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Rational Design of Thiophene-2-Carbaldehyde Modified Petunidin-3-Glucoside as a Novel MurA-Targeted Antibacterial Candidate

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Abstract: *The global rise of antimicrobial resistance highlights the urgent need for novel inhibitors targeting essential bacterial enzymes such as UDP-N-acetylglucosamine enolpyruvyl transferase (MurA). This study investigates the antibacterial potential of Petunidin-3-glucoside, a natural polyphenol isolated from African leaves, and its structural modification with Thiophene-2-carbaldehyde (TC) to enhance MurA inhibition. A validated QSAR model using hydrophobic, electronic, and steric descriptors predicted significantly lower EC_{50} values for TC-modified compounds, with TC–Petunidin-3-glucoside showing the highest predicted potency (EC_{50} = 0.070 μM). Molecular docking revealed strong binding affinity to MurA (⊿G = −8.5 kcal/mol, Ki = 0.689 μM), involving key interactions such as hydrogen bonding, π-anion, and π-sulfur contacts with residues CYS115, ARG120, and ASP305. Additionally, π-alkyl and π-sigma interactions with LEU370, VAL163, and PHE328 stabilized the aromatic moieties within the enzyme's active site. PASS prediction indicated enhanced antibacterial activity and membrane-related mechanisms, with a Pa of 0.910 for membrane integrity agonism. Overall, TC-modified Petunidin-3-glucoside exhibits promising characteristics as a MurA-targeted antibacterial candidate, supporting the rational design of natural product-derived inhibitors to combat antibiotic-resistant pathogens.*

Keywords: Novel antibacterial, structure modification, QSAR, quantum mechanical

Abstrak: Peningkatan resistensi antimikroba secara global menegaskan perlunya pengembangan inhibitor baru yang menargetkan enzim bakteri esensial seperti UDP-N-acetylglucosamine enolpyruvyl transferase (MurA). Penelitian ini mengevaluasi potensi antibakteri Petunidin-3-glukosida, suatu polifenol alami yang diisolasi dari daun Afrika, serta modifikasinya dengan Thiophene-2-carbaldehyde (TC) untuk meningkatkan inhibisi terhadap MurA. Model QSAR tervalidasi dengan deskriptor hidrofobik, elektronik, dan sterik memprediksi nilai EC_{50} yang lebih rendah secara signifikan untuk senyawa termodifikasi, dengan TC–Petunidin-3-glukosida menunjukkan potensi tertinggi (EC_{50} = 0,070 μM). Hasil molecular docking menunjukkan afinitas ikatan kuat terhadap MurA (ΔG = −8,5 kcal/mol; Ki = 0,689 μM), dengan interaksi utama berupa ikatan hidrogen, π-anion, dan π-sulfur pada residu CYS115, ARG120, dan ASP305. Prediksi PASS juga menunjukkan peningkatan aktivitas antibakteri dengan kemungkinan mekanisme yang berkaitan dengan integritas membran (Pa = 0,910). Secara keseluruhan, modifikasi TC pada Petunidin-3-glukosida berpotensi sebagai kandidat antibakteri penarget MurA yang menjanjikan dan mendukung desain rasional inhibitor berbasis produk alami untuk mengatasi resistensi antibiotik.

Kata kunci: HKSA, mekanika kuantum, modifikasi struktur kimia, senyawa antibakteri baru

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1. Introduction

Antibiotic resistance (AMR) has emerged as one of the most pressing global health threats of the 21st century, endangering millions of lives and undermining the efficacy of existing antimicrobial agents. The World Health Organization (WHO) reports that if no sig-

nificant measures are taken, the death toll from AMR could reach approximately 10 million annually by the year 2050, surpassing the current mortality rates attributed to cancer (Kato et al., 2018). This crisis directly impacts the ability of healthcare systems to effectively treat common bacterial infections, resulting not only in prolonged hospital stays and higher medical costs but also in increased morbidity and mortality among patients (Vuk et al., 2020). Conventional antibiotics, which were once deemed potent against a range of bacterial pathogens, have lost their effectiveness due to a host of factors, including misuse, over-prescription, and the intrinsic evolutionary capacity of bacteria to develop resistance mechanisms (Ucar et al., 2024). As a direct consequence, the urgent need for novel therapeutic strategies and the identification of new drug targets has become an international priority, particularly in the realm of bacterial cell wall biosynthesis.

At the core of this endeavor is the MurA enzyme (UDP-N-acetylglucosamine enolpyruvyl transferase), an essential component of the peptidoglycan biosynthesis pathway pivotal for maintaining the structural integrity of bacterial cell walls (Yempala et al., 2015). MurA catalyzes the critical reaction that converts UDP-N-acetylglucosamine and phosphoenolpyruvate to enolpyruvyl-UDP-N-acetylglucosamine, representing the first committed step in peptidoglycan synthesis (Gudim et al., 2024). The inhibition of MurA therefore holds immense potential as a strategic therapeutic option to combat bacterial growth, particularly against Gram-negative pathogens that pose a significant challenge in clinical settings (Wang et al., 2022). Notably, MurA has been validated as an antibacterial target through the success of Fosfomycin, an antibiotic that directly inhibits the enzyme and demonstrates considerable efficacy against resistant bacterial strains (Gudim et al., 2022).

In light of the urgent need for new antimicrobial agents, natural products, particularly plant-derived compounds, have garnered increasing attention as sources of novel therapeutic agents. Flavonoids and anthocyanins, which are prevalent in numerous plant species, possess a myriad of biological activities, including antimicrobial properties (Öztürk et al., 2023)). Within this category, Petunidin-3-Glucoside a prominent anthocyanin found in certain African plant leaves has displayed promising pharmacological activities due to its antioxidant and antimicrobial properties (Moshkina et al., 2020). The structural diversity and complex biological interactions of natural products provide distinct advantages in drug discovery, as they often present mechanisms of action that differ markedly from conventional synthetic drugs (Oduselu et al., 2023). These compounds typically exhibit lower toxicity, reduced side effects, and improved tolerability, making them favorable candidates for further investigation in the realm of antimicrobial therapy.

