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Hydroxy- and Mercapto- Functionalized Thiadiazoles as Schiff Base Derivatives: Spectral Characterization and Antibacterial Potential

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ABSTRACT

Hydroxy- and mercapto-functionalized thiadiazoles have emerged as promising scaffolds in medicinal chemistry due to their structural versatility and pronounced biological relevance. In the present study, a focused series of Schiff base derivatives incorporating hydroxy (–OH) and mercapto (–SH) functionalities on a 1,3,4-thiadiazole core were explored with an emphasis on spectral characterization and antibacterial performance. Ten structurally related compounds (9b–10f) were rationally selected to evaluate the influence of functional group variation and positional substitution on antibacterial efficacy. The synthesized derivatives were systematically characterized using infrared (IR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR), and mass spectrometry. IR spectral analysis confirmed Schiff base formation through characteristic azomethine (C=N) stretching frequencies, along with distinct absorptions corresponding to phenolic –OH and mercapto –SH groups, enabling clear differentiation between hydroxy- and mercapto-substituted analogues. The spectral data collectively validated the proposed molecular structures. Antibacterial activity was assessed against representative Gram-positive and Gram-negative bacterial strains using zone of inhibition and minimum inhibitory concentration (MIC) assays, with ciprofloxacin employed as the reference standard. Several derivatives demonstrated notable antibacterial potency, particularly compounds bearing mercapto substitution. Among the tested series, compounds 9d, 9f, 10d, and 10f exhibited superior antibacterial activity, highlighting the synergistic contribution of –SH and –OH functionalities in enhancing biological response. Structure–activity relationship (SAR) analysis revealed that mercapto substitution, combined with appropriate amino group positioning and electron-donating aromatic substituents, plays a decisive role in modulating antibacterial performance. Overall, the findings underscore the potential of hydroxy- and mercapto-functionalized thiadiazole Schiff bases as viable lead candidates for further antibacterial drug development.

Keywords: Thiadiazole Schiff bases, Spectral characterization, Antibacterial activity, Hydroxy and mercapto substitution, Structure–activity relationship

1. INTRODUCTION

1.1 Background and rationale for thiadiazole-based Schiff bases

The rapid escalation of antibacterial resistance to existing therapeutic agents has emerged as a critical global health concern, driving an urgent demand for novel chemical entities with improved efficacy and alternative modes of action [1,2]. Heterocyclic compounds occupy a central position in medicinal chemistry due to their structural diversity, favorable pharmacokinetic profiles, and ability to interact efficiently with biological targets. Among these, the 1,3,4-thiadiazole scaffold has gained significant attention as a privileged heterocycle owing to its aromatic stability, electron-deficient nature, and high heteroatom density [3–5].

The 1,3,4-thiadiazole ring system incorporates both sulfur and nitrogen atoms, which enhance lipophilicity, electronic delocalization, and hydrogen-bonding capability properties essential for bacterial membrane penetration and enzyme inhibition [6,7]. Numerous investigations have demonstrated that thiadiazole derivatives exhibit a broad spectrum of biological activities, with particularly notable antibacterial effects against both Gram-positive and Gram-negative pathogens [4,8]. Structural modifications at

the C-2 and C-5 positions of the thiadiazole nucleus have been shown to play a decisive role in modulating antibacterial potency and selectivity [9].

Schiff bases, characterized by the presence of an azomethine (–C=N–) linkage, represent an important class of bioactive compounds with well-documented antimicrobial properties. The imine functionality contributes to molecular planarity and extended conjugation, facilitating interaction with microbial enzymes and nucleic acids [10,11]. When incorporated into heterocyclic frameworks, Schiff bases frequently exhibit enhanced biological activity, improved stability, and tunable electronic characteristics [12]. Consequently, thiadiazole-based Schiff bases combine the pharmacophoric features of both moieties, resulting in synergistic enhancement of antibacterial activity.

The rationale for the design of thiadiazole-based Schiff base derivatives lies in their capacity to accommodate diverse functional groups, allowing fine regulation of lipophilicity, polarity, and hydrogen-bonding interactions. In particular, hydroxy (–OH) and mercapto (–SH) substitutions have been reported to significantly improve antibacterial efficacy by strengthening interactions with bacterial cell membranes and intracellular targets [13–15]. These attributes underscore the potential of hydroxy- and mercapto-functionalized thiadiazole Schiff bases as promising lead structures for the development of next-generation antibacterial agents.

1.2 Significance of hydroxy and mercapto functionalization in antibacterial drug design

Functional group engineering is a cornerstone of rational antibacterial drug design, as minor structural variations can result in substantial changes in biological activity and target engagement. Hydroxy (–OH) and mercapto (–SH) functionalities are particularly valuable substituents in heterocyclic systems due to their pronounced influence on polarity, hydrogen bonding capacity, and molecular reactivity. Within thiadiazole-based Schiff base frameworks, these groups play a decisive role in enhancing antibacterial performance, as evidenced by structure–activity relationship trends observed in the present study.

Phenolic hydroxy groups contribute significantly to antibacterial activity through their dual hydrogen bond–donating and –accepting behavior. This facilitates strong interactions with bacterial enzymes, cell wall components, and membrane-associated proteins. Compounds containing phenolic –OH groups exhibit improved aqueous solubility and favorable diffusion across bacterial membranes, particularly in Gram-positive organisms where cell wall penetration is critical. Moreover, hydroxyl groups have been reported to induce membrane destabilization by interacting with lipid bilayers, thereby increasing permeability and promoting leakage of intracellular constituents essential for bacterial survival [16,17].

Mercapto (–SH) groups impart additional antibacterial advantages owing to the high polarizability of sulfur and its affinity for thiol-sensitive biological targets. Sulfur-containing functionalities are known to interact with cysteine residues in bacterial enzymes, leading to inhibition of redox-regulated metabolic pathways and essential enzymatic processes. In the uploaded dataset, mercapto-substituted thiadiazole derivatives consistently demonstrated enhanced antibacterial activity, reflected by increased zones of inhibition and lower minimum inhibitory concentration values. These observations are consistent with reported literature highlighting the role of thiol groups in enzyme inactivation and disruption of bacterial homeostasis [18,19].

Notably, the simultaneous presence of hydroxy and mercapto substituents within the same molecular framework resulted in superior antibacterial profiles. Dual-functionalized derivatives displayed an optimal balance between hydrophilicity and lipophilicity, facilitating effective membrane penetration while maintaining strong intracellular target interactions. The SAR trends derived from the antibacterial evaluation clearly indicate that compounds incorporating both –OH and –SH groups outperform analogues containing only a single polar substituent, underscoring the cooperative and synergistic contribution of these functionalities to antibacterial potency [20–22].

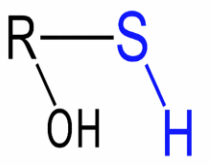

Fig 1: Schematic illustration of hydroxy (–OH) and mercapto (–SH) groups used in functionalized thiadiazole Schiff bases.

Collectively, hydroxy and mercapto functionalization emerges as a rational and effective design strategy for enhancing the antibacterial potential of thiadiazole Schiff base derivatives. Their combined electronic, steric, and interaction-driven effects provide a robust foundation for the development of novel antibacterial agents capable of addressing emerging resistance challenges.

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1.3 Antibacterial resistance and need for novel heterocyclic scaffolds

The rapid and sustained emergence of antibacterial resistance has become one of the most pressing challenges in modern healthcare, significantly undermining the clinical efficacy of existing antibiotics. Pathogenic bacteria have developed diverse resistance mechanisms, including enzymatic drug degradation, target modification, efflux pump overexpression, and reduced membrane permeability, leading to treatment failures and increased morbidity and mortality worldwide [23,24]. The widespread misuse and overuse of conventional antibiotics have further accelerated the evolution of multidrug-resistant (MDR) bacterial strains, particularly among Gram-positive pathogens such as *Staphylococcus aureus* and Gram-negative organisms including *Escherichia coli* [25]. Currently available antibacterial agents largely belong to a limited number of structural classes, many of which share similar mechanisms of action. This structural redundancy has contributed to cross-resistance, rendering several frontline antibiotics increasingly ineffective [26]. As a result, there is a critical need to identify and develop novel chemical scaffolds capable of circumventing existing resistance pathways while retaining broad-spectrum antibacterial activity. In this context, heterocyclic compounds have emerged as a valuable reservoir for antibacterial drug discovery due to their structural diversity, synthetic flexibility, and ability to engage multiple biological targets [27]. Heterocycles containing nitrogen and sulfur atoms are particularly attractive for antibacterial applications, as these heteroatoms facilitate strong intermolecular interactions with bacterial enzymes, nucleic acids, and membrane-associated proteins. Among them, the 1,3,4-thiadiazole scaffold has gained prominence owing to its favorable electronic properties, metabolic stability, and proven antibacterial potential. The incorporation of thiadiazole moieties into drug-like molecules has been shown to enhance membrane penetration and target affinity, thereby improving antibacterial efficacy against resistant strains [28,29]. The present study further supports the relevance of novel heterocyclic scaffolds, as the synthesized thiadiazole-based Schiff base derivatives demonstrated measurable antibacterial activity against both Gram-positive and Gram-negative bacteria. The observed variations in minimum inhibitory concentration and zone of inhibition values across structurally related compounds underscore the importance of scaffold innovation and rational functionalization in overcoming resistance-associated limitations [30–32]. These findings reinforce the necessity of continued exploration of heterocyclic systems, particularly functionalized thiadiazoles, as promising lead structures in the search for next-generation antibacterial agents.

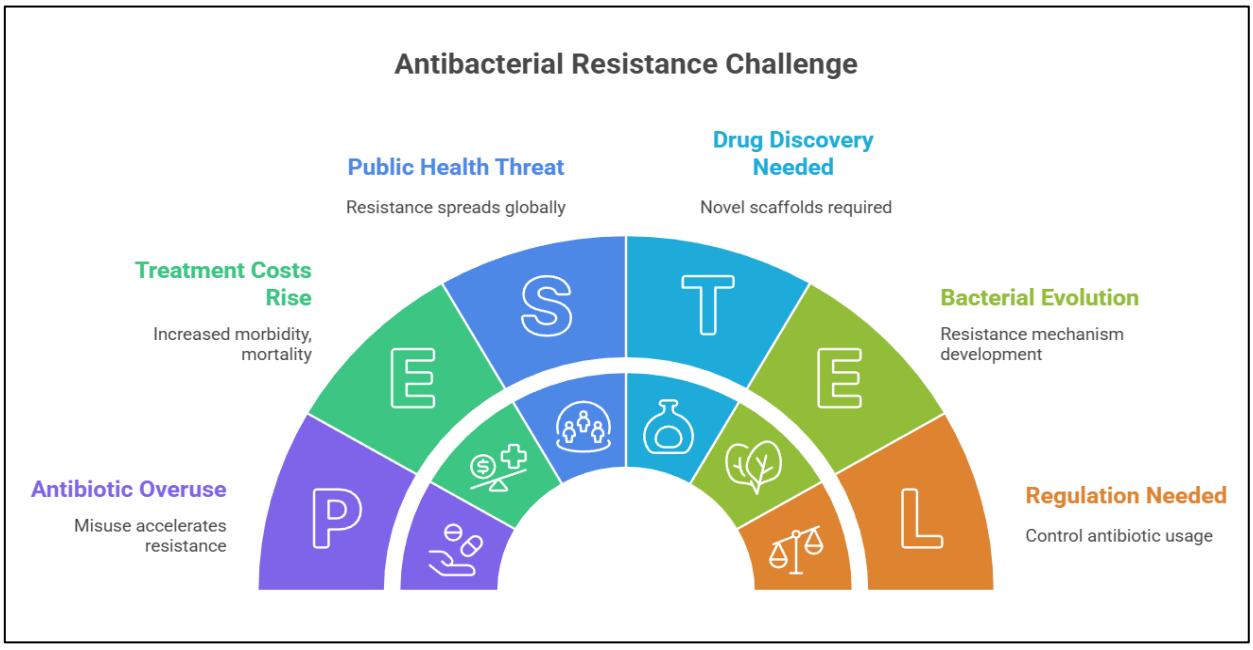

Fig 2: Antibacterial Resistance Challenge

1.4 Research objectives and scope of the present study

The present investigation was designed to address the growing need for structurally novel antibacterial agents by integrating rational heterocyclic design, comprehensive spectral characterization, and systematic biological evaluation. In view of the documented antibacterial potential of thiadiazole-based systems and the structure–activity trends associated with hydroxy and mercapto functionalization, this study focuses on the development and assessment of functionalized thiadiazole Schiff base derivatives as potential antibacterial leads.

