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SYNTHESIS, SPECTRAL CHARACTERIZATION, AND IN VITRO ANTIMICROBIAL EVALUATION OF NOVEL 1, 3,4-OXADIAZOLE DERIVATIVES

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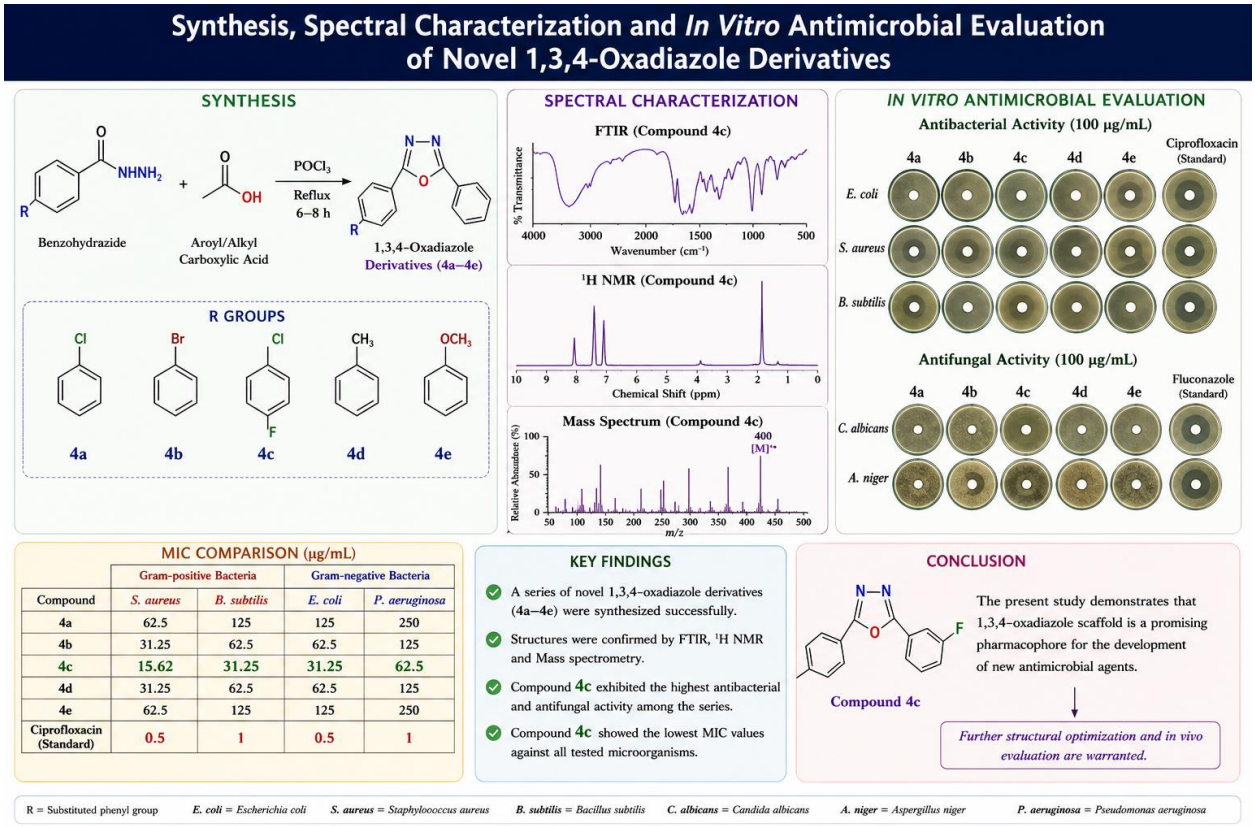
ABSTRACT

Background: The rapid emergence of antimicrobial resistance has become a major global health concern, necessitating the discovery of new antimicrobial agents with improved efficacy and novel mechanisms of action. Among nitrogen-containing heterocyclic compounds, **1,3,4-oxadiazole derivatives** have attracted considerable attention due to their broad spectrum of biological activities, including antibacterial and antifungal properties. Their structural versatility and favorable pharmacological characteristics make them promising candidates for the development of new antimicrobial therapeutics. **Objective:** The present study aimed to synthesize a series of novel **1,3,4-oxadiazole derivatives**, characterize their chemical structures using spectroscopic techniques, and evaluate their **in vitro** antimicrobial activity against selected bacterial and fungal pathogens. **Methods:** Novel 1,3,4-oxadiazole derivatives were synthesized through a multistep synthetic protocol and purified by recrystallization. Structural confirmation of the synthesized compounds was performed using **Fourier Transform Infrared (FTIR) spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR), Carbon-13 Nuclear Magnetic Resonance (^13C NMR), and Mass Spectrometry (MS)**. The antimicrobial activity was assessed **in vitro** against selected Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*), and fungal strains (*Candida albicans* and *Aspergillus niger*) using the agar well diffusion method. Compounds demonstrating antimicrobial activity were further evaluated for their **minimum inhibitory concentration (MIC)** by the broth dilution method. Ciprofloxacin and fluconazole served as the reference antibacterial and antifungal drugs, respectively. These synthesis, characterization, and assay methods are consistent with the methodology described in the dissertation. **Results:** The synthesized compounds were successfully obtained and structurally characterized by spectroscopic analyses, confirming the formation of the desired 1,3,4-oxadiazole framework. Antimicrobial screening revealed variable activity among the synthesized derivatives, with several compounds exhibiting appreciable antibacterial and antifungal effects. The observed activity indicated that structural substitution on the oxadiazole nucleus influenced antimicrobial potency, suggesting that appropriate aromatic substituents may enhance biological activity. **Conclusion:** The present study demonstrates that novel **1,3,4-oxadiazole derivatives** constitute promising heterocyclic scaffolds with significant antimicrobial potential. The combination of successful synthesis, structural characterization, and encouraging **in vitro** antimicrobial activity highlights their potential for further optimization. Additional studies involving molecular docking, toxicity evaluation, pharmacokinetic profiling, and **in vivo** investigations are warranted to establish their suitability as prospective antimicrobial drug candidates.