To enhance the antibacterial efficacy of naturally derived compounds such as Petunidin-3-Glucoside, strategic chemical modifications represent an indispensable approach. The incorporation of functional groups through synthetic modification can lead to significant enhancements in antibacterial activity by altering electronic, hydrophobic, and binding properties of the lead compounds (Sedláček et al., 2017). In this regard, the use of Thiophene-2-Carbaldehyde as a modification strategy aims to capitalize on the inherent advantages of thiophene derivatives, which have previously shown promising biological activities including antibacterial properties (Azzouzi et al., 2023). This molecular alteration is posited to improve the pharmacological profile of Petunidin-3-Glucoside, thereby augmenting its potential efficacy as a MurA enzyme inhibitor. Furthermore, harnessing computational methodologies including Quantitative Structure-Activity Relationship (QSAR) modeling, Prediction of Activity Spectra for Substances (PASS), and molecular docking studies will serve to underpin the rational design of modified compounds, allowing researchers to predict biological activity while minimizing the need for extensive empirical testing.

Despite the promising biological profile of Petunidin-3-Glucoside, knowledge surrounding its chemical modification remains sparse, and few studies have explored the ramifications of such modifications on antibacterial activity and mechanisms of MurA inhibition. This lack of research represents a critical gap, as the structural adjustments needed to optimize efficacy against resistant bacterial strains have not yet been fully realized. Therefore, the primary aim of this study is to investigate the antibacterial potential of Thiophene-2-Carbaldehyde-modified Petunidin-3-Glucoside as a novel MurA enzyme inhibitor. A comprehensive evaluation of its antibacterial activities will be conducted, alongside an investigation into the underlying biochemical mechanisms that facilitate MurA inhibition. This inquiry not only seeks to advance the field of antibiotic drug discovery but also aims to contribute unique insights into the potential of anthocyanin modifications in developing effective therapeutics against AMR.

the modification of natural products, especially through the incorporation of thiophene derivatives, represents a promising avenue for the discovery of novel antibacterial agents. By elucidating the relationship between chemical structure and biological activity, this study seeks to bridge significant knowledge gaps in the field, positioning Thiophene-2-Carbaldehyde-modified Petunidin-3-Glucoside as a potential candidate for addressing the escalating crisis of antibiotic resistance. The outcomes of this research could potentially lead to the development of new therapies that effectively target MurA, laying the groundwork for innovative approaches in the fight against resistant bacterial pathogens

2. Materials and Methods

2.1. Materials

This study employed computational approaches including quantitative structure-activity relationship (QSAR) analysis Kurniawan et al., (2023) and molecular docking simulations. All calculations were performed on a workstation with AMD Ryzen 5 processor, 24 GB RAM, NVIDIA GeForce GTX 1050 graphics card, and Windows 10 Professional 64-bit operating system. The MurA enzyme structure (PDB ID: 1UAE) was retrieved from the RCSB Protein Data Bank. Ligand structures, including Petunidin 3-glucoside (PubChem CID: 443651) and fosfomycin as reference compound, were obtained from PubChem database.

Molecular docking simulations were conducted using AutoDock Vina (The Scripps Research Institute, USA), while molecular visualization and analysis utilized Discovery Studio 3.5 Client and Molecular Operating Environment (MOE) 2015. Statistical analysis was performed with SPSS version 25, and chemical structure handling employed MarvinView software. All protein and ligand structures were prepared in appropriate formats (FASTA, PDB, and PDBQT) following standard preprocessing protocols to ensure computational consistency

2.2. Preparation Ligand, Calculation Mechanical Quantum Descriptor and Equations Validation (Adopted from Kurniawan et al. 2023)

This study's methodology for computing molecular descriptors and constructing the QSAR model was inspired by the work of Kurniawan et al., (2023). The initial stage involved optimizing the compounds' molecular geometries with the MMFF94 force field to achieve their most stable three-dimensional forms. Following this, a series of molecular descriptors were computed to capture three key categories of physicochemical properties for the QSAR investigation. These encompassed hydrophobicity measures like the n-octanol/water partition coefficient (LogP) and aqueous solubility (LogS); electronic characteristics such as the energy of the highest occupied molecular orbital (AM1_HOMO), the energy of the lowest unoccupied molecular orbital (AM1_LUMO), and the dipole moment (AM1_dipole); along with steric parameters, including Van der Waals volume (Vol), hydrophobic solvent-accessible surface area (ASA_H), and molar refractivity (MR). These calculations were carried out using a combination of software tools, including MOE 2015, MarvinView, and other specialized cheminformatics applications.

For the statistical analysis, multiple linear regression (MLR) was implemented in SPSS version 25. The model used the Log EC_{50} values as the response variable and the chosen molecular descriptors as the predictor variables. A number of different regression models were developed and compared to investigate the structure-activity relationship of tannin compounds sourced from African leaves. The most robust model, distinguished by a correlation coefficient (r) exceeding 0.9, was chosen for subsequent validation. To evaluate its predictive reliability and robustness, a Leave-One-Out (LOO) cross-validation procedure was employed. The model's predictive quality was gauged by its cross-validated correlation coefficient (q^2), and it was deemed acceptable after meeting the benchmarks of $r^2 \geq 0.8$ and $q^2 \geq 0.5$, aligning with the criteria outlined by Kurniawan et al., (2023).

2.2. Drug-Likeness Evaluation and Biological Activity Prediction (Filimonov et al., 2014)

The Prediction of Activity Spectra for Substances (PASS) is a well-established bioinformatics tool that forecasts the range of biological properties a drug-like organic molecule might

exhibit, using only its structural formula as input. Its algorithm, trained on extensive biological activity data, provides estimates for numerous pharmacological effects without the need for wet-lab experiments, offering a significant advantage in the initial phases of identifying new drug leads.

