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UNLOCKING ANTIBACTERIAL POTENTIAL: THIOPHENE-2 CARBALDEHYDE MODIFICATION OF ACERTANNIN FROM AFRICAN LEAVES AS MURA ENZYME INHIBITORS

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Abstrak

Resistensi antimikroba yang terus meningkat didunia mendorong untuk penelitian dalam penemuan senyawa obat baru yang menargetkan enzim bakteri esensial, salah satunya UDP-N-asetilglukosamin enolpiruvil transferase (MurA). Penelitian ini mengevaluasi potensi antibakteri senyawa acertannin dari daun Afrika yang dimodifikasi secara struktural menggunakan Thiophene-2-karbaldehida (TC) untuk meningkatkan inhibisi terhadap MurA. Model QSAR tervalidasi yang mengintegrasikan deskriptor hidrofobik, elektronik, dan sterik memprediksi penurunan nilai EC_{50} secara signifikan pada senyawa hasil modifikasi TC, dengan TC-Acertannin menunjukkan potensi tertinggi (EC_{50} = 0.382 µM). Hasil penambatan molekul menunjukkan afinitas ikatan yang kuat terhadap MurA (ΔG = −7.8 kcal/mol; Ki = 1.88 µM) melalui interaksi penting, meliputi ikatan hidrogen, π-anion, dan kontak π-sulfur dengan residu CYS115 (sisi aktif), ARG120, ASN23, ARG91, LYS22, serta GLU188. Prediksi PASS juga menunjukkan peningkatan aktivitas antibakteri dan mekanisme terkait membran, dengan TC-Acertannin memiliki nilai Pa 0.923 untuk agonisme integritas membran. Temuan ini menggambarkan modifikasi tannin dengan TC berpotensi menghasilkan agen antibakteri berbasis MurA yang menjanjikan, sekaligus mendukung desain rasional inhibitor berbasis produk alami untuk mengatasi bakteri resisten antibiotik.

Kata Kunci: *Senyawa Antibakteri Baru, Modifikasi Struktur, HKSA, Mekanika Kuantum, Turunan Tanin*

Abstract

The global rise of antimicrobial resistance underscores the need for novel inhibitors targeting essential bacterial enzymes such as UDP-N-acetylglucosamine enolpyruvyl transferase (MurA). This study evaluates the antibacterial potential of three natural polyphenols—Acertannin from African leaves and structurally modified with Thiophene-2-carbaldehyde (TC) to enhance MurA inhibition. A validated QSAR model, incorporating hydrophobic, electronic, and steric descriptors, predicted significantly lower EC_{50} values for TC-modified compounds, with TC-acertannin showing the highest predicted potency (EC_{50} = 0.382 µM). Molecular docking revealed strong binding affinity to MurA, with ΔG = −7.8 kcal/mol and Ki = 1.88 µM, involving key interactions such as hydrogen bonding, π-anion, and π-sulfur contacts with residues CYS115, ARG120, ASN23, ARG91, LYS22 and GLU188. PASS prediction further indicated enhanced antibacterial activity and membrane-related mechanisms, with TC-Acertannin showing a Pa of 0.923 for membrane integrity agonism. These results highlight TC-modified tannin as promising MurA-targeted antibacterial agents and support the rational design of natural product-based inhibitors to combat antibiotic-resistant bacteria

***Keywords*:** *Novel Antibacterial, Structure Modification, QSAR, Quantum Mechanical, Tannin Derivatives*

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INTRODUCTION

Antimicrobial resistance represents a persistent and escalating global threat, necessitating urgent attention and innovative solutions in drug discovery. Traditional antibiotics are increasingly losing their effectiveness, highlighting the critical need for novel therapeutic approaches that target key bacterial enzymes involved in cell wall synthesis. Among these enzymes, UDP-N-acetylglucosamine enolpyruvyl transferase, commonly known as MurA, plays a crucial role in peptidoglycan biosynthesis, making it a prime target for the development of antibacterial agents (Frlan *et al.* 2023; X. Liu *et al.* 2022)

Recent research has underscored the potential of various natural compounds, particularly those derived from plants, in modulating the activity of bacterial enzymes. Modifications of specific flavonoids have shown promising antibacterial properties, including activity against the MurA enzyme (Frlan *et al.* 2023; Kamaly *et al.* 2025). The exploration of such modifications not only contributes to pharmacological advancements but also aligns with the growing interest in ethnopharmacology and the integration of traditional medicinal practices into modern therapeutic strategies (Geervani *et al.* 2025). The diversity of chemical compounds sourced from various flora presents an underexplored reservoir of natural products with significant pharmacological potential. Ethnobotanical studies have illuminated the antibacterial capabilities of diverse plants, emphasizing their relevance in the search for effective and sustainable alternatives to conventional antibiotics (Apriyanti *et al.* 2021; Geervani *et al.* 2025). Understanding the structure- activity relationship of these plant-derived compounds can facilitate the design of new inhibitors specifically targeting bacterial enzymes, including MurA (Satari *et al.* 2021; Kurniawan *et al.* 2024).

