EJPMR, 2026, 13(6), 613-623 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

RATIONAL DESIGN, SYNTHESIS AND SPECTROSCOPIC CHARACTERIZATION OF NOVEL IMINE-LINKED THIADIAZOLE DERIVATIVES AND THEIR IN VITRO ANTIBACTERIAL EVALUATION

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DOI: https://doi.org/10.5281/zenodo.20526232

How to cite this Article: Hridesh Singh Chauhan*, Dr. Manish Thimmaraju, Dr. Jitendra Kumar Malik, Dr. Vivek Gupta, Gyan Singh, Utkarsh Sharma. (2026). Rational Design, Synthesis and Spectroscopic Characterization of Novel Imine-Linked Thiadiazole Derivatives and Their In Vitro Antibacterial Evaluation. European Journal of Pharmaceutical and Medical Research, 13(6), 613–623.

Article Received on 05/05/2026 **Article Revised on 25/05/2026** **Article Published on 03/06/2026**

ABSTRACT

A series of novel imine-linked 1,3,4-thiadiazole derivatives were rationally designed and synthesized via Schiff base condensation to explore their antibacterial potential. The synthetic strategy aimed to integrate the pharmacologically active thiadiazole nucleus with an azomethine (–CH=N–) linkage to modulate electronic properties and enhance biological activity. The synthesized compounds were characterized by FT-IR, ^{1}H NMR and mass spectrometry. The analytical and spectral data confirmed the proposed structures and indicated satisfactory purity of the compounds. *In vitro* antibacterial activity was evaluated against Gram-positive strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *and Streptococcus mutans*) and the Gram-negative strain (*Escherichia coli*) using agar diffusion and broth micro-dilution methods. Ciprofloxacin was employed as the reference standard. Among the synthesized derivatives, eight compounds exhibited significant antibacterial activity. Compounds TZ3 and TZ5 demonstrated the highest zones of inhibition and TZ5 and TZ10 shown lowest minimum inhibitory concentration (MIC) values, indicating superior antibacterial efficacy within the series. Preliminary structure–activity relationship analysis suggested that substituent variations significantly influenced antimicrobial potency. These findings identify imine-linked thiadiazole derivatives, particularly TZ3, TZ5 and TZ10 as promising antibacterial lead molecules for further optimization.

KEYWORDS: 1,3,4-Thiadiazole; Schiff base synthesis; Antibacterial activity; MIC; Spectral characterization.

INTRODUCTION

Recent global assessments have demonstrated that bacterial antimicrobial resistance constitutes a significant and increasing contributor to global morbidity and mortality, emphasizing its substantial clinical and economic burden. The expanding prevalence of drug-resistant pathogens underscores the urgent need for innovative therapeutic strategies and structurally novel antibacterial agents to mitigate the escalating resistance crisis.[1]

According to the latest report by the World Health Organization, approximately one in six laboratory-confirmed bacterial infections worldwide in 2023 were resistant to antibiotic treatment, with resistance increasing in over 40% of monitored pathogen–drug combinations between 2018 and 2023**.** Drug-resistant Gram-negative pathogens, particularly *Escherichia coli* and *Klebsiella pneumoniae*, pose a significant global threat, with more than 40% of E. coli and 55% of K. pneumoniae isolates showing resistance to third-generation cephalosporins, exceeding 70% in some African regions**.** These findings highlight the escalating

global burden of antimicrobial resistance and underscore the urgent need for the development of novel and effective antibacterial agents.[2]

The failure of conventional antibiotics is increasingly driven by bacterial evolution of diverse resistance mechanisms, such as production of antibiotic-degrading enzymes, target modification, and efflux pumps that prevent drugs from reaching their targets. In addition, biofilm formation shields bacterial communities from effective antibiotic penetration, reducing clinical efficacy. Beyond inherited resistance, non-genetic factors such as dormant persister cells also tolerate antibiotic exposure and contribute to recurrent or chronic infections.[3-5]

The urgent need for novel antibacterial agents has redirected drug discovery efforts toward structurally versatile and biologically privileged scaffolds. Among these, heterocyclic compounds have emerged as fundamental building blocks in modern medicinal chemistry.[6]

In the context of antibacterial therapy, heterocyclic scaffolds are frequently associated with inhibition of key bacterial enzymes such as DNA gyrase, topoisomerase IV, and cell wall–synthesizing transpeptidases.[7-8]

The structural adaptability of heterocycles enables fine-tuning of lipophilicity, electronic distribution, and steric parameters, thereby optimizing membrane permeability and overcoming resistance mechanisms, particularly in Gram-negative pathogens.[9-10]

In thiadiazole-containing heterocyclic systems, the type and positional orientation of substituents significantly affect electronic delocalization, dipolar character, and lipophilic balance, thereby modulating molecular recognition at bacterial targets and influencing membrane permeability.[11]

Introduction of conjugated linkers such as imine (-C=N-) functionalities further alters electron density distribution and may enhance ligand–target interactions through hydrogen bonding and π–π stacking mechanisms.[12]