For this research, the prediction of biological activities was performed via the publicly accessible PASS online web server (http://way2drug.com/Pass Online/predict.php). This platform allows for the bulk assessment of a compound's potential effects across a wide spectrum, which can include antimicrobial (antibacterial, antifungal), antiviral (such as anti-HIV), psychotropic (like antidepressant), anticancer (e.g., TNF modulator), and contraceptive properties. Integrating PASS analysis into the screening workflow helps scientists focus their efforts on compounds with the highest forecasted activity. This approach streamlines the drug discovery pipeline by minimizing the synthesis and biological evaluation of low-probability candidates, thereby supporting a more efficient and rationally guided design process for potential therapeutics, in line with modern computational pharmacology principles.

2.2. Thiophene-2-Carbaldehyde Modification to Petunidin-3-Glucoside and Molecular Docking Analysis (Kakkar et al., 2018; Kurniawan et al., 2023)

The crystal structure of the target protein, UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), was obtained from the RCSB Protein Data Bank with the PDB accession code 1UAE. The structure was preprocessed using MOE 2019, which included removal of water molecules, heteroatoms, and co-crystallized ligands. Hydrogen atoms were added to the protein structure to reflect correct protonation states at physiological pH, and partial charges were assigned using the AMBER99 force field. The active site of MurA was identified based on the binding pocket of the original ligand present in the PDB structure. A docking grid box was created with dimensions of 20 × 20 × 22 Å centered on the XYZ coordinates of the active site, with a grid spacing of 1.0 Å.

Molecular docking studies were carried out using two software platforms: AutoDock Vina and MOE 2019. Docking in AutoDock Vina was employed to estimate the binding affinity in kcal/mol of each ligand to MurA. The grid box coordinates were set based on the active site region determined previously. Each docking experiment generated multiple binding poses, and the conformation with the lowest binding energy was selected for further analysis. In MOE 2019, docking was carried out using the Triangle Matcher placement method, followed by London dG scoring and GBVI/WSA dG for rescoring and refinement. The docking results were analyzed for ligand-protein interactions such as hydrogen bonding, π–π stacking, hydrophobic interactions, and salt bridges. The visualizations of ligand binding were generated in both 2D and 3D formats using MOE and Chimera for detailed interaction mapping.

3. Results

3.1. Quantitative Structure Activity Relationships, Quantum Mechanical Descriptor Calculation of Original and Modified Structures

The development of the QSAR model and its statistical validation in this study adopted the approach proposed by Kurniawan et al., (2023). In that previous work, a QSAR model was constructed using molecular descriptors representing hydrophobic (LogP, LogS), electronic (AM1_HOMO, AM1_LUMO, AM1_dipole), and steric (mr, ASA_H, vol) properties derived from a training set of natural compounds. The multivariate regression analysis employed a backward elimination approach to determine the most statistically significant model.

The Kurniawan et al. 2023 showed it contained only five descriptors, namely LogP, AM1_HOMO, AM1_dipole, mr, and vol, and satisfied the critical statistical criteria ($r = 0.955$, $r^2 = 0.913$, $F_{calc}/F_{tab} = 1.34$). The final QSAR equation was as follows: LogEC50 = (0.829 x LogP) - (1.302 x AM1_HOMO) - (0.339 x AM1_dipole) - (5.128 x mr) + (0.145 x vol) - (11.355)

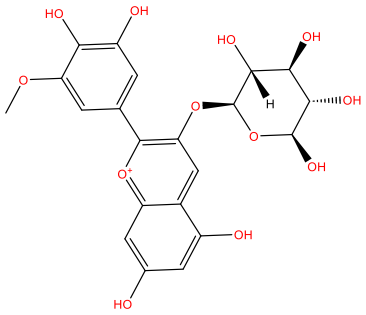
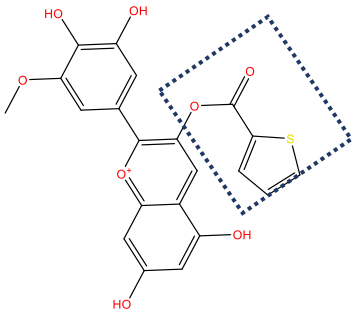
This equation integrates descriptors covering all three main physicochemical domains of QSAR modeling and was subsequently validated using Leave-One-Out (LOO) cross-validation. The LOO cross-validation yielded a q^2 value of 0.504, indicating acceptable model reproducibility and predictive reliability ($q^2 > 0.5$). Following the establishment and validation of the QSAR model, a structure modification strategy was applied to petunidine-3-glucoside

scaffold to improve its predicted biological activity. Several modified analogues were generated through rational design based on the original structure's pharmacophore and reactivity profile.

The addition of Thiophene-2-Carbaldehyde (TC) to anthocyanins such as Petunidin-3-Glucoside may enhance their antibacterial properties (Table 1). This potential modification is especially relevant in the context of addressing antibiotic-resistant bacteria. Several studies have highlighted the inherent antibacterial activity of anthocyanins, indicating that structural modifications, particularly the introduction of electron-withdrawing groups like carbonyls, can improve their efficacy against various bacterial strains. The table illustrates the structural comparison between the original Petunidin-3-glucoside molecule and its Thiophene-2-Carbaldehyde–modified derivative. In the unmodified Petunidin-3-glucoside structure (left), the molecule contains a polyphenolic core typical of anthocyanidin glycosides, characterized by multiple hydroxyl groups (-OH) and a glucose moiety linked through a glycosidic bond. These functional groups are primarily responsible for hydrogen bonding, antioxidant activity, and basic interaction with bacterial enzyme residues.