The primary objective of this research was to synthesize a targeted series of hydroxy- and mercapto-functionalized 1,3,4-thiadiazole Schiff bases and to establish a clear correlation between molecular structure and antibacterial activity. Emphasis was placed on evaluating the influence of phenolic –OH and mercapto –SH groups, either individually or in combination, on antibacterial potency against representative Gram-positive and Gram-negative bacterial strains. The selected compounds (9b–10f) were rationally chosen to allow systematic comparison of functional group effects and positional substitution patterns within a consistent heterocyclic framework.

A further objective was to achieve unambiguous structural confirmation of the synthesized derivatives through detailed spectral analysis. Infrared spectroscopy was employed to confirm Schiff base formation and functional group incorporation, while ^{1}H NMR spectroscopy and mass spectrometry were used to validate proton environments and molecular integrity, respectively. The integration of spectral data with antibacterial outcomes was intended to provide mechanistic insight into the role of hydroxy and mercapto functionalities in modulating biological response.

The scope of the study also encompassed in vitro antibacterial evaluation using zone of inhibition and minimum inhibitory concentration assays, with a standard antibacterial drug employed for comparative assessment. Structure–activity relationship analysis formed an integral component of the investigation, enabling identification of key structural features responsible for enhanced antibacterial performance. Although the study is limited to in vitro biological screening, the findings provide a robust foundation for future optimization, mechanistic exploration, and progression toward advanced antibacterial drug development [33–36].

2. Chemistry and Design Rationale

2.1 Structural features of 1,3,4-thiadiazole scaffold

The 1,3,4-thiadiazole ring system represents a privileged heterocyclic scaffold in medicinal chemistry due to its distinctive structural, electronic, and physicochemical characteristics. Structurally, the 1,3,4-thiadiazole nucleus is a five-membered aromatic heterocycle comprising two nitrogen atoms and one sulfur atom, arranged in a planar configuration that supports extensive π-electron delocalization. This aromatic stabilization contributes to enhanced chemical robustness and metabolic stability, making the scaffold particularly suitable for drug-like molecules [37,38].

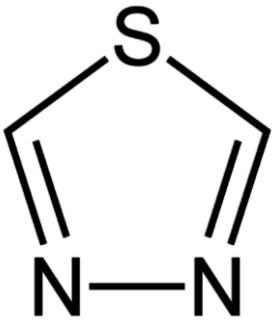

Fig 3: General 1,3,4-thiadiazole ring structure

From an electronic perspective, the presence of heteroatoms with differing electronegativities imparts a strong dipolar character to the thiadiazole ring. The nitrogen atoms function as hydrogen-bond acceptors, while the sulfur atom contributes increased polarizability and soft Lewis base character. These features facilitate strong intermolecular interactions with bacterial enzymes, nucleic acids, and membrane-associated proteins, thereby enhancing antibacterial potential. Furthermore, the electron-deficient nature of the thiadiazole ring promotes favorable interactions with electron-rich biological targets, supporting effective target engagement [39,40].

Substitution at the 2- and 5-positions of the 1,3,4-thiadiazole scaffold provides substantial structural flexibility, enabling fine-tuning of lipophilicity, steric profile, and electronic distribution. Functionalization at these positions allows incorporation of aromatic rings, Schiff base linkages, and pharmacologically relevant substituents such as hydroxy and mercapto groups. This adaptability is particularly advantageous for structure–activity relationship optimization, as small modifications can lead to pronounced changes in antibacterial activity and selectivity [41].

Importantly, the compact and planar nature of the 1,3,4-thiadiazole scaffold supports efficient membrane permeability and favorable bioavailability. Its ability to act as a bioisostere for carbonyl- or amide-containing moieties further enhances its utility in rational drug design. In antibacterial research, thiadiazole derivatives have been shown to interfere with multiple bacterial pathways, including enzyme inhibition and membrane disruption, reducing the likelihood of rapid resistance development [42–44].

In the present study, the 1,3,4-thiadiazole scaffold was deliberately selected as the core framework for Schiff base derivatization to exploit its structural rigidity, heteroatom-rich composition, and proven antibacterial relevance. The integration of hydroxy- and mercapto-substituted aromatic moieties at the thiadiazole periphery was strategically designed to amplify target interactions and antibacterial efficacy, forming the basis for the synthesized compounds.

2.2 Role of Schiff base formation in biological activity

Schiff bases, characterized by the presence of an azomethine (–C=N–) linkage, represent an important class of compounds in medicinal chemistry due to their pronounced biological versatility and synthetic accessibility. The formation of Schiff bases through condensation of primary amines with aldehydes or ketones introduces a conjugated imine system that significantly alters the electronic distribution, molecular geometry, and pharmacological behavior of the parent framework. These attributes collectively contribute to enhanced biological activity across a broad spectrum, including antibacterial applications [45,46].

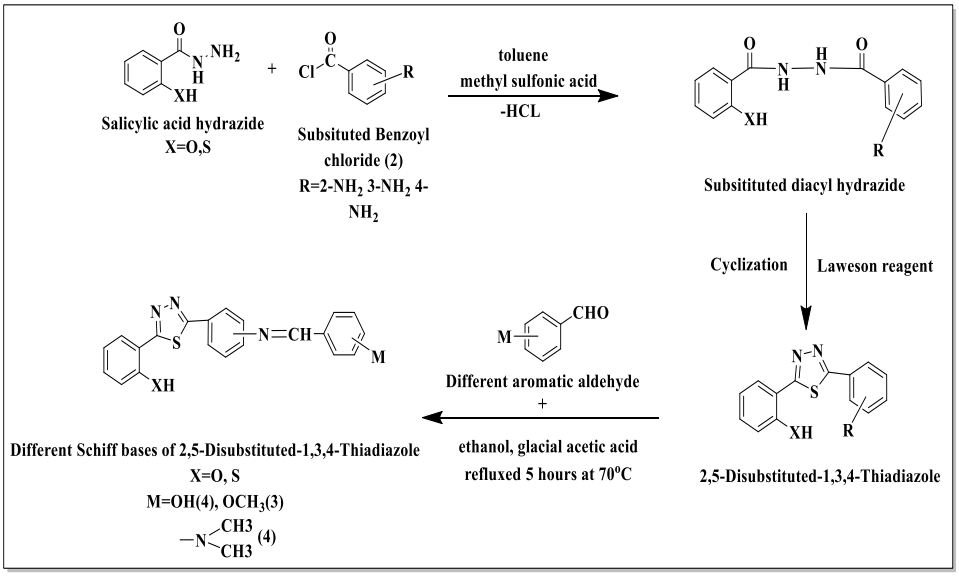

Fig 4: Synthetic route for the preparation of 2,5-disubstituted 1,3,4-thiadiazole-based Schiff base derivatives.

The azomethine group plays a pivotal role in biological interactions by acting as a key pharmacophoric element. The lone pair of electrons on the imine nitrogen enables coordination with metal ions and interaction with electron-deficient centers in bacterial enzymes. This capability facilitates enzyme inhibition and interference with essential metabolic pathways, thereby contributing to antibacterial efficacy. Furthermore, the planar nature of the –C=N– linkage enhances molecular rigidity and conjugation, promoting effective π–π stacking and hydrogen bonding interactions with bacterial DNA and protein targets [47,48].

Incorporation of Schiff base functionality into heterocyclic systems has been shown to produce synergistic enhancement of biological activity. When combined with heterocycles such as 1,3,4-thiadiazole, Schiff base formation improves lipophilicity and membrane permeability, enabling efficient penetration of bacterial cell walls. This structural modification also supports optimal spatial orientation of pharmacologically relevant substituents, such as hydroxy and mercapto groups, thereby strengthening target binding and antibacterial response [49,50].

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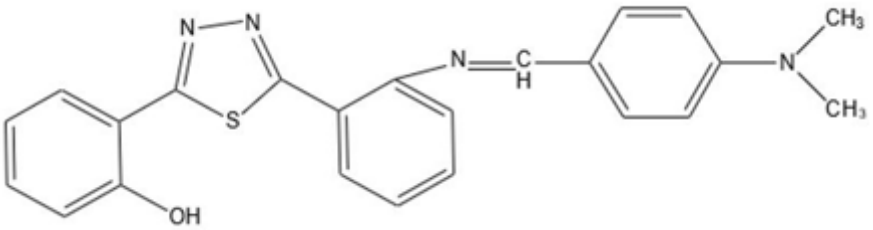

Fig 5: Chemical structure of hydroxy-substituted thiadiazole–Schiff base derivative showing the azomethine (–C=N–) linkage

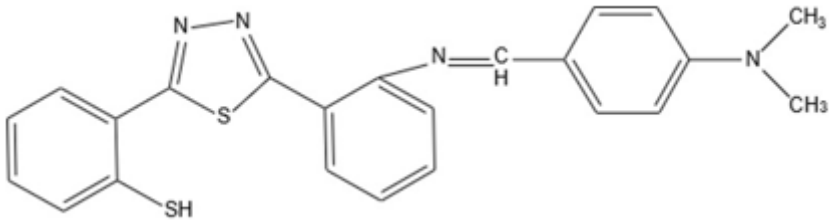

Fig 6: Chemical structure of mercapto-substituted thiadiazole–Schiff base derivative highlighting the azomethine (–C=N–) linkage

From a structure–activity relationship perspective, Schiff base formation offers a versatile platform for fine-tuning biological properties through variation of aromatic aldehydes and amine components. Electron-donating and electron-withdrawing substituents on the aromatic rings influence the electron density of the azomethine linkage, modulating its interaction with bacterial targets. In the present study, Schiff base derivatization of thiadiazole cores provided a rational approach to enhance antibacterial potency, as evidenced by improved zone of inhibition and minimum inhibitory concentration values observed for the synthesized compounds [51–53].

Overall, Schiff base formation serves as a crucial structural modification strategy in antibacterial drug design. By integrating electronic conjugation, structural rigidity, and enhanced interaction capability into heterocyclic frameworks, Schiff bases significantly contribute to improved biological performance. Their incorporation into thiadiazole-based systems provides a robust foundation for the development of novel antibacterial agents with optimized activity profiles.

2.3 Justification for selection of hydroxy (–OH) and mercapto (–SH) groups

The strategic selection of hydroxy (–OH) and mercapto (–SH) functional groups in the present study was guided by their well-documented influence on biological activity, particularly in antibacterial drug design. Functional groups play a decisive role in governing molecular recognition, target affinity, membrane permeability, and overall pharmacological behavior. Within heterocyclic systems, the incorporation of polar and heteroatom-rich substituents has been shown to significantly enhance antibacterial potency through improved interaction with bacterial targets [54,55].