Accordingly, systematic structural tailoring of the thiadiazole core provides a rational framework for optimizing antimicrobial potency and selectivity.[13]

Thiadiazole nucleus is a five-membered heteroaromatic ring containing two nitrogen atoms and one sulfur atom within a $C_2H_2N_2S$ framework. The electronic behavior of the ring is governed by the high electro negativity of nitrogen atoms and the polarizable sulfur center. The extended conjugation within the thiadiazole framework enhances resonance stabilization and maintains rigidity of the ring system.[14-15] Thiadiazole occurs in four positional isomeric forms-1,2,3, 1,2,4, 1,2,5 and 1,3,4 depending on the arrangement of nitrogen and sulfur atoms within the five-membered ring. Among these, the 1,3,4-isomer is the most widely investigated owing to its greater thermodynamic stability and synthetic feasibility. The specific heteroatom orientation in the 1,3,4-configuration promotes improved π-electron delocalization, thereby enhancing overall aromatic stabilization. The thiadiazole ring shows distinct spectral signatures useful for structural verification. In IR spectra, the azomethine (C=N) stretch generally appears around 1600–1650 cm^{-1}, whereas C–S vibrations are observed at lower frequencies. In NMR analysis, the electron-deficient nature of adjacent nitrogen atoms causes noticeable deshielding effects in both proton and carbon signals, supporting structural confirmation of synthesized derivatives.[16]

Recognized for its multifunctional pharmacological potential, the 1,3,4-thiadiazole scaffold has been extensively explored in drug discovery. Various substituted derivatives have demonstrated marked antimicrobial effects against diverse bacterial and fungal strains, with several compounds exhibiting superior inhibitory performance relative to conventional reference agents.[17-18]

Apart from their antimicrobial activity, 1,3,4-thiadiazole derivatives also show notable anti-inflammatory effects. Several designed compounds have been found to reduce protein denaturation and lower inflammation levels, with results comparable to commonly used standard drugs.[19]

Beyond its antimicrobial and anti-inflammatory applications, the 1,3,4-thiadiazole scaffold has also been extensively investigated for its anticancer potential. Various substituted derivatives have demonstrated significant cytotoxic activity against diverse cancer cell lines, highlighting their promise as prospective lead compounds in antitumor drug development.[20-21]

The imine (–C=N–) group is an important pharmacophore in antibacterial drug design owing to its electron-deficient character and ability to form hydrogen bonds or coordinate with microbial enzymes. The azomethine nitrogen can interact with active sites or chelate metal ions, interfering with vital bacterial processes. When linked to the 1,3,4-thiadiazole nucleus, this functionality may further enhance activity, as the heteroatom-rich thiadiazole ring supports favorable electronic interactions and membrane permeability. Together, the –C=N– linkage and 1,3,4-thiadiazole core contribute to improved binding affinity and antibacterial effectiveness.[22]

Collectively, these findings support the rational design of imine-linked 1,3,4-thiadiazole derivatives as promising structurally optimized candidates for the development of novel and effective antibacterial agents to combat emerging drug resistance.

MATERIALS AND METHODS

Chemicals and Reagents

All reagents and solvents were of analytical grade and used as received without further purification unless otherwise stated. Substituted benzoic acids, hydrazine hydrate (99%), substituted aromatic aldehydes and glacial acetic acid were obtained from commercial suppliers and employed directly in the synthetic procedures.

Lawesson's reagent was utilized as a thionating and cyclization agent for the construction of the 1,3,4-thiadiazole nucleus. Absolute ethanol and dry toluene were used as reaction solvents, while ethyl acetate and n-hexane were employed as eluents for thin-layer chromatography (TLC).

Melting points were determined in open capillary tubes using a digital melting point apparatus and are reported uncorrected. The progress of the reactions was monitored by TLC performed on pre-coated silica gel 60 F_{254} aluminum plates, and the chromatograms were visualized under ultraviolet (UV) light at 254 nm.

All glassware was thoroughly oven-dried prior to use. Reflux reactions were carried out in round-bottom flasks fitted with water-cooled condensers and maintained under continuous magnetic stirring to ensure homogeneous mixing.

The general synthetic pathway adopted for the synthesis of the target 2,5-disubstituted-1,3,4-thiadiazole Schiff base derivatives is illustrated in Figure 1.