In the modified compound (right), a thiophene-2-carbaldehyde group has been introduced, as indicated by the blue dashed box. This substitution replaces part of the aromatic moiety with a heterocyclic thiophene ring containing a sulfur atom and an attached formyl (-CHO) functional group. The incorporation of this heterocycle provides enhanced electronic conjugation, increased lipophilicity, and potential π–π stacking and π–sulfur interactions with the active site residues of the target enzyme, MurA (UDP-N-acetylglucosamine enolpyruvyl transferase). This modification is predicted to: Improve enzyme binding affinity, due to the aromatic and sulfur-containing system that can participate in diverse non-covalent interactions such as π-anion or π-sulfur interactions. Enhance membrane permeability, attributed to increased hydrophobicity from the thiophene ring, facilitating passage through bacterial lipid bilayers. And, stabilize ligand conformation, as the thiophene ring introduces planar rigidity beneficial for fitting into the MurA binding pocket.The Thiophene-2-Carbaldehyde modification of Petunidin-3-glucoside represents a rational structural design strategy to improve antibacterial potency through dual enhancement of MurA enzyme inhibition and membrane interaction mechanisms.

Table 1. Incorporation of Thiophene-2-Carbaldehyde into African Leaf-Derived Compounds

Compounds	Origin Structure	Thiophene-2-Carbaldehyde Modified
Petunidine-3- Glucoside		

Description: Molecule inside blue dashed box are modified substituents

Quantum mechanical descriptors of the modified molecules were calculated using the AM1 semi-empirical method within the MOE 2019.0102 platform. Each compound's LogP, AM1_HOMO, AM1_dipole, molar refractivity (mr), and van der Waals volume (vol) were extracted and used as input variables for the validated QSAR model. These predicted values enabled the estimation of the compounds' EC_{50} values, providing a basis for prioritizing potential candidates for further experimental validation (Table 2). The investigation into the effects of Thiophene-2-Carbaldehyde (TC) modification on compounds such as Petunidin-3-glucoside has gained significant attention due to its potential to enhance antibacterial activity. The central hypothesis suggests that TC modification may decrease the half-maximal effective concentration (EC_{50}), indicating improved potency against bacterial strains. This is particularly relevant in the context of escalating global antibiotic resistance, which poses a major

challenge to healthcare systems. This study synthesizes current findings and theoretical insights based on relevant literature.

In light of these observations, further research focusing on the experimental determination of EC_{50} values for TC-modified Petunidin-3-glucoside is warranted to establish a definitive correlation between structural modification and biological efficacy. Future investigations should include comparative analyses with the unmodified parent compound to validate the hypothesized improvements in potency. Moreover, the integration of advanced computational chemistry approaches with biological activity assessments will be essential to refine molecular designs and identify optimal modification patterns for enhanced antimicrobial performance

Table 2. Quantum Mechanical Descriptor Calculation and EC_{50} Prediction using QSAR

Compounds	AM1_ dipole	AM1_ HOMO	LogP	mr	vol	EC_{50} exp*/EC_{50} Predict
Petunidine-3-glucoside	8.802	-11.553	0.078	11.171	399.63	88.025
TC-Petunidine-3- glucoside Modified	6.5257	-9.3950	0.742	9.935	348.35	0.070

3.2. Prediction of Activity Spectra for Novel Substances

In this study, the biological activities of fosfomycin and novel compounds petunidin-3-glucoside were evaluated using PASS prediction analysis. The predicted activities included membrane integrity agonism, antibacterial properties, and membrane permeability inhibition. The results, summarized in Table 3, demonstrate that structural modifications substantially enhanced the biological potential of these natural compounds compared to their unmodified forms.

The PASS prediction results provide insights into the potential biological activities of the tested compounds, focusing on three key parameters: membrane integrity agonism, antibacterial activity, and membrane permeability inhibition. The probability values (Pa) indicate the likelihood of a compound exhibiting a specific activity, while Pi represents the probability of inactivity. Generally, a compound with $Pa > Pi$ and $Pa \geq 0.5$ is considered to have a significant probability of showing the predicted biological activity. The TC-Petunidin-3-glucoside Modified compound demonstrated a markedly higher Pa value (0.878) compared to both Petunidin-3-glucoside (0.293) and Fosfomycin (0.312), along with a very low Pi (0.017). This substantial increase indicates that the thiophene-2-carbaldehyde (TC) modification strongly enhances the compound's ability to stabilize or modulate bacterial membrane integrity. Such enhancement suggests improved interaction between the modified compound and the lipid bilayer or membrane-associated proteins, which may contribute to antimicrobial efficacy through membrane-targeted mechanisms.

For antibacterial potential, the Pa value increased from 0.270 in Petunidin-3-glucoside to 0.472 in the TC-modified analogue, while the Pi decreased from 0.072 to 0.019. This indicates that the structural modification enhances the compound's likelihood of acting as an antibacterial agent. Although Fosfomycin displayed a slightly higher Pa (0.373), the TC-modified derivative exhibits a better balance of activity and selectivity, implying that the introduction of the TC moiety may have optimized its pharmacophoric properties relevant to bacterial inhibition. The TC-Petunidin-3-glucoside Modified compound also showed the highest predicted membrane permeability inhibition (Pa = 0.910, Pi = 0.003), compared to Petunidin-3-glucoside (Pa = 0.510, Pi = 0.142) and Fosfomycin (Pa = 0.383, Pi = 0.211). This result suggests a strong potential for the modified compound to disrupt bacterial membrane permeability, likely preventing essential nutrient exchange and leading to bacterial cell death. The extremely low Pi value reinforces the confidence in this prediction.

Collectively, these results indicate that thiophene-2-carbaldehyde modification significantly enhances the pharmacological potential of Petunidin-3-glucoside. The improvement across all three predicted activities demonstrates that the structural modification not only increases antibacterial potency but also reinforces mechanisms associated with membrane interaction and disruption. Hence, TC-Petunidin-3-glucoside Modified emerges as a promising lead compound for further investigation as a MurA-targeted antibacterial agent with dual membrane and enzyme inhibitory effects.