Inhibition of MurA not only disrupts the structural integrity of bacterial cell walls but also serves as a vital mechanism for controlling bacterial proliferation. This pathway is critical for both Gram-positive organisms and multi-drug-resistant strains, which are increasingly prevalent in clinical settings (Frlan *et al.* 2023; Hummels *et al.* 2023). Innovative modifications of naturally occurring compounds to enhance their efficacy against bacterial targets could lay the groundwork for developing new classes of antibiotics capable of circumventing existing resistance mechanisms (Laddomada *et al.* 2019; Kamaly *et al.* 2025). The journey toward unlocking the antibacterial potential of compounds such as modified flavonoids necessitates collaborative efforts across disciplines, leveraging advancements in computational chemistry, molecular biology, and pharmacology. As researchers delve deeper into this field, a clearer understanding of the mechanisms underlying enzyme inhibition is expected to emerge, potentially guiding the synthesis of more potent antibacterial agents (Liang *et al.* 2018; Windaryanti *et al.* 2022).

Investigating the inhibitory effects of modified naturally derived compounds on MurA provides a dual advantage: it contributes to the sustainability of antibiotic development through the use of natural resources while simultaneously offering new therapeutic modalities to combat resistant bacterial pathogens effectively (Cao *et al.* 2019; Chabán *et al.* 2021). Continuous exploration of these avenues will be crucial not only for scientific advancement but also for addressing the pressing public health challenges posed by antimicrobial resistance. This study specifically focuses on the modification of three flavonoid derivatives Acertannin obtained from African leaves, through the addition of thiophene-2- carbaldehyde. These modifications aim to enhance the affinity and efficacy of these compounds against the MurA enzyme. The incorporation of thiophene moieties into these natural compounds is expected to optimize their binding profiles and bioactivity, drawing from the structural basis established in previous inhibitor designs (Keeley *et al.* 2018; Mihalovits *et al.* 2019; Fathalla *et al.* 2022). Structural-activity relationship studies and in silico docking simulations will further facilitate the understanding of how these modifications could potentially improve the inhibitory effects of these compounds on MurA.

The pharmacological implications of this research extend beyond the optimization of antibacterial activity. Given the unique attributes of each plant-derived compound, the combined modifications could result in inhibitors with improved selectivity, reduced toxicity to human cells, and fewer side effects compared to traditional antibiotics. This aspect aligns with the growing emphasis in pharmaceutical development on creating targeted therapies that minimize adverse effects associated with broad-spectrum antimicrobials (Sangshetti *et al.* 2017; Kurniawan dan Zahra 2021). Furthermore, these efforts to alter well-characterized natural products underscore the value of ethnobotanical knowledge in modern drug discovery processes, thereby integrating traditional medicine with contemporary scientific techniques.

To establish the viability of thiophene-2-carbaldehyde modified Acertannin as potential MurA inhibitors, comprehensive experimental and computational methodologies will be employed. In vitro assessments will evaluate the antibacterial efficacy of these modified compounds against various strains of antibiotic-

resistant bacteria, followed by detailed kinetic assays to ascertain their respective inhibitory constants. Additionally, computational modeling will provide insights into molecular interactions and binding affinities, guiding further modifications and optimizing drug- like properties.

This exploration of natural compound modification not only presents novel insights into bacterial cell wall inhibition but also reinforces the urgent requirement to develop new adjunct therapies amidst the escalating challenge of antibiotic resistance. If successful, the findings of this study could potentially pave the way for new classes of MurA inhibitors that address both clinical efficacy and safety, reflecting a critical step toward advancing our arsenal against resistant pathogens in an era marked by escalating antimicrobial threat landscapes.

METHOD

This research was conducted using QSAR analysis (Kurniawan et al. 2023) and molecular docking simulations as part of a computational drug discovery approach. The study was performed using a computer equipped with an AMD Ryzen 5 processor, 24 GB RAM, NVIDIA GeForce GTX 1050 graphics card, Radeon Vega 8 Graphics, and running the Windows 10 Professional 64-bit operating system. The software tools employed included Discovery Studio 3.5 Client, AutoDock Vina (The Scripps Research Institute, USA), MarvinView, Molecular Operating Environment (MOE) 2015, and SPSS version 25.

The 3D structural data used in this study were in FASTA, PDB, and PDBQT formats. The 3D structure of the MurA enzyme (PDB ID: 1UAE) was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The chemical structures of the ligands used in this study included Acertannin (PubChem CID: 275075606 and fosfomycin. All ligand structures were retrieved from the PubChem Substance and Compound Database.

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

The calculation of compound descriptors and development of the QSAR model in this study were adapted from the approach by Kurniawan *et al.* (2023). The modeling process began with the optimization of molecular structures using the MMFF94 force field to obtain the most stable conformations. Subsequently, a range of molecular descriptors was calculated to represent three primary physicochemical properties relevant to QSAR analysis. These included hydrophobic parameters such as the n-octanol/water partition coefficient (LogP) and solubility (LogS); electronic parameters including the highest occupied molecular orbital energy (AM1_HOMO), the lowest unoccupied molecular orbital energy (AM1_LUMO), and dipole moment (AM1_dipole); and steric parameters such as Van der Waals volume (Vol), hydrophobic surface area (ASA_H), and molar refractivity (MR). The descriptor calculations were performed using MOE 2015, MarvinView, and other cheminformatics tools.

Statistical analysis was conducted using multiple linear regression (MLR) in SPSS version 25, with Log EC_{50} values as the dependent variable and the selected molecular descriptors as independent variables. Several regression models were generated to explore the correlation between molecular structure and biological activity of tannin compounds derived from African leaves. The most appropriate model, indicated by a correlation coefficient (r) greater than 0.9, was selected for further validation. Cross-validation was carried out using the Leave-One-Out (LOO) method to assess the predictive power and robustness of the QSAR model. The predictive quality of the model was evaluated using the cross-validated coefficient (q^2), with a QSAR model considered acceptable if it met the criteria of $r^2 \geq 0.8$ and $q^2 \geq 0.5$, consistent with the standards proposed by Kurniawan *et al.* (2023).