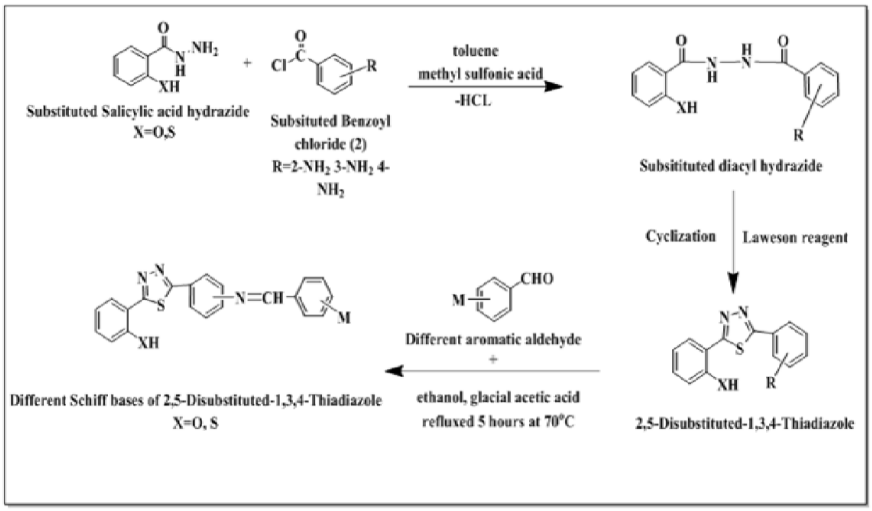

Fig. 1: General synthetic pathway for the synthesis of 2,5-disubstituted-1,3,4-thiadiazole derivatives via cyclization of substituted diacyl hydrazides followed by Schiff base formation with aromatic aldehyde.

The structural variations of the synthesized thiadiazole derivatives (TZ1–TZ10), including different heteroatom substitutions and aromatic aldehyde substituents, are summarized in Table 1

Table 1: Substituent variations in the synthesized 2,5-disubstituted-1,3,4-thiadiazole Schiff base derivatives (TZ1–TZ10), indicating the heteroatom (X = O or S), positional substitution of the aminophenyl group (Y), and aromatic aldehyde substituents (Z).

SN.	COMPOUNDS	X	Y	Z
1	TZ1	S	NH_2(2)	-$N(CH_3)_2$(4)
2	TZ2	O	NH_2(3)	-$N(CH_3)_2$(4)
3	TZ3	S	NH_2(3)	-$N(CH_3)_2$(4)
4	TZ4	O	NH_2(4)	-$N(CH_3)_2$(4)
5	TZ5	S	NH_2(4)	-$N(CH_3)_2$(4)
6	TZ6	O	NH_2(2)	-OH(4)
				-OCH_3(3)
7	TZ7	S	NH_2(2)	-OH(4)
				-OCH_3(3)
8	TZ8	O	NH_2(3)	-OH(4)
				-OCH_3(3)
9	TZ9	S	NH_2(3)	-OH(4)
				-OCH_3(3)
10	TZ10	S	NH_2(4)	-OH(4)
				-OCH_3(3)

Step–I: Synthesis of ortho, meta and para amino substituted diacyl hydrazides (Intermediate I)
Salicylic acid hydrazide (1.52 gm) or thiosalicylic acid hydrazide (1.68 gm) was dissolved in 25 mL of dry toluene in a round-bottom flask equipped with a reflux condenser and magnetic stirring. To this stirred solution, o-, m-, or p-aminobenzoyl chloride (1.20 ml) was added drop wise. Methylsulfonic acid (0.5 ml) was added as a catalyst, and the reaction mixture was refluxed for 4–6 hours.

The progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate: hexane (7:3) as the mobile phase. After completion, the reaction mixture was allowed to cool to room temperature and poured onto crushed ice with continuous stirring to neutralize the liberated hydrogen chloride.

The resulting precipitate was filtered, washed thoroughly with cold water to remove residual acid, and dried under vacuum. The crude product was purified by recrystallization from ethanol to afford the corresponding substituted diacyl hydrazide intermediates (Intermediate I) in good yield.[23]

Step–II: Synthesis of 2,5-Disubstituted-1,3,4-Thiadiazole Derivatives (Cyclization Step)
The synthesized substituted diacyl hydrazide (1.0 m.mol) was dissolved in dry toluene (20 ml) in a round-bottom flask equipped with a reflux condenser and magnetic stirring. To this solution, Lawesson's reagent[24] (0.24 g) was added portion-wise.

The reaction mixture was refluxed for 5–6 hours and the progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate: n-hexane (3:7) as the mobile phase.

After completion of the reaction, the solvent was removed under reduced pressure. The resulting crude residue was cooled to room temperature and poured into crushed ice. The solid obtained was filtered, washed with cold water, and dried.

The crude product was purified by recrystallization from ethanol to afford the corresponding 2,5-disubstituted-1,3,4-thiadiazole derivative as a solid in good yield.[25]

Step–III: Formation of imine (–C=N–) derivatives via nucleophilic condensation of 2,5-disubstituted-1,3,4-thiadiazole amines with substituted benzaldehydes
The synthesized 2,5-disubstituted-1,3,4-thiadiazole amine (0.25 gm) was dissolved in absolute ethanol (20 ml) in a 100 ml round-bottom flask equipped with magnetic stirring.

To this stirred solution, the appropriate substituted aromatic aldehyde (benzaldehyde 0.15 ml) was added, followed by the addition of glacial acetic acid (0.2 ml, 2–3 drops) as a catalyst.

The reaction mixture was refluxed for 4–6 h and the progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate:n-hexane (4:6) as the mobile phase.