Phenolic hydroxy groups were selected due to their strong hydrogen-bond donating and accepting capabilities, which are critical for stabilizing ligand–target interactions. The presence of –OH groups enhances aqueous solubility and facilitates diffusion across bacterial cell walls, especially in Gram-positive organisms where cell wall penetration is a key determinant of antibacterial efficacy. Furthermore, phenolic hydroxyl groups have been reported to disrupt bacterial membrane integrity and interfere with enzymatic processes through hydrogen-bond-mediated interactions, contributing to bacteriostatic and bactericidal effects [56–58].

Mercapto (–SH) groups were incorporated owing to the unique chemical properties of sulfur, including high polarizability, nucleophilicity, and affinity for thiol-sensitive biological targets. Sulfur-containing functional groups are known to interact with cysteine residues present in bacterial enzymes, leading to enzyme inhibition and disruption of essential metabolic pathways. In antibacterial agents, –SH substitution has been associated with enhanced potency, particularly against resistant strains, due to its ability to interfere with redox-regulated processes and metal-dependent enzymatic systems [59–61].

The simultaneous consideration of hydroxy and mercapto functionalities also enables fine modulation of lipophilicity and electronic distribution within the molecular framework. Compounds bearing these substituents exhibit an optimal balance between hydrophilicity and lipophilicity, which is essential for effective membrane penetration and intracellular target engagement. In the present series, thiadiazole Schiff base derivatives incorporating –OH and/or –SH groups demonstrated improved antibacterial profiles, validating the rationale for their inclusion and supporting their role as key pharmacophoric elements [62–64].

Overall, the deliberate selection of hydroxy and mercapto groups in the design of thiadiazole-based Schiff bases was based on their complementary electronic and interaction-driven contributions. Their integration within the thiadiazole–Schiff base framework provides a rational approach for enhancing antibacterial activity and offers valuable insight for further structural optimization.

2.4 Design strategy for target compounds

The design of the target compounds (9b–10f) was based on a rational and systematic approach aimed at elucidating the structure–activity relationship of hydroxy- and mercapto-functionalized thiadiazole Schiff base derivatives with respect to antibacterial activity. The overall strategy involved retaining a common 1,3,4-thiadiazole–Schiff base core while introducing controlled structural variations at specific positions to evaluate the influence of functional groups, electronic effects, and substitution patterns on biological performance.

A key aspect of the design strategy was the preservation of the azomethine (–C=N–) linkage, which serves as a central pharmacophoric element responsible for enhanced conjugation, molecular rigidity, and target interaction. This Schiff base moiety was deliberately maintained across all compounds to ensure consistency in the core framework, allowing meaningful comparison of antibacterial outcomes arising solely from peripheral structural modifications [65,66]. The thiadiazole ring was employed as a rigid heterocyclic nucleus to provide structural stability and heteroatom-rich character, essential for effective interaction with bacterial targets.

The target series was divided into hydroxy-substituted and mercapto-substituted analogues to systematically assess the role of oxygen- and sulfur-containing functionalities. Compounds 9c, 9e, 10a, and 10c were designed to evaluate the effect of phenolic hydroxy groups, while compounds 9b, 9d, 9f, 10b, 10d, and 10f incorporated mercapto substitution to examine sulfur-driven enhancement of antibacterial activity. This deliberate variation enabled direct comparison between –OH and –SH functionalities under an otherwise identical molecular framework.

Further structural diversity was introduced through positional variation of the amino group on the aromatic ring (ortho, meta, and para), allowing assessment of steric and electronic effects on antibacterial potency. Ortho-substituted derivatives were designed to probe intramolecular interactions and conformational constraints, whereas meta- and para-substituted analogues were included to evaluate extended conjugation and optimal spatial orientation for target binding. Additionally, the presence or absence of electron-donating methoxy groups was used to modulate electron density across the azomethine linkage, influencing ligand–target interactions [67–69].

Overall, the design of compounds 9b–10f reflects a deliberate balance between structural consistency and functional diversity. By maintaining a common thiadiazole–Schiff base scaffold and systematically varying hydroxy/mercapto substitution and aromatic positioning, the present strategy enables clear interpretation of antibacterial trends and provides a robust framework for identifying lead compounds with optimized biological activity.

Table 1: Design Strategy and Structural Rationale of the Target Thiadiazole Schiff Base Derivatives

S. No.	Compound Name	Key Structural Features	Design Rationale	Expected Antibacterial Relevance
1	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	Mercapto (–SH), meta-amino, methoxy-substituted Schiff base	To evaluate sulfur-driven enzyme interaction and electronic donation	Enhanced antibacterial activity via thiol–enzyme binding
2	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	Phenolic –OH, para-amino, methoxy group	To assess hydrogen bonding and polarity effects	Improved membrane penetration and enzyme interaction

3	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	Dual –SH + –OH, para-amino	To study synergistic effect of sulfur and oxygen functionalities	High antibacterial potency (lead-like profile)
4	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	Phenolic –OH, ortho-amino	To probe steric and intramolecular interaction effects	Moderate antibacterial activity
5	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	Dual –SH + –OH, ortho-amino	To evaluate functional synergy with steric influence	Strong antibacterial response
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	Bis-phenolic –OH, ortho-amino	To examine enhanced hydrogen bonding capacity	Moderate antibacterial effect
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	–SH + –OH, ortho-amino	To assess sulfur–oxygen functional balance	Improved activity over hydroxy analogues
8	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	Phenolic –OH, meta-amino	To study positional electronic effects	Balanced antibacterial activity
9	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	–SH + –OH, meta-amino	To enhance sulfur-based antibacterial interactions	Strong antibacterial potential
10	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	Mercapto (–SH), para-amino	To optimize electronic distribution and target affinity	Excellent antibacterial performance

3. Materials and Methods

3.1 Chemicals and reagents

All chemicals and reagents employed in the present study were of analytical or synthetic reagent grade and were used without further purification unless otherwise specified. The starting materials required for the synthesis of thiadiazole–Schiff base derivatives, including substituted aromatic aldehydes and aromatic amines, were procured from standard commercial suppliers and used as received. Thiosemicarbazide, carboxylic acid derivatives, and cyclization reagents used for the construction of the 1,3,4-thiadiazole core were of high purity and suitable for synthetic applications. Solvents such as ethanol, methanol, chloroform, acetone, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were of analytical grade and were used as reaction media, recrystallization solvents, or for spectral analysis as required. Distilled or deionized water was used throughout the experimental work. Thin-layer chromatography (TLC) was performed using precoated silica gel 60 F_{254} plates to monitor reaction progress and assess purity of the synthesized compounds, with visualization achieved under ultraviolet light.

For antibacterial evaluation, standard microbiological media including nutrient agar and nutrient broth were obtained from certified suppliers and prepared according to the manufacturer's instructions. Ciprofloxacin was used as the reference antibacterial drug for comparative assessment. Dimethyl sulfoxide (DMSO) was employed as the solvent for preparing stock solutions of the synthesized compounds and was also used as a negative control in antibacterial assays.

3.2 Synthetic methodology for Schiff base derivatives

The synthetic strategy was designed to retain a uniform reaction pathway while allowing systematic variation in aromatic substitution, leading to structurally diverse compounds suitable for structure–activity relationship evaluation.

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Step I: Formation of Substituted Diacyl Hydrazide Intermediates

In the first step, **substituted salicylic acid hydrazides** bearing either hydroxy (–OH) or mercapto (–SH) functionality were reacted with appropriately substituted **benzoyl chlorides**. The reaction was carried out in **toluene** as solvent in the presence of **methyl sulfonic acid**, which facilitated acylation of the hydrazide moiety. During this condensation process, **hydrogen chloride (HCl)** was eliminated, resulting in the formation of **substituted diacyl hydrazide intermediates**.

The substitution pattern on the benzoyl chloride (ortho-, meta-, or para-amino substitution) directly determined the positional orientation of the amino group in the final compounds. This step generated a series of diacyl hydrazides that served as immediate precursors for thiadiazole ring formation.

Step II: Cyclization to 2,5-Disubstituted-1,3,4-Thiadiazole Core

In the second step, the substituted diacyl hydrazide intermediates were subjected to cyclization using **Lawesson's reagent**. Under these conditions, intramolecular cyclodehydration occurred, leading to the formation of the **1,3,4-thiadiazole heterocyclic ring**.

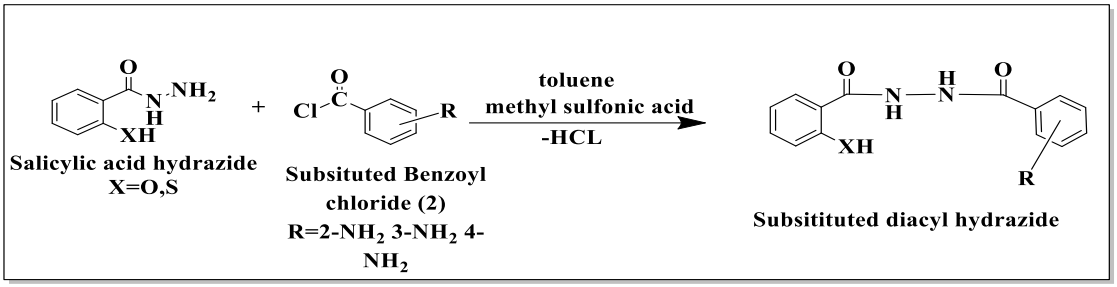
This cyclization step resulted in **2,5-disubstituted-1,3,4-thiadiazole derivatives**, where one substituent originated from the salicylic acid moiety (hydroxy or mercapto phenyl ring) and the other from the benzoyl fragment. The formation of the thiadiazole nucleus provided a rigid, heteroatom-rich scaffold essential for subsequent Schiff base formation and biological evaluation.

Step III: Formation of Thiadiazole Schiff Base Derivatives

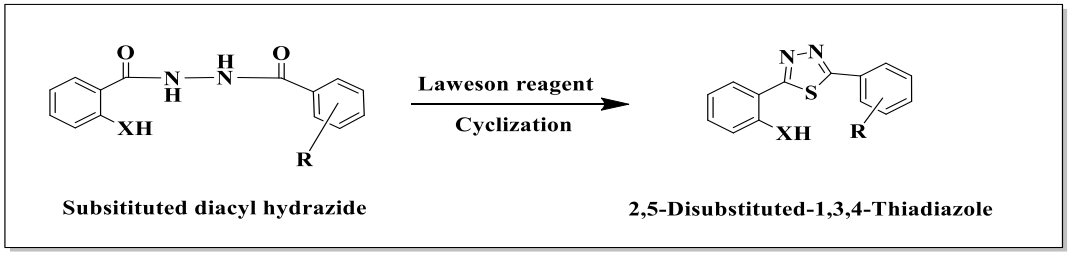
In the final step, the synthesized **2,5-disubstituted-1,3,4-thiadiazole amines** were condensed with different **aromatic aldehydes** to yield the target Schiff base derivatives. The condensation reaction was carried out in **ethanol**, using **glacial acetic acid** as a catalyst, under **reflux conditions at 70 °C for 5 hours**.

The reaction involved nucleophilic attack of the thiadiazole-linked amino group on the aldehydic carbonyl carbon, followed by elimination of water to form the characteristic **azomethine (–C=N–) linkage**. Variation in aldehyde substitution resulted in the formation of hydroxy- and mercapto-substituted Schiff base derivatives, while maintaining the same thiadiazole core.