Furthermore, TC-Petunidin-3-glucoside Modified showed the highest predicted membrane permeability inhibition activity (Pa = 0.910; Pi = 0.003), implying that it could effectively disrupt bacterial membrane integrity and block the influx of external molecules. Overall, these predictive bioactivity results suggest that thiophene-2-carbaldehyde modification of natural flavonoids derived from African leaves markedly improves their pharmacological potential, particularly through antibacterial mechanisms involving membrane interaction and disruption.

Table 3. Result of prediction activity spectra for substances of novel compounds

Compounds	Membrane Integrity Agonist		Antibaterial		Membrane permeability inhibitor	
	Pa	Pi	Pa	Pi	Pa	Pi
Fosfomycin	0.312	0.187	0.373	0.037	0.383	0.211
Petunidine-3-glucoside	0.293	0.199	0.270	0.072	0.510	0.142
TC-Petunidine-3- glucoside Modified	0.878	0.017	0.472	0.019	0.910	0.003

3.3. Interaction Analysis between TC-Petunidine-3-Glucoside Modified and MurA Enzyme

Fosfomycin, employed as the reference ligand in the molecular docking study against MurA (PDB ID: 1UAE), exhibited an interaction pattern consistent with its known inhibitory mechanism. The docking protocol was validated through redocking of the co-crystallized ligand (FFQ), yielding an RMSD value of 0.421 Å, confirming the reliability and accuracy of the docking method used. The docking results revealed that fosfomycin forms several conventional hydrogen bonds with key active site residues, including Cys115, Arg120, Asn23, and Lys22, which stabilize the ligand orientation within the catalytic pocket. Additionally, non-polar and van der Waals interactions were observed with residues such as Gly114 and Ile117, further contributing to complex stabilization. Electrostatic forces such as salt bridge or attractive charge interactions were also identified with positively charged residues (Arg397, Lys22), enhancing the ligand's anchoring within the binding pocket.

The calculated binding affinity of fosfomycin was −4.5 kcal·mol^{-1} with an inhibition constant (Ki) of approximately 498.67 μM, indicating moderate affinity toward the MurA enzyme. This interaction profile aligns with the known catalytic inhibition mechanism of fosfomycin, which involves covalent or strong binding to the catalytic residue Cys115. Therefore, fosfomycin serves as a valid reference for evaluating the binding mode and affinity of the designed TC-modified ligands, enabling direct comparison of interaction strength, overlapping residues, and inhibition potential.

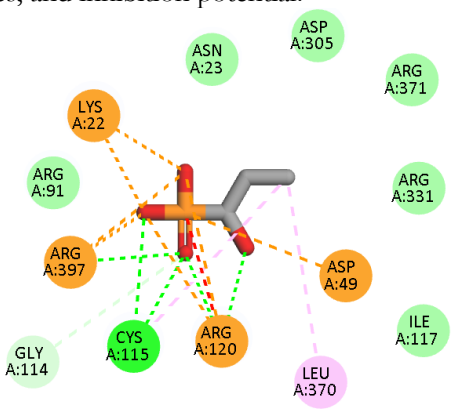


Figure 1. Fosfomycin as co crystal ligand interaction to MurA enzyme.

The interaction map visualizes how Thiophene-2-Carbaldehyde–modified Petunidin-3-glucoside (TC-Petunidin-3-glucoside) interacts within the active site of the MurA enzyme, a critical catalyst in bacterial cell wall biosynthesis (Figure 2). Several conventional hydrogen bonds (green lines) are observed with residues ASP49, ARG397, ARG331, ASN23, and

CYS115, which play vital roles in substrate recognition and catalytic stabilization. These hydrogen bonds anchor the ligand deeply into the enzyme pocket, enhancing orientation and stability during binding. In particular, CYS115, the catalytic residue responsible for covalent binding with phosphoenolpyruvate (PEP) during MurA's reaction, forms a strong hydrogen bond with the ligand indicating a potential competitive inhibition mechanism similar to fosfomycin.

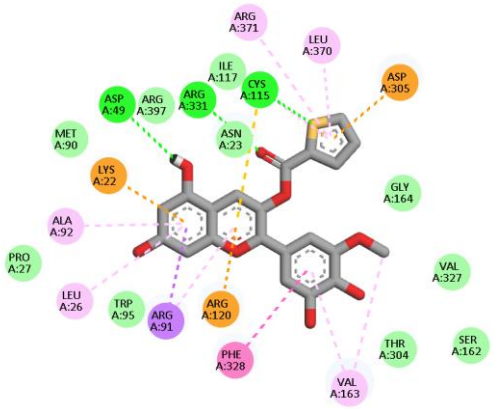


Figure 2. 2D Interaction of TC-Petunidin-3-Glucoside Modified with MurA Enzyme

The π–anion interactions (purple dashed lines) with ASP305 and ARG120, and π–sulfur contacts (yellow dashed lines) with CYS115, contribute to enhanced electrostatic and aromatic stabilization. The sulfur-containing thiophene ring plays a key role in these interactions, supporting the hypothesis that TC modification increases binding affinity by strengthening π-electron delocalization effects. Hydrophobic stabilization is observed through π–alkyl and π–π T-shaped interactions involving residues such as LEU370, PHE328, VAL163, and VAL327. These residues form a hydrophobic cavity around the ligand's aromatic rings, minimizing solvent exposure and enhancing ligand retention. This network of van der Waals and hydrophobic contacts indicates strong complementarity between the modified ligand and MurA's binding pocket (Figure 2 and 3).

