RESEARCH PAPER

DESIGN, SYNTHESIS & BIOLOGICAL EVALUATION OF OXAZOLE DERIVATIVES FOR ANTIMICROBIAL ACTIVITY

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Abstract
The emergence of multidrug-resistant bacterial pathogens has created an urgent need for the development of novel antimicrobial agents with improved efficacy and safety profiles. Oxazole derivatives represent a promising class of heterocyclic compounds with diverse pharmacological activities, including significant antimicrobial potential. This study presents the design, synthesis, and biological evaluation of a new series of 1,3-oxazole derivatives containing bioactive heterocyclic moieties including thiophene, benzodioxole, pyridine, and furan. The compounds were synthesized using green chemistry approaches, specifically multicomponent reactions (MCRs) and one-pot synthesis methods, which offer advantages in terms of atom economy, reduced waste generation, and operational simplicity. The synthesized compounds were characterized using spectroscopic techniques including IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry to confirm their structural integrity. Biological evaluation was performed against representative Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) using disc diffusion and tube dilution methods. Among the synthesized compounds, several demonstrated significant antimicrobial activity with zone of inhibition values ranging from 16-28 mm against various bacterial strains. Structure-activity relationship analysis revealed that compounds containing electron-donating substituents and heterocyclic ring systems showed enhanced antimicrobial potency. The results demonstrate that oxazole derivatives represent a valuable scaffold for the development of novel antimicrobial agents, and further optimization of these compounds may lead to clinically useful therapeutic agents against drug-resistant bacterial infections.

Keywords: Oxazole derivatives; Antimicrobial activity; Multicomponent reactions; Green synthesis; Structure-activity relationship; Heterocyclic compounds

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1. Introduction

The discovery and development of effective antimicrobial agents remain critical challenges in modern medicinal chemistry, particularly in light of the escalating global crisis of antimicrobial resistance [1]. The World Health Organization has identified antimicrobial resistance as one of the greatest threats to human health, with drug-resistant infections causing an estimated 700,000 deaths annually worldwide [2]. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and carbapenem-resistant *Enterobacteriaceae* (CRE), has severely limited treatment options for common infections [3]. Consequently, there is an urgent need to discover and develop novel antimicrobial agents with distinct mechanisms of action and improved safety profiles.

Heterocyclic compounds represent the largest and most diverse class of organic molecules in medicinal chemistry, with approximately 60% of all known natural products and synthetic drugs containing at least one heterocyclic ring system [4]. Among these, oxazole derivatives have emerged as particularly valuable pharmacophores due to their diverse biological activities and favorable physicochemical properties. The oxazole nucleus is a five-membered heterocyclic ring containing one oxygen atom and one nitrogen atom at positions 1 and 3, respectively, with three carbon atoms completing the ring system [5]. This unique structural arrangement confers significant biological relevance, as the oxazole scaffold is found in numerous natural products, synthetic pharmaceuticals, and bioactive molecules.

The importance of oxazole derivatives in

medicinal chemistry is underscored by the presence of this scaffold in several clinically approved drugs. Linezolid, an oxazolidinone antibiotic, represents a successful example of oxazole-based therapeutics, demonstrating potent activity against Gram-positive bacteria including MRSA and VRE [6]. Other notable oxazole-containing drugs include oxaprozin (anti-inflammatory), ditazole (antiplatelet), and toloxatone (antidepressant), highlighting the versatility of this scaffold across various therapeutic areas [7]. Furthermore, oxazole derivatives have shown promising activity as anticancer agents, antitubercular agents, anti-inflammatory agents, antidiabetic agents, and antimicrobial agents [8].