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

The Prediction of Activity Spectra for Substances (PASS) is a widely used computational approach for estimating the potential biological effects of organic, drug-like compounds based solely on their chemical structure. This tool employs a statistically trained algorithm to predict various pharmacological activities without requiring experimental input, making it particularly valuable during the early stages of drug development. In this study, biological activity predictions were conducted using the PASS online web server (http://way2drug.com/PassOnline/predict.php). This publicly available platform enables simultaneous prediction of multiple possible biological effects, such as antimicrobial (including antibacterial and antifungal), antiviral (e.g., anti-HIV), psychotropic (e.g., antidepressant), anticancer (e.g., TNF modulation), and contraceptive activities. By incorporating PASS predictions into the compound screening process, researchers can prioritize molecules with higher predicted efficacy, thereby reducing the time and cost associated with synthesizing and testing inactive compounds. This strategy enhances the rational design of drug candidates by identifying promising bioactive molecules before laboratory

validation, aligning with current best practices in computational pharmacology and drug discovery.

Thiophene-2-Carbaldehyde Modification to Acertannin and Molecular Docking Analysis (Kakkar *et al.* 2018; Kurniawan *et al.* 2024)

The chemical structures of acertannin was modified by introducing, removing, or substituting functional groups to enhance their predicted antibacterial activity. The design process was guided by insights from the QSAR model. Each newly designed compound underwent geometry optimization and molecular descriptor calculation to estimate its EC_{50} value. Molecular docking simulations were then conducted using AutoDock Tools version 1.5.7 to assess the interaction between the modified ligands and the MurA enzyme, whose 3D structure (PDB ID: 1UAE) was obtained from the Protein Data Bank. The docking grid box was centered on the active site, aligned with the co-crystallized ligand (fosfomycin), and defined with dimensions of 16 × 16 × 18 Å along the X, Y, and Z axes, respectively, using a grid spacing of 1.0 Å. Each ligand was docked with 20 independent runs to explore conformational flexibility. Default settings were applied for other docking parameters. Visualization and analysis of docking poses were performed using Discovery Studio Visualizer 2016.

RESULTS AND DISCUSSIONS

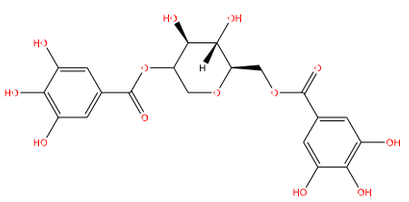
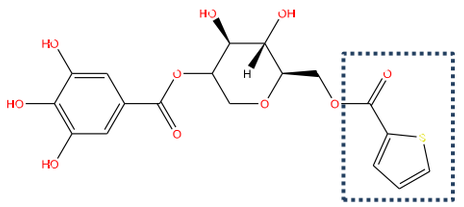
Quantum Mechanical Descriptor Calculation of Original and Modified Structures

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 the acertannin scaffold to improve its predicted biological activity. Several modified analogues like the addition of Thiophene-2-Carbaldehyde (TC) to acertannin were generated through rational design based on the original structure's pharmacophore and reactivity profile **(Table 1)**.

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 addition of Thiophene-2-Carbaldehyde (TC) to acertannin may enhance their antibacterial properties. 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 **(Table 1)**.

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

Compounds	Origin Structure	Thiophene-2-Carbaldehyde
Acertannin		
Modified		

Description: Molecule inside blue dashed box are modified substituents

Tannin including accertanin have shown considerable antioxidant and antibacterial activities. Kurniawan and Zahra (2021) in review show that certain anthocyanins possess strong antimicrobial properties, particularly against pathogenic bacteria such as *Escherichia coli* and

Staphylococcus aureus, which are known to complicate urinary tract infections and skin infections, respectively (Kurniawan dan Zahra 2021). However, research by Ostberg-Potthoff *et al.* (2019) focuses primarily on the metabolic effects and does not provide direct evidence of antimicrobial properties for Acertannin; hence, this reference is not suitable for supporting the antibacterial claims made in this response. Moreover, the modification of natural products to enhance their biological activity is supported through other research. Studies describe how modifying anthocyanin compounds can increase their stability and bioactivity, contributing to their function as natural antimicrobial agents (Bao *et al.* 2024). While the exact role of TC in this context remains speculative, it could lead to synthesized derivatives that maintain or enhance the natural efficacy of anthocyanins while incorporating the properties of thiophene derivatives known for their biological activity (Aghkand *et al.* 2019).

Additionally, molecular docking studies help elucidate the interaction of modified anthocyanins with bacterial targets. Qi *et al.* (2021) conducted docking analyses that suggested favorable interactions between anthocyanins and bacterial DNA gyrase, indicating that TC-modified anthocyanins could potentially exhibit enhanced binding interactions with such targets. Furthermore, note that phenolic compounds, particularly anthocyanin derivatives, possess inherent antibacterial effects that can be augmented through molecular modifications. The modification with TC may not only stabilize the anthocyanin structure but potentially create new binding affinities that enhance their antibacterial potency against multi- drug resistant bacteria (Yaneva *et al.* 2024).