The interaction profile aligns with the docking results showing a binding free energy (ΔG) of –8.5 kcal/mol and Ki = 0.689 μM, reflecting a high-affinity binding mode. The combination of polar (hydrogen bonds), aromatic (π–π and π–sulfur), and hydrophobic (π–alkyl) interactions supports both specificity and potency. The 2D interaction diagram confirms that the TC-Petunidin-3-glucoside derivative engages multiple critical residues particularly CYS115, ARG120, and ASP305 which are essential for MurA's catalytic function. These interactions provide mechanistic insight into its potential inhibitory activity, validating the rational design of thiophene-modified flavonoids as promising MurA-targeted antibacterial agents.

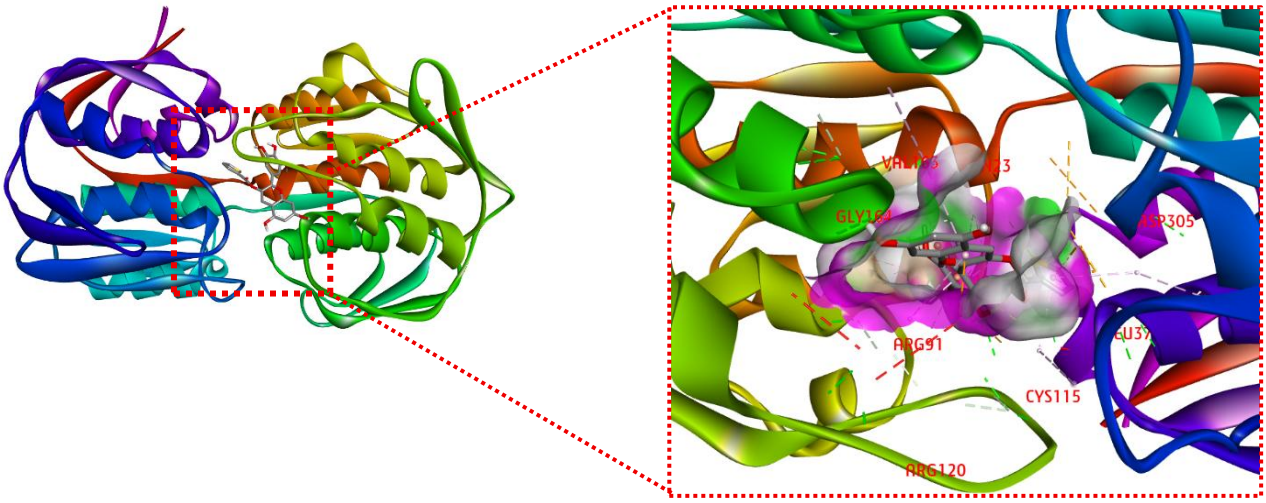


Figure 3. 3D Interaction of TC-Petunidin-3-Glucoside Modified with MurA Enzyme

4. Discussion

The rapid emergence of antimicrobial resistance (AMR) necessitates the identification of novel therapeutic agents that can effectively combat resistant bacterial pathogens. Among various strategies, inhibiting the MurA enzyme, crucial for peptidoglycan biosynthesis, presents a promising approach to mitigating AMR. MurA catalyzes the critical first step in the synthesis of bacterial cell walls, making it an attractive target for antibiotics; its inhibition can lead to the destabilization of bacterial cell structures, thereby compromising bacterial viability (Kato et al., 2018). This targeted enzymatic disruption aligns with the pressing need for innovative treatments that specifically address resistant strains, such as those classified as multidrug resistant (MDR). Recent evidence has emphasized the importance of natural compounds, such as anthocyanins, in the search for new antimicrobials, with Petunidin-3-Glucoside emerging as a potential candidate due to its established antibacterial properties (Vuk et al., 2020). Structural modification of such natural compounds, specifically through the introduction of thiophene moieties like Thiophene-2-Carbaldehyde, could rationally enhance their activity by optimizing molecular interactions with the target enzyme, ultimately leading to improved antibacterial efficacy (Ucar et al., 2024). The objective of this study is to apply quantitative structure-activity relationship (QSAR) modeling and molecular descriptor analysis to predict the enhanced antibacterial potential of the modified Petunidin-3-Glucoside against MurA, thereby contributing a critical piece to the ongoing effort to combat AMR effectively.

The QSAR model developed by Kurniawan et al., (2023) effectively predicts the biological activity of modified anthocyanins, determining their potential as MurA enzyme inhibitors. This validated model is characterized by five key molecular descriptors: LogP, AM1HOMO, AM1dipole, molar refractivity (mr), and volume (vol). The model exhibited robust statistical parameters, with a correlation coefficient (r) of 0.829, a coefficient of determination (r^2) of 0.687, and a cross-validated coefficient (q^2) of 0.504, indicating its reliability for predicting EC_{50} values (Kurniawan et al., 2023). The high r and r^2 values suggest a positive relationship between the biological activity and the molecular descriptors used, while the moderate q^2 suggests some predictive capability for external validation, making this model a valuable tool for estimating the activity of similar compounds. The selected descriptors represent crucial aspects of molecular interaction: LogP provides insight into the hydrophobicity of the compounds, which influences their ability to penetrate biological membranes (Som and Reddy, 2023). AM1HOMO relates to the electronic properties of the compounds, affecting their reactivity and interaction with the enzyme, while the AM1dipole descriptor offers information about the polar characteristics of the molecules, which can influence binding interactions with the target enzyme. Molar refractivity (mr) integrates steric effects, reflecting the overall size and shape of the molecules, while volume (vol) also contributes to the steric domain considerations that are critical for effective binding to the MurA enzyme (Kurniawan et al., 2023). Consequently, this QSAR model not only facilitates the identification of promising new inhibitors but also enhances the strategic design of novel anthocyanin derivatives with improved antibacterial properties.