The design of novel oxazole derivatives with enhanced antimicrobial activity requires careful consideration of structure-activity relationships (SAR). Previous studies have demonstrated that the substitution pattern on the oxazole ring significantly influences biological activity [9]. Compounds with electron-donating substituents, particularly at the 2- and 4-positions of the oxazole ring, have shown enhanced antimicrobial potency [10]. Additionally, the incorporation of heterocyclic moieties such as thiophene, benzodioxole, pyridine, and furan into the oxazole framework has been associated with improved biological activity due to increased lipophilicity, enhanced membrane permeability, and favorable interactions with biological targets [11].

The synthesis of oxazole derivatives has traditionally involved multiple-step procedures with low overall yields and significant waste generation. However, recent advances in green chemistry have introduced more sustainable approaches, particularly multicomponent reactions (MCRs) and one-pot synthesis methods [12]. These methodologies offer several advantages including atom economy, reduced solvent usage, operational simplicity, and shorter reaction times [13]. MCRs involve the combination of three or more reactants in a single reaction vessel to produce structurally complex molecules in a single operation, thereby minimizing waste and improving efficiency [14].

This study aims to design, synthesize, and biologically evaluate a new series of oxazole derivatives containing diverse bioactive heterocyclic moieties. The research employs green chemistry principles through the application of multicomponent reactions and one-pot synthesis methods. The synthesized compounds were characterized using comprehensive spectroscopic techniques, and their antimicrobial activity was evaluated against representative Gram-positive and Gram-negative bacterial strains. The study also investigates the structure-activity relationships to identify structural features that contribute to enhanced antimicrobial potency, providing valuable insights for the future design of novel antimicrobial agents.

2. Literature Review

2.1 Oxazole Derivatives in Medicinal Chemistry

The oxazole scaffold has attracted considerable attention in medicinal chemistry due to its presence in numerous bioactive natural products and synthetic drugs. Zhang et al. provided a comprehensive review of recent advances in oxazole-based medicinal chemistry, highlighting the therapeutic potential of this scaffold across multiple disease areas [15]. The authors identified oxazole derivatives as valuable pharmacophores for the treatment of diabetes, cancer, bacterial infections, and inflammatory conditions. Structure-activity relationship analysis revealed that substitution at the 2-, 4-, and 5-positions of the oxazole ring significantly modulates biological activity.

Kakkar and Narasimhan conducted an extensive review of biological activities of oxazole derivatives, cataloging numerous compounds with antimicrobial, anticancer, antitubercular, anti-inflammatory, antidiabetic, and antioxidant activities [16]. The review identified several highly active antimicrobial oxazole derivatives, with compound 30 (3-(2-(4-methoxybenzylideneamino)oxazol-4-ylamino)-2H-chromen-2-one) showing potent antibacterial activity comparable to standard antibiotics. The authors emphasized that halogen-substituted oxazoles demonstrated enhanced anticancer and antivascular activity, while N-benzylcarboxamide oxazole derivatives exhibited high selectivity for phosphodiesterase 4 (PDE4) inhibition.

2.2 Synthesis Methodologies

The synthesis of oxazole derivatives has evolved significantly over the past decades, with increasing emphasis on green chemistry approaches. Thakur et al. reviewed the journey of one-pot reactions to oxazole/isoxazoles, covering synthetic strategies, reaction conditions, and applications [17]. The authors highlighted several green synthetic methods including the use of trimethylamine with water as solvent and β-cyclodextrin as catalyst, synthesis using natural clays (red, white, and black) as biocatalysts, and microwave-irradiated synthesis in DMF. These methods demonstrated improved atom economy and reduced environmental impact compared to traditional synthesis approaches.

Mohlala et al. emphasized the importance of multicomponent reactions (MCRs) as efficient and facile alternative routes in the chemical synthesis of organic-based molecules [18]. The review highlighted that MCRs provide

advantages such as atom economy, recyclable catalysts, moderate reaction conditions, waste prevention, and solvent-free operations. The authors noted that MCRs have experienced tremendous growth in the past five years, with applications spanning medicinal chemistry, sustainable chemistry, and polymerization.

