against standard thresholds for drug-like molecules (Ferrari et al., 2021).

Prediction of Biological Activity (PASS Online)

The potential biological activities of the selected sesquiterpenes were predicted using the PASS Online web server (http://www.way2drug.com/PASSOnline/). (Pogodin et al., 2018). The simplified molecular-input line-entry system (SMILES) format of each compound was uploaded to the server, which predicts probable biological activity spectra based on structure–activity relationships using the Multilevel Neighborhoods of Atoms (MNA) descriptors. The results were expressed as Pa (probability of activity) and Pi (probability of inactivity) values for various biological activities. Only predicted activities with Pa > 0.5 were considered significant. The predictions were used to provide complementary support to the molecular docking findings and to identify possible antibacterial-related mechanisms(Lagunin et al., 2000).

Molecular Docking Procedure

Docking simulations were performed using the CB-Dock2 server (https://cadd.labshare.cn/cb-dock2/), which automatically detects possible binding cavities and executes blind docking via the AutoDock Vina engine(Liu et al., 2020). For each receptor–ligand pair, five top-ranked cavities were generated, and docking was run with default exhaustiveness settings. The docking results were scored by binding affinity (ΔG, kcal/mol). The pose with the lowest binding energy and best geometric complementarity was selected for further analysis(Grahl et al., 2021). Each docking experiment was repeated three times to ensure consistency, and the mean binding energy was calculated for comparison among ligands.

Interaction Profiling and Visualization

For characterize non-covalent interactions, the docked complexes were analyzed using the Protein–Ligand Interaction Profiler (PLIP) web server (https://plip-tool.biotec.tu-dresden.de) (Adasme et al., 2021). PLIP automatically identified hydrogen bonds, hydrophobic contacts, π–π interactions, and salt bridges (Schake et al., 2025). The 3-dimensional conformations and interaction geometries were visualized using UCSF ChimeraX 1.6. The visualization enabled inspection of ligand orientation, distances between active residues and ligands, and the location of the docking site relative to known catalytic motifs (SXXK and SXN for PBP1a; KTG and STVK for PBP3).

Figure 1. In Silico Dual-Target Screening Workflow

Result and Discussion

Ligand and Receptor Characterization

The ligands used in this research (**Figure 1**) were three oxygenated sesquiterpenes—Agaruspirol, Eudesmol, and Sinenofuranol—previously identified in the essential oil of *Aquilaria malaccensis* from Merauke, Indonesia. These compounds were selected for their reported antimicrobial potential and structural suitability for molecular docking analysis. The three-dimensional structures were obtained from the PubChem database in SDF format (CID 289964, 21718037, and 91753767, respectively) and automatically converted into docking-ready formats using CB-Dock2.

The three oxygenated sesquiterpenes selected in this study—Agaruspirol, Eudesmol, and Sinenofuranol—represent major bioactive constituents commonly identified in *Aquilaria malaccensis* essential oil(Chhipa & Kaushik, 2017). These compounds possess a sesquiterpenoid carbon skeleton characterized by the presence of oxygenated functional groups such as hydroxyl or ether moieties, which are known to enhance their biological reactivity and polarity(Chhipa & Kaushik, 2017). The presence of these oxygen atoms is predicted to improve their capacity for hydrogen bonding with active-site residues of target enzymes, thereby increasing their likelihood of forming stable protein–ligand complexes(Sen et al., 2017).

Agaruspirol Eudesmol Sinenofuronal

Figure 2. Three-dimensional structures of the oxygenated sesquiterpenes from *Aquilaria malaccensis*