After completion of the reaction, the mixture was allowed to cool to room temperature. The resulting precipitate was filtered, washed with cold ethanol (5 ml) and dried under vacuum.

The crude product was recrystallized from ethanol (10-15 ml) to afford the corresponding Schiff base derivatives as crystalline solids in good yield.[26]

The physico-analytical properties of the synthesized compounds, including molecular formula, melting point, Rf value, percentage yield, and λmax values, are presented in Table 2.

Table 2: Physico-anlytical properties of newly synthesized 2,5-disubstituted-1,3,4-thiadoazole derivatives.

SN	Compound	Molecular Formula	M.Wt. *	Melting point**	Colour	R_f Value	% Yield	λ_{max}***
1	TZ1	$C_{23}H_{20}N_4S_2$	416	171 -172	Magenta red	0.33	61%	264
2	TZ2	$C_{23}H_{20}N_4OS$	400	195 -196	Light yellow	0.64	68%	338
3	TZ3	$C_{23}H_{20}N_4S_2$	416	185 -186	Light brown	0.30	62%	273
4	TZ4	$C_{23}H_{20}N_4OS$	400	209 -210	Light yellow	0.61	58%	338
5	TZ5	$C_{23}H_{20}N_4S_2$	416	212 -213	Light brown	0.36	61%	266
6	TZ6	$C_{22}H_{17}N_3O_3S$	403	212 -213	Light yellow	0.59	71%	340
7	TZ7	$C_{22}H_{17}N_3O_2S_2$	419	201 -202	Magenta red	0.32	64%	230
8	TZ8	$C_{22}H_{17}N_3O_3S$	403	215 -216	Cream color	0.58	59%	338
9	TZ9	$C_{22}H_{17}N_3O_2S_2$	419	212 -213	Magenta red	0.28	66%	226
10	TZ10	$C_{22}H_{17}N_3O_2S_2$	419	219 -220	Magenta red	0.28	63%	266

*Molecular Weight in g/mol, ** Melting point in (°C), *** λ_{max} in nm.

SPECTRAL ANALYSIS OF COMPOUND (TZ1-TZ10)

Compound TZ1

IR Spectrum (KBr, cm^{-1}): 2995.36 (aliphatic C–H), 1624.53 (C=N, azomethine), 1496.32 (Ar C=C), 1270.30 (C–N dimethyamino), 1300.53, (C-N aromatic amine), 763.46 (Ar–H bending), 645.58 (C–S), 556.45 (thiadiazole ring vibrations).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.02 (1H, br s, –SH), 8.84 (1H, s, –CH=N–), 7.82–6.78 (12H, m**,** Ar–H)**,** 2.92 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 415 (M^+)

Compound TZ2

IR (KBr, cm^{-1}): 3448 (Ar-OH), 3058 (Ar–C–H), 2924 (C–H), 1618 (C=N), 1585 (C=C), 1512 (C=N, thiadiazole), 1256 (C–N), 1178 (C–O), 832 (p-disubstituted Ar), 745 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.18 (1H, s, Ar–OH), 8.86 (1H, s, CH=N), 7.85–6.75 (12H, m, Ar–H), 3.04 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 399 (M^+)

Compound TZ3

IR (KBr, cm^{-1}): 2572 (w, S–H), 3056 (Ar–C–H), 2923 (C–H, CH_3), 1619 (C=N, azomethine), 1587 (C=C, aromatic), 1513 (C=N, thiadiazole), 1260 (C–N), 834 (p-disubstituted benzene), 749 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.96 (1H, br s, SH), 8.88 (1H, s, CH=N), 7.82–7.48 (8H, m, Ar–H), 7.18 (2H, d, J = 8.8 Hz, Ar–H), 6.76 (2H, d, J = 8.8 Hz, Ar–H), 3.03 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 415 (M^+)

Compound TZ4

IR (KBr, cm^{-1}): 3438 (br, O–H), 3054 (Ar–C–H), 2921 (C–H), 1618 (C=N, azomethine), 1586 (C=C, aromatic), 1514 (C=N, thiadiazole), 1257 (C–N), 1180 (C–O, phenolic), 832 (p-disubstituted Ar), 750 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.22 (1H, s, OH), 8.87 (1H, s, CH=N), 7.83–7.50 (8H, m, Ar–H), 7.17 (2H, d, J = 8.8 Hz, Ar–H), 6.78 (2H, d, J = 8.8 Hz, Ar–H), 3.04 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 399 [M+]

Compound TZ5

IR (KBr, cm^{-1}): 2574 (w, S–H), 3056 (Ar–C–H), 2923 (C–H), 1620 (C=N, azomethine), 1587 (C=C, aromatic), 1512 (C=N, thiadiazole), 1261 (C–N), 833 (p-disubstituted Ar), 743 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.94 (1H, br s, SH), 8.89 (1H, s, CH=N), 7.84–7.52 (8H, m, Ar–H), 7.18 (2H, d, J = 8.8 Hz, Ar–H), 6.77 (2H, d, J = 8.8 Hz, Ar–H), 3.03 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 415 [M+]

Compound TZ6

IR (KBr, cm^{-1}): 3452 (br, O–H), 3058 (Ar–C–H), 2934 (C–H, OCH_3), 1617 (C=N, azomethine), 1584 (C=C, aromatic), 1516 (C=N, thiadiazole), 1264 (Ar–O–CH_3), 1186 (C–O, phenolic), 1032 (C–O–C), 832 (Ar–H), 741 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.34 (1H, s, OH), 9.88 (1H, s, OH), 8.86 (1H, s, CH=N), 7.84–6.82 (12H, m, Ar–H), 3.80 (3H, s, OCH_3).