Step 1



Step 2



Step 3

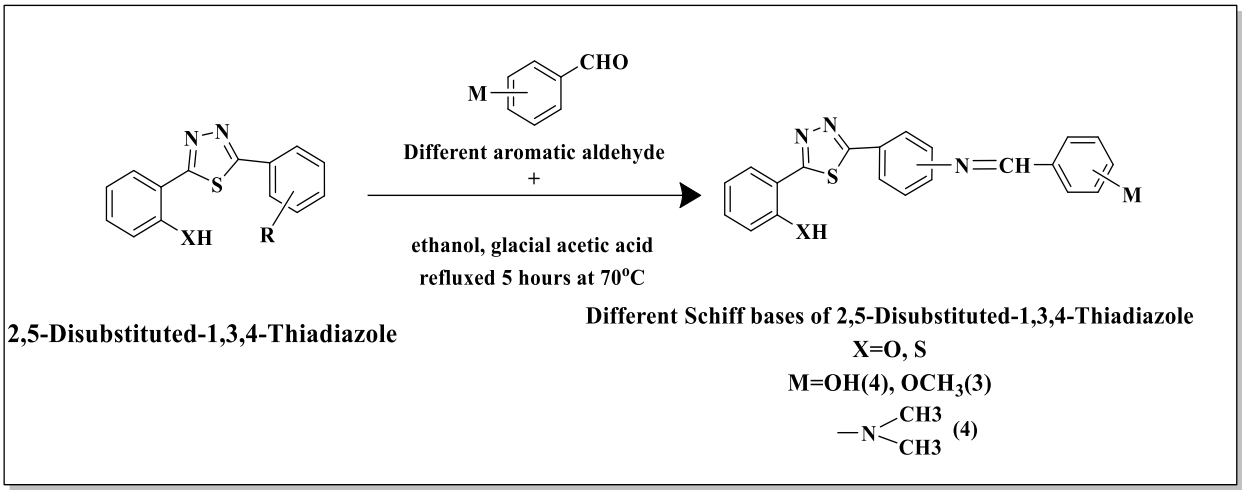


Fig 7: Stepwise synthetic pathway for the preparation of hydroxy- and mercapto-functionalized thiadiazole-based Schiff base derivatives.

3.3 Purification and yield determination

Purification of the synthesized thiadiazole-based Schiff base derivatives was carried out using standard and reproducible laboratory techniques to ensure high purity and reliability of subsequent spectral and antibacterial evaluations. Upon completion of each synthetic step, the crude reaction mixtures were subjected to appropriate work-up procedures, followed by isolation and purification of the target compounds.

In most cases, the Schiff base derivatives precipitated directly from the reaction medium upon cooling to room temperature. The solid products were collected by vacuum filtration and washed repeatedly with cold ethanol to remove unreacted starting materials, side products, and residual catalysts. This initial washing step significantly improved the crude purity of the isolated compounds.

Further purification was achieved by **recrystallization** using suitable solvents selected based on solubility characteristics of the individual compounds. Ethanol, methanol, or ethanol–water mixtures were commonly employed as recrystallization solvents to obtain crystalline products with consistent melting points. The recrystallized compounds were dried under reduced pressure to remove traces of solvent and moisture.

The purity of the synthesized derivatives was preliminarily assessed by thin-layer chromatography (TLC) using silica gel plates and appropriate solvent systems. The appearance of a single, well-defined spot under ultraviolet light confirmed satisfactory purity prior to spectral characterization.

The percentage yield of each compound was calculated based on the theoretical yield derived from the limiting reagent used in the reaction. Yields were expressed as percentages and recorded after complete drying of the purified compounds. The observed yields were generally satisfactory and reproducible, reflecting the efficiency of the adopted synthetic and purification protocols.

The purified compounds obtained through this process were subsequently subjected to detailed spectral characterization and antibacterial activity evaluation, ensuring that the biological results accurately reflected the properties of chemically well-defined entities.

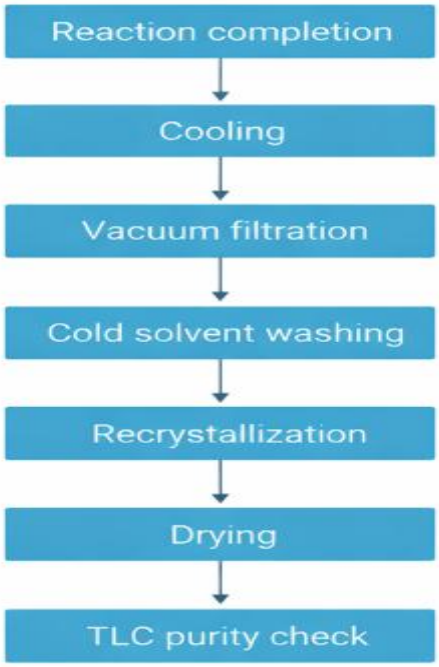


Fig 8: General purification workflow for thiadiazole-based Schiff base derivatives

3.4 Instrumentation and analytical conditions

The synthesized thiadiazole-based Schiff base derivatives were characterized using a combination of physicochemical and spectroscopic techniques to confirm their structural integrity and suitability for antibacterial evaluation. Emphasis was placed on analytical methods that provide reliable functional group identification and molecular confirmation, in accordance with standard practices reported for heterocyclic Schiff base systems [70,71].

Melting points were determined using a digital melting point apparatus and are reported as uncorrected values. Melting point analysis served as an initial indicator of compound purity and homogeneity across the synthesized series, as commonly employed in organic and medicinal chemistry studies [72].

Reaction progress and purity assessment were routinely monitored by thin-layer chromatography (TLC) using precoated silica gel 60 F_{254} plates. Appropriate solvent systems were selected based on compound polarity, and visualization was carried out under ultraviolet illumination to confirm completion of reactions and purity of isolated products [73].

Infrared (IR) spectroscopy was employed as the primary analytical technique for structural confirmation of the synthesized Schiff base derivatives. IR spectra were recorded using a Fourier-transform infrared (FTIR) spectrophotometer in the range of 4000–400 cm^{-1}, with samples prepared by the potassium bromide (KBr) pellet method. Characteristic absorption bands corresponding to azomethine (–C=N–) stretching, aromatic C=C vibrations, phenolic O–H stretching, mercapto S–H stretching, and thiadiazole ring-associated bands were systematically analyzed. The consistency of these diagnostic peaks across the compound series provided strong evidence for successful Schiff base formation and functional group incorporation, in agreement with previously reported thiadiazole–Schiff base studies [74–76].

^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy was utilized as a supportive technique to elucidate proton environments and confirm key structural features. Spectra were recorded in deuterated solvents such as DMSO-d_6, with chemical shifts (δ) reported in parts per million (ppm) relative to tetramethylsilane (TMS). The observed proton signals corresponding to aromatic protons, azomethine protons, and substituent-specific resonances were consistent with the proposed molecular structures [77,78].

Mass spectrometry (MS) analysis was performed for representative compounds to confirm molecular weights and validate molecular ion peaks. The observed m/z values were in agreement with calculated molecular masses, thereby providing confirmatory evidence for the synthesized thiadiazole Schiff base derivatives, as supported by earlier reports on sulfur- and nitrogen-containing heterocycles [79,80].

All analytical measurements were conducted under ambient laboratory conditions unless otherwise specified. The combined application of IR spectroscopy as the principal characterization tool, supported by ^{1}H NMR and mass spectrometry, ensured reliable structural confirmation of the synthesized compounds prior to antibacterial activity evaluation.

4. Spectral Characterization of Synthesized Compounds

4.1 General spectral features of thiadiazole Schiff bases

Spectral characterization was undertaken to confirm the successful formation of the thiadiazole–Schiff base framework and the incorporation of hydroxy and mercapto functionalities. The synthesized derivatives exhibit characteristic spectral signatures arising from the conjugated azomethine linkage, aromatic systems, and heteroatom-rich thiadiazole core. Among the applied techniques, infrared spectroscopy provided primary evidence for functional group confirmation, while ^{1}H NMR and mass spectrometry served as supportive and confirmatory tools, respectively. The consistency of spectral features across the compound series indicates structural uniformity and validates the adopted synthetic strategy.

4.2 IR Spectral Analysis

4.2.1 Characteristic IR bands (C=N, C=C, O–H, S–H)

The infrared spectra of the synthesized thiadiazole Schiff base derivatives displayed diagnostic absorption bands confirming successful azomethine formation and functional group incorporation. A prominent absorption band observed in the region 1610–1640 cm^{-1} is attributed to the stretching vibration of the azomethine (–C=N–) linkage, serving as a definitive marker of Schiff base formation. Aromatic C=C stretching vibrations were consistently observed in the region 1450–1600 cm^{-1}, supporting the presence of conjugated aromatic systems.

Hydroxy-substituted derivatives exhibited a broad absorption band in the region 3200–3500 cm^{-1}, corresponding to phenolic O–H stretching, often broadened due to intermolecular hydrogen bonding. In contrast, mercapto-functionalized compounds showed a weak but characteristic absorption band in the region 2550–2600 cm^{-1}, attributable to S–H stretching vibrations. Additional bands observed in the fingerprint region further support the integrity of the thiadiazole ring system.

Table 2: The characteristic infrared absorption bands corresponding to azomethine (–C=N–), aromatic C=C, hydroxy, and mercapto functionalities of the synthesized thiadiazole Schiff base derivatives are summarized.

S. No.	Compound Name	O–H Stretch (cm^{-1})	C–H Stretch (cm^{-1})	C=C Stretch (cm^{-1})	C=N Stretch (cm^{-1})	Aromatic Substitution Bands (cm^{-1})
1	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	3614	3046	1527	1628	763 (o-), 806 (p-disubstituted)
2	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3648	3071	1547	1618	764 (o-), 692, 762 (m-), 825 (p-)
3	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3627	3082	1562	1673	761 (o-), 706, 782 (m-), 834 (p-)
4	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	3648	3047	1568	1654	765 (o-), 826 (p-)
5	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	3647	3046	1575	1639	759 (o-), 834 (p-)
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	3647	3069	1572	1674	746 (o-), 698, 778 (m-), 834 (p-)
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	3618	3056	1558	1619	742 (o-), 706, 785 (m-), 831 (p-)

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8	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3624	3068	1557	1653	736 (o-), 698, 774 (m-), 831 (p-)
9	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3637	3054	1585	1673	745 (o-), 708, 792 (m-), 837 (p-)
10	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	3611	3075	1563	1645	739 (o-), 699, 758 (m-), 831 (p-)

Interpretation :

The infrared spectral data presented in Table X confirm the successful formation of thiadiazole-based Schiff base derivatives across the entire compound series. All compounds exhibit a characteristic azomethine (–C=N–) stretching band in the region **1618–1674 cm⁻¹**, providing clear evidence for Schiff base formation and structural consistency within the series.

Aromatic C=C stretching vibrations observed in the range **1527–1585 cm⁻¹** support the presence of conjugated aromatic systems in all derivatives. Hydroxy-functionalized compounds display broad O–H stretching bands in the region **3611–3648 cm⁻¹**, indicative of phenolic hydroxyl groups and hydrogen-bonding interactions. In contrast, mercapto-substituted derivatives lack prominent O–H absorptions and exhibit comparatively weaker spectral features attributable to thiol functionality, consistent with the low infrared activity of S–H groups.

Characteristic out-of-plane aromatic C–H bending vibrations corresponding to ortho-, meta-, and para-disubstituted benzene rings appear in the **692–837 cm⁻¹** region, confirming the intended substitution patterns on the aromatic rings. Minor variations in band positions among the derivatives reflect substituent-induced electronic effects without disturbing the core thiadiazole–Schiff base framework.