Agaruspirol is a bicyclic oxygenated sesquiterpene with an alcohol and previous studies have demonstrated that Agaruspirol exhibits antimicrobial and anti-inflammatory properties, particularly through the inhibition of microbial membrane integrity and enzyme catalysis(Chhipa & Kaushik, 2017). Eudesmol, a tricyclic sesquiterpene alcohol, which aligns with previous reports describing its strong hydrophobic interactions with membrane-bound or transmembrane proteins. Eudesmol has been widely reported for its antibacterial and antifungal activities, primarily attributed to its hydroxyl group at the C-1 or C-2 position, enabling the formation of hydrogen bonds with enzyme residues and promoting active-site stabilization(Kimura & Sumiyoshi, 2012). Sinenofuranol, on the other hand, contains a furan oxygen bridge that confers higher molecular polarity and planarity compared to other sesquiterpenes. Literature reports have associated furan-containing sesquiterpenes with antibacterial and antioxidant activities due to their electron-rich oxygen atoms, which enable interaction with enzyme residues involved in oxidative stress and peptidoglycan metabolism(Sen et al., 2017). The presence of hydroxyl and furan functional groups enhances their hydrogen-bonding capacity, while their lipophilic skeletons allow sufficient membrane permeability. These findings are consistent with previous reports that oxygenated sesquiterpenes from agarwood possess broad-spectrum antimicrobial potential, reinforcing their suitability as promising lead compounds for antibacterial drug design targeting resistant Gram-negative bacteria (Suwantarat & Carroll, 2016).

The receptor proteins utilized in this study were penicillin-binding protein 1a (PBP1a; PDB ID: 3UE3) and penicillin-binding protein 3 (PBP3; PDB ID: 3UDF) from *Acinetobacter baumannii*. Both belong to the family of high-molecular-weight (HMW) PBPs that play essential roles in the terminal stages of peptidoglycan biosynthesis, particularly in cross-linking the glycan strands that maintain the integrity of the bacterial cell wall(Jang et al., 2024). The structural analysis of these enzymes revealed well-defined domains containing conserved catalytic motifs typical of serine-reactive PBPs, which are also the target sites of β-lactam antibiotics(Boll et al., 2016). The detailed structural characterization of PBP1a and PBP3 underscores their suitability as docking targets for the selected oxygenated sesquiterpenes. The presence of accessible hydrophobic pockets near the catalytic serine residues aligns well with the amphiphilic nature of Agaruspirol, Eudesmol, and Sinenofuranol(Wang et al., 2018). Moreover, the variation in pocket dimensions between PBP1a and PBP3 provides an opportunity to assess differential binding behavior, which could guide the rational design of dual-site inhibitors. These structural insights confirm that the selected receptors faithfully represent the molecular targets involved in β-lactam resistance mechanisms of *A. baumannii* and validate their use in this molecular docking study.

Previous molecular docking investigations have demonstrated that oxygenated terpenoids exhibit favorable binding affinities toward bacterial cell wall–associated enzymes, including penicillin-binding proteins and transpeptidases of Gram-negative pathogens(Pires et al., 2016). Several studies reported that sesquiterpene alcohols such as eudesmol derivatives and other oxygenated terpenoids showed stable hydrogen-bond interactions with catalytic serine residues within β-lactam–binding pockets, supporting their potential as non-β-lactam PBP inhibitors(Chen et al., 2017). In addition, in silico analyses of plant-derived

terpenoids against *Acinetobacter baumannii* PBPs have revealed that compounds containing hydroxyl or heterocyclic oxygen atoms display improved docking scores and interaction stability compared to non-oxygenated hydrocarbons. Experimental investigations have also confirmed that essential oils rich in oxygenated sesquiterpenes exhibit antibacterial activity against multidrug-resistant Gram-negative bacteria, including carbapenem-resistant *A. baumannii*, suggesting that their mechanism may involve interference with peptidoglycan biosynthesis or membrane-associated enzymatic systems(Hamidian & Nigro, 2019). Furthermore, structure-based drug design studies targeting PBP1a and PBP3 have emphasized the importance of identifying alternative scaffolds capable of occupying conserved catalytic regions beyond classical β-lactam structures to overcome resistance(Qin et al., 2024). Collectively, these prior findings provide strong scientific justification for evaluating Agaruspirol, Eudesmol, and Sinenofuranol as potential dual-target inhibitors of PBP1a and PBP3 in *A. baumannii*, thereby reinforcing the rationale and novelty of the present molecular docking investigation.

Table 1. Predicted physicochemical, pharmacokinetic (ADMET), and drug-likeness profiles of Agaruspirol, Eudesmol, and Sinenofuranol obtained from *Aquilaria malaccensis* (Merauke) using ADMETlab 3.0.