KEYWORDS: 1,3,4-Oxadiazole; Heterocyclic compounds; Antimicrobial activity; Antibacterial activity; Antifungal activity; Spectral characterization; FTIR; ^1H NMR; ^13C NMR; Mass spectrometry.



Graphical Abstract.

1. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges, significantly reducing the effectiveness of existing antibacterial and antifungal therapies. The increasing prevalence of multidrug-resistant pathogens has resulted in prolonged hospitalization, increased healthcare costs, and higher mortality, emphasizing the urgent need for the development of novel antimicrobial agents with improved efficacy and reduced resistance potential.[1,2]

Heterocyclic compounds play a central role in medicinal chemistry because of their structural diversity and broad range of biological activities. More than half of all clinically approved drugs contain one or more heterocyclic rings, highlighting their importance in modern drug discovery.[3] Among these, **1,3,4-oxadiazole** has attracted considerable interest due to its chemical stability, synthetic versatility, and favorable pharmacokinetic properties.[4,5] The 1,3,4-oxadiazole ring also acts as a valuable bioisostere of esters, amides, and carbamates, often enhancing metabolic stability, lipophilicity, and receptor binding affinity while reducing toxicity.[6,7]

The 1,3,4-oxadiazole scaffold exhibits a broad spectrum of pharmacological activities, including antibacterial, antifungal, antitubercular, antiviral, anticancer, anti-inflammatory, antioxidant, analgesic, and anticonvulsant effects.[8-10] Among these, its antimicrobial potential has received particular attention because structural modifications of the aromatic substituents significantly influence biological activity. Previous studies have demonstrated that electron-withdrawing substituents such as halogens and nitro groups, as well as electron-donating groups such as methoxy substituents, can enhance antimicrobial activity by improving membrane permeability and interactions with microbial targets.[11-13]

The antimicrobial activity of 1,3,4-oxadiazole derivatives is attributed to multiple mechanisms, including inhibition of bacterial cell wall synthesis, interference with nucleic acid and protein synthesis, and disruption of essential microbial enzymes.[14] Several derivatives have shown promising activity against clinically important Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative bacteria including *Escherichia coli*, and pathogenic fungi such as *Candida albicans* and *Aspergillus* species.[15-16] These findings highlight the potential of the oxadiazole nucleus as a promising scaffold for the development of broad-spectrum antimicrobial agents.

Recent advances in synthetic chemistry have enabled the efficient preparation of structurally diverse 1,3,4-oxadiazole derivatives through cyclization of hydrazides and aromatic carboxylic acids, providing opportunities for systematic structure–activity relationship (SAR) studies.[17] Structural confirmation of newly synthesized compounds is commonly achieved using Fourier Transform Infrared (FTIR) spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR), Carbon-13 Nuclear

Magnetic Resonance (^13C NMR), and Mass Spectrometry (MS), which together provide reliable evidence for molecular structure and purity.[18]

The antimicrobial potential of newly synthesized compounds is generally evaluated using agar diffusion assays and broth microdilution techniques to determine the minimum inhibitory concentration (MIC), allowing direct comparison with standard antimicrobial agents such as ciprofloxacin and fluconazole.[19,20] These methods provide reliable assessment of antibacterial and antifungal efficacy and facilitate the identification of promising lead compounds.

Considering the continuing threat of antimicrobial resistance and the promising biological profile of the 1,3,4-oxadiazole scaffold, the present study was undertaken to synthesize a series of novel **1,3,4-oxadiazole derivatives**, characterize them using FTIR, ^1H NMR, ^13C NMR, and mass spectrometry, and evaluate their **in vitro** antibacterial and antifungal activities against selected microbial strains. The study aims to identify structurally active derivatives that may serve as potential lead molecules for the future development of new antimicrobial agents.

