Design, Synthesis, and Mechanistic Pharmacological Evaluation of Novel Quinoline–1,3,4-Oxadiazole Hybrid Molecules as Therapeutic Agents Against Multidrug-Resistant Infections

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ABSTRACT

Background The rapid emergence of multidrug-resistant (MDR) bacterial strains poses a critical global health challenge, significantly reducing the efficacy of existing antimicrobial agents such as fluoroquinolones and β-lactams. Quinoline derivatives are well-established antimicrobial pharmacophores, while 1,3,4-oxadiazole scaffolds are known to enhance biological activity and pharmacokinetic properties. Hybridization of these two moieties offers a promising strategy to develop potent agents against resistant pathogens.

Objective To design, synthesize, and evaluate novel quinoline–1,3,4-oxadiazole hybrid molecules for their antimicrobial activity against MDR bacterial strains and to investigate their possible mechanisms of action.

Methods A series of ten quinoline–oxadiazole hybrid compounds (QO-1 to QO-10) were synthesized via cyclization of quinoline-based hydrazide intermediates under reflux conditions. The structures were confirmed using IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Antimicrobial activity was assessed against MDR strains of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa using broth microdilution method to determine minimum inhibitory concentration (MIC). Ciprofloxacin was used as a standard reference drug. Mechanistic studies included membrane permeability assay, DNA binding analysis (UV-visible spectroscopy), and DNA gyrase inhibition assay. Cytotoxicity was evaluated using MTT assay on HEK-293 cell lines.

Results All synthesized compounds exhibited moderate to significant antimicrobial activity. Among them, compounds QO-4, QO-7, and QO-9 demonstrated the most potent effects. MIC values ranged from 1.56 to 25 µg/mL against tested strains. QO-7 showed highest activity with MIC: S. aureus: 1.56 µg/mL, E. coli: 3.12 µg/mL, P. aeruginosa: 6.25 µg/mL, showing comparable activity to ciprofloxacin (MIC: 0.78–1.56 µg/mL). Mechanistic evaluation revealed increased membrane permeability (up to 65% leakage of intracellular material), significant DNA binding affinity (hypochromic shift ~18%), and DNA gyrase inhibition (IC_{50} = 2.8 µM for QO-7). Cytotoxicity studies indicated low toxicity, with cell viability >85% at 50 µg/mL, suggesting a favorable safety profile.

Conclusion The synthesized quinoline–1,3,4-oxadiazole hybrids exhibited promising antimicrobial activity against MDR pathogens, with QO-7 emerging as the most potent candidate. The mechanism of action is likely mediated through bacterial membrane disruption and DNA gyrase inhibition. These findings highlight the potential of these hybrid molecules as lead compounds for the development of new antimicrobial agents.

Keywords: Quinoline, 1,3,4-oxadiazole, Multidrug resistance, Antimicrobial activity, DNA gyrase inhibition, Hybrid molecules.

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1. Introduction

The global rise of antimicrobial resistance (AMR) has emerged as one of the most pressing public health challenges of the 21st century. Multidrug-resistant (MDR) bacterial infections are increasingly associated with higher morbidity, mortality, prolonged hospital stays, and significant economic burden. According to the World Health Organization, AMR is projected to cause millions of deaths annually by 2050 if effective interventions are not developed. The diminishing efficacy of conventional antibiotics, including fluoroquinolones, β-lactams, and aminoglycosides, has necessitated the urgent development of novel antimicrobial agents with improved efficacy and distinct mechanisms of action.

Among various heterocyclic scaffolds explored in medicinal chemistry, quinoline derivatives have received considerable attention due to their broad spectrum of biological activities. Quinoline-based compounds, such as Ciprofloxacin, exert potent antibacterial effects primarily through inhibition of bacterial DNA gyrase and topoisomerase IV, thereby interfering with DNA replication and transcription. However, the extensive clinical use of such agents has led to the rapid emergence of resistant strains, limiting their long-term therapeutic utility. Structural modification of the quinoline nucleus is therefore a promising strategy to overcome resistance and enhance antimicrobial potency.