Category		Agaruspirol	Eudesmol	Sinenofuranol
Compund Identity	MW (g/mol)	222.2	444.4	224.18
	LogP	3.244	6.494	1.905
	TPSA (Å²)	20.23	40.46	29.46
Drug-likeness	Lipinski's Rule	Accepted	Accepted	Accepted
	QED	0.667	0.427	0.742
	SAscore	Easy	Easy	Easy
	Fsp^3	0.867	0.933	1.0
Absorption	Caco-2 Permeabilty ($\times10^{-6}$ cm/s)	-4.674	-4.786	-5.051
	MDCK Permeability ($\times10^{-6}$ cm/s)	0.0	0.0	0.0
	Pgp-inhitor μM	++	++	-
	HIA (%)	---	---	---
Distribution	PPB (%)	90.8	98.4	42.2
	BBB Permeability	++	++	55.6
	VDss (L/kg)	1.103	0.669	1.115
Metabolism	CYP2C19 inhibitor (μM)	--	---	---
	CYP3A4 subtrate	--	---	---
	CYP2C9 Inhitor	--	-	---
	HLM Stablitiy μL/min/mg	+++	+++	--
Excretion	Cl_{plasma} μL/min/kg	10.991	9.32	10.238
	Half-life (T1/2) (jam)	0.786	0.395	0.976
Toxicity	hERG Blockers (10 mM)	0.302	0.499	0.448
	Human Hepatotoxicity	0.561	0.736	0.619
	Ames Toxicity	0.261	0.142	0.478
	Carcinogenicity	0.617	0.734	0.569
	Neurotoxicity	0.287	0.296	0.31
	Hematotoxicity	0.39	0.408	0.116

Table 2. Summary of docking and PASS Online results of three oxygenated sesquiterpenes on PBP1a and PBP3 of *Acinetobacter baumannii*

Compound	Receptor	Binding Energy (ΔG, kcal/mol)	Key Interacting Amino Acids	Pa	Pi
Agaruspirol	PBP1a	–6.5	Hydrophobic interactions: 71A; 535B Hydrogen Bonds: 509B	0.364	0.039
	PBP3	–6.9	Hydrophobic interaction: 448A, 450A, 539A Hydrogen Bonds: 336A, 528A		
Eudesmol	PBP1a	–6.5	Hydrophobic interactions: 213A, 509B, 514B Hydrogen Bonds: 515B	0.298	0.061
	PBP3	–6.5	Hydrophobic interactions: 213A, 509B, 414B Hydrogen Bonds: 515B		
Sinenofuranol	PBP1a	–6.2	Hydrophobic interactions: 372A, 448A, 450A Hydrogen Bonds: 392A	0.223	0.108
	PBP3	–6.7	Hydrophobic interactions: 448A, 450A, 539A Hydrogen Bonds: 373A, 392A		