Mass Spectrum (m/z): 402 [M+]

Compound TZ7

IR (KBr, cm^{-1}): 3446 (br, O–H), 2570 (w, S–H), 3056 (Ar–C–H), 2932 (C–H, OCH_3), 1619 (C=N, azomethine), 1586 (C=C, aromatic), 1514 (C=N, thiadiazole), 1262 (Ar–O–CH_3), 1184 (C–O, phenolic), 1034 (C–O–C), 832 (Ar–H), 746 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.98 (1H, br s, SH), 10.26 (1H, s, OH), 8.88 (1H, s, CH=N), 7.85–6.80 (12H, m, Ar–H), 3.79 (3H, s, OCH_3).

Mass Spectrum (m/z): 418 [M+]

Compound TZ8

IR (KBr, cm^{-1}): 3456 (br, O–H), 3055 (Ar–C–H), 2923 (C–H, OCH_3), 1628 (C=N, azomethine), 1575 (C=C, aromatic), 1519 (C=N, thiadiazole), 1261 (Ar–O–CH_3), 1180 (C–O, phenolic), 1035 (C–O–C), 831 (Ar–H), 752 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.35 (1H, s, OH), 9.82 (1H, s, OH), 8.78 (1H, s, CH=N), 7.76–6.76 (12H, m, Ar–H), 3.78 (3H, s, OCH_3).

Mass Spectrum (m/z): 402 [M+]

Compound TZ9

IR (KBr, cm^{-1}): 3449 (br, O–H), 2575 (w, S–H), 3051 (Ar–C–H), 2939 (C–H, OCH_3), 1615 (C=N, azomethine), 1576 (C=C, aromatic), 1524 (C=N, thiadiazole), 1272 (Ar–O–CH_3), 1188 (C–O, phenolic), 1036 (C–O–C), 838 (Ar–H), 753 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.81 (s, 1H, Ar–SH), 10.25 (s, 1H, Ar–OH), 8.69 (s, 1H, CH=N), 7.82–6.88 (m, 11H, Ar–H), 3.73 (s, 3H, OCH_3).

Mass Spectrum (m/z): 418 [M+]

Compound TZ10

IR (KBr, cm^{-1}): 3458 (br, O–H), 2566 (w, S–H), 3051 (Ar–C–H), 2936 (C–H, OCH_3), 1625 (C=N, azomethine), 1581 (C=C, aromatic), 1526 (C=N, thiadiazole), 1275 (Ar–O–CH_3), 1185 (C–O, phenolic), 1034 (C–O–C), 838 (Ar–H bending), 752 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.38 (s, 1H, Ar–SH), 10.20 (s, 1H, Ar–OH), 8.73 (s, 1H, CH=N), 7.90–6.75 (m, 11H, Ar–H), 3.83 (s, 3H, OCH_3).

Mass Spectrum (m/z): 418 [M+]

BIOLOGICAL EVALUATION

In Vitro Antibacterial Activity by Agar Well Diffusion Method

The synthesized compounds were evaluated for their *in vitro* antibacterial activity using the agar well diffusion method as previously described in standard antimicrobial screening procedures.[27,29] The test organisms included Gram-positive strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus mutans*, along with the Gram-negative strain *Escherichia coli*.

Fresh bacterial cultures were prepared in nutrient broth and incubated at 37°C for 18–24 h. Mueller–Hinton agar plates were prepared and uniformly inoculated with the respective bacterial suspension using a sterile cotton swab following established guidelines for antimicrobial susceptibility testing.[28] Wells of approximately 6 mm diameter were aseptically punched into the agar medium using a sterile cork borer.

The test compounds were dissolved in dimethyl sulfoxide (DMSO) and introduced into the wells at a fixed concentration. **Ciprofloxacin** was used as the standard reference drug, while the solvent served as the negative control.

The plates were incubated at 37°C for 24 h, and antibacterial activity was determined by measuring the diameter of the zone of inhibition (ZOI) in millimeters. The procedure was carried out in accordance with standardized antimicrobial testing protocols.[27, 29] All experiments were performed in triplicate and results were expressed as mean ± standard deviation (Mean ± SD).

The experimental workflow followed for antibacterial screening using the agar well diffusion method is represented in Figure 2.