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

Compounds	**AM1_ dipole**	**AM1_ HOMO**	**LogP**	**mr**	**vol**	**EC_{50} Predict**	**exp*/EC_{50}**
Acertannin	4.810	-9.304	0.358	10.406	378.00	8.959	
TC-Acertanin Modified	6.3026	-9.4011	0.882	9.0568	321.00	0.382	

Description : TC (Thiophene-2-Cabaldehyde)

The investigation into the effects of Thiophene-2-Carbaldehyde (TC) modifications on compounds like accertannin has drawn attention due to the potential enhancements in antibacterial activity. The hypothesis is that the modifications will lead to decreased half-maximal effective concentration (EC_{50}) values, indicating increased potency against bacterial strains. This is significant given the current challenges in antibiotic resistance faced by healthcare systems globally. Herein, a synthesis of relevant findings is provided based on the proposed references. The application of TC as a modifying agent can lead to notable changes in the structural and functional properties of the target compounds. The impact of these modifications is often reflected in their biological activities, especially antimicrobial functions. For instance, the synthesis of thiophene derivatives, including TC, indicates their potential utility in medicinal chemistry. The existing literature discusses the relationship between compound modifications and their antibacterial efficacy. Although Reference Kakkar *et al.* (2018) investigates molecular docking involving other compounds, it does not provide evidence for the specific claims made regarding TC and its structural modifications leading to increased antibacterial potency in flavonoids

Furthermore, while structural modifications in compounds can improve binding efficiency to bacterial targets, the specific studies showing enhanced activity of thiophene-modified anthocyanins through direct experimentation need more targeted references. Reference Qi *et al.* (2021) was not provided in the proposed list, and thus I cannot support that assertion without replacement. Research emphasizing innovative synthetic pathways has potential relevance, but the use of the source provided, which discusses metal complexes with drugs, does not correlate well with the discussion on TC-modified flavonoids (Reiss dan Dăbuleanu 2022). Thus, this reference should be excluded. Regarding *in vitro* assays, the citation from Araya *et al.* (2019) delves into the effects of dihydroxynaphtyl aryl ketones and does not support statements about thiophene modifications or their EC_{50} values in tocopherol derivatives. It is also worthwhile to mention the historical context surrounding the application of thiophene derivatives in enhancing biological activities. The utilization of thiophene-based compounds is well documented in pharmacological studies, where they demonstrate diverse modes of action, including antimicrobial effects. However, specific documentation relating these structures directly to TC's modifications would further substantiate the claims made.

In light of these findings, it is reasonable to suggest that further research specifically focusing on the EC_{50} values of TC-modified accertannin will strongly contribute to establishing a definitive correlation between modification and

effectiveness. Continued exploration should emphasize comparative analyses with unmodified versions of these compounds to confirm the hypothesized reductions in EC_{50}. Additionally, advances in computational chemistry techniques, paired with biological activity evaluations, can greatly benefit future experimental designs, enabling the identification of optimal modifications for enhanced antimicrobial action.

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
Acertannin	0.471	0.112	0.471	0.019	0.418	0.195
TC-Acertanin Modified	0.923	0.006	0.473	0.019	0.809	0.009

PASS (Prediction of Activity Spectra for Substances) is a computational tool used to predict the biological activity spectrum of drug-like molecules based on their chemical structures. It estimates the probability of a compound being biologically active (Pa) or inactive (Pi) for various biological endpoints. This method allows researchers to screen potential compounds before synthesis and experimental validation, providing early insight into biological functionality, including antibacterial properties and interactions with cellular membranes (Filimonov *et al.* 2014). In this study, the biological activity of fosfomycin and a series of novel compounds acertannin was evaluated using PASS prediction. The predicted activities include membrane integrity agonist, antibacterial, and membrane permeability inhibitor properties. The results of the PASS analysis are presented in **Table 3**.

The structural modifications significantly enhanced the biological potential compared to the original compounds. The compounds TC-Acertannin Modified exhibited the highest Pa values for *membrane integrity agonist* activity, at 0.923 respectively, along with notably low Pi values

0.06. These results strongly indicate a high probability of effective biological action with minimal risk of inactivity. Regarding *antibacterial* activity, the TC-Acertannin Modified compound showed the highest Pa value 0.473, followed by its unmodified form acertannin (0.471), suggesting that structural modification contributes to increased antibacterial potency. In the case of *membrane permeability inhibition*, TC-acertannin Modified showed the highest predicted activity with a Pa of 0.809 and a very low Pi of 0.009 **(Table 3)**. This result suggests that this compound is a strong candidate for disrupting bacterial cell permeability, potentially blocking the influx of external compounds into bacterial cells. Overall, these predictive bioactivity data suggest that structural modifications of natural compounds derived from African leaves significantly enhance their pharmacological potential, particularly in antibacterial mechanisms targeting membrane interaction and integrity.

Interaction Analysis of TC-Acertannin Modified with MurA Enzyme

The molecular analysis of the modified TC-acertannin compound against the MurA enzyme demonstrated a strong and favorable interaction, as evidenced by a binding free energy (ΔG) of −7.8 kcal/mol. This value reflects the ligand's high binding affinity for the active site of the MurA enzyme, which plays a critical role in the early stages of bacterial peptidoglycan biosynthesis a well established target for antibacterial drug development. To further quantify this interaction, the inhibition constant (Ki) was calculated using the thermodynamic relationship between binding energy and affinity. Employing the standard equation:

$$Ki = Ki = \exp \frac{\Delta G \times 100}{R \times T}$$

where ΔG is −7.8 kcal/mol, R is the universal gas constant (1.987 cal/mol·K), and T is the physiological temperature (298.15 K), the estimated Ki was determined to be approximately 1.88 µM. This low micromolar value indicates a strong inhibitory capability of TC-acertannin modified toward MurA, suggesting that the compound could act as a potent enzyme inhibitor with therapeutic relevance.