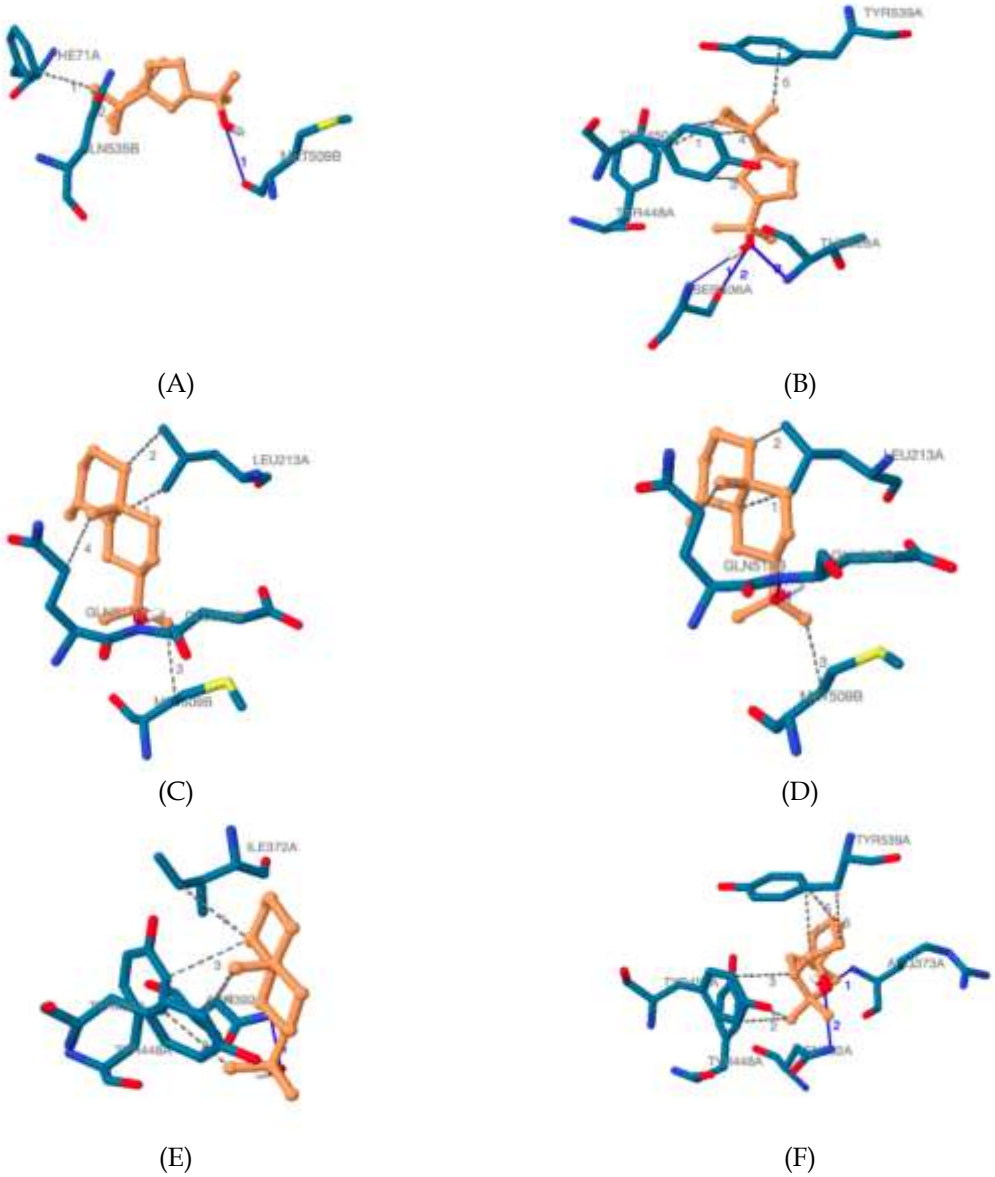

Figure 2. Predicted docking interactions of oxygenated sesquiterpenes with *Acinetobacter baumannii* PBP3 (3UDF, left) and PBP1a (3UE3, right). (A, B) Agaruspirol; (C, D) Eudesmol; (E, F) Sinenofuranol. Hydrogen bonds are represented by dashed lines between the ligands (orange) and the interacting amino acid residues (blue)

ADMET and Pharmacokinetic Properties

The ADMET profiling of Agaruspirol, Eudesmol, and Sinenofuranol was conducted to predict their pharmacokinetic behavior and drug-likeness before docking simulations. The *compound identity* parameters, including molecular weight (MW), LogP, and topological polar surface area (TPSA), indicate that all three ligands comply with the Lipinski's rule of five, suggesting good oral bioavailability(Xiong et al., 2021a). Agaruspirol and Sinenofuranol possess relatively low molecular weights (222.2 and 224.18 g/mol, respectively) and moderate LogP values (3.24 and 1.90), consistent with optimal membrane permeability and solubility balance. In contrast, Eudesmol displays higher molecular weight (444.4 g/mol) and lipophilicity (LogP 6.49), which may enhance hydrophobic interactions within target protein pockets but slightly limit aqueous solubility. The TPSA values below 50 Å² for all compounds support favorable passive diffusion across bacterial membranes.

In terms of *drug-likeness*, all compounds satisfy the Lipinski and Veber criteria, with acceptable quantitative estimate of drug-likeness (QED) values (0.427–0.742) and low synthetic accessibility scores, indicating that these sesquiterpenes are structurally feasible for chemical synthesis and potential drug optimization. The high Fsp^3 ratios (≥0.86) also suggest good three-dimensionality(Xiong et al., 2021b), which is associated

with higher clinical success rates compared to flat aromatic molecules.

