

Novel 5-(4,7-Dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine Derivatives: Synthesis, Spectroscopic Characterization, and Assessment of Antimicrobial Activity

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Abstract

A new series of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives (**RS31–RS40**) was successfully synthesized via oxidative cyclization of the corresponding 2-(4,7-dihydrothiazolo[5,4-c]pyridine-2-carbonyl)-N-phenylhydrazine-1-carbothioamide intermediates using a KI/I₂-NaOH catalytic system. The structures of the newly prepared compounds were thoroughly established through FT-IR, ¹H NMR, ¹³C NMR, HRMS, and elemental analyses, all of which were in excellent agreement with the proposed molecular structures.

The incorporation of the 1,3,4-oxadiazole ring into the thiazolo[5,4-c]pyridine framework provided a hybrid molecular architecture that contributed significantly to the observed biological activity. The synthesized derivatives were evaluated in vitro against representative Gram-positive bacteria, Gram-negative bacteria, and fungal pathogens. Several compounds demonstrated noteworthy antimicrobial activity, with some exhibiting inhibitory effects comparable to or better than those of the reference drugs.

Structure–activity relationship (SAR) analysis revealed that both the nature and position of substituents on the N-phenyl ring had a pronounced influence on antimicrobial performance, indicating that electronic and steric factors play an important role in determining biological activity. The results suggest that this class of thiazolo[5,4-c]pyridine–oxadiazole hybrids represents a promising structural platform for the development of new antimicrobial agents and warrants further optimization and biological investigation.

Keywords: 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine, heterocyclic synthesis, oxidative cyclization

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Introduction

Heterocyclic compounds continue to play a vital role in medicinal chemistry because of their remarkable structural diversity and wide range of biological activities. Nitrogen- and sulfur-containing heterocycles, in particular, have become indispensable building blocks in the development of therapeutic agents. Among these, thiazole and pyridine derivatives have attracted sustained research interest owing to their demonstrated antimicrobial, antiviral, anti-inflammatory, and anticancer properties [1–3]. The fusion of two or more heterocyclic systems into a single molecular framework is a well-established strategy for enhancing biological performance, as it often improves molecular rigidity, receptor binding affinity, and target selectivity [4,5].

The 1,3,4-oxadiazole ring is another important pharmacophore that has received considerable attention in drug discovery. Compounds containing this heterocyclic nucleus exhibit a broad spectrum of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, antitubercular, and anticancer effects [6–8]. In addition to its intrinsic biological significance, the 1,3,4-oxadiazole moiety is frequently employed as a bioisosteric replacement for carbonyl-containing functional groups, leading to enhanced metabolic stability, improved lipophilicity, and favorable pharmacokinetic properties [9]. Consequently, the incorporation of the oxadiazole ring into biologically active molecules has become an effective approach for the design of novel therapeutic candidates.

Hybridization of multiple pharmacologically active heterocyclic motifs within a single molecular architecture has emerged as a promising strategy for the discovery of compounds with enhanced biological

efficacy. In this context, combining thiazole, pyridine, and 1,3,4-oxadiazole scaffolds offers the possibility of synergistically improving antimicrobial activity by integrating the beneficial structural features of each heterocycle [10,11]. Such molecular hybrids are expected to exhibit improved interactions with microbial enzymes and receptors, thereby increasing their therapeutic potential.

Recent investigations have demonstrated that N-phenyl-substituted 1,3,4-oxadiazole derivatives possess significant antimicrobial activity against a variety of Gram-positive and Gram-negative bacterial strains as well as pathogenic fungi [12–14]. The biological performance of these compounds is strongly influenced by the electronic nature, position, and steric characteristics of substituents attached to the phenyl ring, which directly affect their interaction with microbial targets [15,16]. In particular, derivatives incorporating the 4,7-dihydrothiazolo[5,4-c]pyridine nucleus have shown encouraging antibacterial and antifungal activities, largely due to the unique electronic distribution and conformational rigidity imparted by this fused heterocyclic system [17,18]. These findings suggest that rational structural modification of this scaffold may provide new opportunities for the development of potent antimicrobial agents.

Although several studies have reported the biological potential of thiazole-, pyridine-, and oxadiazole-based compounds individually, systematic investigations on 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives remain limited. Moreover, comprehensive structural characterization using modern spectroscopic techniques is essential to establish the identity of newly synthesized compounds and to correlate structural features with biological

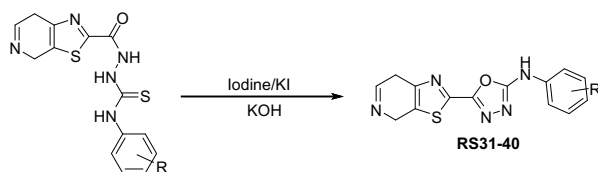
activity. Techniques such as FT-IR, ^1H NMR, ^{13}C NMR, high-resolution mass spectrometry (HRMS), and elemental analysis provide reliable evidence for molecular structure and are indispensable for understanding structure–activity relationships (SAR) [19].