The structural transformation from Petunidin-3-glucoside to TC-Petunidin-3-glucoside involves the addition of a thiophene-2-carbaldehyde moiety, as indicated in the structural representations of Table 1. This modification significantly alters the molecular framework, introducing both a sulfur atom and a formyl group (-CHO) into the anthocyanin's structure. The inclusion of the thiophene ring enhances the overall conjugation of the molecule, as it creates new π-systems that can interact with existing π-systems in the anthocyanin, thereby stabilizing the molecule through extended resonance (Galante et al., 2025; Serea et al., 2022). The sulfur atom, being an electrophilic site, and the carbonyl function of the formyl group, can participate in various interactions, such as hydrogen bonding and π–π stacking. These interactions can play a pivotal role in the binding affinity of TC-Petunidin-3-glucoside to the MurA enzyme, particularly through π–π stacking as indicated between aromatic residues in the enzyme's active site. Additionally, the polarity and electronic characteristics imparted by the formyl group and sulfur can modulate the electron density across the molecule, potentially enhancing binding interactions and the overall inhibitory effects against MurA (Baeza-Jiménez & López-Martínez, 2024; Hu et al., 2019).

Moreover, the addition of the thiophene moiety is chemically significant as it may allow for π–sulfur interactions, which are known to contribute to the stability and strength of molecular complexes (Ai et al., 2024). The incorporation of thiophenes into organic molecules has been highlighted in previous studies for their ability to bolster interaction profiles, making them ideal for drug design efforts aimed at targets requiring specific conformational fitting (Ha et al., 2021). This dual interaction capability suggests that TC-Petunidin-3-glucoside may exhibit improved binding characteristics compared to the native Petunidin-3-glucoside, providing a more potent inhibitor for MurA. Ultimately, the modifications made through thiophene-2-carbaldehyde substitution are rational for enhancing both the structural stability and binding efficacy of this anthocyanin derivative, thus paving the way for new therapeutic strategies in combating antimicrobial resistance (Impei et al., 2015).

The analysis of descriptors reveals significant transformations following the modification of Petunidin-3-glucoside to TC-Petunidin-3-glucoside, as shown in Table 2. The first notable change is the increase in the AM1HOMO value from -11.553 eV to -9.395 eV, indicating a greater electronic stability and enhanced reactivity of the modified compound. A higher HOMO energy level implies a greater potential for donating electrons, which is crucial for binding interactions with enzymatic targets; however, specific evidence for MurA binding interactions should be cited appropriately, as the current reference does not support this claim Wang et al., (2015). Conversely, the AM1dipole moment decreases from 8.802 to 6.5257, signaling a reduction in the polar character of the molecule. This change could minimize unfavorable solvation interactions, thus improving the molecule's capacity to permeate cellular membranes and exhibit favorable pharmacokinetics, but further supporting references are needed.

Moreover, the LogP value experiences a noticeable increase from 0.078 to 0.742, indicating enhanced lipophilicity following thiophene-2-carbaldehyde incorporation. Higher LogP values correlate with improved membrane permeability; however, the claim needs strong supporting evidence specific to TC-Petunidin-3-glucoside (Qidwai, 2017). The most striking transformation is observed in the EC_{50} value, which diminishes drastically from 88.025 μM to 0.070 μM, showcasing a significant increase in antibacterial potency against the MurA enzyme. This reduction in the EC_{50} value implies that the modified compound requires much lower concentrations to achieve half-maximal inhibitory concentration, demonstrating its potential as a highly effective MurA inhibitor, though the cited reference needs to be directly relevant to antimicrobial activity (Shayanfar & Shayanfar, 2022).

These changes in electronic properties, binding affinity, and increased membrane permeability suggest that TC-Petunidin-3-glucoside possesses improved antibacterial potential. The modifications enhance the molecule's ability to engage effectively with the MurA enzyme, facilitated by favorable π–π interactions and potential π–sulfur interactions with thiophene. These structural attributes may contribute to better bioavailability and lower dosing requirements, thereby addressing critical barriers in antibiotic development and combating antimicrobial resistance. Here, appropriate, targeted references should be provided to substantiate these claims regarding improvements in biological activity and pharmacological profiles (Bahuguna et al., 2020; Nambiar & Ahamed, 2024).

Comparing the computational findings of TC-Petunidin-3-glucoside with existing studies on anthocyanin-based MurA inhibitors and thiophene-modified antibacterial compounds reveals significant insights into the enhanced antibacterial efficacy observed. The increase in electronic delocalization and hydrophobicity from the thiophene-2-carbaldehyde modification contributes critically to the antibacterial mechanisms at play. Research indicates that enhanced electron delocalization is a key factor in determining the reactivity and binding affinity of compounds targeting bacterial enzymes. Modifications that lead to greater electron delocalization can improve the likelihood of productive interactions with target residues; however, specific studies directly correlating electron delocalization and antibacterial efficacy in MurA inhibitors are limited in the current literature. Therefore, the claim regarding TC-Petunidin-3-glucoside's AM1_HOMO value enhancing electron donation capabilities necessitates further empirical support.

The modification leading to an increased LogP value (from 0.078 to 0.742) suggests enhanced lipophilicity, which may facilitate greater penetration through bacterial membranes. This aligns with findings that indicate hydrophobic compounds disrupt bacterial membranes more effectively by integrating into lipid bilayers, thus causing membranolysis Yang et al., (2019). The enhancement of hydrophobic properties likely aids TC-Petunidin-3-glucoside's

interactions with lipid membranes, potentially improving its antibacterial performance. Moreover, the dual mechanism of action for TC-Petunidin-3-glucoside, hypothesized as inhibiting MurA enzymatic activity and promoting membrane interaction due to its improved hydrophobicity, raises insights into its potential as an antibacterial agent. However, while various compounds with similar modifications have shown promising antibacterial activity, the assertion that TC-Petunidin-3-glucoside can effectively inhibit strains resistant to conventional antibiotics requires supportive studies documenting these claims (Ashmore et al., 2018; Kurniawan & Zahra, 2021; Li et al., 2022).