The 1,3,4-oxadiazole ring system represents another important pharmacophore widely incorporated in drug design. This five-membered heterocycle is known for its favorable physicochemical properties, including metabolic stability, hydrogen bonding capability, and improved lipophilicity. Compounds containing the 1,3,4-oxadiazole moiety have demonstrated diverse pharmacological activities such as antimicrobial, anti-inflammatory, anticancer, and antiviral effects. Importantly, the oxadiazole ring can act as a bioisostere for amide and ester functionalities, thereby enhancing drug-like characteristics and biological activity (Keri et al., 2015; Verma et al., 2020).

The concept of molecular hybridization**,** which involves the combination of two or more pharmacophores into a single molecular framework, has gained prominence in recent years as an effective strategy for drug development. Hybrid molecules often exhibit enhanced biological activity, reduced toxicity, and the ability to act on multiple biological targets simultaneously. In the context of antimicrobial drug discovery, hybridization of quinoline and 1,3,4-oxadiazole scaffolds is particularly attractive, as it may lead to synergistic effects and improved activity against resistant bacterial strains. Previous studies have reported that such hybrids exhibit significant antibacterial activity, potentially through dual mechanisms involving enzyme inhibition and membrane disruption (Desai et al., 2019).

Despite these promising findings, there remains a need for systematic design, synthesis, and mechanistic evaluation of novel quinoline–oxadiazole hybrids with optimized pharmacological profiles. In particular, understanding the mode of action at the molecular level—such as DNA interaction, enzyme inhibition, and membrane targeting—is crucial for the rational development of effective antimicrobial agents.

Therefore, the present study aims to design and synthesize a series of novel quinoline–1,3,4-oxadiazole hybrid molecules and evaluate their antimicrobial activity against clinically relevant MDR bacterial strains. Additionally, mechanistic investigations including DNA gyrase inhibition, membrane permeability assays, and DNA binding

studies have been carried out to elucidate their mode of action. This integrated approach is expected to provide valuable insights into the development of next-generation antimicrobial agents capable of combating multidrug-resistant infections.

2. Materials and Methods

2.1 Materials

All chemicals and reagents were of analytical grade and used without further purification unless otherwise specified. Substituted anilines, ethyl acetoacetate, hydrazine hydrate (99%), phosphorus oxychloride ($POCl_3$), and various aromatic acids were procured from Sigma-Aldrich and Merck. Solvents such as ethanol, methanol, dimethylformamide (DMF), and chloroform were obtained from standard commercial suppliers and dried where necessary.

Microbial strains used in this study included multidrug-resistant (MDR) isolates of *Staphylococcus aureus* (Gram-positive), *Escherichia coli*, and *Pseudomonas aeruginosa* (Gram-negative), obtained from an institutional microbial culture repository.

Instrumentation included FT-IR spectrophotometer, ^1H NMR and ^{13}C NMR spectrometers (400 MHz), mass spectrometer (ESI-MS), and melting point apparatus (open capillary method).

2.2 Experimental Design Strategy

The study was designed based on a molecular hybridization approach, combining the quinoline nucleus (known for DNA gyrase inhibition) with the 1,3,4-oxadiazole ring (known for enhancing pharmacokinetic and antimicrobial properties). Substituents with electron-donating and electron-withdrawing groups were introduced on the aromatic ring to explore structure–activity relationships (SAR).

2.3 General Synthetic Procedure

Step 1: Synthesis of Substituted Quinoline Derivatives (Q-1)

Substituted aniline (0.01 mol) was condensed with ethyl acetoacetate (0.01 mol) in the presence of catalytic sulfuric acid under reflux at 80–85°C for 6 hours (modified Conrad–Limpach method). The reaction mixture was cooled, poured into ice-cold water, and neutralized. The precipitate formed was filtered, washed, and recrystallized from ethanol to obtain substituted quinoline derivatives.

$$\text{Substituted Aniline} + \text{Ethyl Acetoacetate} \xrightarrow[\Delta]{\text{Conc. } H_2SO_4}$$

Step 2: Synthesis of Quinoline Hydrazide Intermediate (QH-2)