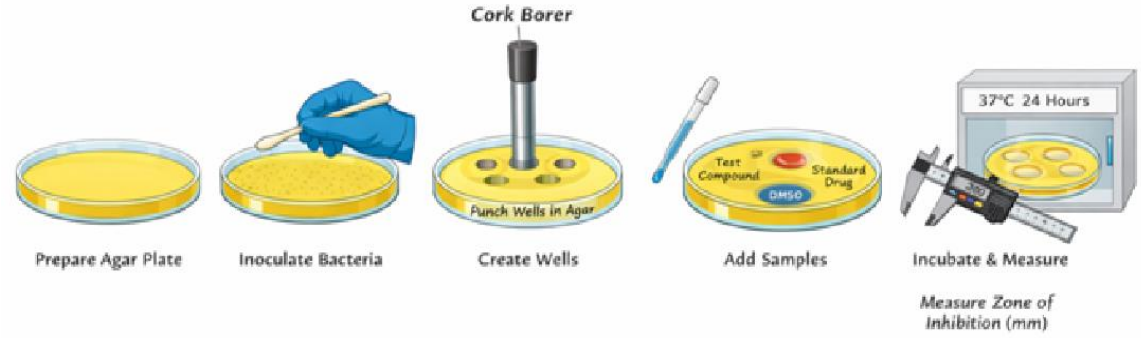

Figure 2: Experimental Workflow of Agar Well Diffusion Technique for Determination of Zone of Inhibition (ZOI).

Determination of Minimum Inhibitory Concentration (MIC) by Broth Micro-dilution Method

Antibacterial activity of the synthesized thiadiazole derivatives was quantified by the broth micro dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[30]

Fresh overnight cultures of *S. aureus* were adjusted to 0.5 McFarland standard and diluted to achieve a final inoculum density of approximately 5×10^5 CFU/ml per well, as recommended for standardized antimicrobial susceptibility testing.[30,31]

Serial two-fold dilutions of the test compounds (0.5–256 µg/ml) were prepared in sterile Mueller–Hinton broth in sterile 96-well micro titer plates following established broth dilution protocols.[31,32]

After addition of the bacterial inoculum, plates were incubated at 37 °C for 18–24 h under aerobic conditions. The lowest concentration of the compound showing no visible bacterial growth was recorded as the minimum inhibitory concentration (MIC). Appropriate controls included broth-only sterility controls and growth controls containing inoculated broth without test compound.[30, 32]

RESULT AND DISCUSSION

The antibacterial activity of the synthesized thiazole–Schiff base derivatives (TZ1–TZ10) was evaluated against four bacterial strains, namely *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans* and *Staphylococcus epidermidis* using the agar diffusion method. The results were expressed as Zone of Inhibition (ZOI) in mm ± SD and compared with the standard drug Ciprofloxacin (100 µg/disc).

Among the tested compounds, TZ5 exhibited the highest antibacterial activity with a ZOI of 23.0 ± 0.60 mm against *S. aureus*, indicating strong efficacy against Gram-positive bacteria. This was followed by TZ3, which showed significant inhibition **(**21.1 ± 0.58 mm**)** against *S. epidermidis*. Compounds TZ10 and TZ9 also demonstrated considerable activity against *E. coli* with inhibition zones of 20.5 ± 0.59 mm and 19.8 ± 0.56 mm, respectively.

Most of the synthesized derivatives showed comparatively better activity against *E. coli* and Gram-positive strains, suggesting a broad-spectrum antibacterial potential. However, all synthesized compounds exhibited lower activity compared to the standard drug. Ciprofloxacin showed the highest inhibition overall, with a maximum ZOI of 31.0 ± 1.18 mm against *S. aureus*.

The variation in antibacterial activity among the synthesized compounds may be attributed to structural differences and the presence of different substituents on the aromatic ring, which influence electron distribution, lipophilicity and interaction with bacterial cell targets. The enhanced activity observed in TZ5 suggests that specific substituent patterns significantly improve antibacterial potency.

Overall, the results indicate that the synthesized thiadiazole derivatives possess promising antibacterial activity, with TZ5 emerging as the most potent compound among the tested series, although still less active than Ciprofloxacin.

A comparative ranking of antibacterial activity based on maximum zone of inhibition values is shown in Table 3.

Table 3: Comparative antibacterial activity of synthesized compounds (TZ1–TZ10) and Ciprofloxacin based on maximum ZOI values.

Rank	Compound	Best ZOI (mm ± SD)	Bacteria
1	Ciprofloxacin	31.0 ± 1.18	*S. aureus*
2	TZ_5	23.0 ± 0.60	*S. aureus*
3	TZ_3	21.1 ± 0.58	*S. mutans*
4	TZ_{10}	20.5 ± 0.59	*E. coli*
5	TZ_9	19.8 ± 0.56	*E. coli*
6	TZ_4	18.4 ± 0.58	*E. coli*
7	TZ_7	18.3 ± 0.58	*E. coli*
8	TZ_8	18.1 ± 0.58	*E. coli*
9	TZ_1	18.0 ± 0.59	*E. coli*
10	TZ_2	17.1 ± 0.56	*E. coli*
11	TZ_6	16.4 ± 0.58	*S. aureus*

The maximum antibacterial activity of synthesized compounds against the tested bacterial strains is summarized in Table 4.

