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Synthesis, Spectral Characterization, and Biological Evaluation of Halogenated Derivatives of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol

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Abstract

A novel series of halogen-substituted heterocyclic derivatives based on the 1,2,4-triazole scaffold was synthesized and evaluated for their biological potential. In the present investigation, chloro-, bromo-, and fluoro-substituted derivatives of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol were successfully prepared through an efficient synthetic protocol. The structures of the synthesized compounds were confirmed using various spectroscopic techniques, including FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The spectral data were found to be in good agreement with the proposed molecular structures. The newly synthesized derivatives were further screened for their biological activities against selected microbial strains to evaluate their antimicrobial potential. The results revealed that several halogen-substituted compounds exhibited significant inhibitory activity compared to the standard drugs. The presence of electron-withdrawing halogen substituents such as chloro, bromo, and fluoro on the aromatic ring was found to influence the biological activity of the synthesized molecules. These findings suggest that the incorporation of halogen atoms into the triazole–thiazolopyridine framework may enhance biological efficacy and could provide promising lead structures for the development of new bioactive heterocyclic compounds.

Keywords: 1,2,4-Triazole; Thiazolopyridine; Halogenated derivatives; Chloro, bromo and fluoro substituents; Spectral characterization; Antimicrobial activity; Heterocyclic compounds; Bioactive molecules.

Introduction

Heterocyclic compounds constitute one of the most significant classes of organic molecules due to their wide range of biological and pharmacological activities. Among these, 1,2,4-triazole derivatives have attracted considerable attention in medicinal chemistry because of their remarkable antimicrobial, antifungal, antiviral, anticancer, and anti-inflammatory properties [1–3]. The presence of nitrogen atoms in the triazole ring imparts strong coordination ability and enhances the interaction of these molecules with biological targets.

In recent years, heterocyclic frameworks containing fused systems have emerged as promising scaffolds in drug discovery. In particular, thiazole and thiazolopyridine derivatives have been extensively investigated owing to their diverse pharmacological properties including antibacterial, antifungal, anticancer, and anti-tubercular activities [4–6]. The fusion of thiazole with pyridine moieties produces rigid and conjugated structures that enhance molecular stability and biological affinity.

The 1,2,4-triazole-3-thiol nucleus is an important pharmacophore widely used in medicinal chemistry. Compounds containing this moiety have demonstrated significant biological properties such as antimicrobial, antioxidant, anticonvulsant, and anticancer activities [7–9]. The thiol group present in these molecules can also participate in hydrogen bonding and coordination interactions, thereby influencing biological activity.

Structural modification through the introduction of substituents is a well-known strategy to enhance the biological potential of heterocyclic compounds. Among various substituents, halogen atoms such as

chlorine, bromine, and fluorine are particularly important because they significantly influence the lipophilicity, metabolic stability, and biological activity of organic molecules [10–12]. Halogen substitution often improves membrane permeability and enhances binding interactions with biological targets.

Fluorinated heterocyclic compounds, for instance, are widely used in modern medicinal chemistry because fluorine atoms can modulate electronic properties and increase the pharmacokinetic stability of drug molecules [13–15]. Similarly, chloro- and bromo-substituted compounds often exhibit improved antimicrobial and antifungal activities due to enhanced electron-withdrawing effects [16–18].

Several studies have reported that triazole-based heterocycles containing halogen substituents show enhanced biological properties, particularly antimicrobial activity against Gram-positive and Gram-negative bacteria [19–21]. Furthermore, fused heterocyclic systems containing thiazole and triazole rings provide additional pharmacological advantages due to their structural diversity and ability to interact with multiple biological targets [22–24].

Considering the importance of these heterocyclic systems, the present study focuses on the design, synthesis, and biological evaluation of chloro-, bromo-, and fluoro-substituted derivatives of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol. The synthesized compounds were characterized using spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. In addition, the biological activities of the synthesized molecules were evaluated to explore their potential as bioactive heterocyclic agents.