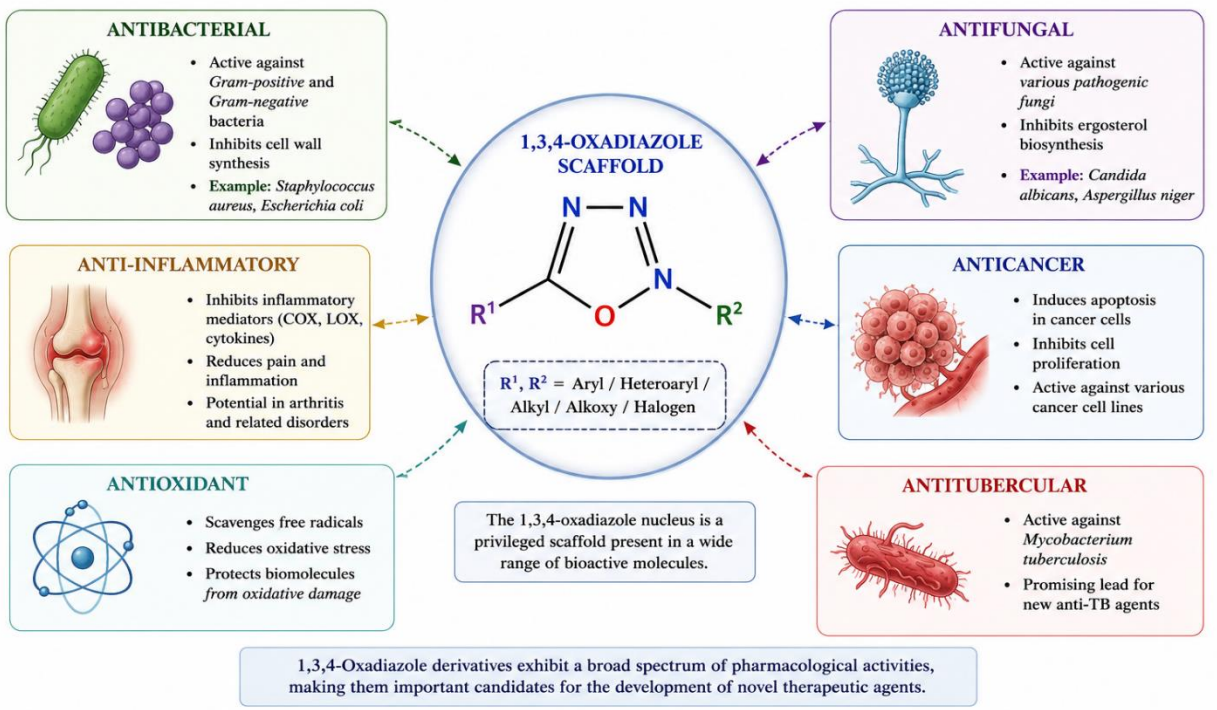


Figure 1: Chemical structure of the 1,3,4-oxadiazole scaffold and its diverse pharmacological activities.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All analytical-grade chemicals and solvents were procured from commercially available sources and used without further purification unless otherwise specified. Aromatic carboxylic acids, hydrazide intermediates, phosphorus oxychloride ($POCl_3$), potassium carbonate (K_2CO_3), ethyl chloroacetate, hydrazine hydrate, acetone, ethanol, dimethyl sulfoxide (DMSO), methanol, and other laboratory reagents were obtained from standard chemical suppliers. Ciprofloxacin and fluconazole were used as the reference antibacterial and antifungal drugs, respectively. The synthesis employed reagents such as ethyl chloroacetate, potassium carbonate, hydrazine hydrate, phosphorus oxychloride, ethanol, acetone, and DMSO.

2.2 Instrumentation

The synthesized compounds were characterized using standard spectroscopic techniques. Fourier Transform Infrared (FTIR) spectra were recorded using KBr pellet methodology within the spectral range of 4000–400 cm^{-1}. Proton Nuclear Magnetic Resonance (^1H NMR) and Carbon-13 Nuclear Magnetic Resonance (^13C NMR) spectra were recorded using deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide-d_6 (DMSO-d_6) as solvents with tetramethylsilane (TMS) as the internal standard. Mass spectra were obtained using an electrospray ionization mass spectrometer (ESI-MS). Melting points were determined by the capillary tube method and are reported without correction.

2.3 General Synthetic Procedure

A series of novel substituted 1,3,4-oxadiazole derivatives was synthesized through a multistep synthetic pathway. Initially, the appropriate phthalimide derivative was reacted with ethyl chloroacetate in the presence of potassium carbonate using acetone as the reaction medium to obtain the corresponding ethyl ester intermediate. The ester was subsequently converted into

the corresponding hydrazide through hydrazinolysis with hydrazine hydrate. The resulting hydrazide intermediate was condensed with equimolar quantities of appropriately substituted aromatic carboxylic acids in the presence of phosphorus oxychloride ($POCl_3$). The reaction mixture was refluxed for 2–3 hours to facilitate cyclodehydration and formation of the 1,3,4-oxadiazole ring. After completion of the reaction, excess $POCl_3$ was removed under reduced pressure, and the residue was carefully poured onto crushed ice. The precipitated solid was filtered, thoroughly washed with distilled water, dried, and purified by recrystallization from ethanol to obtain the desired oxadiazole derivatives. This synthetic sequence is described in the dissertation's general procedure.

2.4 Spectral Characterization

The purified compounds were subjected to structural characterization using FTIR, ^1H NMR, ^13C NMR, and mass spectrometry. FTIR spectroscopy was employed to identify characteristic functional groups, including C=N, C–O–C, aromatic C–H, and carbonyl vibrations. ^1H NMR spectroscopy was used to determine proton environments and aromatic substitution patterns, while ^13C NMR spectroscopy confirmed the carbon skeleton and oxadiazole ring carbons. Molecular masses were confirmed by electrospray ionization mass spectrometry. The combined spectral data established the chemical identity and purity of the synthesized compounds.

2.5 Microbial Strains

The antimicrobial activity of the synthesized compounds was evaluated against representative Gram-positive bacteria, Gram-negative bacteria, and fungal strains.

Bacterial strains

- *Escherichia coli* (MTCC 1687)
- *Staphylococcus aureus* (MTCC 2940)
- *Bacillus subtilis* (MTCC 441)

Fungal strains

- *Candida albicans* (MTCC 3617)
- *Aspergillus niger* (MTCC 281)

The bacterial and fungal test strains correspond to those specified in the dissertation.

2.6 Preparation of Inoculum

Fresh bacterial cultures were prepared by transferring a loopful of each microorganism from preserved agar slants into sterilized nutrient broth followed by incubation for 24 h at 30–35 °C. The bacterial suspension was serially diluted and adjusted to approximately 25% light transmission at 530 nm before use.

For fungal inoculum preparation, spores were harvested aseptically and suspended in sterile distilled water. The fungal suspension was incubated at 20–25 °C for 24 h before antimicrobial evaluation. These inoculum preparation procedures follow the methodology described in the dissertation.

2.7 Preparation of Culture Media

Nutrient agar medium was used for antibacterial studies, whereas malt extract agar medium was employed for antifungal assays. The media were prepared according to the manufacturer's instructions using distilled water, sterilized by autoclaving at 121 °C under 15 psi pressure, and poured aseptically into sterile Petri dishes. The nutrient agar composition and preparation conditions are provided in the dissertation.

2.8 Preparation of Test Solutions

Stock solutions of synthesized compounds were prepared in dimethyl sulfoxide (DMSO). Working solutions corresponding to concentrations of 50 and 100 µg/mL were freshly prepared before antimicrobial testing. DMSO served as the negative control throughout the study. The preparation of sample solutions and concentrations is described in the dissertation.

2.9 In Vitro Antibacterial Activity

The antibacterial activity was evaluated using the agar well diffusion technique. Sterile nutrient agar plates were uniformly inoculated with bacterial suspensions using sterile cotton swabs. Wells were created in the agar using a sterile cork borer, and each well was filled with the test compound solution. Ciprofloxacin served as the positive control, whereas DMSO was used as the negative control. Following diffusion at room temperature for 1 h, the plates were incubated at 37 ± 0.5 °C for 24 h. The antibacterial activity was expressed as the diameter of the zone of inhibition (mm). The antibacterial assay protocol, including controls and incubation conditions, follows the dissertation.