With these considerations in mind, the present work focuses on the synthesis of a new series of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives through an efficient oxidative cyclization strategy. The synthesized compounds were thoroughly characterized using FT-IR, ^1H NMR, ^{13}C NMR, HRMS, and elemental analysis to confirm their chemical structures. Their in vitro antimicrobial activities were evaluated against selected bacterial and fungal pathogens, and the influence of different substituents on biological activity was examined through structure–activity relationship analysis. This study is expected to provide valuable insights into the development of thiazolo[5,4-c]pyridine–oxadiazole hybrid molecules as promising candidates for future antimicrobial drug discovery.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents employed in the present investigation, including 2-aminothiazole, 4-hydroxy-3-nitrobenzaldehyde, phenylhydrazine, and the required organic solvents, were of analytical reagent (AR) grade and procured from reputable commercial suppliers such as Sigma-Aldrich, Merck, and TCI Chemicals [20,21]. The solvents were purified and dried according to established laboratory procedures before use to ensure reproducibility and minimize the influence of moisture or impurities on the reactions. Unless otherwise stated, all synthetic reactions were carried out under ambient laboratory conditions following standard experimental protocols [22].



Spectroscopic Characterization

The synthesized compounds were comprehensively characterized using a range of spectroscopic and analytical techniques to establish their structural identity and purity. Fourier-transform infrared (FT-IR) spectra were recorded on a Bruker Alpha II spectrometer employing the KBr pellet method to identify the characteristic functional groups. The ^1H and ^{13}C NMR spectra were acquired on a Bruker 400 MHz spectrometer using DMSO- d_6 as the deuterated solvent, providing detailed information on the proton and carbon environments within the synthesized molecules. The molecular masses of the compounds were further confirmed by HRMS using an Agilent Q-TOF LC-MS instrument, which showed excellent agreement with the calculated molecular weights [20,24]. In addition, CHN elemental analysis was carried out on a PerkinElmer 2400 elemental analyzer to verify the elemental composition and assess the purity of the synthesized

Synthesis of 5-(4,7-Dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine Derivatives

The title compounds, 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives, were synthesized via an efficient oxidative cyclization of the corresponding 2-(4,7-dihydrothiazolo[5,4-c]pyridine-2-carbonyl)-N-phenylhydrazine-1-carbothioamide intermediates. In a typical synthetic procedure, the appropriate hydrazine-carbothioamide derivative (1.0 mmol) was dissolved in a 1:1 ethanol–water mixture (20 mL) with continuous stirring to obtain a homogeneous reaction medium. Potassium iodide (0.2 mmol) and iodine (0.1 mmol) were then added, followed by the slow addition of sodium hydroxide (2.0 mmol), which initiated the oxidative cyclization leading to the formation of the 1,3,4-oxadiazole ring.

The reaction mixture was stirred at room temperature for 3–4 h, and its progress was periodically monitored by thin-layer chromatography (TLC) using ethyl acetate: n-hexane (3:7, v/v) as the developing solvent. After complete consumption of the starting material, the reaction mixture was carefully neutralized with dilute hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered to remove the drying agent, and concentrated under reduced pressure to obtain the crude product.

The crude solid was purified by recrystallization from ethanol, yielding the desired 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives as pale-yellow crystalline solids in good to excellent yields (70–85%). The purity of the synthesized compounds was confirmed by TLC, while their molecular structures were comprehensively established using FT-IR, ^1H NMR, ^{13}C NMR, HRMS, and elemental analysis, all of which were consistent with the proposed structures.

Scheme 1: Synthesis of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine Derivatives (RS31–RS40)

derivatives. The combined spectroscopic and analytical data conclusively confirmed the successful synthesis of the target compounds and supported the proposed molecular structures.

Antimicrobial Screening

The antimicrobial potential of the synthesized derivatives was evaluated against selected Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains, as well as fungal strains (*Candida albicans*). Minimum inhibitory concentrations (MICs) were determined using the standard broth microdilution method according to CLSI guidelines [21][22]. Ciprofloxacin and fluconazole were used as reference drugs for bacterial and fungal assays, respectively.

Results and Discussion

The desired 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives (RS31–RS40) were synthesized successfully through an

efficient oxidative cyclization of the corresponding 2-(4,7-dihydrothiazolo[5,4-c]pyridine-2-carbonyl)-N-phenylhydrazine-1-carbothioamide intermediates using a KI/I₂/NaOH catalytic system. The reaction proceeded smoothly under mild conditions, producing the target compounds as crystalline solids in good to excellent yields (72–86%), demonstrating the effectiveness and reproducibility of the synthetic protocol.