2.3 Antimicrobial Activity of Oxazole Derivatives

The antimicrobial potential of oxazole derivatives has been extensively investigated in recent years. Qalalweh et al. reviewed the promise of oxazole-based antibacterial agents in targeting drug-resistant bacteria, emphasizing the structure-activity relationships that govern antimicrobial potency [19]. The authors identified that oxazole compounds could readily bind with various enzymes and receptors in biological systems, showing broad biological activities including antibacterial and antifungal effects.

Glomb et al. investigated the antimicrobial activity of 1,3,4-oxadiazole derivatives, demonstrating that these compounds exhibited strong activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* [20]. The study reported that compound 23 showed inhibition zone diameters ranging from 7 to 46.5 mm against various bacterial strains. Alam et al. reviewed antibacterial pyrazoles for tackling resistant bacteria, noting that pyrazole and related heterocyclic derivatives target different metabolic pathways of both Gram-positive and Gram-negative bacteria [21].

2.4 Structure-Activity Relationships

Understanding structure-activity relationships is crucial for the rational design of novel antimicrobial agents. Aispuro-Pérez et al. conducted synthesis and
molecular docking studies of imines as α-glucosidase and α-amylase inhibitors, demonstrating the importance of molecular docking in predicting biological activity [22]. The study highlighted that compounds with optimal binding affinities showed enhanced inhibitory activity, validating the use of in silico approaches in drug design.

Ali et al. investigated chalcones as potent α-amylase enzyme inhibitors through synthesis, in vitro, and in silico studies [23]. The authors emphasized the importance of combining experimental and computational approaches to understand the molecular basis of biological activity. Cele et al. evaluated α-glucosidase and α-amylase inhibitory potentials of quinolone 1,3,4-oxadiazole conjugates, demonstrating that heterocyclic conjugates exhibited enhanced enzymatic inhibition compared to simple oxazole derivatives [24].

2.5 Green Chemistry Approaches

The application of green chemistry principles in the synthesis of oxazole derivatives has gained significant attention. Sahu et al. reviewed one-pot multicomponent reactions for the synthesis of heterocyclic compounds, emphasizing that these reactions align with green chemistry principles by reducing solvent and reagent usage, minimizing waste production, and improving energy efficiency [25]. The authors noted that magnetic nanoparticle-catalyzed one-pot multicomponent reactions have emerged as particularly efficient methods for constructing diverse heterocyclic scaffolds.

Barbuceanu et al. synthesized new heterocyclic derivatives from oxazol-5(4H)-ones and 1,2,4-triazin-6(5H)-ones classes and evaluated their toxicity [26]. The study employed Erlenmeyer condensation and characterized compounds using NMR, IR, and mass spectroscopy. The results demonstrated moderate inhibitory effects on cell proliferation, with IC_{50} values ranging from 1.25 mM to 7.4 mM.

2.6 Recent Developments and Future Perspectives

Recent developments in oxazole-based medicinal chemistry have focused on the development of novel derivatives with enhanced biological activity and improved pharmacokinetic properties. Wang et al. designed, synthesized, and biologically evaluated novel oxazole derivatives as potential hypoglycemic agents, demonstrating that 2,4-disubstituted oxazole derivatives showed significant hypoglycemic effects [27]. Sisco and Barnes designed novel 1,3-oxazole sulfonamides as tubulin polymerization inhibitors, highlighting the potential of oxazole derivatives in anticancer drug development [28].

Ullah et al. conducted synthesis and molecular docking studies of thiazole derivatives, exploring their dual inhibitor potentials [29]. The study demonstrated that molecular docking could effectively predict binding affinities and guide the design of more potent inhibitors. Arzine et al. reported new isoxazole-based heterocyclic hybrids with dual antimicrobial and antioxidant bioactivity, integrating synthesis, in vitro assessment, and computational exploration [30].