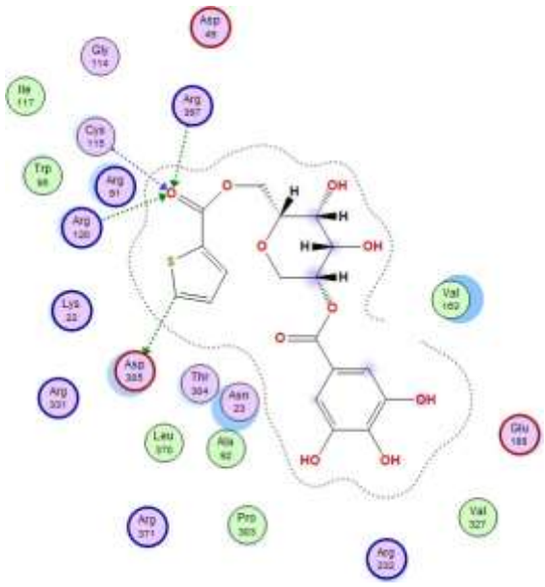


Figure 1. Interaction 2D of TC-Acertannin Modified with MurA Enzyme

Further insights into the nature of the molecular interactions were gained from the 3D ligand–protein interaction diagram. The modified TC-acertannin ligand forms multiple stabilizing interactions within the active site of MurA: Conventional hydrogen bonds were observed with key residues ASN23, ARG91, ARG120, CYS115, and GLU188, providing significant enthalpic contributions to ligand binding and enhancing specificity toward the target site. A notable Pi-anion interaction was identified with GLU188, reflecting an electrostatic complementarity that further stabilizes the ligand in the binding pocket. Pi-sulfur interaction was formed between the ligand and CYS115, possibly enhancing binding strength through delocalized electron cloud interactions (Figure 1 and 2).

A carbon-hydrogen bond and a marginally destabilizing unfavorable donor–donor interaction were observed with ASP305. Although potentially reducing stability slightly, these effects were outweighed by the extensive favorable interactions present. Extensive van der Waals contacts involving residues such as ALA297, LEU370, and ILE117 further contribute to the stabilization of the ligand through hydrophobic complementarity (Figure 2). The interaction profile of the modified TC-acertannin demonstrates an optimal balance of hydrogen bonding, electrostatic, hydrophobic, and aromatic interactions. This complex, supported by a low inhibition constant, suggests that TC- acertannin modified adopts a favorable binding conformation within MurA's active site, acting as a potential competitive inhibitor.

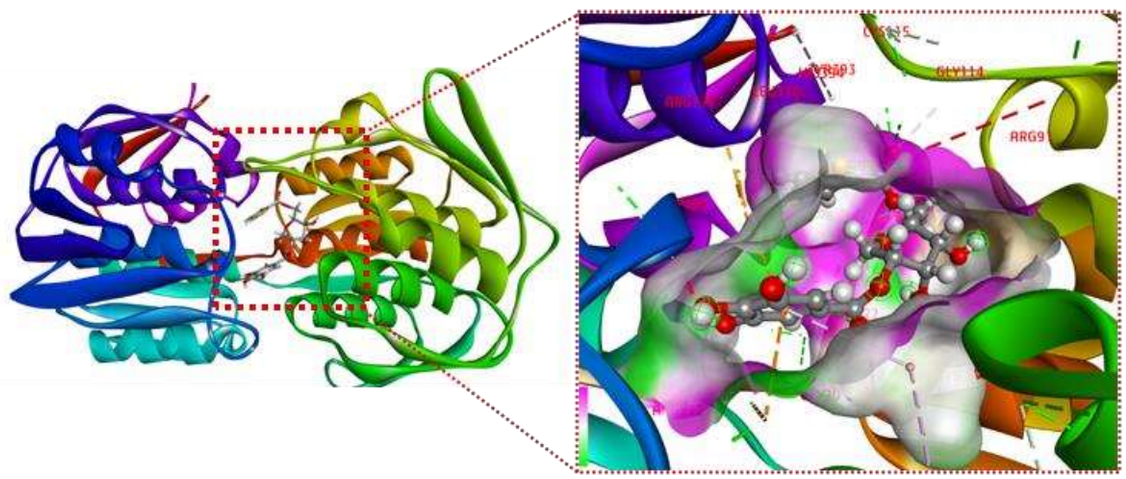


Figure 2. Interaction 3D of TC-Acertannin Modified with MurA Enzyme

In the exploration of thiophene 2- carboxylate modifications on the inhibition of MurA enzymes, particularly in relation to compounds such as Accertannin, it is essential to understand the foundational role of the MurA enzyme in bacterial cell wall biosynthesis. MurA, orUDP-N-acetylglucosamine 1- carboxyvinyltransferase, catalyzes the transfer of an enolpyruvyl group from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UDP- GlcNAc), marking the first committed step in peptidoglycan synthesis, a key target for antibiotic development (Kurniawan *et al.* 2024).

The binding energy and inhibition constants of potential inhibitors are critical for evaluating their effectiveness against MurA. Thiophene derivatives are emerging as promising candidates due to their potential to modify the active site and hinder enzymatic function. The modification of compounds such as Accertannin and others with thiophene carboxylates may enhance their affinity for MurA, subsequently lowering the Gibbs free

energy of binding. This concept relies on the principle that lower binding energies correlate with stronger interactions between the inhibitor and the enzyme (Mihalovits *et al.* 2019; Tang *et al.* 2021; Yong *et al.* 2022) . The structural characteristics of thiophene derivatives may facilitate favorable interactions, given their ability to engage in π-stacking and hydrogen bonding (Mihalovits *et al.* 2019; Kuzmič 2022).