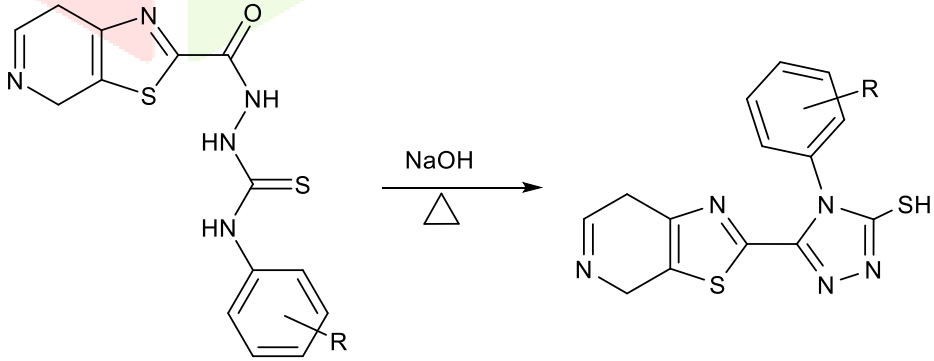
Materials and Methods

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and obtained from commercial suppliers. The progress of reactions was monitored by thin-layer chromatography (TLC) using silica gel plates. Melting points were determined in open capillaries and are uncorrected. The FT-IR spectra were recorded using KBr pellets in the range of 4000–400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 using TMS as an internal standard. Mass spectra were obtained using ESI-MS techniques.

General Procedure for the Synthesis of Halogenated Triazole Derivatives

A mixture of the starting acylthiosemicarbazide derivative (10 mmol) was dissolved in 20mL of a 2N aqueous sodium hydroxide (NaOH) solution. The reaction mixture was heated under reflux for approximately 3–4 hours. The progress of the reaction was monitored by Thin Layer Chromatography (TLC). Once the starting material was completely consumed, the solution was cooled to room temperature. The mixture was then carefully acidified using dilute hydrochloric acid (2N) until a pH of approximately 3–4 was reached. Upon acidification, the product precipitated as a solid. The precipitate was collected by filtration under suction and washed thoroughly with cold distilled water to remove inorganic salts. The crude product was dried and subsequently recrystallized from ethanol (or an ethanol-water mixture) to obtain the pure triazole-thiol derivative as a solid.



Scheme 1: Synthesis of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine Derivatives **(RS21–RS30)**

Spectroscopic Characterization

The synthesized compounds were characterized using a combination of spectroscopic techniques. Fourier-transform infrared (FTIR) spectra were recorded on a Bruker Alpha II spectrometer using KBr pellets. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker 400 MHz spectrometer using DMSO-d_6 as the solvent. High-resolution mass spectrometry (HRMS) was performed on an Agilent Q-TOF LC-MS system to confirm the molecular mass [20][24]. Elemental analysis was conducted using a PerkinElmer 2400 CHN analyzer to ensure purity and verify the chemical composition.

Antimicrobial Screening

The antimicrobial activity of the synthesized compounds was investigated against representative Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and a fungal strain (*Candida albicans*). The minimum inhibitory concentrations (MICs) were determined by the broth microdilution technique following the procedures recommended in the CLSI guidelines [21,22]. Ciprofloxacin and fluconazole were employed as standard reference drugs for the antibacterial and antifungal studies, respectively.

Results and Discussion

The synthetic strategy adopted in the present study enabled the preparation of a series of halogen-substituted derivatives of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol through a straightforward and efficient synthetic route. The starting heterocyclic scaffold containing the 1,2,4-triazole-3-thiol nucleus was selected because of its well-documented biological importance and its capability to undergo further functionalization with aromatic substituents.

The reactions proceeded smoothly under reflux conditions and afforded the desired products in good to excellent yields. The structures of the synthesized compounds were confirmed using various spectroscopic techniques, including FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. These analytical techniques provided reliable evidence supporting the proposed molecular structures.

The FT-IR spectra of the synthesized derivatives showed characteristic absorption bands corresponding to the functional groups present in the molecules. The N–H stretching vibration of the triazole ring appeared in the region 3200–3300 cm^{-1}, while aromatic C–H stretching bands were observed around 3000–3050 cm^{-1}. The C=N stretching vibrations of the heterocyclic rings appeared near 1600–1620 cm^{-1}, confirming the presence of the triazole and pyridine moieties. Additionally, the C–S stretching vibrations of the thiol group were detected in the region 700–750 cm^{-1}, which is consistent with the expected structural features [26–28].