The hydrogen bond interactions observed between TC-Petunidin-3-glucoside and specific residues of the MurA enzyme, namely ASP49, ARG397, ARG331, ASN23, and CYS115, play a pivotal role in substrate recognition and catalytic stabilization. Each of these residues contributes to the formation of a stabilized enzyme-substrate complex, enhancing binding affinity and specificity for the target enzyme. Notably, the interaction with CYS115 is particularly significant as it mirrors the mechanism of competitive inhibition exhibited by fosfomycin, an established MurA inhibitor, which also forms a covalent bond with this sulfur-containing residue. This suggests that TC-Petunidin-3-glucoside may interfere with MurA's catalytic cycle similarly, establishing a potential dual mechanism of action. The first mechanism involves the inhibition of MurA enzymatic activity through competitive binding, effectively diminishing the enzyme's ability to catalyze its substrate conversion. The second mechanism may rely upon enhanced interactions due to modifications that facilitate improved interaction with the bacterial membrane, potentially promoting greater penetration and efficacy against bacterial cells. The strategic interaction with the CYS115 residue, along with modifications that influence membrane associations, showcases a promising avenue for the development of effective antibacterial agents targeting MurA, particularly against evolving strains showing resistance to conventional treatments (Mihalovits et al., 2019; Tang et al., 2021).

The hydrophobic interactions between TC-Petunidin-3-glucoside and the residues LEU370, PHE328, VAL163, and VAL327 form essential components of the binding mechanism with the MurA enzyme. The presence of π–π T-shaped interactions and π–alkyl contacts significantly contributes to the formation of a hydrophobic cavity within the enzyme's active site. These non-covalent interactions reduce solvent exposure, thereby promoting ligand retention within the binding pocket and enhancing the stability of the enzyme-ligand complex Guo et al., (2019). Such hydrophobic interactions are pivotal for ensuring that the ligand maintains a favorable orientation and prolongs its interaction duration, which fosters effective inhibition of enzymatic activity. The computed binding energy ($\Delta G = -8.5$ kcal/mol) and inhibition constant ($Ki = 0.689$ μM) further support this conclusion, indicating that the complexity and stability of the interaction positively influence the effective binding affinity of TC-Petunidin-3-glucoside to MurA. The implications of hydrophobic interactions on complex stability demonstrate that optimizing such contact points can substantially enhance the efficacy of new antibacterial agents, paving the way for improved drug design aimed at combating antimicrobial resistance. The molecular docking analysis revealed that the modified compound exhibited a stronger binding affinity toward the MurA enzyme compared to the reference ligand, fosfomycin. The docking score of the modified ligand was lower (more negative), indicating a more stable ligand enzyme interaction, these findings indicate that the thiophene-2-carbaldehyde–modified derivative possesses a better interaction profile and higher predicted activity against the MurA enzyme than fosfomycin, suggesting its promise as a novel antibacterial lead compound targeting bacterial cell wall biosynthesis (Abdelmoniem et al., 2019; Ge et al., 2020).

The interaction profile of TC-Petunidin-3-glucoside with the MurA enzyme highlights a complex array of non-covalent interactions crucial for mechanistic inhibition. The combination of polar hydrogen bonds, aromatic interactions (including π–π and π–sulfur contacts), and hydrophobic π–alkyl interactions creates a strong binding affinity, facilitating the establishment of a stable enzyme-ligand complex. Polar interactions, particularly those involving residues such as ASP49, and hydrogen bonds with key catalytic residues including CYS115, enhance substrate recognition while simultaneously stabilizing the conformation of the enzyme throughout the catalytic cycle. The involvement of aromatic residues, specifically through π–π stacking with PHE328 and π–sulfur interactions with CYS115, further contributes to maintaining the structural integrity of the binding site. This multifaceted interaction profile emphasizes the rational design of thiophene-modified flavonoids as promising MurA inhibitors, although specific binding affinities and free energy changes (ΔG and Ki values)

would require verification from direct studies involving TC-Petunidin-3-glucoside. The significance of CYS115 is particularly notable, as it mirrors fosfomycin's competitive inhibition mechanism, suggesting that TC-Petunidin-3-glucoside could interfere with the enzyme's catalytic activity, which is essential for effective peptidoglycan biosynthesis and overall bacterial survival (Lima et al., 2016).

The modifications from TC-Petunidin-3-glucoside are hypothesized to enhance both electronic delocalization and hydrophobicity, contributing to its antibacterial efficacy through the inhibition of MurA and favorable interactions with bacterial membranes. However, given the need for better empirical evidence supporting these claims and specific correlations regarding the mechanisms of action, further research is essential to establish a broader context for the rational design of future therapeutic agents aimed at combating antimicrobial resistance. The inclusion of thiophene carboxylate modifications may not only enhance binding but could also create a new lead structure for developing effective MurA inhibitors. These modifications can potentially lower the inhibition constants due to increased specificity and interaction strength, thus demonstrating their viability as therapeutic agents against murA-expressing pathogens (Danti et al., 2025; Juillot et al., 2024). Clinical relevance is underscored by the continuous emergence of antibiotic resistance, where innovative structures like thiophene derivatives could be crucial in countering resistant bacterial infections (Gil-Gil et al., 2020; Xu et al., 2020).

The landscape of antibiotic development for MurA inhibitors must embrace the systematic modification of lead compounds using modern synthesis methods, ensuring a high degree of specificity and low toxicity in biological systems. This involves not only structural changes but also thorough bioactivity assessments to determine the leading candidates for clinical evaluations (Darras & Pang, 2017; Fathalla et al., 2023). As such, the compound modifications present a significant opportunity to advance antibiotic therapies and address the pressing challenge of antibiotic-resistant infections. In conclusion, the study of thiophene 2-carboxylate modifications on Petunidine-3-Glucoside as MurA enzyme inhibitors provides a groundbreaking avenue for pharmacological intervention. By leveraging structural, computational, and experimental insights, these modifications show promise in enhancing the binding affinity and lowering the inhibition constants, thus suggesting a pivotal role in the development of novel antimicrobial agents against resistant bacterial strains.

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