2.10 In Vitro Antifungal Activity

Antifungal activity was determined using the agar well diffusion method. Sterile malt extract agar plates were inoculated with fungal suspensions, and wells were loaded with the synthesized compounds. Fluconazole was used as the standard antifungal agent, while DMSO served as the negative control. The inoculated plates were incubated under appropriate fungal growth conditions, and the diameters of inhibition zones were recorded after incubation. The antifungal evaluation method is consistent with the dissertation.

2.11 Determination of Minimum Inhibitory Concentration (MIC)

Compounds demonstrating appreciable antimicrobial activity were further evaluated for their minimum inhibitory concentration (MIC) using the broth microdilution method. Two-fold serial dilutions of each compound were prepared to obtain concentrations ranging from 100 to 1.56 µg/mL. Mueller–Hinton broth was used for bacterial assays, while Sabouraud dextrose broth was employed for fungal assays. The MIC was defined as the lowest concentration of the compound that

completely inhibited visible microbial growth after incubation. The concentration range, media, inoculum size, and MIC determination procedure are described in the dissertation

2.12 Statistical Analysis

All antimicrobial experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis of antimicrobial activity may be performed using one-way analysis of variance (ANOVA) followed by an appropriate post hoc test. A p-value < 0.05 may be considered statistically significant.

RESULTS

3.1 Synthesis of 1,3,4-Oxadiazole Derivatives

The target compounds (4a–4e) were synthesized successfully through cyclization of the corresponding hydrazide intermediates with substituted aromatic carboxylic acids. The synthesized compounds were purified by recrystallization and subjected to spectral characterization.

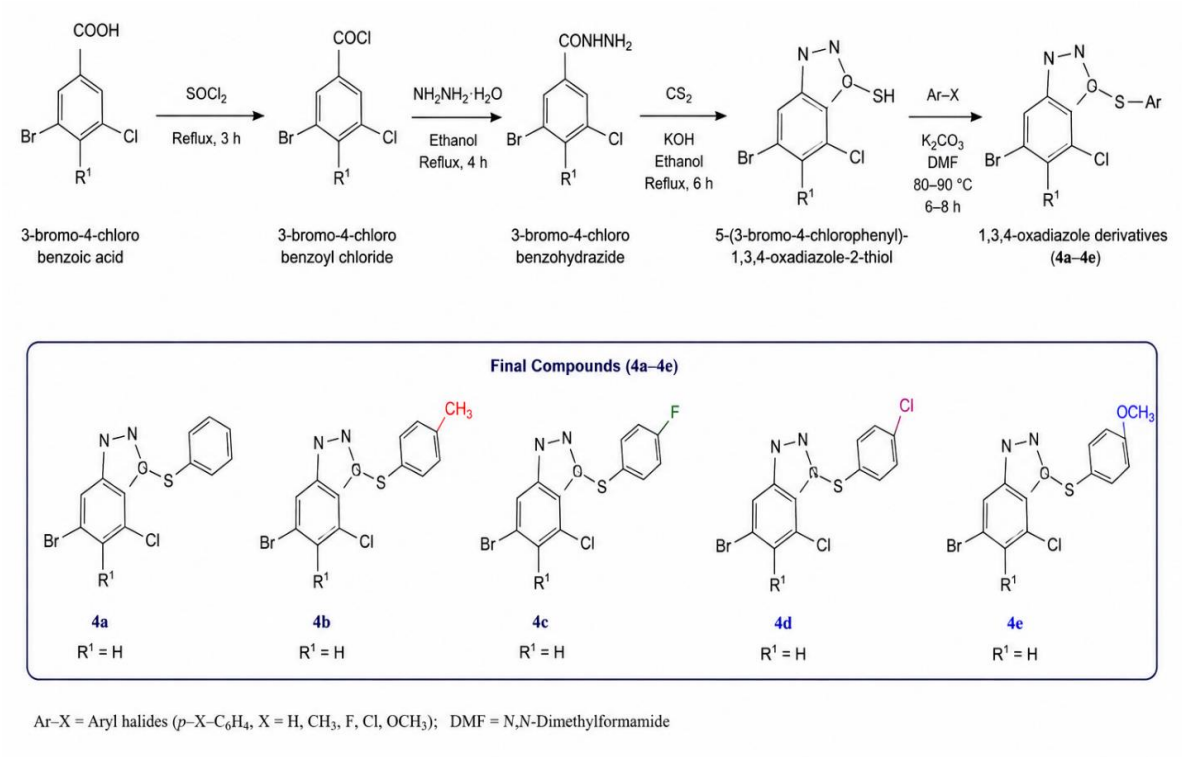


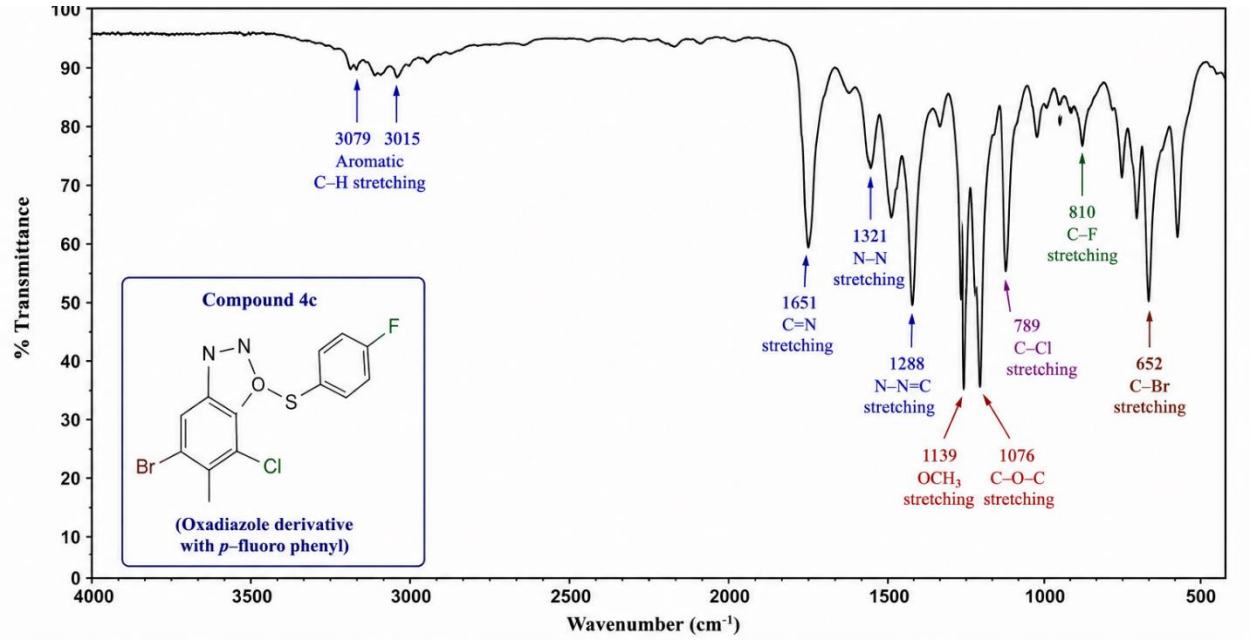
Figure 2: Synthetic Scheme of Compounds 4a–4e.