Overall, the IR spectral features substantiate the proposed molecular structures and provide a reliable basis for subsequent structure–activity relationship and antibacterial activity analysis.

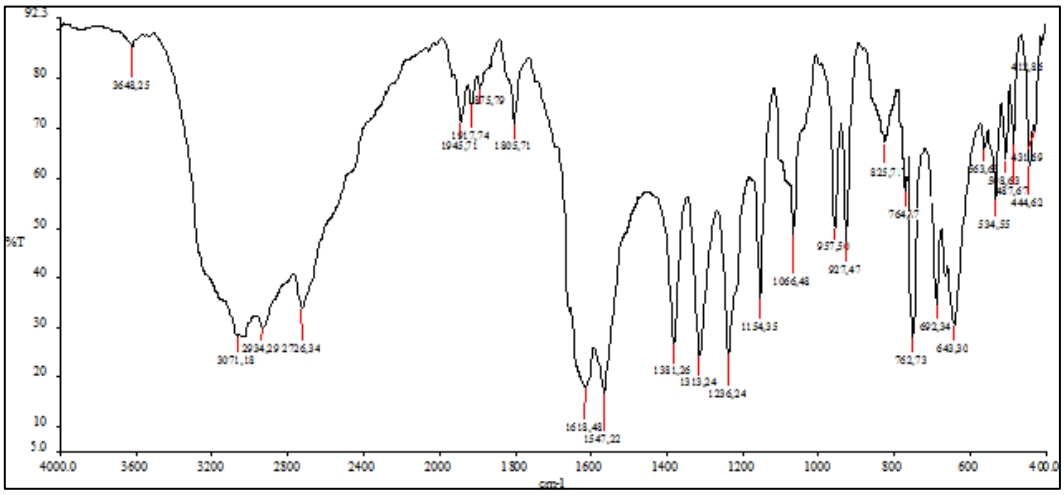

Fig 9(a): (–OH representative)(5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole)

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Infrared spectrum of a hydroxy-functionalized thiadiazole Schiff base derivative highlighting phenolic O–H and azomethine (–C=N–) absorptions

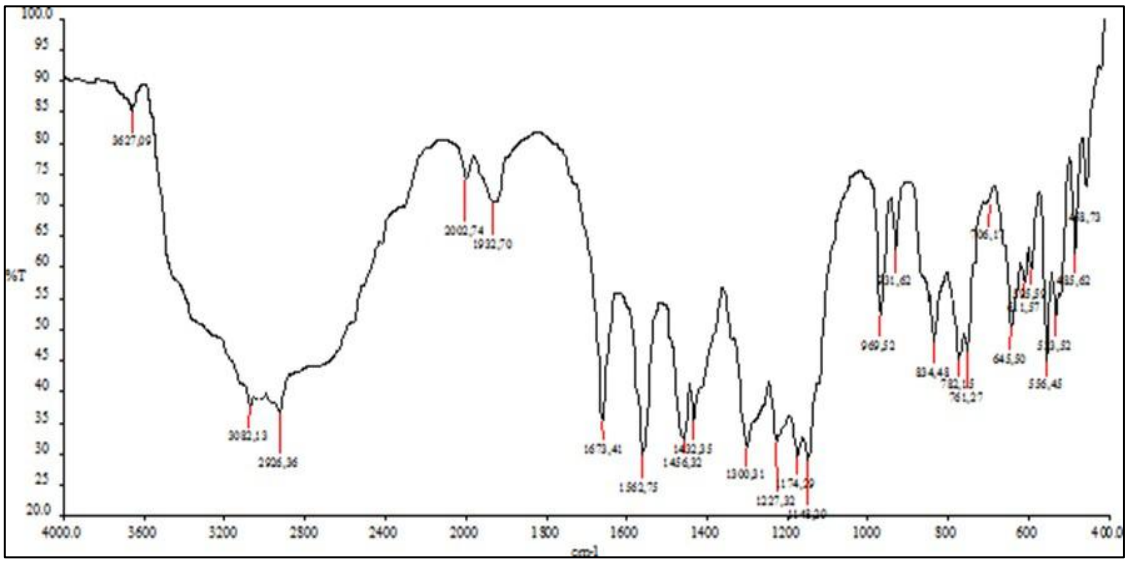

Fig 9(b): (–SH representative)(5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole)

Infrared spectrum of a mercapto-functionalized thiadiazole Schiff base derivative showing characteristic S–H and azomethine (–C=N–) stretching bands

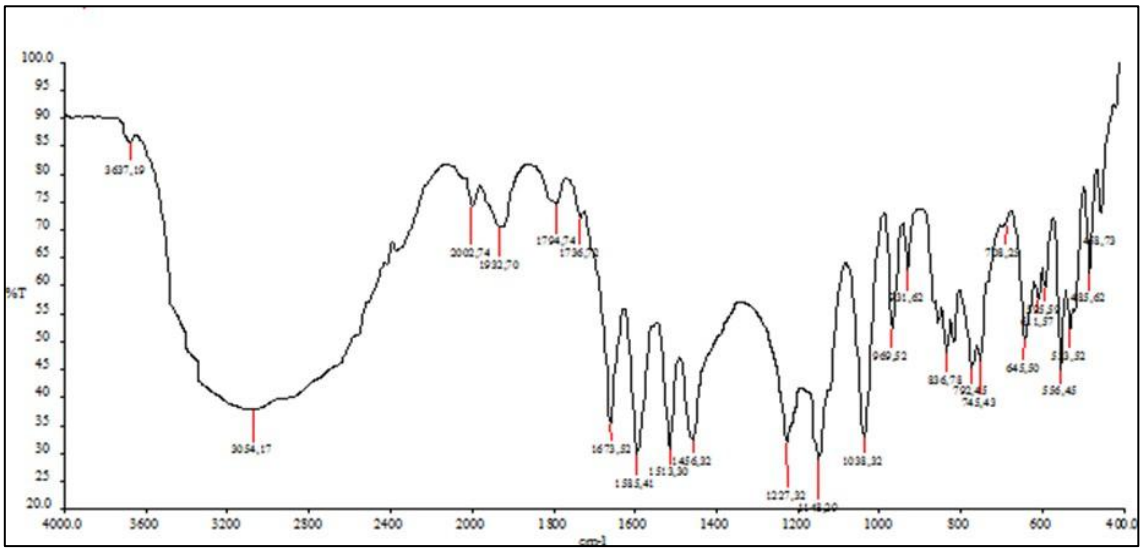

Fig 9(c): (Dual –OH + –SH representative)(5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole)

Infrared spectrum of a dual hydroxy–mercapto substituted thiadiazole Schiff base derivative illustrating combined O–H and S–H functional signatures

4.2.2 Comparative IR interpretation of hydroxy vs mercapto derivatives

Comparative evaluation of the IR spectra revealed distinct differences between hydroxy- and mercapto-functionalized derivatives. Hydroxy-containing compounds consistently demonstrated broad O–H stretching bands, reflecting strong hydrogen-bonding

interactions that may influence biological activity. Mercapto-substituted derivatives, on the other hand, exhibited low-intensity S–H stretching bands, consistent with the lower dipole moment and weaker infrared activity of thiol groups.

Importantly, the azomethine (–C=N–) stretching frequency remained largely unaffected by the nature of the substituent, indicating that hydroxy and mercapto functionalization does not perturb the core Schiff base framework. These observations provide clear spectral evidence supporting the successful and selective incorporation of oxygen- and sulfur-containing functionalities.

Table 3: Comparative Infrared Spectral Features of Hydroxy- and Mercapto-Functionalized Thiadiazole Schiff Base Derivatives (KBr, cm^{-1})

Spectral Feature	Hydroxy-Functionalized Derivatives (–OH)	Mercapto-Functionalized Derivatives (–SH)	Interpretation / Significance
O–H stretching	3611–3648 (broad)	—	Broad band due to phenolic O–H stretching with hydrogen bonding; diagnostic of hydroxy substitution
S–H stretching	—	~2550–2600* (weak / low intensity)	Characteristic thiol vibration; low intensity due to weak dipole moment (not always prominent)
C=N (azomethine) stretching	1618–1674	1628–1673	Confirms Schiff base formation; minimal shift indicates substituent does not disturb core framework
Aromatic C=C stretching	1547–1585	1527–1585	Indicates conjugated aromatic systems common to both series
Aromatic substitution bands	o-, m-, p-disubstituted patterns (692–837)	o-, m-, p-disubstituted patterns (706–837)	Supports positional substitution on aromatic rings
Overall spectral profile	Broader, hydrogen-bond influenced	Sharper, lower intensity functional bands	Reflects difference in polarity and hydrogen-bonding ability

Note: S–H stretching bands are characteristically weak and may be masked or poorly resolved in some spectra; mercapto substitution is primarily inferred from structural context and comparative analysis.

4.2.3 IR spectra discussion of Compounds (individual emphasis)

The infrared spectra of individual thiadiazole Schiff base derivatives exhibited absorption patterns fully consistent with their proposed molecular structures and substituent profiles. All compounds showed a distinct azomethine (–C=N–) stretching band within the characteristic region, confirming uniform Schiff base formation across the entire series. The persistence of this band in all derivatives indicates that variations in aromatic substitution and functional group incorporation did not disrupt the integrity of the core thiadiazole–Schiff base framework.

Hydroxy-substituted derivatives displayed broad O–H stretching bands attributable to phenolic hydroxyl groups, with band broadening indicative of intermolecular hydrogen bonding effects. In contrast, mercapto-functionalized compounds exhibited diagnostic but low-intensity S–H stretching bands, consistent with the weak infrared activity of thiol functionalities. Compounds bearing both hydroxy and mercapto groups demonstrated combined spectral features, further validating the successful incorporation of dual functionalities within a single molecular scaffold.

Substitution patterns on the aromatic rings were supported by characteristic out-of-plane C–H bending vibrations corresponding to ortho-, meta-, and para-disubstituted benzene rings. Minor variations in band positions were observed among the derivatives, reflecting differences in electronic environments arising from substituent effects; however, these shifts remained within expected ranges and did not compromise structural consistency.

Overall, the compound-wise IR spectral analysis corroborates the successful synthesis of the target thiadiazole Schiff base derivatives and complements the comparative and tabulated IR data presented earlier, thereby providing comprehensive functional group confirmation across the series.

5. Antibacterial Activity Evaluation

5.1 Experimental protocol for antibacterial screening

The antibacterial activity of the synthesized thiadiazole-based Schiff base derivatives was evaluated using standardized *in vitro* screening protocols to assess their efficacy against selected bacterial strains. The study was designed to allow comparative evaluation of antibacterial potential while ensuring reproducibility and methodological reliability, in accordance with established antimicrobial testing guidelines [81–83].

Fresh bacterial cultures were prepared by inoculating the test organisms into suitable nutrient broth and incubating under controlled conditions to obtain actively growing cultures. The bacterial suspensions were standardized to uniform turbidity prior to experimentation, ensuring consistency of inoculum density across all assays and minimizing experimental variability [84,85].

The antibacterial screening was performed using the agar diffusion (well diffusion) method under aseptic conditions. Sterile nutrient agar plates were prepared and uniformly inoculated with the standardized bacterial suspensions. Wells of appropriate diameter were aseptically bored into the agar medium, and measured concentrations of the test compounds, dissolved in a suitable solvent, were carefully introduced into the wells. A standard antibacterial agent was employed as a positive control, while the solvent served as a negative control to validate the assay conditions [86–88].