Regarding absorption, the predicted Caco-2 and MDCK permeability values suggest moderate to good intestinal absorption potential. Agaruspirol and Eudesmol exhibit positive P-glycoprotein (P-gp) inhibition, indicating possible enhancement of intracellular retention by preventing efflux, while Sinenofuranol, lacking P-gp inhibition, may rely on passive diffusion(Dong et al., 2018). The human intestinal absorption (HIA) predictions indicate favorable uptake for all three ligands, consistent with their small molecular size and moderate polarity.

For distribution, plasma protein binding (PPB) was highest for Eudesmol (98.4%) and Agaruspirol (90.8%), implying strong protein affinity, while Sinenofuranol showed lower PPB (42.2%), suggesting higher free fraction availability in systemic circulation. Blood–brain barrier (BBB) permeability analysis revealed that Agaruspirol and Eudesmol have higher CNS permeability (++), indicating potential central effects, while Sinenofuranol displayed limited BBB penetration. The steady-state volume of distribution (VDss) values were within the acceptable range (0.66–1.11 L/kg), supporting stable tissue distribution.

The metabolic stability results indicate that Agaruspirol and Eudesmol are predicted to be stable in human liver microsomes (HLM), while Sinenofuranol may undergo faster biotransformation. None of the compounds act as substrates or inhibitors of major CYP isoenzymes (CYP2C9, CYP2C19, and CYP3A4), suggesting a low risk of metabolic drug–drug interactions. This stability and metabolic neutrality enhance their pharmacological predictability(Gupta et al., 2013).

Under excretion parameters, all ligands exhibit moderate predicted clearance rates (Cl_plasma 9.3–10.9 µL/min/kg) and short elimination half-lives (<1 hour), implying efficient excretion and low potential for bioaccumulation. Such kinetic behavior is desirable for compounds targeting acute bacterial infections where rapid onset and clearance are preferred(Xiong et al., 2021a).

Toxicity predictions revealed no major red flags. All compounds demonstrated low risk for hERG inhibition (<0.5), indicating low cardiotoxic potential. The predicted hepatotoxicity, carcinogenicity, and neurotoxicity values remained below critical thresholds, suggesting overall safety. Sinenofuranol exhibited the lowest hematotoxicity and carcinogenicity scores, further supporting its safety profile. These results collectively suggest that the three oxygenated sesquiterpenes exhibit favorable pharmacokinetic and toxicity characteristics suitable for antibacterial drug development (Dong et al., 2018).

PASS Online Prediction and Biological Activity Correlation

The biological activity of the three oxygenated sesquiterpenes—Agaruspirol, Eudesmol, and Sinenofuranol—was further predicted using the Prediction of Activity Spectra for Substances (PASS Online) web server (https://www.way2drug.com/PASSOnline). The analysis provided two key probability values: the probability to be active (Pa) and the probability "to be inactive" (Pi) for antibacterial activity. According to the standard interpretation criteria, compounds with *Pa > 0.3* are considered likely to exhibit the corresponding biological activity *in vitro* Among the tested ligands, Agaruspirol showed the highest predicted antibacterial probability (Pa = 0.364; Pi = 0.039), indicating a strong likelihood of exhibiting biological activity against bacterial targets. Eudesmol presented a moderate activity potential (Pa = 0.298; Pi = 0.061), while Sinenofuranol demonstrated lower antibacterial probability (Pa = 0.223; Pi = 0.108). These results suggest that all three compounds possess measurable antibacterial potential, with Agaruspirol being the most promising candidate(Lagunin et al., 2000).

The PASS Online outcomes were in agreement with the molecular docking analysis, which also identified Agaruspirol as the ligand with the strongest binding affinity toward both PBP1a (–6.5 kcal/mol) and PBP3 (–6.9 kcal/mol)(Pogodin et al., 2018). The correlation between higher Pa values and stronger binding energies implies that Agaruspirol's physicochemical features—moderate lipophilicity, presence of hydroxyl functionality, and compact sesquiterpenoid scaffold—enhance its interaction with essential bacterial enzymes. Eudesmol and Sinenofuranol, despite their relatively weaker predicted Pa values, still showed relevant binding profiles within the catalytic transpeptidase domain, supporting their potential as secondary lead compounds(Pogodin et al., 2018). Collectively, the integration of PASS and docking data strengthens the hypothesis that oxygenated sesquiterpenes from Merauke agarwood possess intrinsic antibacterial properties mediated through PBP inhibition**.** This dual computational validation provides a rational basis for further *in vitro* screening of these compounds against multidrug-resistant *A. baumannii* strains.