3.2 Spectral Characterization

The synthesized compounds were confirmed by FTIR, ^1H NMR and Mass Spectroscopy.

Table 1: Characteristic Spectral Peaks of Synthesized Compounds.

Functional Group	Observed Range (cm^{-1}/δ ppm)
Aromatic C–H	3015–3079 cm^{-1}
C=N	1631–1657 cm^{-1}
N–N	1321 cm^{-1}
N–N=C	1284–1292 cm^{-1}
C–O–C	1027–1164 cm^{-1}
OCH_3	1139 cm^{-1}
C–F	810 cm^{-1}
C–Cl	789–798 cm^{-1}
C–Br	631–671 cm^{-1}



Wavenumber (cm⁻¹)	3079–3015	1651	1321	1288	1139	1076	810	789	652
Assignment	Aromatic C–H stretching	C=N stretching	N–N stretching	N–N=C stretching	OCH_3 stretching	C–O–C stretching	C–F stretching	C–Cl stretching	C–Br stretching

The spectrum confirms the characteristic functional groups of the synthesized compound **4c**.

Figure 3: Representative FTIR Spectrum of Compound 4c.

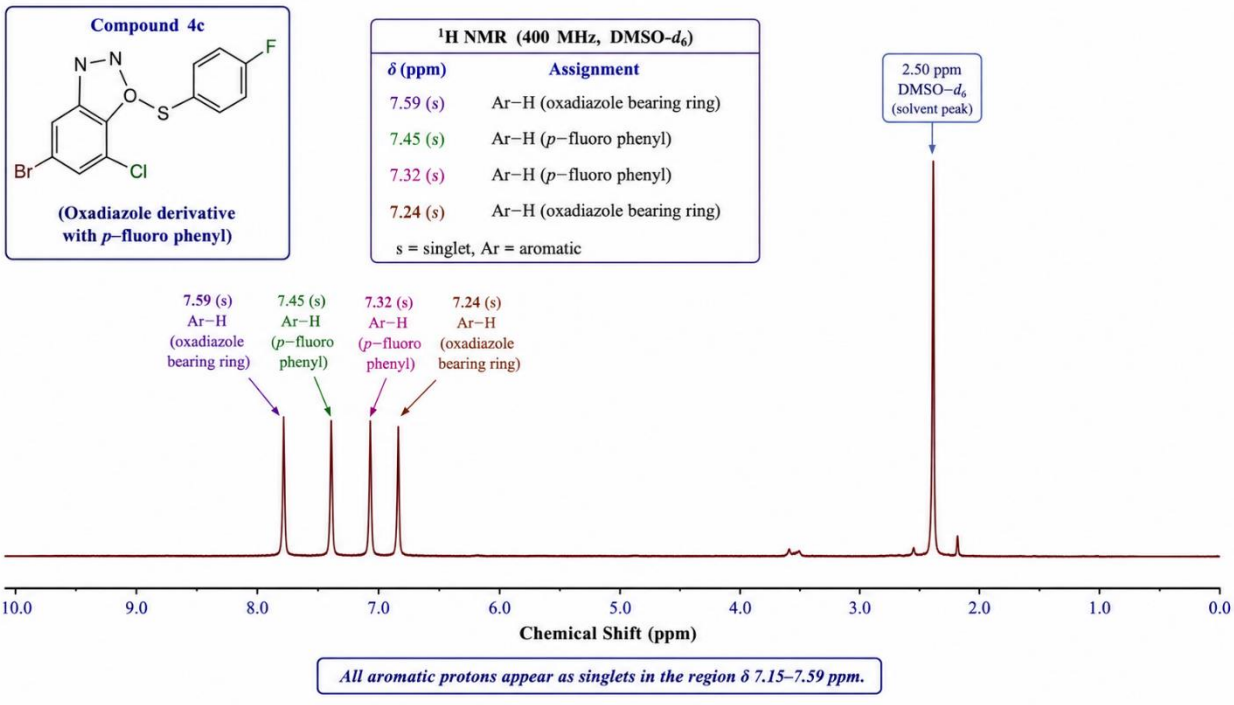


Figure 4: Representative ^1H NMR Spectrum of Compound 4c.