Table 4: Maximum Zone of Inhibition (ZOI) of Synthesized Compounds Compared with Standard Drug.

Compound Code	Maximum ZOI (mm ± SD)	Most Sensitive Organism
TZ1	18.0 ± 0.59	*E. coli*
TZ2	17.1 ± 0.56	*E. coli*
TZ3	21.1 ± 0.58	*S. mutans*
TZ4	18.4 ± 0.58	*E. coli*
TZ5	23.0 ± 0.60	*S. aureus*
TZ6	16.4 ± 0.58	*S. aureus*
TZ7	18.3 ± 0.58	*E. coli*
TZ8	18.1 ± 0.58	*E. coli*
TZ9	19.8 ± 0.56	*E. coli*
TZ10	20.5 ± 0.59	*E. coli*
Ciprofloxacin (100 µg/disc)	**31.0 ± 1.18**	***S. aureus***

MIC-Based Antibacterial Activity Assessment Against *S. aureus*

The minimum inhibitory concentration (MIC) values of the most active synthesized derivatives were determined against *S. aureus*. The results demonstrated that compound TZ5 exhibited the highest antibacterial potency among the tested derivatives with an MIC value of 5 µg/mL, which is comparable to the standard drug Ciprofloxacin (4 µg/mL).

Compounds TZ10 and TZ9 also showed significant antibacterial activity with MIC values of 6 µg/mL and 7 µg/mL, respectively. Compounds TZ1 and TZ3 displayed moderate activity (9 µg/mL), whereas compounds TZ4, TZ7 and TZ8 exhibited comparatively lower activity (>10 µg/mL).

The enhanced activity of compounds may be attributed to favorable electronic and structural features, which possibly improve bacterial cell membrane penetration and interaction with intracellular targets. The observed

MIC values correlate well with the zone of inhibition data, confirming their strong antibacterial potential,

The MIC potency ranking of the most active compounds against *S. aureus* is presented in Table 5

Table 5: MIC Potency Ranking Against *S. aureus* (Based on Broth Micro-dilution Method.

Rank	Compound	MIC (µg/mL)	Relative Activity
1	Ciprofloxacin (Standard)	4	Highest
2	TZ5	5	Very High
3	TZ10	6	High
4	TZ9	7	High
5	TZ1	9	Moderate
6	TZ3	9	Moderate
7	TZ4	>10	Low
8	TZ7	>10	Low
9	TZ8	>10	Low

Integrated Comparative Analysis: ZOI, MIC and SAR Correlation

The antibacterial performance of the synthesized thiadiazole derivatives was comprehensively evaluated using both zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) assays against *Staphylococcus aureus*. A clear correlation between ZOI and MIC values was observed, validating the reliability of the biological results and supporting the proposed structure–activity relationship (SAR).

Among the tested compounds, TZ5 exhibited the highest antibacterial potency, showing a ZOI of 23.0 ± 0.60 mm and an MIC value of 5 µg/ml, second only to the reference drug ciprofloxacin (MIC = 4 µg/mL). The strong activity of TZ5 can be attributed to the presence of a thiol (–SH) group combined with a strong electron-donating dimethylamino substituent. This structural arrangement likely enhances electron density across the aromatic system, improving π–π stacking interactions and facilitating stronger binding with bacterial enzymatic targets.

TZ10 demonstrated comparatively high activity with a ZOI of 19.0 ± 0.60 mm and an MIC value of 6 µg/mL. The presence of methoxy and phenolic groups contributes moderate electron donation and hydrogen-bonding capability, which may enhance molecular interaction with bacterial cell components. However, its activity remained slightly lower than TZ5, suggesting that stronger electron-donating substituents provide superior antibacterial enhancement.

Compounds such as TZ9 (MIC = 7 µg/mL) displayed good activity, whereas derivatives with weaker substituents (TZ4, TZ7, TZ8) exhibited lower potency (MIC >10 µg/mL), corresponding to smaller ZOI values. This trend confirms that substituent electronic properties play a decisive role in determining antibacterial efficacy.

The inverse relationship between MIC and ZOI values further supports the SAR findings, where compounds exhibiting larger inhibition zones correspondingly showed lower MIC values. Overall, the results suggest that the thiadiazole–Schiff base scaffold serves as a biologically active core, while antibacterial potency is significantly influenced by the electronic nature and lipophilicity of the attached substituents.

Activity Correlation Summary

A consistent inverse correlation between ZOI and MIC values was observed across the compound series, indicating that derivatives producing larger inhibition zones required lower concentrations to suppress bacterial growth. This agreement between qualitative (ZOI) and quantitative (MIC) assays strengthens the biological validity of the study.