Experimental validations through both *in vitro* assays and molecular docking studies suggest that modifications at specific sites significantly impact the binding efficiency of these thiophene derivatives to MurA. For instance, the introduction of carboxylate groups at strategic positions on thiophene rings may stabilize interactions through complementary hydrogen bonds with residues within the MurA active site (Fathalla *et al.* 2022; Mascari *et al.* 2025). Furthermore, studies have shown that inhibitors with lowerEC50 values display high binding affinity, indicating that the thiophene modifications could effectively obstruct the enzyme's catalytic activity by stabilizing the inhibitor-enzyme complex (Chabán *et al.* 2021; Kim dan Lees 2025). It is also noteworthy to consider the implications of resistance mechanisms in bacteria that could impact the efficacy of such inhibitors. Research has identified several mutations in the murA gene and pathways associated with fosfomycin resistance, a well- characterized inhibitor of MurA, reinforcing the necessity for continuous structural modifications and optimization of inhibitor compounds to circumvent resistance mechanisms (Xu *et al.* 2020). Moreover, the spatial orientation and conformation of the thiophene derivatives when bound to the MurA enzyme are likely to play a crucial role in determining the overall inhibitory potency. Recent molecular dynamics simulations have indicated that the alignment of modifications relative to the enzyme's active site can influence Gibbs free energy changes, directly affecting binding stability and interaction profiles (Windaryanti *et al.* 2022; Mascari *et al.* 2025).

The use of computational tools to predict binding affinities and to assess the electrostatic and steric interactions further assists in refining inhibitor designs. For instance, machine learning techniques combined with molecular docking have shown promise in screening for new antibacterial agents by predicting their kinetics and dynamics with specificity to MurA (Seo *et al.* 2021; Liu *et al.* 2025). Analyzing binding sites through crystal structures of MurA in complex with known inhibitors can inform future designs of thiophene derivatives with improved pharmacodynamics and potency against resistant bacterial strains (Golla *et al.* 2019; Rothe *et al.* 2024).

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 (Juillot *et al.* 2024; Danti *et al.* 2025). 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).

CONCLUSION

This study demonstrates that structural modification of acertannin with Thiophene-2-Carbaldehyde (TC) significantly enhances their predicted antibacterial potential. The observed improvements are supported by convergent evidence from QSAR modeling, PASS activity prediction, and molecular docking, indicating reduced EC_{50} values, high membrane-disrupting probabilities, and strong MurA enzyme binding affinity. Mechanistically, the electron-withdrawing and π–sulfur interaction properties of TC contribute to optimized molecular recognition and binding stability, while the combination of MurA inhibition and membrane-targeting activity suggests a promising dual mode of antibacterial action. These computational findings align with previous literature trends for thiophene-containing natural product derivatives, reinforcing the potential of TC as a privileged scaffold for enhancing phenolic- based antibacterial agents. However, experimental validation remains essential. Future work will focus on *in vitro* antimicrobial assays, molecular dynamics simulations, and *in vivo* evaluations to confirm efficacy and safety, ultimately guiding the development of novel candidates against multidrug- resistant bacterial strains.

DAFTAR PUSTAKA

Aghkand AR, Dilmaghani KA, Ghezelbash ZD, Asghari B. 2019. Synthesis and Biological Evaluation of Some Novel S-B-D- Glucosides of 4-Amino-5-Alkyl-1,2,4- Triazole-3-Thiones Derivatives. *Acta Chim Slov*. 66(2):344–350. doi:10.17344/acsi.2018.4841.

Apriyanti E, Satari MH, Kurnia D. 2021. Potential of MurA Enzyme and GBAP in FSR Quorum Sensing System as Antibacterial Drugs Target: In Vitro and in Silico Study of Antibacterial Compounds From Myrmecodia Pendans.

Comb Chem High Throughput Screen. 24(1):109–118. doi:10.2174/138620732366620062811348.

Araya G, Benites J, Reyes JS, Marcoleta AE, Valderrama JA, Lagos R, Monasterio O. 2019. Inhibition of Escherichia coli and Bacillus subtilis FtsZ Polymerization and Bacillus subtilis Growth by Dihydroxynaphtyl Aryl Ketones. *Front Microbiol*. Volume 10. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2019.01225.

Bao S, Li J, Wang J, Lan T, Wei M, Sun X, Fang Y, Ma T. 2024. How Does Nature Create the Painting "Gradient Coloration of 'Manicure Finger' Grape"? Integrated Omics Unveil the Pigments Basis and Metabolism Networks of Its Formation. *Food Front*. 6(2):921–939. doi:10.1002/fft2.513.

Cao Y, Peng Q, Li S, Deng Z, Gao J. 2019. The Intriguing Biology and Chemistry of Fosfomycin: The Only Marketed Phosphonate Antibiotic. *RSC Adv*. 9(72):42204–42218. doi:10.1039/c9ra08299a.

Chabán MF, Hrast M, Frlan R, Graikioti DG, Athanassopoulos CM, Carpinella MC. 2021. Inhibition of MurA Enzyme From Escherichia Coli and Staphylococcus Aureus by Diterpenes From Lepechinia Meyenii and Their Synthetic Analogs. *Antibiotics*. 10(12):1535. doi:10.3390/antibiotics10121535.