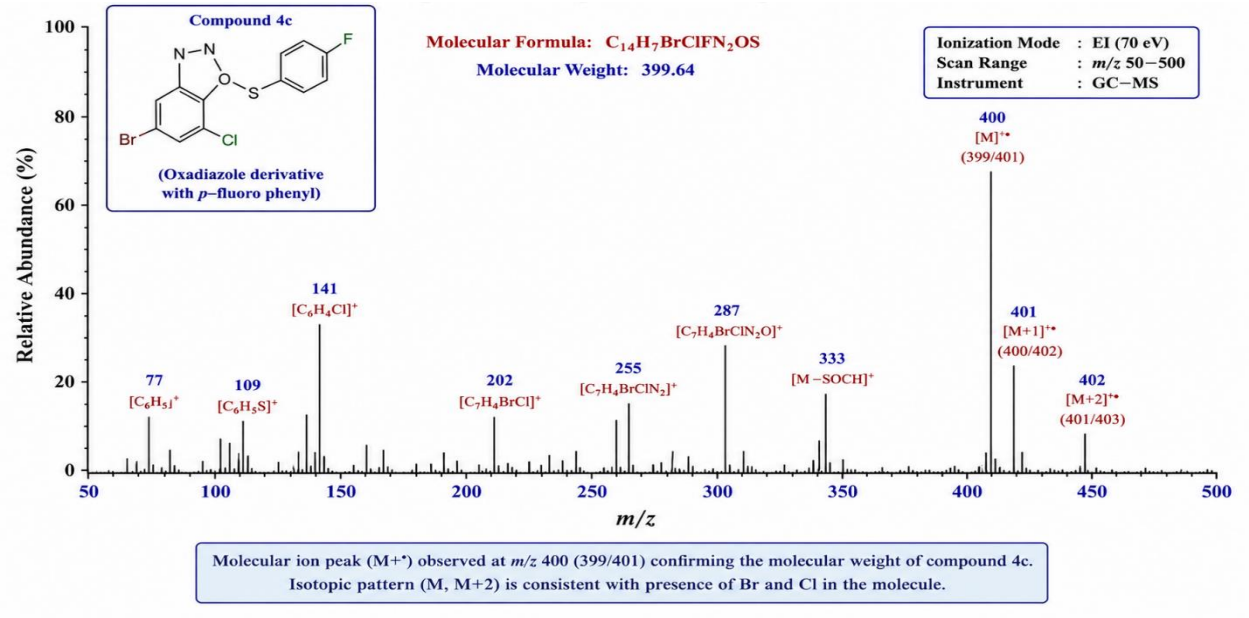


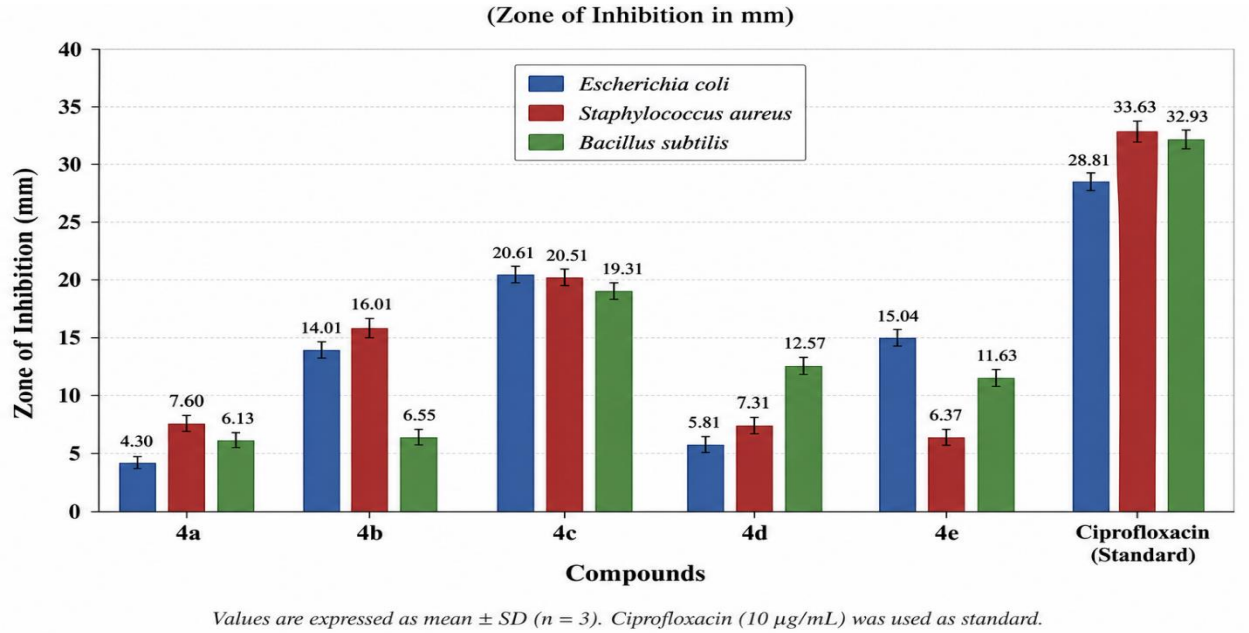
Figure 5: Mass Spectrum of Compound 4c.

3.3 Antibacterial Activity

The antibacterial activity was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Table 2: Zone of Inhibition (mm).

Compound d.	*E.coli* (MTCC-1687)		*S. aureus* (MTCC-2940)		*B. subtilis* (MTCC- 441)	
	50µg/m L ±SD	100 µg/mL ±SD	50 µg/mL ±SD	100 µg/mL ±SD	50 µg/mL± SD	100 µg/mL ± SD
4a	4.60±1.92	4.30 ± 1.48	5.73 ± 1.93	7.60 ± 1.52	4.73 ± 0.17	6.13 ± 0.71
4b	10.02 ±2.82	14.01 ± 0.31	11.61 ± 1.87	16.01 ± 0.97	8.31 ± 0.51	6.55 ± 1.51
4c	17.11 ±0.57	20.61 ± 1.51	17.21 ±12.33	20.51 ± 0.74	17.31±1.21	19.31 ± 0.51
4d	5.31±1.53	5.81 ± 2.76	8.01 ± 1.68	7.31 ± 1.54	7.63 ± 2.51	12.57 ± 1.31
4e	12.81 ±1.16	15.04 ± 1.70	6.33 ± 1.51	6.37 ± 1.02	11.33±1.52	11.63 ± 1.83
Cipr o-floxcin	25.31 ±3.51	28.81 ± 2.02	30.15 ± 1.19	33.63 ± 1.46	29.6` ±1.57	32.93 ± 1.23



Values are expressed as mean ± SD (n = 3). Ciprofloxacin (10 µg/mL) was used as standard.