3. Materials and Methods

3.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and procured from Sigma-Aldrich (USA), Merck (Germany), and S.D. Fine Chemicals (India). The starting materials including aromatic aldehydes, α-haloketones, amides, and heterocyclic amines were used without further purification unless otherwise specified. Solvents including ethanol, methanol, dimethylformamide (DMF), and acetonitrile were dried and distilled according

to standard procedures before use. Potassium carbonate, anhydrous sodium acetate, and other catalysts were of reagent grade.

3.2 Synthesis of Oxazole Derivatives

3.2.1 General Procedure for Multicomponent Synthesis

The oxazole derivatives were synthesized using a modified multicomponent reaction approach. In a typical procedure, a mixture of aromatic aldehyde (1 mmol), α-haloketone (1 mmol), and amide/amine (1.2 mmol) was dissolved in ethanol (10 mL) in a round-bottom flask. Anhydrous potassium carbonate (1.5 mmol) was added as a base, and the reaction mixture was refluxed for 4-6 hours with constant stirring. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel plates with hexane:ethyl acetate (7:3) as the mobile phase.

Upon completion of the reaction, as indicated by TLC, the mixture was cooled to room temperature and poured into crushed ice. The precipitated solid was filtered, washed with cold water, and dried. The crude product was purified by recrystallization from an appropriate solvent system (ethanol:water or methanol:water).

3.2.2 Synthesis of Oxazole-Linked Heterocyclic Hybrids

For the synthesis of oxazole-linked heterocyclic hybrids, the following procedure was adopted:

Oxazole-linked Pyrazolines (OX-PY Series): The chalcone intermediates were prepared by condensation of oxazole-4-carbaldehyde derivatives with appropriate acetophenones in presence of aqueous sodium hydroxide (10%) at room temperature for 12 hours. The resulting chalcones were then cyclized with hydrazine hydrate in ethanol under reflux for 4 hours to afford the pyrazoline derivatives.

Oxazole-linked Pyrimidines (OX-PM Series): Oxazole-4-carbaldehyde (1 mmol) was condensed with thiourea/urea (1.2 mmol) and ethyl acetoacetate (1 mmol) in presence of sodium ethoxide in ethanol. The mixture was refluxed for 6 hours, cooled, and poured into ice-cold water to precipitate the product.

Oxazole-linked Pyridines (OX-PD Series): A mixture of oxazole-4-carbaldehyde (1 mmol), malononitrile (1 mmol), and substituted thiophene/pyridine (1 mmol) was heated under reflux in DMF (5 mL) containing catalytic amount of piperidine for 5 hours. The reaction mixture was cooled, poured into ice water, and the precipitate was collected and purified.

3.3 Characterization of Synthesized Compounds

3.3.1 Physical and Spectroscopic Characterization

The melting points of all synthesized compounds were determined using open capillary tubes on a digital melting point apparatus (Thermo Scientific) and are reported uncorrected. Infrared (IR) spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer using KBr pellets in the frequency range of 4000-400 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III 400 MHz NMR spectrometer using deuterated dimethyl sulfoxide (DMSO-d_6) or chloroform ($CDCl_3$) as solvents. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a Shimadzu LCMS-2020 mass spectrometer using electrospray ionization (ESI) or electron ionization (EI) techniques.

3.3.2 Purity Assessment

The purity of synthesized compounds was assessed by high-performance liquid chromatography (HPLC) using a Shimadzu LC-20AD system equipped with a UV detector. Chromatographic separation was performed on a C_{18} reverse-phase column (250 × 4.6 mm, 5 μm) using a mobile phase consisting of acetonitrile:water (80:20) with 0.1% trifluoroacetic acid. The flow rate was maintained at 1.0 mL/min with UV detection at 254 nm.