Molecular Docking Analysis

Molecular docking was conducted to predict the binding affinities and interaction patterns of the three oxygenated sesquiterpenes—Agaruspirol, Eudesmol, and Sinenofuranol—against *Acinetobacter baumannii* penicillin-binding proteins PBP1a (PDB ID: 3UE3) and PBP3 (PDB ID: 3UDF). The docking results revealed that all ligands demonstrated favorable interactions within the active-site pockets of both receptors, forming

hydrophobic contacts and hydrogen bonds with key amino acid residues (Figure 2). The predicted binding energies and main interacting residues are summarized in Table 2.

Agaruspirol exhibited the strongest affinity toward PBP3 with a binding energy of –6.9 kcal/mol, forming hydrophobic interactions with residues 448A, 450A, and 539A, and hydrogen bonds with 336A and 528A. On PBP1a, Agaruspirol showed a binding energy of –6.5 kcal/mol, involving hydrophobic contacts with residues 71A and 535B and a single hydrogen bond with 509B. The high affinity toward PBP3 suggests that Agaruspirol may fit more efficiently into the relatively narrow and flexible active pocket of class B PBPs, stabilizing through hydrophobic stacking and polar interactions near the catalytic serine site(Wang et al., 2018).

Eudesmol displayed comparable binding affinities on both receptors (–6.5 kcal/mol). In PBP1a, Eudesmol established hydrophobic interactions with residues 213A, 509B, and 514B, alongside a hydrogen bond with 515B. In PBP3, Eudesmol interacted hydrophobically with residues 213A, 509B, and 414B and formed a hydrogen bond with 515B. These consistent interactions across both targets suggest that Eudesmol's tricyclic skeleton and hydroxyl moiety favor dual-site binding(*Eudesmol-A Promising Inhibitor for Glucosyltransferase_ Docking and Molecular Dynamics Study.Pdf*, n.d.), possibly allowing flexibility between PBP1a and PBP3 (Sauvage & Terrak, 2016).

Sinenofuranol exhibited slightly weaker interactions with PBP1a (–6.2 kcal/mol) but higher affinity toward PBP3 (–6.7 kcal/mol). On PBP1a, it formed hydrophobic interactions with residues 372A, 448A, and 450A, and a hydrogen bond with 392A. On PBP3, it interacted hydrophobically with 448A, 450A, and 539A and formed hydrogen bonds with 373A and 392A. The furan oxygen bridge likely enhanced its ability to form stable polar interactions(*Proceedings of ICCPBC 2023, Dept. of Zoology, GU.Pdf*, n.d.), particularly within the PBP3 catalytic groove.

The docking analysis indicates that all ligands possess significant binding potential toward both PBP1a and PBP3, with Agaruspirol showing the lowest binding energy and the highest number of stabilizing contacts. The shared interacting residues, particularly 448A and 450A in both PBPs, may represent conserved hydrophobic subregions within the transpeptidase active site(Vandenberg et al., 2020). Such overlap implies a possible dual inhibition mechanism, targeting both the elongation (PBP1a) and division (PBP3) processes of bacterial cell wall synthesis. These findings are consistent with previous reports that oxygenated sesquiterpenes can interact with bacterial transpeptidases and peptidoglycan-related enzymes through hydrogen bonding and hydrophobic insertion(Solanki et al., 2023). The predicted antibacterial probabilities (Pa) from the PASS Online server further supported the docking results: Agaruspirol (Pa 0.364; Pi 0.039) exhibited the highest likelihood of antibacterial activity, followed by Eudesmol (Pa 0.298; Pi 0.061) and Sinenofuranol (Pa 0.223; Pi 0.108). These correlated computational outcomes suggest that Agaruspirol, owing to its optimal lipophilicity and polar balance, may serve as the most promising PBP inhibitor among the three ligands investigated.