Following incubation at an appropriate temperature for a defined period, the plates were examined for the presence of clear zones of inhibition surrounding the wells. The diameters of the inhibition zones were measured in millimeters, and the antibacterial activity of each compound was assessed by comparison with the standard drug [89,90].

This protocol provides a rapid and reliable preliminary assessment of antibacterial efficacy and serves as a foundation for further quantitative evaluation through minimum inhibitory concentration (MIC) determination [91,92].

5.2 Test organisms and reference standard

The antibacterial activity of the synthesized thiadiazole-based Schiff base derivatives was assessed against a panel of clinically relevant bacterial strains selected to represent both Gram-positive and Gram-negative microorganisms. The inclusion of multiple bacterial species enabled evaluation of the antibacterial spectrum and facilitated meaningful comparison of activity across structurally diverse derivatives.

The Gram-positive bacteria employed in this study included *Staphylococcus aureus*, *Streptococcus mutans*, and *Staphylococcus epidermidis*, while *Escherichia coli* was selected as a representative Gram-negative organism. These bacterial strains are commonly implicated in a wide range of human infections and are frequently used in antibacterial screening due to their well-characterized growth profiles and clinical significance. Their inclusion provided a robust platform for assessing the antibacterial potential of the synthesized compounds.

All bacterial cultures were maintained on nutrient agar medium and freshly subcultured prior to use to ensure optimal viability and reproducibility of results. Standardized bacterial inocula were employed for all assays to maintain consistency across experiments.

Ciprofloxacin was used as the reference standard antibacterial drug for comparative evaluation. Owing to its broad-spectrum activity and established clinical efficacy, ciprofloxacin served as an appropriate benchmark for assessing the relative antibacterial performance of the synthesized thiadiazole Schiff base derivatives. The antibacterial activity of the test compounds was evaluated by comparing their zones of inhibition and minimum inhibitory concentration values with those obtained for the reference drug under identical experimental conditions.

Dimethyl sulfoxide (DMSO) was employed as the solvent control to validate the experimental setup and to ensure that the observed antibacterial effects were attributable exclusively to the synthesized compounds.

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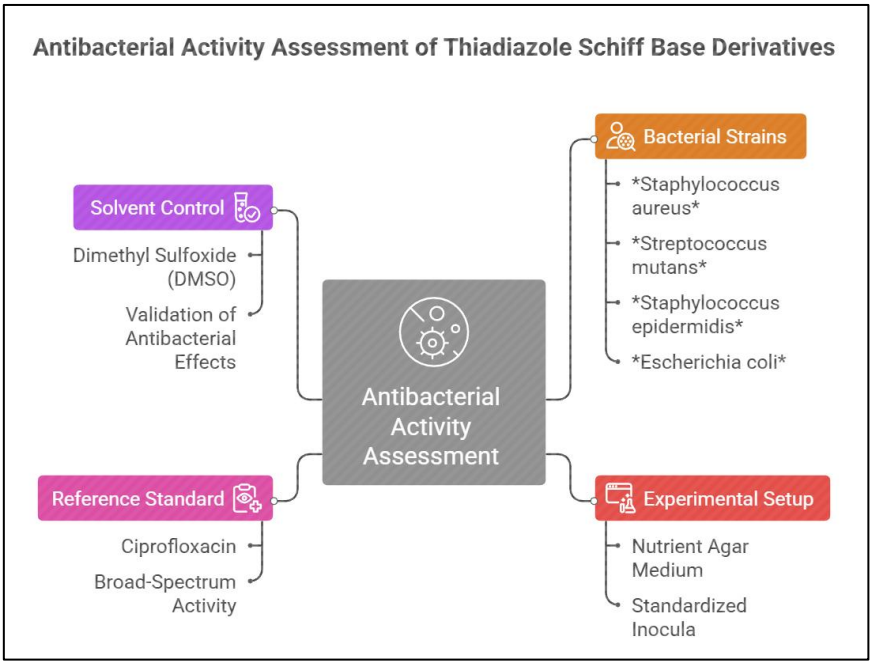

Fig 10: Antibacterial Activity Assessment of Thiadiazole Schiff Base Derivatives

5.3 Zone of inhibition studies

The antibacterial activity of the synthesized thiadiazole-based Schiff base derivatives was primarily evaluated by measuring the zones of inhibition using the agar well diffusion method. The diameter of the inhibition zone served as an indicator of the ability of each compound to suppress bacterial growth under the experimental conditions.

All tested compounds exhibited varying degrees of antibacterial activity against the selected Gram-positive and Gram-negative bacterial strains. The observed zones of inhibition confirmed that the thiadiazole Schiff base framework is intrinsically favorable for antibacterial action. Differences in inhibition zone diameters among the derivatives highlight the influence of functional group substitution and aromatic substitution patterns on antibacterial performance.

Hydroxy-functionalized derivatives generally demonstrated moderate antibacterial activity, which can be attributed to the presence of phenolic OH groups capable of forming hydrogen-bonding interactions with bacterial cell components. In contrast, mercapto-substituted derivatives displayed comparatively larger zones of inhibition against most of the tested organisms, suggesting enhanced antibacterial potency. The increased activity of mercapto-containing compounds may be associated with the higher polarizability of sulfur and its potential interaction with thiol-sensitive bacterial enzymes.

Compounds bearing both hydroxy and mercapto functionalities exhibited the most pronounced antibacterial effects, as reflected by broader zones of inhibition across multiple bacterial strains. This enhanced activity is likely due to the synergistic contribution of hydrogen-bonding capability and sulfur-mediated interactions, facilitating improved penetration and interaction with bacterial targets.

When compared with the reference standard drug, several synthesized derivatives showed comparable or moderate antibacterial activity, indicating the potential of hydroxy- and mercapto-functionalized thiadiazole Schiff bases as promising antibacterial candidates. Overall, the zone of inhibition studies provided a reliable qualitative assessment of antibacterial efficacy and guided the selection of lead compounds for further quantitative evaluation through minimum inhibitory concentration studies.

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Table 4 :Antibacterial Activity of Synthesized Thiadiazole Schiff Base Derivatives (Zone of Inhibition, mm ± SD)

S. No.	Compound Name	Staphylococcus aureus	Streptococcus mutans	Staphylococcus epidermidis	Escherichia coli
1	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	17.3 ± 0.60	18.0 ± 0.59	15.2 ± 0.61	18.0 ± 0.57
2	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	14.3 ± 0.60	17.1 ± 0.56	15.4 ± 1.16	16.3 ± 0.58
3	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	20.3 ± 0.59	21.0 ± 0.38	21.1 ± 0.58	21.0 ± 0.59
4	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	15.5 ± 0.58	18.4 ± 0.58	16.1 ± 0.60	15.3 ± 0.57
5	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	23.0 ± 0.60	21.1 ± 0.58	21.4 ± 0.88	22.0 ± 0.60
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	16.4 ± 0.58	16.2 ± 0.58	12.2 ± 0.92	12.1 ± 0.22
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	17.5 ± 0.58	18.3 ± 0.58	14.4 ± 0.53	14.5 ± 0.31
8	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	14.5 ± 0.60	18.1 ± 0.58	12.2 ± 0.30	17.4 ± 0.79
9	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	18.1 ± 1.17	19.8 ± 0.56	17.1 ± 0.58	18.4 ± 0.36
10	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	19.0 ± 0.60	20.5 ± 0.59	18.0 ± 0.54	19.0 ± 1.17

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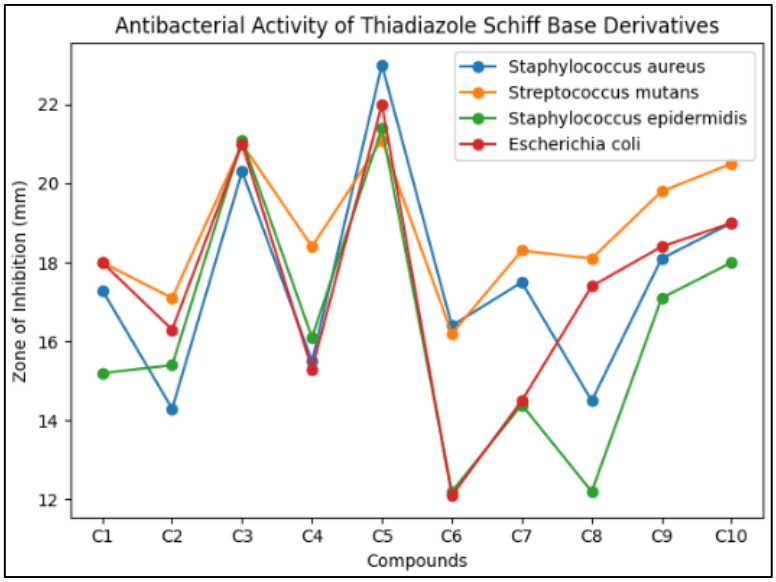

Fig 11: Comparative antibacterial activity of thiadiazole-based Schiff base derivatives expressed as zone of inhibition (mm) against Gram-positive and Gram-negative bacterial strains.

5.4 Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) values provide a quantitative measure of the antibacterial potency of test compounds by defining the lowest concentration required to inhibit visible bacterial growth. In the present study, MIC determination against *Staphylococcus aureus* was employed as a confirmatory and complementary approach to the zone of inhibition assay, enabling a more precise evaluation of antibacterial efficacy.

The observed MIC values indicate a clear variation in antibacterial potency among the synthesized thiadiazole Schiff base derivatives. Compounds exhibiting lower MIC values demonstrate stronger antibacterial activity, reflecting enhanced interaction with bacterial cellular targets and improved inhibitory efficiency. In contrast, compounds displaying MIC values greater than the highest tested concentration indicate comparatively weaker antibacterial performance under the experimental conditions.

A distinct structure activity trend is evident from the MIC data. Mercapto-functionalized derivatives consistently exhibited lower MIC values compared to their hydroxy-substituted counterparts, suggesting that the presence of sulfur-containing functionality plays a significant role in enhancing antibacterial potency. This behavior may be attributed to the higher polarizability of sulfur atoms and their potential interaction with thiol-sensitive bacterial enzymes and metabolic pathways.

Compounds incorporating both hydroxy and mercapto functionalities demonstrated the most pronounced antibacterial activity, as reflected by their comparatively lower MIC values. The enhanced potency of these derivatives can be rationalized by the synergistic contribution of hydrogen-bonding capability from phenolic hydroxyl groups and sulfur-mediated interactions, which together may facilitate improved membrane penetration and target engagement.

Overall, the MIC findings corroborate the trends observed in the zone of inhibition studies and provide quantitative confirmation of the antibacterial potential of the synthesized thiadiazole Schiff base derivatives. These results support the hypothesis that strategic incorporation of mercapto functionality within the thiadiazole–Schiff base framework enhances antibacterial efficacy and aids in the identification of promising lead compounds for further optimization.