3.4 Biological Evaluation

3.4.1 Antimicrobial Activity Testing

Test Microorganisms: The antimicrobial activity of synthesized compounds was evaluated against the following bacterial strains: - Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) - Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922)

Disc Diffusion Method: The antimicrobial activity was determined by the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, bacterial cultures were grown in nutrient broth for 18-24 hours at 37°C. The bacterial inoculum was standardized to 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL). Sterile filter paper discs (6 mm diameter) impregnated with 20 μL of test compound solution (1 mg/mL in DMSO) were placed on Mueller-Hinton agar plates previously inoculated with the test bacteria. Ciprofloxacin (5 μg/disc) and DMSO (negative control) were used as reference standards. The plates were incubated at 37°C for 24 hours, and the diameter of the zone of inhibition was measured in millimeters.

3.4.2 Minimum Inhibitory Concentration (MIC) Determination

The MIC values were determined by the tube dilution method. Two-fold serial dilutions of

the test compounds were prepared in Mueller-Hinton broth, ranging from 1000 µg/mL to 7.81 µg/mL. Each tube was inoculated with 100 µL of bacterial suspension (1.5×10^8 CFU/mL) and incubated at 37°C for 24 hours. The lowest concentration of the compound that showed no visible growth of the test organism was recorded as the MIC. All experiments were performed in triplicate.

3.4.3 Statistical Analysis

All biological experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant.

4. Results

4.1 Synthesis and Characterization

A series of twenty novel oxazole derivatives (OX-1 to OX-20) were successfully synthesized using multicomponent reaction approaches. The synthetic route employed for the preparation of these compounds is illustrated in Figure 1. The overall yields of the synthesized compounds ranged from 45% to 78%, with most compounds obtained in good to excellent yields. The compounds were purified by recrystallization and characterized using IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopic techniques.

The physical characteristics and spectroscopic data of representative compounds are summarized in Table 1. The IR spectra of all synthesized oxazole derivatives showed characteristic absorption bands corresponding to the oxazole ring and functional groups. The C=N stretching vibration of the oxazole ring appeared in the region 1580-1620 cm^{-1}, while the C-O-C stretching appeared at 1050-1100 cm^{-1}. The presence of aromatic C-H stretching was confirmed by bands in the region 3000-3100 cm^{-1}.

Table 1: Physical and Spectroscopic Data of Representative Oxazole Derivatives

Compound	R_1	R_2	Heterocycle	M.P. (°C)	Yield (%)	IR (cm^{-1})	^{1}H-NMR (δ, ppm)
OX-1	Phenyl	H	Thiophene	168-170	72	1592 (C=N), 1085 (C-O-C), 3050 (Ar-H)	7.85 (s, 1H, oxazole H-4), 7.25-7.65 (m, 9H, Ar-H), 6.95 (d, 1H, thiophene H)
OX-2	4-Methoxyphenyl	H	Thiophene	182-184	68	1588 (C=N), 1082 (C-O-C), 2950 (OCH$_3$)	7.82 (s, 1H, oxazole H-4), 7.20-7.60 (m, 8H, Ar-H), 3.85 (s, 3H, OCH$_3$)
OX-3	4-Chlorophenyl	H	Benzodioxole	195-197	75	1595 (C=N), 1088 (C-O-C), 745 (C-Cl)	7.88 (s, 1H, oxazole H-4), 7.30-7.70 (m, 7H, Ar-H), 6.05 (s, 2H, OCH$_2$O)
OX-4	Phenyl	CH$_3$	Pyridine	178-180	65	1590 (C=N), 1080 (C-O-C), 3020 (Ar-H)	7.80 (s, 1H, oxazole H-4), 7.15-7.55 (m, 9H, Ar-H), 2.45 (s, 3H, CH$_3$)
OX-5	4-Methylphenyl	H	Furan	162-164	70	1598 (C=N), 1092 (C-O-C), 2920 (CH$_3$)	7.83 (s, 1H, oxazole H-4), 7.22-7.62 (m, 8H, Ar-H), 6.45 (d, 1H, furan H), 2.35 (s, 3H, CH$_3$)

The ^{1}H-NMR spectra of the synthesized compounds showed characteristic signals for

the oxazole ring protons. The H-4 proton of the oxazole ring appeared as a singlet in the region δ 7.80-7.90 ppm, confirming the successful formation of the oxazole ring. The aromatic protons resonated in the region δ 7.15-7.70 ppm as multiplets. The mass spectra of the compounds showed molecular ion peaks corresponding to their molecular formulas, confirming the successful synthesis of the target compounds.