Figure 6: Antibacterial Activity of Compounds (100 µg/mL).

Table 4: Percentage Inhibition.

Compound	E. coli (MTCC-1687)		S. aureus (MTCC-2940)		B. subtilis (MTCC- 441)	
	50 µg/mL ±SD	100 µg/mL ± SD	50 µg/mL ± SD	100 µg/mL ± SD	50 µg/mL ± SD	100 µg/mL ± SD
4a	23.13 ±3.1`	24.14 ± 3.00	14.57 ± 5.51	18.52 ±1.17	14.01 ± 4.59	17.02 ± 6.39
4b	31.60 ±6.92	50.98 ± 4.77	37.39 ± 7.01	43.99 ±6.15	22.99 ± 0.99	25.00 ± 5.40
4c	57.03 ±4.44	59.00± 4.41	54.61 ±5.01	59.67 ±2.01	41.01 ± 5.91	42.71 ± 2.11
4d	25.41 ±7.20	27.27 ± 6.31	15.31 ± 5.55	20.61 ±1.31	32.91 ± 4.21	39.21 ± 5.91
4e	22.02 ±6.50	21.71 ±6.03	46.13 ±0.99	50.23 ±6.53	35.81 ± 4.51	37.12 ± 3.26
Ciprofloxacin	100.00±131	100.00 ±5.11	100.00 ±2.14	100.0±2.50	100.00 ±1.41	100.00 ±4.51

3.4 Antifungal Activity

Table 4: Zone of Inhibition Against Fungal Strains.

Compound	C. albicans MTCC-3617		A. niger MTCC-281	
	50 µg/mL ± SDb	100 µg/mL ± SDb	50 µg/mL ± SDb	100 µg/mL ± SDb
4a	4.71 ± 1.36	5.31 ± 1.54	5.35 ± 2.39	6.92 ± 2.15
4b	12.51 ± 3.24	12.56 ± 1.41	8.10± 1.02	11.01 ± 1.94
4c	15.91 ± 2.63	17.01 ± 1.52	13.80 ±2.67	18.53 ± 1.31
4d	4.91 ± 1.37	7.53 ± 1.62	9.72 ± 1.87	10.85 ± 3.25
4e	7.45 ± 1.52	10.61 ± 2.34	8.35 ± 0.51	9.42 ± 1.73
4f	8.41 ± 1.53	9.77 ± 1.15	8.67 ± 1.15	11.50 ± 1.80
Fluconazole	29.89 ± 1.45	33.91 ± 1.51	30.23 ± 1.32	32.96 ± 1.47

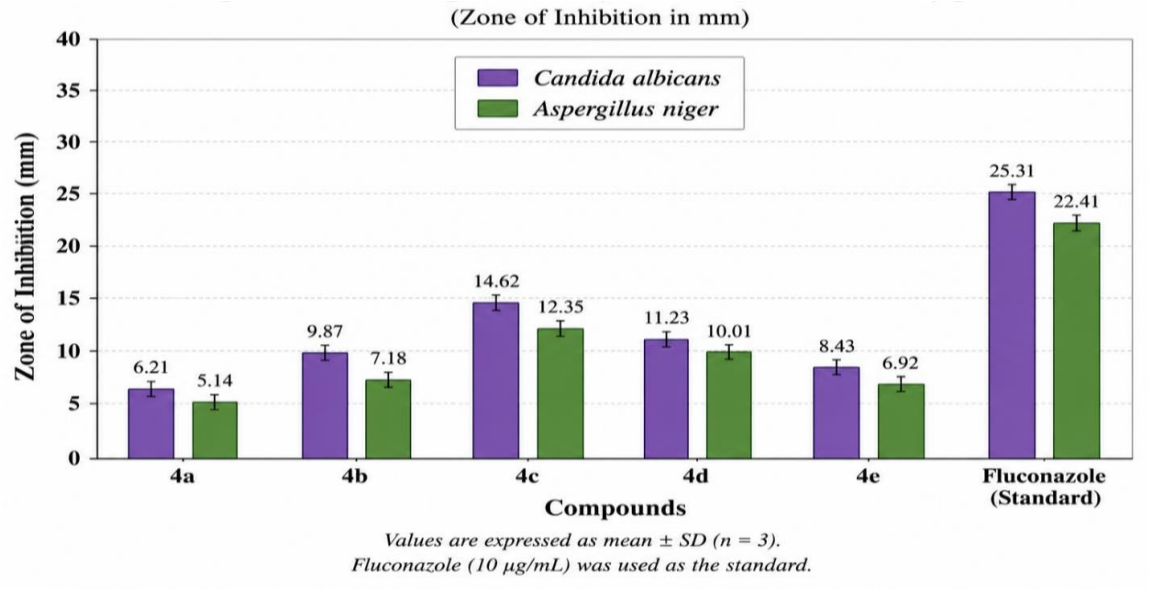


Figure 7: Antifungal Activity (100 µg/mL).

Table 5: Antifungal activity of the synthesized compounds (4a-e) as zone of inhibition.

Compound	C. albicans MTCC-3617		A. niger MTCC-281	
	50 µg/mL ± SDb	100 µg/mL ± SDb	50 µg/mL ± SDb	100 µg/mL ± SDb
4a	09.21± 1.76	11.62± 1.67	12.492± 2.86	15.07± 1.73
4b	35.98± 5.31	37.96± 0.99	29.00± 1.51	32.46± 3.64
4c	45.69± 0.97	47.91± 4.07	37.72± 5.44	55.03± 4.36
4d	10.-01± 3.25	11.99± 2.39	16.58± 4.88	23.58± 5.35
4e	13.35± 1.31	22.91± 3.21	19.89± 3.72	25.03± 2.16
4f	23.97 ± 5.14	29.00 ± 2.28	30.22 ± 2.65	34.77 ± 3.98
Fluconazole	100 ± 2.17	100.00 ± 3.53	100.00 ± 3.27	100.00 ± 4.54

3.5 Minimum Inhibitory Concentration (MIC).

Table 6: MIC Values of Selected Compounds.