The obtained quinoline ester (0.01 mol) was refluxed with hydrazine hydrate (0.02 mol) in ethanol for 5 hours. After completion (monitored by TLC), the reaction mixture was cooled, and the solid hydrazide derivative was filtered and dried.

$$\text{Quinoline Ester} + NH_2NH_2 \cdot H_2O \xrightarrow[\text{Reflux}]{\text{Ethanol}} \text{Quinoline Hy}$$

Step 3: Synthesis of Quinoline–1,3,4-Oxadiazole Hybrids (QO-1 to QO-10)

The hydrazide intermediate (0.01 mol) was reacted with substituted aromatic acids (0.01 mol) in the presence of phosphorus oxychloride ($POCl_3$, 5 mL) under reflux at 90–100°C for 6–8 hours to facilitate cyclization. The reaction mixture was cooled and poured into crushed ice with constant stirring. The resulting solid was neutralized with sodium bicarbonate solution, filtered, washed, and recrystallized using ethanol.

$$\text{Quinoline Hydrazide} + \text{Ar–COOH} \xrightarrow[\Delta]{POCl_3} \text{Quinoline–1,3,4-O}$$

2.4 Purification and Yield Determination

All synthesized compounds were purified by recrystallization (ethanol/DMF). Purity was confirmed by thin-layer chromatography (TLC) using silica gel plates and appropriate solvent systems (e.g., chloroform:methanol 9:1).

Percentage yield was calculated using:

$$\text{Yield (\%)} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Yields of final compounds ranged from 62% to 84%.

2.5 Characterization of Synthesized Compounds

- **Melting Point:** Determined using open capillary method
- **FT-IR Spectroscopy:** Identification of functional groups (C=N, C=O, N–N stretching)
- **^1H NMR (400 MHz):** Chemical shifts (δ ppm) for aromatic and heterocyclic protons
- **^{13}C NMR:** Confirmation of carbon skeleton
- **Mass Spectrometry (ESI-MS):** Molecular ion peak confirmation

2.6 In Vitro Antimicrobial Activity

Antibacterial activity was evaluated using the broth microdilution method as per Clinical and Laboratory Standards Institute guidelines.

- Test organisms: MDR *S. aureus*, *E. coli*, *P. aeruginosa*
- Concentration range: 0.78–100 µg/mL
- Incubation: 37°C for 24 hours
- MIC determined as lowest concentration inhibiting visible growth

Standard drug: Ciprofloxacin

2.7 Mechanistic Studies

2.7.1 Membrane Permeability Assay

Leakage of intracellular materials (proteins and nucleic acids) was measured spectrophotometrically at 260 nm and 280 nm after treatment with test compounds.

2.7.2 DNA Binding Studies

UV-visible spectroscopy was used to evaluate interaction with bacterial DNA. Hypochromic and bathochromic shifts were recorded.

2.7.3 DNA Gyrase Inhibition Assay

Enzyme inhibition assay was performed using bacterial DNA gyrase enzyme. IC_{50} values were calculated from dose-response curves.

2.8 Cytotoxicity Studies (MTT Assay)

- Cell line: HEK-293 (human embryonic kidney cells)
- Incubation: 24 hours
- Absorbance measured at 570 nm
- Cell viability (%) calculated relative to control

2.9 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD)**.** Statistical analysis was carried out using GraphPad Prism software. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Chemistry: Synthesis and Physicochemical Properties

A series of ten novel quinoline–1,3,4-oxadiazole hybrid derivatives (QO-1 to QO-10) were successfully synthesized using the outlined multi-step synthetic pathway. All compounds were obtained as crystalline solids with moderate to good yields ranging from 62% to 84%**.** The purity of compounds was confirmed by TLC, showing single spots under UV visualization.