Comparative Conclusion

The integrated biological evaluation establishes a strong correlation between electronic substituent effects and antibacterial potency. Compounds bearing strong electron-donating groups demonstrated superior ZOI and lower MIC values, indicating enhanced bacterial inhibition. The thiadiazole–azomethine framework appears to be a promising antibacterial pharmacophore, and rational modification of substituents may further optimize antimicrobial efficacy, The structure–activity relationship (SAR) analysis correlating substituent effects with antibacterial activity is summarized in Table 6.

Table 6: Structure–Activity Relationship of Thiadiazole Derivatives (TZ_1–TZ_{10}): a comparative study of ZOI against *S.aureus* (gram+ve) and *E.coli*(gram-ve) strain.

Compound	Key Substituent(s)	Electronic Nature	ZOI (*S. aureus*) mm	ZOI (E. coli) mm	Activity Level
TZ1	–SH	Moderate EDG	17.3	18.0	Moderate
TZ2	–OH	Weak EDG	14.3	17.1	Low–Moderate
TZ3	–SH + EDG	Moderate	20.3	21.0	Good

TZ4	–OH substituted	Weak–Moderate	15.5	18.4	Moderate
TZ5	–SH + $–N(CH_3)_2$	Strong EDG	23.0	21.1	Highest
TZ6	–OH	Moderate	16.4	16.2	Moderate
TZ7	–SH	Moderate	17.5	18.3	Moderate
TZ8	–OH	Weak–Moderate	14.5	18.1	Moderate
TZ9	–SH	Moderate	18.1	19.8	Good
TZ10	–SH + $–OCH_3$ + –OH	Moderate EDG	19.0	20.5	Good

An integrated comparison of ZOI, MIC values, and electronic substituent effects is provided in Table 7,

Table 7: Integrated Antibacterial Activity and SAR Profile of Thiadiazole Derivatives against *S. aureus*: a comparative study of ZOI and MIC.

Compound	Key Substituent(s)	ZOI (mm)	MIC (µg/mL)	Electronic Effect	Activity Rank
Ciprofloxacin	Reference	31.0	4	—	1
TZ5	–SH + $–N(CH_3)_2$	23.0	5	Strong EDG	2
TZ10	–SH + $–OCH_3$ + –OH	19.0	6	Moderate EDG	3
TZ9	–SH	18.1	7	Moderate	4
TZ1	–SH	17.3	9	Moderate	5
TZ3	–SH + EDG	20.3	9	Moderate	6
TZ4	–OH	15.5	>10	Weak	7
TZ7	–SH	17.5	>10	Moderate	8
TZ8	–OH	14.5	>10	Weak	9

Mechanistic Insights into Antibacterial Activity

The antibacterial activity of the synthesized derivatives may be attributed to multiple mechanistic pathways. The thiadiazole ring system is known to interact with bacterial enzymes through coordination and hydrogen bonding interactions. The azomethine (–CH=N–) functionality may facilitate metal ion chelation within microbial systems, potentially disrupting essential enzymatic processes. Additionally, the presence of thiol (–SH) groups could enhance interaction with thiol-sensitive bacterial proteins, leading to enzyme inhibition. Electron-donating substituents further increase electron density across the aromatic system, potentially strengthening π–π stacking interactions with nucleic acid bases or enzyme active sites. Collectively, these structural features may contribute to bacterial growth inhibition.

CONCLUSION AND FUTURE PERSPECTIVES

In the present study, a series of novel thiadiazole-based Schiff base derivatives (TZ1–TZ10) were successfully synthesized and evaluated for their antibacterial potential against Gram-positive and Gram-negative bacterial strains. The biological assessment demonstrated that several derivatives exhibited moderate to significant antibacterial activity, with TZ5 emerging as the most potent compound. The observed zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) values showed a consistent inverse correlation, confirming the reliability of the antibacterial evaluation.

Structure–activity relationship (SAR) analysis revealed that the antibacterial potency is strongly influenced by the electronic nature of substituents attached to the aromatic ring. Compounds bearing strong electron-donating groups, particularly in combination with thiol functionality and azomethine linkage, exhibited enhanced activity. The thiadiazole nucleus, coupled with the –CH=N– pharmacophore, appears to serve as a crucial structural scaffold responsible for antibacterial efficacy.

A mechanistic interpretation suggests that these derivatives may exert their antibacterial action through enzyme inhibition, potentially involving hydrogen bonding, π–π stacking interactions, and coordination with active-site residues of essential bacterial enzymes. The enhanced activity of selected compounds may be attributed to improved electronic complementarity and binding stabilization within the enzymatic cavity.

Overall, the findings of this study highlight the thiadiazole–Schiff base framework as a promising platform for the rational development of new antibacterial agents.

ACKNOWLEDGMENT

The authors gratefully acknowledge P.K. University, Madhya Pradesh, for providing the necessary laboratory infrastructure to carry out the synthetic work. The authors are also thankful to Banaras Hindu University, Varanasi, for facilitating IR and NMR spectral analyses, and to Madurai Kamaraj University, Madurai, for extending mass spectrometric and antimicrobial evaluation facilities.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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