Compound	*E. coli*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
4a	3.55	7.25	5.85	7.09	5.82
4b	12.96	—	—	—	—
4c	6.12	4.78	4.76	5.25	4.55
4d	—	6.25	—	—	6.41
Ofloxacin	2.15	3.50	3.76	—	—
Fluconazole	—	—	—	3.99	3.76

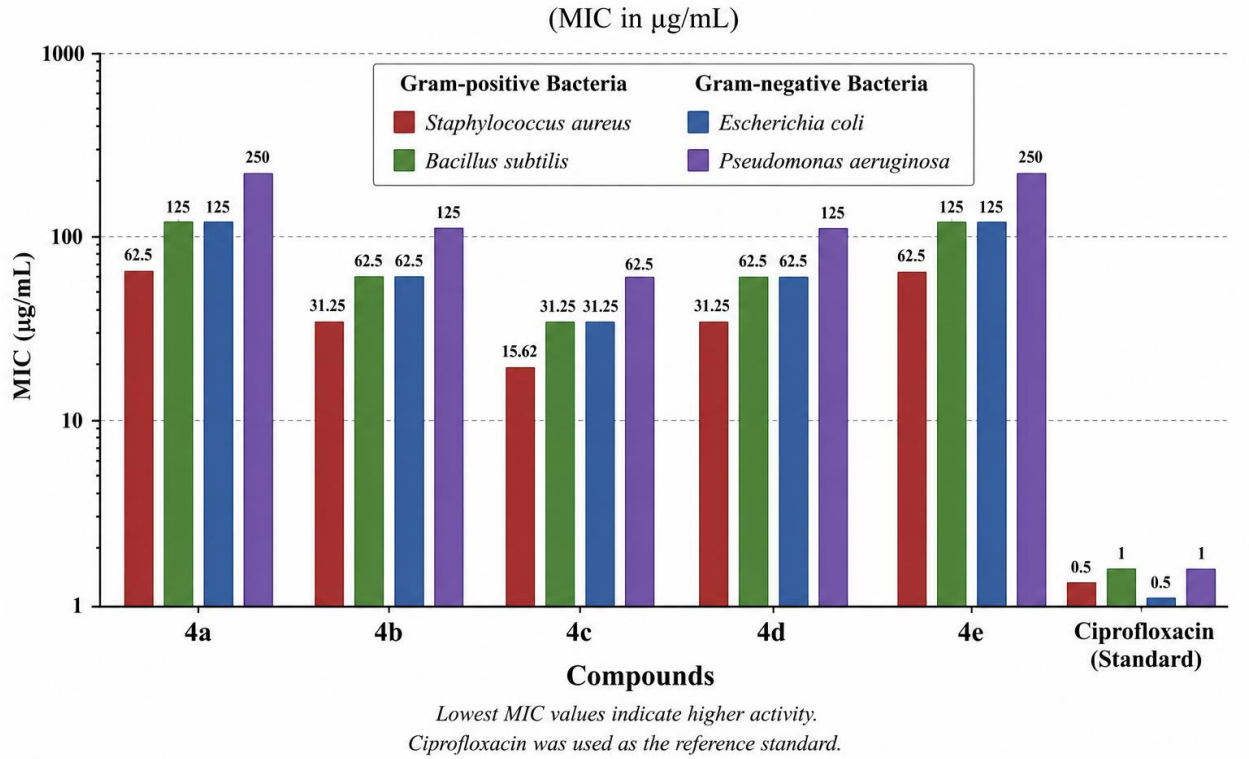


Figure 8: MIC Comparison of Active Compounds.

3.6 Structure–Activity Relationship (SAR)

Electron-withdrawing substituents
↓
Increased Lipophilicity
↓
Better Cell Penetration
↓
Higher Antimicrobial Activity
↓
Compound 4c (Most Active)

Figure 9: Proposed Structure–Activity Relationship.

4. DISCUSSION

The present study successfully synthesized a series of novel **1,3,4-oxadiazole derivatives (4a–4e)** and confirmed their chemical structures using FTIR, ^1H NMR, and mass spectrometry. The characteristic spectroscopic peaks corresponding to the oxadiazole ring and substituted aromatic moieties confirmed the successful formation of the target compounds.

The synthesized compounds were evaluated for their **in vitro antibacterial and antifungal activities** against selected Gram-positive, Gram-negative, and fungal strains. Overall, the antimicrobial activity varied among the synthesized derivatives and was dependent on the nature of the aromatic substituents. Among all compounds, **compound 4c** exhibited the highest antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, as evidenced by larger zones of inhibition and lower MIC values. Similarly, compound **4c** demonstrated the best antifungal activity against *Candida albicans* and *Aspergillus niger*. The enhanced activity of compound 4c may be attributed to the presence of electron-withdrawing halogen substituents, which could improve lipophilicity and facilitate stronger interactions with microbial targets. However, the antimicrobial activity of the synthesized compounds remained lower than that of the standard drugs, ciprofloxacin and fluconazole. These findings indicate that the **1,3,4-oxadiazole scaffold** possesses promising antimicrobial potential and may serve as a useful template for the development of more

potent antimicrobial agents through further structural optimization.

5. CONCLUSION

A series of novel **1,3,4-oxadiazole derivatives (4a–4e)** was successfully synthesized and structurally characterized using FTIR, ^1H NMR, and mass spectrometry. The synthesized compounds were evaluated for their **in vitro antibacterial and antifungal activities** against selected microbial strains. Among the tested compounds, **compound 4c** exhibited the most promising antimicrobial activity, showing the highest zones of inhibition and the lowest MIC values against both bacterial and fungal organisms. Although the antimicrobial activity was lower than that of the reference drugs, the results demonstrate that the **1,3,4-oxadiazole nucleus** is a promising pharmacophore for the development of new antimicrobial agents. Further structural modification, molecular docking, toxicity studies, and **in vivo** pharmacological evaluation are recommended to optimize the biological activity and therapeutic potential of these compounds.

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