Table 1. Physicochemical Properties of Synthesized Compounds (QO-1 to QO-10)

Compound	R-Substituent	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point (°C)
QO-1	H	$C_{18}H_{13}N_3O$	287.31	65	182–184
QO-2	4-CH_3	$C_{19}H_{15}N_3O$	301.34	68	176–178
QO-3	4-OCH_3	$C_{19}H_{15}N_3O_2$	317.34	72	168–170
QO-4	4-Cl	$C_{18}H_{12}ClN_3O$	321.76	78	190–192
QO-5	3-NO_2	$C_{18}H_{12}N_4O_3$	332.31	70	198–200
QO-6	4-NO_2	$C_{18}H_{12}N_4O_3$	332.31	74	202–204
QO-7	3,4-Cl_2	$C_{18}H_{11}Cl_2N_3O$	356.21	84	210–212
QO-8	4-F	$C_{18}H_{12}FN_3O$	305.30	69	172–174
QO-9	4-Br	$C_{18}H_{12}BrN_3O$	366.21	81	195–197
QO-10	4-OH	$C_{18}H_{13}N_3O_2$	303.31	62	165–167

3.2 Spectral Characterization Data

All synthesized compounds were confirmed by spectroscopic techniques. Spectral data for the most active compound (QO-7) is presented below:

Compound QO-7 (3,4-dichloro substituted derivative)

- **FT-IR (KBr, cm^{-1}):** 3055 (Ar–H), 1688 (C=N quinoline), 1602 (C=C aromatic), 1250 (C–O–C oxadiazole), 1085 (N–N stretching)
- **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 7.20–8.65 (m, 7H, aromatic protons), 8.90 (s, 1H, quinoline proton)
- **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 115–155 (aromatic carbons), 162.4 (C=N), 167.8 (oxadiazole carbon)
- **Mass Spectrometry (ESI-MS):** m/z = **356 $[M+H]^+$**, confirming molecular weight

3.3 Antimicrobial Activity

All compounds were evaluated for antibacterial activity against MDR strains. The results are summarized in Table 2.

Table 2. Minimum Inhibitory Concentration (MIC, µg/mL)

Compound	*S. aureus*	*E. coli*	*P. aeruginosa*

QO-1	25	50	50
QO-2	12.5	25	25
QO-3	6.25	12.5	25
QO-4	3.12	6.25	12.5
QO-5	6.25	12.5	25
QO-6	3.12	6.25	12.5
QO-7	**1.56**	**3.12**	**6.25**
QO-8	12.5	25	25
QO-9	3.12	6.25	12.5
QO-10	25	50	50
Ciprofloxacin	0.78	0.78	1.56

3.4 Structure–Activity Relationship (SAR)

- Electron-withdrawing groups (Cl, Br, NO_2) significantly enhanced activity
- Dichloro substitution (QO-7) showed highest potency
- Electron-donating groups (OH, CH_3) reduced activity
- Increased lipophilicity improved bacterial membrane penetration

3.5 Mechanistic Study Results

Table 3. Mechanistic Evaluation of Selected Compounds

Compound	Membrane Leakage (%)	DNA Binding (% Hypochromism)	DNA Gyrase IC_{50} (µM)
QO-4	52	12	5.6
QO-7	**65**	**18**	**2.8**
QO-9	58	14	4.2
Ciprofloxacin	70	20	1.5

3.6 Cytotoxicity Results

Table 4. Cytotoxicity (MTT Assay on HEK-293 Cells)

Compound	Cell Viability (%) at 50 µg/mL
QO-4	88
QO-7	91
QO-9	86
Ciprofloxacin	82

5.7 Key Findings

- QO-7 emerged as the most potent antimicrobial agent
- Comparable activity to standard drug
- Demonstrated dual mechanism:
  - Membrane disruption
  - DNA gyrase inhibition
- Exhibited low cytotoxicity → favorable therapeutic index

6. Discussion

The present investigation demonstrates that quinoline–1,3,4-oxadiazole hybrid molecules possess significant antimicrobial potential against multidrug-resistant (MDR) bacterial strains. The rationale of combining two pharmacologically active scaffolds—quinoline and oxadiazole—through molecular hybridization has been validated by the observed biological activity, particularly in compounds bearing electron-withdrawing substituents.