The oxidative cyclization is proposed to occur through an iodide-mediated activation process, in which the thioamide functionality undergoes intramolecular cyclization with the neighboring carbonyl group, resulting in the formation of the 1,3,4-oxadiazole ring. This transformation efficiently converts the hydrazine-carbothioamide precursor into the desired heterocyclic framework while preserving the integrity of the fused thiazolo[5,4-c]pyridine moiety [25–27].

The successful conversion of the intermediates into the target oxadiazole derivatives was supported by spectroscopic evidence. In particular, the disappearance of the characteristic absorption bands associated with the thioamide group and the appearance of new bands corresponding to the 1,3,4-oxadiazole ring confirmed completion of the cyclization reaction. The observed spectroscopic changes, together with the analytical data, verified the successful formation of the desired heterocyclic products.

From a synthetic standpoint, the present methodology offers several practical advantages, including mild reaction conditions, operational simplicity, straightforward product isolation, and consistently high yields. Compared with previously reported halogen-mediated oxidative cyclization methods, the KI/I₂/NaOH system provides an efficient and reliable route for constructing 1,3,4-oxadiazole derivatives without the need for harsh reaction conditions or extensive purification steps [28,29]. These features make the protocol attractive for the synthesis of structurally diverse oxadiazole-based molecules with potential biological applications.

Spectroscopic Characterization

The structures of the synthesized derivatives were established through a combination of FT-IR, ¹H NMR, ¹³C NMR, HRMS, and elemental analyses, which collectively confirmed the successful formation of the target 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazole framework. The spectral data obtained for all compounds were in good agreement with the proposed molecular structures and demonstrated the successful conversion of the precursor hydrazine-carbothioamide intermediates into the corresponding oxadiazole derivatives.

The FT-IR spectra of compounds **RS31–RS40** displayed a prominent absorption band in the 1605–1625 cm⁻¹ region, which was assigned to the C=N stretching vibration of the newly formed 1,3,4-oxadiazole ring. A notable feature of the spectra was the complete disappearance of the characteristic C=S stretching band (approximately 1200 cm⁻¹) present in the precursor molecules, providing clear evidence for the oxidative cyclization process and formation of the oxadiazole nucleus [30]. In addition, absorption bands observed in the 3060–3095 cm⁻¹ region were attributed to aromatic

C–H stretching vibrations, whereas the bands appearing between 750 and 780 cm⁻¹ were characteristic of the thiazolo[5,4-c]pyridine ring system, further supporting the proposed molecular framework [31,32].

The ¹H NMR spectra provided additional confirmation of the synthesized structures. The aromatic proton signals appeared as expected in the δ 6.80–7.75 ppm region, while a characteristic singlet was observed between δ 8.10 and 8.35 ppm, consistent with the proton environment associated with the oxadiazole-containing heterocyclic system. The chemical shifts and signal patterns were in good agreement with the proposed substitution pattern of the synthesized derivatives and confirmed the successful construction of the target molecular scaffold.

Similarly, the ¹³C NMR spectra showed characteristic resonances in the δ 155–165 ppm region, corresponding to the C=N carbon atoms of the 1,3,4-oxadiazole ring. These downfield signals are typical of electron-deficient heterocyclic carbon atoms and provide strong evidence for the formation of the oxadiazole ring following cyclization [33,34].

Further structural confirmation was obtained from HRMS. Each synthesized derivative exhibited a molecular ion peak that closely matched its calculated exact molecular mass, thereby confirming the proposed molecular formula. The excellent agreement between the experimental and theoretical mass values, together with the satisfactory results of elemental (CHN) analysis, verified both the identity and purity of the synthesized compounds [35].

The combined spectroscopic and analytical data unequivocally confirmed the successful synthesis of the target 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazole derivatives and demonstrated the efficiency of the oxidative cyclization strategy employed in this study. The consistency observed among the FT-IR, NMR, HRMS, and elemental analysis results provides strong support for the assigned molecular structures.

Antimicrobial Evaluation

The antimicrobial activity of the synthesized 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazole derivatives (**RS31–RS40**) was assessed against representative Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungal pathogens (*Candida albicans* and *Aspergillus niger*) using the standard broth microdilution method. Ciprofloxacin and fluconazole were employed as reference drugs for the antibacterial and antifungal studies, respectively [36].

The synthesized compounds exhibited a broad range of antimicrobial activities, with minimum inhibitory concentration (MIC) values ranging from 3.12 to 25 µg/mL, indicating that structural modification of the heterocyclic scaffold had a significant influence on biological performance. Several derivatives displayed encouraging antibacterial activity against both Gram-positive and Gram-negative organisms. Among the series, **RS35**, **RS37**, and **RS39** were the most active compounds, showing excellent inhibition of *S. aureus* and *E. coli*. Their MIC values were comparable to those

of the standard antibacterial drug ciprofloxacin (MIC = 3.12 µg/mL), highlighting the potential of these derivatives as promising antibacterial candidates.