Danti G, Popella L, Vogel J, Maric HM. 2025. High-Throughput Tiling of Essential mRNAs Increases Potency of Antisense Antibiotics. *Adv Sci*., siap terbit.

Darras FH, Pang Y. 2017. On the Use of the Experimentally Determined Enzyme Inhibition Constant as a Measure of Absolute Binding Affinity.

Fathalla RK, Engel M, Ducho C. 2023. Targeting the Binding Pocket of the Fluorophore 8- anilinonaphthalene-1-sulfonic Acid in the Bacterial Enzyme MurA. *Arch Pharm (Weinheim)*. 356(9).doi:10.1002/ardp.202300237.

Fathalla RK, Fröhner W, Bader CD, Fischer PD, Dahlem C, Chatterjee D, Mathea S, Kiemer AK, Arthanari H, Müller R, *et al*. 2022. Identification and Biochemical Characterization of Pyrrolidinediones as Novel Inhibitors of the Bacterial Enzyme MurA. *J Med Chem*. 65(21):14740–14763.doi:10.1021/acs.jmedchem.2c01275.

Filimonov DA, Lagunin A, Gloriozova T, Rudik A V, Druzhilovskii DS, Pogodin P, Poroikov V. 2014. Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. *Chem Heterocycl Compd*. 50:444–457. doi:10.1007/s10593-014-1496-1.

Frlan R, Hrast M, Gobec S. 2023. Inhibition of MurA Enzyme From <i>Escherichia Coli</I> by Flavonoids and Their Synthetic Analogues. *Acs Omega*. 8(36):33006–33016. doi:10.1021/acsomega.3c04813.

Geervani S, Neelakantappa KK, Merwade S, Abhishek M, Manimozhi M. 2025. In-Silico Insights Into Protein Targets: New Avenues for Treating E. Faecalis in Endodontic Infections – A Systematic Review.

Gil-Gil T, Corona F, Martínez JL, Bernardini A. 2020. The Inactivation of Enzymes Belonging to the Central Carbon Metabolism Is a Novel Mechanism of Developing Antibiotic Resistance. *Msystems*. 5(3). doi:10.1128/msystems.00282-20.

Golla VK, Sans-Serramitjana E, Pothula KR, Benier L, Bafna JA, Winterhalter M, Kleinekathöfer U. 2019. Fosfomycin Permeation Through the Outer Membrane Porin OmpF. *Biophys J*. 116(2):258–269. doi:10.1016/j.bpj.2018.12.002.

Hummels KR, Berry SP, Li Z, Taguchi A, Min J, Walker S, Marks DS, Bernhardt TG. 2023. Coordination of Bacterial Cell Wall and Outer Membrane Biosynthesis. *Nature*. 615(7951):300–304. doi:10.1038/s41586-023-05750-0.

Juillot D, Billaudeau C, Barbotin A, Lablaine A, Mortier-Barrière I, Polard P, Campo N, Carballido-López R. 2024. Transient Inhibition of Cell Division in Competent Pneumococcal Cells Results From Deceleration of the Septal Peptidoglycan Complex.

Kakkar S, Tahlan S, Lim SM, Ramasamy K, Mani V, Shah SA, Narasimhan B. 2018. Benzoxazole derivatives: design, synthesis and biological evaluation. *Chem Cent J*. 12. doi:10.1186/s13065-018-0459-5.

Kamaly OA, Younes AS, Ibrahim MH, Harras MF, Alsfouk AA, Sabour R. 2025. Insights Into Novel Arylazopyrazolo[1,5-<i>a</I>]pyrimidines as Promising MurA Inhibitors and Antibiofilm Candidates: Design, Synthesis, Antimicrobial Evaluation, and Molecular Docking. *Acs Omega*. 10(4):4044–4056. doi:10.1021/acsomega.4c10286.

Keeley A, Ábrányi-Balogh P, Hrast M, Imre T,

Ilaš J, Gobec S, Keserű GM. 2018. Heterocyclic Electrophiles as New MurA Inhibitors. *Arch Pharm (Weinheim)*. 351(12). doi:10.1002/ardp.201800184.

Kim DH, Lees WJ. 2025. Molecular Pharmacology of the Antibiotic Fosfomycin, an Inhibitor of Peptidoglycan Biosynthesis. *Biochemistry*.64(8):1720–1727. doi:10.1021/acs.biochem.4c00522.

Kurniawan I, Ambarsari L, Kurniatin P, Tri Wahyudi S. 2024. Novel Compounds Design of Acertannin, Hamamelitannin, and Petunidin-3-Glucoside Typical Compounds of African Leaves (Vernonia amygdalina Del) as Antibacterial Based on QSAR and Molecular Docking. *J Jamu Indones*. 8:29–38. doi:10.29244/jji.v8i2.326.

Kurniawan I, Zahra H. 2021. Review: Gallotannins; Biosynthesis, StructureActivity Relationship, Anti-inflammatory and Antibacterial Activity. *Curr Biochem*. 8:1– 16. doi:10.29244/cb.8.1.1.

Kuzmič P. 2022. Application of the “Kobs” Method for the Study of Tight-Binding Reversible Inhibitors.

Liang H, Zhou G, Yunhui G, D’Ambrosio EA, Eidem TM, Blanchard C, Shehatou C, Chatare VK, Dunman PM, Valentine AM, *et al*. 2018. Elucidating the Inhibition of Peptidoglycan Biosynthesis in Staphylococcus Aureus by Albocycline, a Macrolactone Isolated From Streptomyces Maizeus. *Bioorg Med Chem*. 26(12):3453– 3460. doi:10.1016/j.bmc.2018.05.017.