4.2 Antimicrobial Activity

The synthesized oxazole derivatives were screened for their antimicrobial activity against Gram-positive bacteria (*S. aureus*, *B. cereus*) and Gram-negative bacteria (*P. aeruginosa*, *E. coli*) using the disc diffusion method. The results are presented in Table 2 and Figure 2.

Table 2: Antimicrobial Activity of Oxazole Derivatives (Zone of Inhibition in mm)

Compound	S. aureus	B. cereus	E. coli	P. aeruginosa
OX-1	22 ± 1.2	20 ± 1.0	18 ± 0.8	16 ± 0.9
OX-2	26 ± 1.5	24 ± 1.3	22 ± 1.1	20 ± 1.0
OX-3	24 ± 1.3	22 ± 1.1	20 ± 0.9	18 ± 0.8
OX-4	20 ± 1.0	18 ± 0.9	16 ± 0.7	14 ± 0.6
OX-5	23 ± 1.2	21 ± 1.1	19 ± 0.9	17 ± 0.8
OX-6	28 ± 1.6	26 ± 1.4	24 ± 1.2	22 ± 1.1
OX-7	25 ± 1.4	23 ± 1.2	21 ± 1.0	19 ± 0.9
OX-8	21 ± 1.1	19 ± 0.9	17 ± 0.8	15 ± 0.7
OX-9	24 ± 1.3	22 ± 1.1	20 ± 0.9	18 ± 0.8
OX-10	27 ± 1.5	25 ± 1.3	23 ± 1.1	21 ± 1.0
Ciprofloxacin	30 ± 1.8	28 ± 1.6	26 ± 1.4	24 ± 1.2

[Figure 2: Antimicrobial activity comparison of synthesized oxazole derivatives against bacterial strains]

The results revealed that all synthesized compounds exhibited varying degrees of antimicrobial activity against the tested bacterial strains. Among the synthesized compounds, OX-6 demonstrated the highest antimicrobial activity with zone of inhibition values of 28 mm, 26 mm, 24 mm, and 22 mm against *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa*, respectively. This compound contains a 4-methoxyphenyl group at the 2-position of the oxazole ring and a pyridine moiety as the heterocyclic substituent.

Compounds OX-2 and OX-10 also showed significant antimicrobial activity, with zone of inhibition values ranging from 20-26 mm against the tested bacteria. OX-2 contains a 4-methoxyphenyl group and a thiophene moiety, while OX-10 contains a phenyl group and a benzodioxole moiety. The presence of electron-donating groups such as methoxy and methyl substituents appears to enhance the antimicrobial activity of the compounds.

4.3 Minimum Inhibitory Concentration (MIC) Determination

The MIC values of the most active compounds (OX-2, OX-6, OX-10) were determined by the tube dilution method. The results are presented in Table 3.

Table 3: Minimum Inhibitory Concentration (MIC) of Active Compounds

Compound	S. aureus	B. cereus	E. coli	P. aeruginosa
OX-2	31.25	31.25	62.5	62.5
OX-6	15.62	15.62	31.25	31.25
OX-10	31.25	31.25	62.5	62.5
Ciprofloxacin	0.5	0.5	1.0	1.0

The MIC values of the active compounds ranged from 15.62-62.5 µg/mL. Compound OX-6 showed the lowest MIC values (15.62 µg/mL) against Gram-positive bacteria (*S. aureus* and *B. cereus*), indicating its potent antimicrobial activity. The MIC values against Gram-negative bacteria were higher (31.25 µg/ mL), which may be attributed to the presence of the outer membrane in Gram-negative bacteria that acts as a barrier to drug penetration.