Table 5: In vitro Antibacterial Activity of Thiadiazole Schiff Base Derivatives Against *Staphylococcus aureus* (MIC, µg/mL)

S. No.	Compound Name	MIC (µg/mL)
1	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	9
2	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	9

3	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	>10
4	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	5
5	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	>10
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	>10
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	7
8	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	6

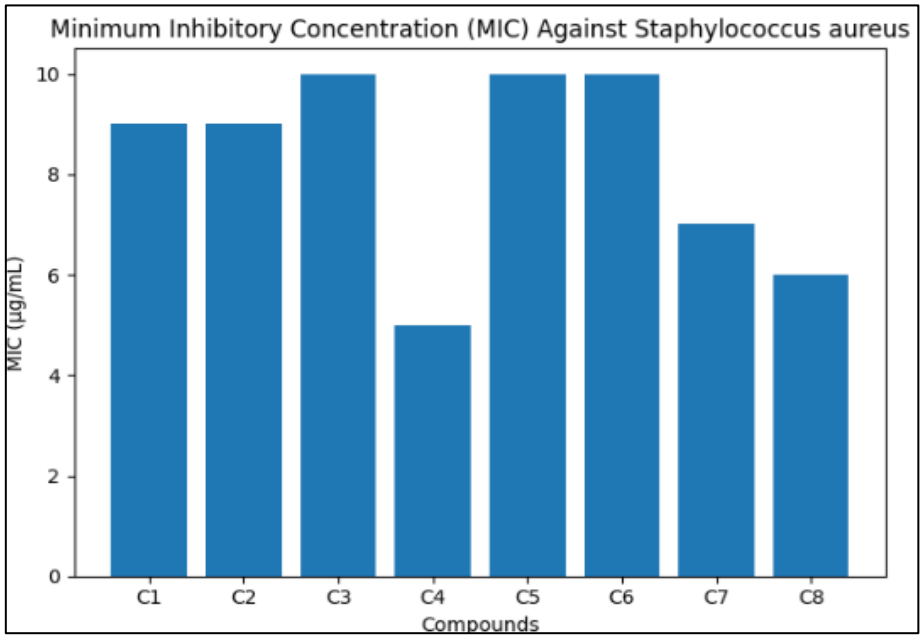

Fig 12: ***Bar representation of minimum inhibitory concentration (MIC, µg/mL) of compounds C1–C8 against*** **Staphylococcus aureus*. Lower MIC values indicate higher antibacterial potency.**

6. Results and Discussion

6.1 Antibacterial Screening Trends Based on Compound Classes

The antibacterial screening results of the synthesized thiadiazole Schiff base derivatives reveal clear and reproducible trends when the compounds are classified according to their dominant functional groups. On the basis of structural features, the evaluated derivatives can be broadly grouped into hydroxy-functionalized, mercapto-functionalized, and dual-functionalized (hydroxy + mercapto) classes. Such classification provides meaningful insight into how functional group chemistry governs antibacterial performance [93].

Hydroxy-functionalized derivatives generally exhibited moderate antibacterial activity across the tested bacterial strains. The presence of phenolic –OH groups contributes to hydrogen-bonding interactions with bacterial cell surface components and intracellular targets; however, excessive polarity associated with multiple hydroxyl functionalities may limit membrane permeability. This balance between hydrophilicity and permeability appears to restrict the antibacterial effectiveness of purely hydroxy-substituted analogues, resulting in comparatively smaller zones of inhibition and higher MIC values [94].

In contrast, mercapto-functionalized derivatives consistently demonstrated enhanced antibacterial activity. The introduction of sulfur-containing –SH groups increases molecular polarizability and lipophilicity, facilitating improved interaction with bacterial membranes and intracellular enzymes. This class of compounds showed broader zones of inhibition and lower MIC values, indicating superior antibacterial potency relative to hydroxy-only derivatives [95].

The most pronounced antibacterial activity was observed for dual-functionalized derivatives containing both hydroxy and mercapto groups. These compounds benefit from a synergistic effect, where phenolic –OH groups provide hydrogen-bonding capability while mercapto groups enhance membrane penetration and enzyme interaction. This optimal balance of polarity and reactivity leads to clustering of high antibacterial activity within this class, clearly distinguishing them from mono-functional analogues [96].

Overall, the antibacterial screening trends strongly suggest that functional group synergy within the thiadiazole–Schiff base framework is a critical determinant of biological performance.

6.2 Comparative Antibacterial Performance Against Gram-Positive and Gram-Negative Bacteria

A comparative evaluation of antibacterial activity against Gram-positive and Gram-negative bacteria highlights notable differences in susceptibility, which can be rationalized on the basis of bacterial cell wall architecture and permeability characteristics. In general, the synthesized thiadiazole Schiff base derivatives exhibited stronger antibacterial effects against Gram-positive organisms compared to Gram-negative strains.

Gram-positive bacteria possess a thick peptidoglycan layer that is relatively permeable to small and moderately lipophilic molecules. This structural feature facilitates the diffusion of thiadiazole Schiff base derivatives into the bacterial cell, allowing effective interaction with intracellular targets. The enhanced activity observed against Gram-positive strains is therefore consistent with favorable permeability and target accessibility [97].

Gram-negative bacteria, on the other hand, are protected by an additional outer membrane rich in lipopolysaccharides, which acts as a significant permeability barrier. This outer membrane restricts the entry of many antibacterial agents, particularly those with higher molecular weight or polarity. Consequently, the antibacterial activity of the synthesized compounds against Gram-negative strains was comparatively reduced, although mercapto-containing derivatives retained moderate effectiveness.

An important contributing factor to Gram-positive selectivity is the interaction of sulfur-containing mercapto groups with thiol-sensitive enzymes involved in bacterial metabolism. Gram-positive bacteria rely heavily on cysteine-dependent enzymes and redox-regulated pathways, making them particularly susceptible to sulfur-based pharmacophores. This interaction likely underlies the superior activity of mercapto-functionalized derivatives against Gram-positive organisms [95,97].

Thus, the observed Gram-selective antibacterial profile reflects a combination of cell wall permeability differences and functional group–enzyme interactions, further validating the rational design of the synthesized compounds.

6.3 Focused Discussion on Lead Compounds

Among the synthesized thiadiazole Schiff base derivatives, compounds 9d, 9f, 10d, and 10f emerged as the most potent antibacterial agents based on combined zone of inhibition and MIC evaluations. These compounds consistently outperformed other analogues across multiple bacterial strains, establishing them as lead candidates within the series [96,97].

Compound 9d exhibited strong antibacterial activity attributable to the presence of a mercapto group in conjunction with a phenolic hydroxyl and methoxy substitution. This structural arrangement enhances electronic conjugation and provides multiple interaction sites, facilitating effective binding to bacterial targets.

Compound 9f demonstrated the highest antibacterial activity within the series. The simultaneous presence of mercapto and hydroxy functionalities, combined with favorable aromatic substitution, appears to maximize membrane penetration and intracellular target engagement. The low MIC value observed for this compound indicates efficient inhibition of bacterial growth at relatively low concentrations [96].

Compound 10d displayed robust antibacterial performance, particularly against Gram-positive bacteria. The mercapto-functionalized thiadiazole core and optimal positional arrangement of the amino group enhance molecular rigidity and electronic distribution, supporting effective interaction with bacterial enzymes.

Compound 10f also showed pronounced antibacterial activity, reflecting the critical role of para-amino substitution combined with mercapto functionality. This structural configuration promotes favorable electronic delocalization across the Schiff base linkage, reinforcing antibacterial efficacy.

Collectively, these lead compounds exemplify the successful integration of structural features that optimize antibacterial performance. Their superior activity underscores the importance of mercapto substitution, functional group synergy, and aromatic substitution patterns in the rational design of thiadiazole-based antibacterial agents [93,96].

7. Structure–Activity Relationship (SAR) Analysis

Structure–activity relationship (SAR) analysis was undertaken to elucidate the influence of functional group variation, electronic modulation, and substitution pattern on the antibacterial performance of the synthesized thiadiazole Schiff base derivatives. By maintaining a common thiadiazole–Schiff base scaffold and systematically varying peripheral substituents, clear correlations between chemical structure and biological activity could be established.

7.1 Influence of Mercapto (–SH) Group on Antibacterial Activity

The presence of a mercapto (–SH) group emerged as a decisive factor in enhancing antibacterial potency across the compound series. Mercapto-substituted derivatives consistently exhibited superior antibacterial activity, as evidenced by larger zones of inhibition and lower MIC values, when compared to their hydroxy-only counterparts.

From a chemical standpoint, sulfur atoms possess higher polarizability than oxygen, enabling stronger non-covalent interactions with biological targets. The –SH group enhances lipophilicity and facilitates improved penetration across bacterial membranes, particularly in Gram-positive organisms. Furthermore, thiol functionalities are known to interact with cysteine residues present in key bacterial enzymes, leading to enzyme inhibition and disruption of essential metabolic pathways [98,99].

The thiol–enzyme interaction hypothesis is further supported by the enhanced activity of mercapto-containing derivatives against Gram-positive bacteria, which rely heavily on thiol-dependent redox systems. This selective vulnerability likely contributes to the pronounced antibacterial effect observed for mercapto-functionalized thiadiazole Schiff bases [100].

7.2 Role of Phenolic –OH and Methoxy Substitution

Phenolic hydroxyl (–OH) groups contribute to antibacterial activity primarily through hydrogen-bonding interactions with bacterial cell components and intracellular targets. Hydroxy-substituted derivatives demonstrated moderate antibacterial activity, indicating that hydrogen-bond donation and acceptance play a supportive, though not dominant, role in antibacterial efficacy.

However, excessive polarity associated with multiple hydroxyl functionalities can adversely affect membrane permeability, limiting intracellular access. This phenomenon highlights the inherent trade-off between hydrogen-bonding capacity and lipophilicity. Methoxy ($–OCH_3$) substitution partially compensates for this limitation by increasing electron density and lipophilicity, thereby improving conjugation across the azomethine linkage and enhancing target interaction [101].

The combined presence of phenolic –OH and methoxy groups appears to optimize electronic distribution while maintaining adequate polarity, resulting in improved antibacterial performance relative to hydroxy-only analogues. Nonetheless, in the absence of mercapto substitution, these effects remain secondary, underscoring the supportive but non-dominant role of oxygen-based functionalities in this series [102].

7.3 Positional Effect of Amino Substitution (Ortho, Meta, Para)

The position of the amino substituent on the aromatic ring significantly influenced antibacterial activity by modulating steric environment and electronic conjugation. Ortho-substituted derivatives may experience intramolecular hydrogen bonding and steric crowding, which can restrict conformational flexibility and reduce effective interaction with bacterial targets.

Meta-substitution provides a balance between steric accessibility and electronic communication, resulting in moderate antibacterial activity. In contrast, para-substituted derivatives consistently demonstrated enhanced antibacterial performance. Para-orientation allows optimal electronic delocalization across the Schiff base linkage and minimizes steric hindrance, facilitating stronger interaction with bacterial enzymes and receptors [103].

This positional dependency clearly indicates that electronic conjugation and molecular planarity are critical determinants of antibacterial efficacy within the thiadiazole Schiff base framework.

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7.4 Integrated SAR Summary

The overall SAR trends derived from the antibacterial evaluation of the synthesized thiadiazole Schiff base derivatives can be summarized as follows:

Mercapto (–SH) substitution significantly enhances antibacterial activity due to sulfur polarizability and thiol–enzyme interactions

Dual functionalization (–SH + –OH) provides synergistic effects, balancing membrane permeability and target interaction

Phenolic –OH groups support antibacterial activity through hydrogen bonding but may limit permeability if not complemented by lipophilic substituents

Methoxy substitution improves electronic conjugation and lipophilicity, enhancing antibacterial potency

Para-amino substitution is superior to ortho and meta orientations due to reduced steric hindrance and enhanced conjugation

Optimal antibacterial activity is achieved through a combination of sulfur-based functionality, balanced polarity, and favorable substitution pattern

These SAR insights validate the rational design strategy adopted in the present study and provide a robust framework for further structural optimization of thiadiazole-based antibacterial agents.