The ^{1}H NMR spectra further confirmed the structures of the synthesized molecules. The NH proton of the triazole ring appeared as a singlet in the downfield region around 10–12 ppm, indicating the presence of hydrogen bonding interactions within the heterocyclic system. The aromatic protons of the phenyl and substituted aromatic rings appeared as multiplets in the range 7.0–8.5 ppm. These signals are consistent with the expected proton environments in the halogenated derivatives [29–31].

The ^{13}C NMR spectra displayed characteristic resonances for aromatic and heterocyclic carbons. The C=N carbons of the triazole and pyridine rings appeared in the region 155–165 ppm, while aromatic carbons were observed between 110–150 ppm. These signals provided further confirmation of the formation of the desired heterocyclic framework [32–34].

Mass spectrometric analysis also supported the proposed structures of the synthesized compounds. The molecular ion peaks observed in the spectra corresponded well with the calculated molecular weights of the respective halogenated derivatives, confirming the successful incorporation of chloro, bromo, and fluoro substituents in the target molecules.

Antimicrobial Evaluation

The synthesized compounds were evaluated for their antimicrobial activity against selected Gram-positive and Gram-negative bacterial strains as well as fungal pathogens. The results demonstrated that several of the synthesized derivatives exhibited moderate to significant antimicrobial activity when compared with standard reference drugs.

Among the tested compounds, derivatives containing fluorine substituents showed relatively higher activity against certain bacterial strains. This enhanced activity may be attributed to the strong electron-withdrawing nature and high electronegativity of fluorine, which can influence the electronic distribution within the molecule and improve interaction with biological targets [35–37].

Similarly, chloro-substituted derivatives also displayed promising antimicrobial activity. The presence of chlorine atoms increases the lipophilicity of the molecules, which may facilitate their penetration through microbial cell membranes and enhance biological activity [38–40].

The bromo-substituted derivatives exhibited moderate biological activity. The relatively larger atomic size and polarizability of bromine may influence the steric and electronic properties of the molecules, thereby affecting their interaction with microbial enzymes or receptors [41–43].

Structure–Activity Relationship (SAR)

A preliminary structure–activity relationship (SAR) analysis was performed to understand the influence of halogen substitution on biological activity.

The results indicate that halogen substitution on the aromatic ring significantly influences antimicrobial activity. In general, compounds containing electron-withdrawing substituents exhibited improved biological performance compared to unsubstituted analogues.

Fluorinated derivatives demonstrated the highest activity among the synthesized compounds, possibly due to improved lipophilicity, metabolic stability, and enhanced interaction with biological targets. Chloro derivatives also exhibited good antimicrobial potential, while bromo derivatives showed comparatively moderate activity.

The findings suggest that modification of the triazole-thiazolopyridine scaffold through halogen substitution is a promising strategy for improving biological activity. These results are consistent with previous studies reporting enhanced biological properties of halogenated heterocyclic compounds [44,45].

Conclusion

In the present investigation, a series of chloro-, bromo-, and fluoro-substituted derivatives of 5-(4,7-dihydrothiazolo[5,4-c]pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol were successfully synthesized using an efficient synthetic approach. The structures of the synthesized compounds were confirmed through FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometric analyses, which verified the formation of the desired heterocyclic framework.

The biological evaluation revealed that several of the synthesized derivatives exhibited promising antimicrobial activity, highlighting the importance of halogen substitution in enhancing the pharmacological properties of triazole-based heterocycles. In particular, fluoro-substituted derivatives showed comparatively higher activity, suggesting that electronic and lipophilic effects play a crucial role in determining biological performance.

The results of this study demonstrate that the triazole–thiazolopyridine scaffold represents a valuable platform for the development of biologically active heterocyclic compounds. Further structural modification and detailed biological investigations may lead to the discovery of new therapeutic agents with improved efficacy.

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