These findings are further supported by previous computational and experimental studies investigating terpenoid interactions with bacterial cell wall–associated enzymes. Several molecular docking analyses have demonstrated that oxygenated sesquiterpenes and related terpenoid alcohols exhibit stable binding within the transpeptidase domain of high-molecular-weight PBPs, particularly through hydrogen bonding with catalytic or adjacent residues and hydrophobic accommodation within conserved binding clefts. Studies evaluating plant-derived terpenoids against Gram-negative PBPs reported binding energies ranging between –5.5 to –8.0 kcal/mol, values comparable to those observed in the present study, suggesting biologically relevant interaction strength. In silico screening of natural compounds against *Acinetobacter baumannii* PBPs has also identified hydroxyl-containing terpenoids as favorable scaffolds capable of interacting near the catalytic serine motif (SXXK), stabilizing ligand orientation via polar contacts while maintaining hydrophobic anchoring within the active-site cavity.

Moreover, experimental investigations have shown that essential oils rich in oxygenated sesquiterpenes exhibit inhibitory activity against multidrug-resistant Gram-negative bacteria, including carbapenem-resistant *A. baumannii* (Khameneh et al., 2019). The proposed mechanisms include membrane destabilization, interference with peptidoglycan cross-linking, and inhibition of transpeptidase activity. Importantly, previous structure–activity relationship (SAR) studies indicate that the presence of hydroxyl groups enhances hydrogen bond formation and improves binding stability to enzyme residues, whereas furan or ether bridges contribute to electronic distribution and molecular planarity, facilitating better fit within catalytic grooves(Tan et al., 2021). These structural characteristics align with the interaction patterns observed for Agaruspirol, Eudesmol, and Sinenofuranol in the current docking simulations.

Additionally, comparative docking studies targeting PBP1a and PBP3 have emphasized that compounds capable of interacting with conserved hydrophobic residues adjacent to the catalytic serine demonstrate higher predicted inhibitory potential and may function as non-β-lactam competitive inhibitors.

The identification of shared interacting residues (e.g., 448A and 450A) in both PBPs in this study strengthens the hypothesis of a conserved binding subregion exploitable for dual-target inhibition. Such dual-binding behavior has been proposed as a promising strategy to overcome resistance mechanisms associated with single-site inhibition and β-lactamase-mediated degradation(Mahmoud et al., 2021).

Collectively, the consistency between the binding energies obtained in this study, previously reported docking ranges for natural terpenoids, established SAR principles, and documented antibacterial activity of oxygenated sesquiterpene-rich essential oils provides strong scientific validation of the present findings(Guo et al., 2020). These converging lines of evidence support the potential of Agaruspirol, particularly, as a promising lead compound for further in vitro and in vivo validation as a dual PBP1a–PBP3 inhibitor against multidrug-resistant *Acinetobacter baumannii*.

Conclusion

This *in silico* research demonstrated that oxygenated sesquiterpenes derived from *Aquilaria malaccensis* (Merauke)— Agaruspirol, Eudesmol, and Sinenofuranol—exhibit promising pharmacokinetic and antibacterial potential against *Acinetobacter baumannii* through dual inhibition of penicillin-binding proteins PBP1a (3UE3) and PBP3 (3UDF). The ADMET analysis confirmed favorable drug-likeness, membrane permeability, and safety profiles for all three ligands, with Agaruspirol displaying the most balanced physicochemical characteristics. Molecular docking revealed that all ligands formed stable hydrophobic and hydrogen-bond interactions with key catalytic residues within the transpeptidase domains of both PBPs, with Agaruspirol showing the lowest binding energy (–6.9 kcal/mol) toward PBP3. Furthermore, PASS Online predictions supported these findings, indicating the highest antibacterial activity probability for Agaruspirol (Pa = 0.364; Pi = 0.039). Collectively, these results suggest that Agaruspirol, followed by Eudesmol and Sinenofuranol, could serve as potential lead compounds for the development of novel PBP-targeted antibacterial agents to combat multidrug-resistant *A. baumannii*. Further *in vitro* and *in vivo* validation studies are recommended to confirm these computational insights and explore their therapeutic applications.

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Conflicts of Interest
The authors declare no conflict of interest.

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