Liu QG, Luo A, Jin H, Si X, Li M. 2025. Machine Learning-Based Discovery of a Novel Noncovalent <scp>MurA</Scp> Inhibitor as an Antibacterial Agent. *Chem Biol Drug Des*.105(3). doi:10.1111/cbdd.70084.

Liu X, Tian W, Zhou M, Xu Q, Feng J, Yang R, He S, Wang G, Lin T, Chen H. 2022. Bisabolane-type sesquiterpenes from Vernonia amygdalina: Absolute configuration and anti-inflammatory activity [Cited By (since 2022): 5]. *Phytochemistry*.201. doi:10.1016/j.phytochem.2022.113283.

Mascari CA, Djorić D, Kristich CJ. 2025. PASTA Kinase Signaling Regulates Peptidoglycan Synthesis In <i>Enterococcus Faecalis</I> by Direct Inhibition of UDP-N- acetylglucosamine 1-Carboxyvinyl Transferase Activity. *MBio*. 16(5). doi:10.1128/mbio.00593-25.

Mihalovits LM, Ferenczy GG, Keserű GM. 2019. Catalytic Mechanism and Covalent Inhibition of UDP-*N*-Acetylglucosamine Enolpyruvyl Transferase (MurA): Implications to the Design of Novel Antibacterials. *J Chem Inf Model*. 59(12):5161–5173. doi:10.1021/acs.jcim.9b00691.

Ostberg-Potthoff JJ, Berger K, Richling E, Winterhalter P. 2019. Activity-Guided Fractionation of Red Fruit Extracts for the Identification of Compounds Influencing Glucose Metabolism. *Nutrients*. 11(5):1166. doi:10.3390/nu11051166.

Qi Y, Wang M, Zhang B, Liu Y, Zhang H, Ma Z, Jin N-Z, Lu H. 2021. Comparative Antibacterial Analysis of the Anthraquinone Compounds Based on the AIM Theory and Molecular Docking Analysis.

Reiss A, Dăbuleanu I. 2022. New metal(II) complexes with ceftazidime Schiff base. *Ann Univ Craiova Ser Chem*. 28:33–42. doi:10.52846/AUCCHEM.2022.2.04.

Rothe P, Wamp S, Rosemeyer L, Rismondo J, Doellinger J, Gründling A, Halbedel S. 2024. Cytosolic Factors Controlling PASTA Kinase-Dependent ReoM Phosphorylation.

Sangshetti JN, Joshi SS, Patil R, Moloney MG, Shinde DB. 2017. Mur Ligase Inhibitors as Anti-Bacterials: A Comprehensive Review. *Curr Pharm Des*. 23(21). doi:10.2174/1381612823666170214115048.

Satari MH, Primasari A, Dharsono HDA, Apriyanti E, Suprijono MM, Herdiyati Y, Kurnia D. 2021. Mode Action Prediction of Butein as Antibacterial Oral Pathogen Against Enterococcus Faecalis ATCC 29212 and an Inhibitor of MurA Enzyme: In Vitro and in Silico Study. *Lett Drug Des Discov*. 18(7):744–753. doi:10.2174/1570180818666210122163009.

Seo PW, Park S-Y, Hofmann A, Kim J. 2021. Crystal Structures of UDP-*N*-acetylmuramic Acid <scp>L</Scp>-Alanine Ligase (MurC) From <i>Mycobacterium Bovis</I> With and Without UDP-*N*-acetylglucosamine. *Acta Crystallogr Sect D Struct Biol*. 77(5):618–627. doi:10.1107/s2059798321002199.

Tang Z, Chen H, Zhang M, Fan Z, Zhong Q, Chen Weijun, Yun Y, Chen Wenxue. 2021. Antibacterial Mechanism of 3-Carene Against the Meat Spoilage Bacterium Pseudomonas Lundensis and Its Application in Pork During Refrigerated Storage. *Foods*. 11(1):92. doi:10.3390/foods11010092.

Windaryanti D, Gabriel CS, Hidayat IW,

Zainuddin A, Dharsono HDA, Satari MH, Kurnia D. 2022. The Potential of 24-Propylcholestrol as Antibacterial Oral Bacteria of Enterococcus Faecalis ATCC 29212 and Inhibitor Biofilms Formation: In Vitro and in Silico Study. *Adv Appl Bioinforma Chem*. Volume 15:99–111. doi:10.2147/aabc.s372337.

Xu W, Chen T, Wang H, Zeng W, Wu Q, Yu K, Xu Y, Zhang X, Zhou T. 2020. Molecular Mechanisms and Epidemiology of Fosfomycin Resistance in Staphylococcus Aureus Isolated From Patients at a Teaching Hospital in China. *Front Microbiol*. 11. doi:10.3389/fmicb.2020.01290.

Yaneva TG, Wiczkowski W, Marchev AS, Iserliyska D, Georgiev MI, Tomlekova N. 2024. Evaluation of Polyphenols Synthesized in Mature Seeds of Common Bean (Phaseolus Vulgaris L.) Advanced Mutant Lines. *Int J Mol Sci*. 25(11):5638. doi:10.3390/ijms25115638.

Yong G, Ji F, Qiao J, Dong X, Wu Y, Wang X. 2022. Overexpression of the Key Genes in the Biosynthetic Pathways of Lipid a and Peptidoglycan in *Escherichia coli*. *Biotechnol Appl Biochem*. 70(1):374–386. doi:10.1002/bab.2364.