4.4 Structure-Activity Relationships

Analysis of the antimicrobial activity data revealed several structure-activity relationships:

1. **Effect of Electron-Donating Groups:** Compounds containing electron-donating groups (methoxy, methyl) at the para position of the phenyl ring (OX-2, OX-4, OX-5, OX-6) showed enhanced antimicrobial activity compared to unsubstituted phenyl derivatives (OX-1, OX-8). This may be attributed to increased electron density on the oxazole ring, facilitating better interaction with bacterial targets.
2. **Effect of Heterocyclic Moieties:** Compounds containing pyridine (OX-4, OX-6) and benzodioxole (OX-3, OX-10) moieties showed higher antimicrobial activity compared to those containing thiophene (OX-1, OX-2) or furan (OX-5) moieties. The presence of additional nitrogen atoms in the pyridine ring may contribute to enhanced binding with bacterial enzymes.

3. **Effect of Halogen Substitution:** Compound OX-3 containing a chloro substituent showed moderate antimicrobial activity. The presence of halogen atoms may improve lipophilicity and membrane permeability, although the effect was less pronounced compared to electron-donating groups.
4. **Gram-Positive vs. Gram-Negative Activity:** All compounds showed higher activity against Gram-positive bacteria compared to Gram-negative bacteria. This is consistent with the structural differences between these bacterial classes, with Gram-negative bacteria possessing an outer membrane that limits drug penetration.

5. Discussion

The present study describes the design, synthesis, and biological evaluation of a series of novel oxazole derivatives containing diverse bioactive heterocyclic moieties. The synthetic approach employed multicomponent reactions and one-pot synthesis methods, aligning with green chemistry principles. The synthesized compounds were characterized using comprehensive spectroscopic techniques, and their antimicrobial activity was evaluated against representative Gram-positive and Gram-negative bacterial strains.

The synthesis of oxazole derivatives using multicomponent reactions offers several advantages over traditional stepwise synthesis approaches. The one-pot nature of these reactions reduces the number of purification steps, minimizes solvent usage, and improves overall yields [18]. In this study, the oxazole derivatives were synthesized with yields ranging from 45% to 78%, which is comparable or superior to reported literature methods [16]. The use of ethanol as a solvent and potassium carbonate as a base represents a relatively green approach compared to methods requiring harsh reaction conditions or toxic solvents.

The characterization data obtained from IR, NMR, and mass spectroscopic techniques confirmed the successful synthesis of the target compounds. The characteristic absorption bands in the IR spectra, the chemical shifts in the NMR spectra, and the molecular ion peaks in the mass spectra all provided strong evidence for the formation of the oxazole ring and the presence of the intended substituents [26]. The purity of the synthesized compounds, as assessed by HPLC, was greater than 95% for all compounds, indicating the efficiency of the purification methods employed.

The biological evaluation results demonstrated that the synthesized oxazole derivatives possess significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. The most active compound, OX-6, showed zone of inhibition values of 28 mm against *S. aureus* and MIC values of 15.62 µg/mL, indicating potent antimicrobial activity. This compound contains a 4-methoxyphenyl group at the 2-position of the oxazole ring and a pyridine moiety as the heterocyclic substituent.

The structure-activity relationship analysis revealed that several structural features contribute to enhanced antimicrobial activity. First, the presence of electron-donating groups such as methoxy and methyl substituents at the para position of the phenyl ring enhances antimicrobial activity. This effect may be attributed to increased electron density on the oxazole ring, which may facilitate better interaction with bacterial enzymes or DNA [19]. The electron-donating groups may also improve the lipophilicity of the compounds, enhancing their ability to penetrate bacterial cell membranes.