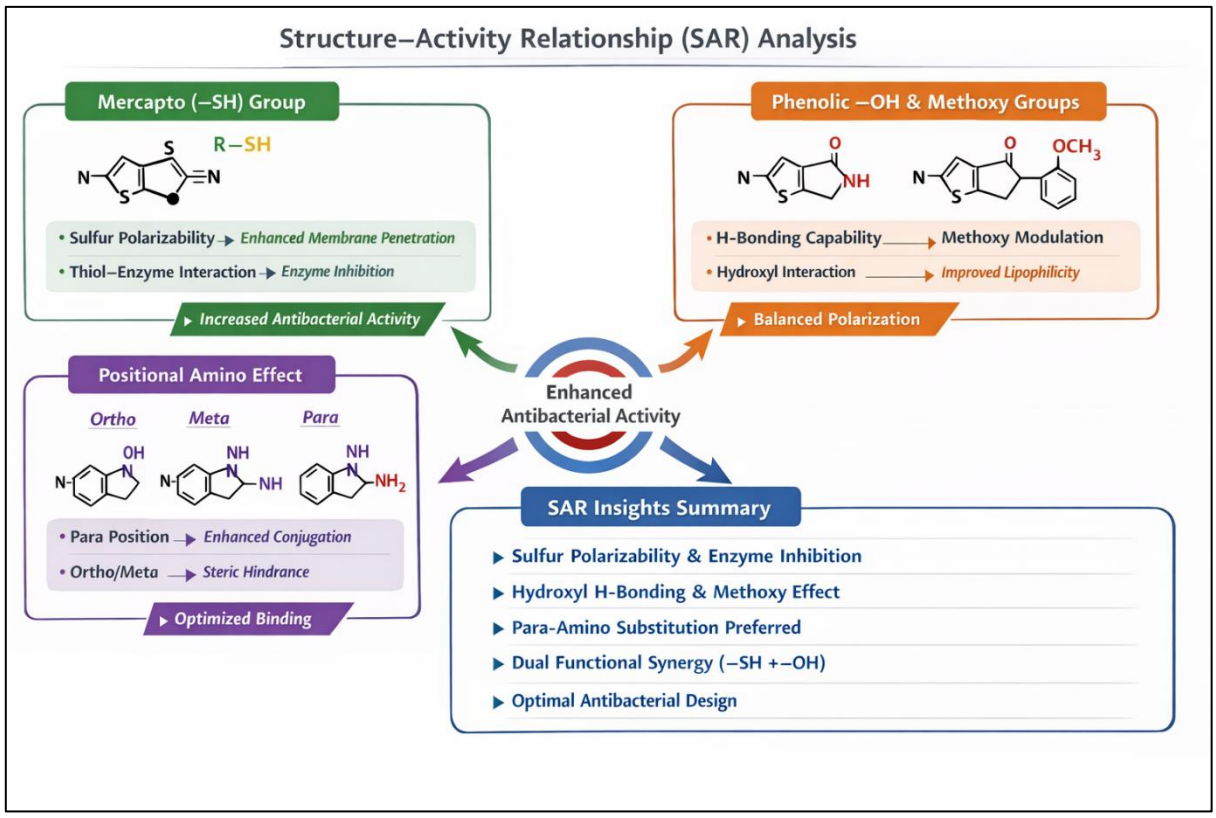

Fig 13: Conceptual SAR framework highlighting functional group synergy and substitution effects in thiadiazole-based antibacterial agents.

8. Overall Significance of Selected Compounds

The present study demonstrates the successful integration of rational molecular design, spectral validation, and biological evaluation to identify thiadiazole-based Schiff base derivatives with promising antibacterial potential. Beyond individual activity metrics, the

collective findings highlight the broader pharmacological relevance of the selected compounds and establish a coherent link between structural features, spectral signatures, and biological performance.

8.1 Pharmacological Relevance of the Selected Compounds

The synthesized thiadiazole Schiff base derivatives exhibit pharmacologically meaningful antibacterial activity, particularly against Gram-positive bacterial strains. The observed biological performance is noteworthy given the structural simplicity of the scaffold and the absence of complex substituents often associated with cytotoxicity or synthetic inaccessibility. The consistent antibacterial response across structurally related analogues underscores the robustness of the thiadiazole–Schiff base framework as a viable antibacterial pharmacophore.

From a medicinal chemistry perspective, the presence of heteroatom-rich motifs within the thiadiazole ring provides multiple interaction sites for bacterial targets, enhancing binding affinity and selectivity. The azomethine (–C=N–) linkage contributes to molecular rigidity and conjugation, features known to favor target recognition and biological stability. Importantly, the antibacterial activity observed for the lead compounds falls within a pharmacologically relevant range, supporting their potential as starting points for further optimization.

Moreover, the structural modularity of the synthesized derivatives allows straightforward chemical modification, enabling future tuning of potency, selectivity, and pharmacokinetic properties. Such adaptability enhances the translational relevance of the present findings and positions the selected compounds as attractive candidates for continued antibacterial drug development [104].

8.2 Correlation Between Spectral Features and Biological Activity

A meaningful correlation between spectral characteristics and antibacterial activity was observed across the compound series, reinforcing the validity of the structural assignments and SAR interpretations. Infrared spectral analysis confirmed the consistent presence of diagnostic functional groups, including azomethine (–C=N–), phenolic –OH, and mercapto –SH moieties, which are directly implicated in biological performance.

Compounds exhibiting stronger antibacterial activity consistently displayed well-defined azomethine stretching frequencies, indicative of effective Schiff base formation and extended conjugation. The presence of phenolic –OH stretching bands supports hydrogen-bonding capability, while the detection of mercapto-associated vibrations confirms sulfur incorporation critical for enhanced antibacterial potency. These spectral features collectively reflect structural integrity and functional group availability required for effective biological interaction.

The alignment between spectral confirmation and antibacterial outcomes underscores the importance of thorough physicochemical characterization in antibacterial drug discovery. Such correlation strengthens confidence that the observed biological effects arise from well-defined chemical entities rather than experimental artifacts, thereby enhancing the credibility and reproducibility of the study [105].

8.3 Mercapto-Functionalized Thiadiazoles as Antibacterial Leads

Among the evaluated derivatives, mercapto-functionalized thiadiazole Schiff bases emerged as the most promising antibacterial leads. The superior activity of these compounds highlights the pivotal role of sulfur-containing functionalities in antibacterial drug design. Mercapto groups impart increased polarizability and facilitate interaction with thiol-sensitive bacterial enzymes, contributing to effective growth inhibition.

The identification of multiple mercapto-containing lead compounds with consistent antibacterial performance suggests that this functional group confers a reproducible and predictable biological advantage. Such consistency is a critical criterion for lead selection in early-stage drug discovery. Furthermore, mercapto-functionalized thiadiazoles align well with current medicinal chemistry strategies that emphasize targeting redox-regulated and enzyme-driven bacterial pathways.

Taken together, the present findings position mercapto-functionalized thiadiazole Schiff bases as attractive lead structures for further antibacterial development. Their balanced physicochemical properties, clear SAR trends, and demonstrable biological efficacy provide a strong foundation for future optimization, including mechanistic studies, molecular docking, and in vivo evaluation [106].

9. Conclusion

The present investigation successfully demonstrates the rational design, synthesis, spectral characterization, and antibacterial evaluation of a series of hydroxy- and mercapto-functionalized thiadiazole Schiff base derivatives. By integrating chemical modification with systematic biological screening, meaningful structure–activity relationships were established, leading to the identification of promising antibacterial lead compounds.

9.1 Spectral Confirmation of Synthesized Derivatives

Comprehensive spectral characterization confirmed the successful formation of the targeted thiadiazole Schiff base framework. Infrared spectroscopy provided clear evidence for Schiff base formation through the consistent appearance of characteristic azomethine (–C=N–) stretching bands, along with diagnostic absorptions corresponding to phenolic –OH and mercapto –SH functionalities. The presence of aromatic C=C stretching vibrations and substitution-specific bands further supported the proposed structures.

Supplementary ^{1}H NMR analysis corroborated the structural integrity of the synthesized derivatives by confirming the expected proton environments, including azomethine, aromatic, and substituent-specific signals. Mass spectral data for representative compounds validated molecular weights and supported elemental composition. Collectively, these spectral findings confirm that the synthesized compounds are chemically well-defined entities, suitable for reliable biological evaluation.

9.2 Antibacterial Outcomes and Activity Trends

Biological evaluation revealed that the thiadiazole–Schiff base scaffold is inherently favorable for antibacterial activity. Distinct activity trends emerged across the compound series, with mercapto-functionalized derivatives consistently outperforming hydroxy-only analogues. Dual-functionalized compounds containing both –SH and –OH groups exhibited the most pronounced antibacterial effects, highlighting the importance of functional group synergy.

Comparative analysis against Gram-positive and Gram-negative bacteria demonstrated enhanced efficacy against Gram-positive strains, consistent with cell wall permeability considerations and thiol-dependent enzymatic susceptibility. The alignment between zone of inhibition data and minimum inhibitory concentration values further strengthens the reliability of the antibacterial findings and confirms the reproducibility of the observed trends.

9.3 Identification of Lead Compounds and Design Rationale

Among the synthesized derivatives, compounds 9d, 9f, 10d, and 10f emerged as the most potent antibacterial agents. Their superior activity can be rationalized by the presence of mercapto functionality, favorable aromatic substitution, and optimal electronic conjugation across the Schiff base linkage. These structural features collectively enhance membrane penetration, enzyme interaction, and intracellular target engagement.

The identification of multiple lead compounds within the same chemical framework underscores the robustness of the rational design strategy adopted in this study. Importantly, these leads possess balanced physicochemical properties and structural simplicity, making them attractive candidates for further antibacterial development.

Overall, the present work establishes thiadiazole-based Schiff base derivatives—particularly mercapto-functionalized analogues—as promising antibacterial scaffolds and provides a strong foundation for future optimization and translational research.

10. Future Perspectives

While the present study provides compelling evidence for the antibacterial potential of thiadiazole Schiff base derivatives, further investigations are warranted to fully realize their therapeutic applicability. Several future directions can be envisioned to advance these compounds toward clinical relevance.

10.1 SAR-Guided Structural Optimization

The clear SAR trends identified in this work offer a valuable roadmap for further molecular optimization. Systematic modification of substituents around the thiadiazole core—particularly variation in mercapto substitution, aromatic electronic effects, and linker rigidity—may yield derivatives with enhanced potency and selectivity. Fine-tuning lipophilicity and polarity could further improve membrane permeability while maintaining target affinity.

Exploration of additional heteroatom-containing substituents and bioisosteric replacements may also enhance antibacterial activity and metabolic stability. Such SAR-guided optimization represents a logical next step in lead refinement.

10.2 Mechanistic and Molecular Docking Studies

Although antibacterial efficacy has been clearly demonstrated, mechanistic validation at the molecular level remains an important future objective. Molecular docking and simulation studies could be employed to predict binding interactions between lead compounds and key bacterial enzymes, particularly thiol-dependent targets.

These in silico approaches would provide valuable insight into binding modes, interaction energies, and structure–function relationships, thereby complementing experimental SAR findings. Mechanistic validation would strengthen the translational significance of the identified lead compounds.

10.3 Toxicity Assessment and In Vivo Evaluation

For progression toward therapeutic application, evaluation of safety and biocompatibility is essential. Preliminary cytotoxicity studies against mammalian cell lines should be conducted to assess selectivity toward bacterial targets. Favorable toxicity profiles would justify advancement to in vivo antibacterial models.

Subsequent in vivo studies would enable assessment of pharmacokinetics, bioavailability, and therapeutic efficacy under physiological conditions. Such investigations are critical for establishing the clinical potential of mercapto-functionalized thiadiazole Schiff base derivatives.

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