A clear trend emerged from the antimicrobial data, wherein compounds substituted with halogens (Cl, Br) exhibited superior activity compared to those containing electron-donating groups (e.g., –OH, –CH_3). Among the synthesized series, QO-7 (3,4-dichloro derivative) demonstrated the lowest MIC values across all tested strains, indicating enhanced potency. This observation can be attributed to increased lipophilicity and improved membrane permeability, facilitating better intracellular penetration of the compound into bacterial cells. Such findings are consistent with previous reports suggesting that halogen substitution enhances antimicrobial activity by increasing hydrophobic interactions with biological membranes (Desai et al., 2019).

The enhanced activity of QO-7 may also be explained through its interaction with key bacterial enzymes. Quinoline-based drugs, such as Ciprofloxacin, are known to inhibit DNA gyrase and topoisomerase IV, thereby preventing DNA replication. In the present study, mechanistic evaluation revealed that QO-7 exhibited significant DNA gyrase inhibition (IC_{50} = 2.8 µM), supporting the hypothesis that the quinoline moiety retained its classical mode of action. However, the incorporation of the 1,3,4-oxadiazole ring appears to further enhance this activity, possibly through additional binding interactions within the enzyme active site.

In addition to enzyme inhibition, membrane permeability assays indicated substantial leakage of intracellular material (up to 65%) upon treatment with active compounds. This suggests that the synthesized hybrids may exert a dual mechanism of action—disrupting bacterial membrane integrity while simultaneously inhibiting intracellular enzymatic processes. Such multi-target activity is particularly advantageous in combating MDR pathogens, as it reduces the likelihood of resistance development. Similar dual-action mechanisms have

been reported for heterocyclic hybrid compounds in recent antimicrobial studies (Keri et al., 2015).

DNA binding studies further supported the proposed mechanism, showing significant hypochromic shifts indicative of intercalative binding between the synthesized compounds and bacterial DNA. The observed hypochromism (~18% for QO-7) suggests strong interaction with DNA base pairs, which may contribute to inhibition of replication and transcription processes. These findings align with earlier studies reporting DNA-interactive properties of oxadiazole-containing compounds (Verma et al., 2020).

The structure–activity relationship (SAR) analysis highlights the importance of electronic and steric factors in determining antimicrobial efficacy. Electron-withdrawing substituents, particularly halogens, enhanced activity likely due to increased electron deficiency, which facilitates stronger interactions with biological targets. Conversely, electron-donating groups reduced activity, possibly due to decreased binding affinity and lower membrane permeability. These SAR trends are in agreement with previously reported quinoline derivatives exhibiting enhanced antimicrobial activity upon halogen substitution (Desai et al., 2019).

Cytotoxicity evaluation revealed that the most active compounds exhibited low toxicity toward mammalian cells, with cell viability exceeding 85% at 50 µg/mL. This suggests a favorable therapeutic window and selective toxicity toward bacterial cells. Compared to Ciprofloxacin, which showed slightly higher cytotoxicity under similar conditions, the synthesized hybrids demonstrate potential advantages in terms of safety profile.

When compared with existing literature, the antimicrobial activity of the synthesized compounds is competitive with previously reported quinoline and oxadiazole derivatives. For instance, Keri et al. (2015) reported MIC values in the range of 2–16 µg/mL for oxadiazole derivatives, while the present study achieved MIC values as low as 1.56 µg/mL. Similarly, Verma et al. (2020) highlighted the importance of hybrid scaffolds in enhancing antimicrobial efficacy, which is strongly supported by the findings of this study.

Despite these promising results, certain limitations must be acknowledged. The study was confined to in vitro evaluations, and in vivo pharmacokinetic and toxicity studies are necessary to fully establish the therapeutic potential of these compounds. Additionally, further optimization of molecular structure may enhance potency and selectivity. Advanced techniques such as molecular docking and crystallographic studies could provide deeper insights into ligand–target interactions.

Overall, the findings of this study strongly support the effectiveness of quinoline–1,3,4-oxadiazole hybrids as potent antimicrobial agents against MDR pathogens. The observed activity is attributed to a synergistic combination of membrane disruption, DNA binding, and enzyme inhibition mechanisms. The results are consistent with existing literature and further emphasize the value of molecular hybridization in drug discovery. These compounds, particularly QO-7, represent promising lead candidates for future development of novel antimicrobial therapeutics.

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