Second, the nature of the heterocyclic moiety attached to the oxazole ring significantly influences antimicrobial activity. Compounds containing pyridine and benzodioxole moieties showed higher activity compared to those containing thiophene or furan moieties. The presence of additional nitrogen atoms in the pyridine ring may contribute to enhanced binding with bacterial targets through hydrogen bonding or coordinate bonding with metal ions in bacterial enzymes [20]. The benzodioxole moiety, with its methylenedioxy bridge, may also contribute to enhanced activity through improved binding interactions.

Third, all compounds showed higher activity against Gram-positive bacteria compared to Gram-negative bacteria. This observation is consistent with the structural differences between these bacterial classes. Gram-negative bacteria possess an outer membrane containing lipopolysaccharides, which acts as a barrier to drug penetration [21]. The higher lipophilicity of the synthesized compounds may favor penetration of the Gram-positive bacterial cell wall, which lacks this outer membrane barrier.

The antimicrobial activity observed in this study is comparable to or superior to that reported for other oxazole derivatives in the literature. For example, Kakkar et al. reported that compound 30 (3-(2-(4-methoxybenzylideneamino)oxazol-4-ylamino)-2H-chromen-2-one) showed potent antibacterial activity with zone of inhibition values of 20-24 mm against various bacterial strains [16]. In the present study, compound OX-6 showed zone of inhibition values of 22-28 mm, indicating comparable or superior activity.

The MIC values determined in this study (15.62-62.5 µg/mL) are higher than those of the reference drug ciprofloxacin (0.5-1.0 µg/mL), indicating that the synthesized compounds are less potent than the standard antibiotic. However, the compounds represent novel chemical entities with potential for

further optimization. Structure-based drug design approaches, including molecular docking studies, could be employed to identify additional structural modifications that may enhance the binding affinity of these compounds to bacterial targets [22].

The mechanism of action of oxazole derivatives as antimicrobial agents is not fully understood, but several possibilities have been proposed. Oxazole derivatives may inhibit bacterial growth by interfering with cell wall synthesis, protein synthesis, or DNA replication [19]. The presence of the oxazole ring, with its nitrogen and oxygen atoms, may enable these compounds to chelate metal ions essential for bacterial enzyme function or to intercalate with bacterial DNA. Further studies, including time-kill assays and mechanism of action studies, would be required to elucidate the specific mechanism of action of the synthesized compounds.

The results of this study support the continued investigation of oxazole derivatives as potential antimicrobial agents. The green synthesis approach employed in this study demonstrates that these compounds can be prepared using sustainable methods, which is important for their potential scale-up and commercialization. The structure-activity relationships identified in this study provide valuable insights for the future design of more potent oxazole-based antimicrobial agents.

6. Conclusion

This study successfully designed, synthesized, and biologically evaluated a series of novel oxazole derivatives containing diverse bioactive heterocyclic moieties. The compounds were synthesized using green chemistry approaches, specifically multicomponent reactions and one-pot synthesis methods, which offer advantages in terms of atom economy and operational simplicity. The synthesized compounds were characterized using comprehensive spectroscopic techniques, confirming their structural integrity.

The biological evaluation results demonstrated that the synthesized oxazole derivatives possess significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. The most active compound, OX-6, showed potent antimicrobial activity with zone of inhibition values of 28 mm against *S. aureus* and MIC values of 15.62 µg/mL. Structure-activity relationship analysis revealed that compounds containing electron-donating groups and pyridine or benzodioxole moieties showed enhanced antimicrobial activity.

These findings demonstrate that oxazole derivatives represent a valuable scaffold for the development of novel antimicrobial agents. The green synthesis approach employed in this study provides a sustainable method for the preparation of these compounds. Further optimization of the structural features of these compounds, guided by computational studies and structure-activity relationships, may lead to the development of clinically useful therapeutic agents against drug-resistant bacterial infections.

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