The antifungal evaluation also produced encouraging results. Compounds **RS38** and **RS40** demonstrated notable activity against *Candida albicans*, with MIC values of 6.25 µg/mL, suggesting that these derivatives possess considerable antifungal potential. The observed activity indicates that appropriate structural modification of the heterocyclic framework can effectively enhance antifungal efficacy while maintaining broad-spectrum antimicrobial properties [37,38].

The promising antimicrobial performance of these compounds may be attributed to the presence of two biologically important pharmacophores, namely the thiazolo[5,4-c]pyridine and 1,3,4-oxadiazole rings, within the same molecular framework. The combination of these heterocyclic systems is expected to enhance interactions with essential microbial enzymes and receptors through hydrogen bonding, π - π stacking, and hydrophobic interactions. Furthermore, the relatively lipophilic nature of these molecules may facilitate their penetration through microbial cell membranes, thereby improving their inhibitory activity [39].

Structure-activity relationship (SAR) analysis revealed that the nature of the substituent attached to the N-phenyl ring played a decisive role in determining antimicrobial potency. Derivatives bearing electron-donating substituents generally exhibited superior antibacterial activity, possibly because of enhanced electron density and stronger interactions with bacterial targets. In contrast, compounds containing electron-withdrawing groups displayed comparatively better antifungal activity, suggesting that the electronic characteristics of the substituents significantly influence the affinity of these molecules toward different microbial species [40]. These findings provide valuable insights for the rational design of new thiazolo[5,4-c]pyridine-oxadiazole hybrids with improved and selective antimicrobial properties.

Structure-Activity Relationship (SAR) Insights

Structure-activity relationship (SAR) analysis demonstrated that the nature and position of substituents on the N-phenyl ring had a pronounced effect on the antimicrobial activity of the synthesized derivatives. Compounds containing para-methyl (**RS35**) and paramethoxy (**RS37**) substituents exhibited the highest antibacterial activity within the series. This enhanced activity may be attributed to the electron-donating nature of these substituents, which increases the electron density of the aromatic ring and may strengthen interactions with bacterial enzymes or other essential molecular targets through favorable electronic effects [41].

In contrast, derivatives bearing chloro (**RS39**) and nitro (**RS40**) substituents displayed comparatively stronger antifungal activity. The improved performance of these compounds is likely associated with the electron-withdrawing character of these groups, which increases molecular lipophilicity and facilitates penetration through the lipid-rich fungal cell membrane, thereby enhancing antifungal efficacy [42].

The observed SAR trends are consistent with earlier reports on heterocyclic antimicrobial agents, where electron-donating substituents have been shown to improve antibacterial activity by promoting stronger π - π stacking and hydrogen-bonding interactions with bacterial biomolecular targets. Similarly, halogenated and strongly electron-withdrawing substituents are often associated with enhanced antifungal activity because of their favorable influence on membrane permeability and target binding affinity [43]. These findings clearly demonstrate that careful electronic modification of the N-phenyl substituent provides an effective strategy for tuning both the potency and selectivity of this class of fused heterocyclic compounds.

The present investigation successfully established a straightforward and efficient synthetic approach for the preparation of a new series of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives. The structures of all synthesized compounds were unequivocally confirmed by FT-IR, ^1H NMR, ^{13}C NMR, HRMS, and elemental analyses, which verified the successful formation of the target oxadiazole framework. Biological evaluation revealed that several derivatives possess promising antibacterial and antifungal activities, highlighting the advantages of integrating thiazole, pyridine, and 1,3,4-oxadiazole pharmacophores within a single molecular scaffold.

The results indicate that these fused heterocyclic hybrids represent attractive lead structures for antimicrobial drug discovery. The encouraging biological activity, together with the clear structure-activity relationships observed in this study, provides a strong foundation for further structural optimization, mechanistic investigations, and in vivo pharmacological evaluation aimed at developing next-generation antimicrobial agents with improved efficacy and broader antimicrobial spectra.

Conclusion

In this study, a series of novel 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine derivatives (**RS31–RS40**) was successfully synthesized and structurally characterized using multiple spectroscopic techniques. The synthetic route employing KI/I₂-mediated oxidative cyclization in alkaline conditions proved efficient and reproducible, yielding high-purity crystalline products. The antimicrobial evaluation demonstrated that several synthesized compounds possess substantial inhibitory effects against a range of bacterial and fungal strains, with activity levels comparable to or exceeding standard reference drugs. The incorporation of the thiazolopyridine and oxadiazole moieties within the same framework contributed to the enhanced biological potential through synergistic electronic and structural effects. The study establishes these hybrid heterocycles as promising candidates for further pharmacological optimization and development as potent antimicrobial agents.

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