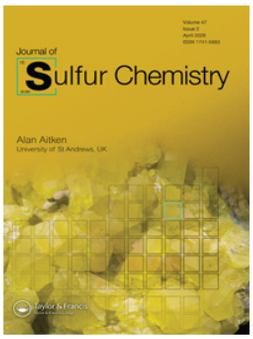

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Pyridine hybrid 1,2,4-triazole-5-thione–Schiff base derivatives: synthesis, characterization, antimicrobial, and antioxidant activity evaluations

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ABSTRACT

Antimicrobials are fundamental agents in the control and treatment of infections; however, increasing antimicrobial resistance severely reduces therapeutic efficacy and exacerbates the global burden of disease and mortality. In this context, a novel series of pyridine-containing 1,2,4-triazole-5-thione Schiff base derivatives (6a–g) were synthesized in good to excellent yields and evaluated for their antimicrobial and antioxidant potential. The structural elucidation of the synthesized compounds was accomplished through FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Antimicrobial activity was assessed using both disc diffusion and minimum inhibitory concentration (MIC) methods against a panel of Gram-positive, Gram-negative bacteria, and the yeast *Candida albicans*. Compounds 6b, 6d, and 6 g showed potent antibacterial activity against *Streptococcus pyogenes* ATCC 19615 with low MIC values (19.531 µg/mL), while compound 6 g demonstrated broad-spectrum activity, also inhibiting *Staphylococcus aureus* and *C. albicans*. In antioxidant assays, compound 6f exhibited the highest radical scavenging activity in the DPPH assay, whereas compound 6d showed superior reducing power in the FRAP assay (290.16 mg TE/g). The results indicated that electron-donating (*e.g.* methoxy) and electron-withdrawing (*e.g.* nitro) groups on the aromatic ring significantly influenced biological activity. These findings highlight compounds 6d and 6 g as promising candidates for further pharmacological development owing to their multifunctional bioactivity profiles.

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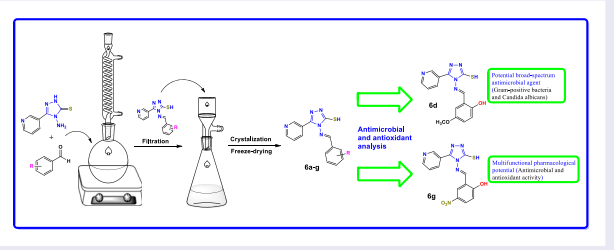


1. Introduction

Antimicrobial agents inhibit the growth of, or eradicate, bacteria, fungi, viruses, and parasites, and remain indispensable in treating infectious diseases. They are grouped by target organism and mechanism of action, with the principal classes being antibiotics, antifungals, antivirals, and antiparasitics [1,2].

Antimicrobial resistance (AMR) is now a major global public-health threat, undermining effective therapy and driving morbidity and mortality. Misuse and overuse of antimicrobials in human medicine and agriculture are key drivers of resistance [3–5]. High antimicrobial consumption in healthcare correlates with elevated resistance among pathogens [4,6], while use in livestock has fostered resistant bacteria transmissible to humans via the food chain [7,8].

Also, antioxidants are essential in safeguarding biological systems against oxidative stress, a condition associated with the pathogenesis of numerous disorders, including cancer and cardiovascular diseases. [9]. They function primarily by scavenging free radicals and reactive oxygen species (ROS), thereby preventing cellular damage [10,11]. Therefore, discovering new compounds that can protect against reactive species that may form within cells could have therapeutic benefits [12,13].

Pyridine represents a key subclass of azaheterocycles, with its derivatives demonstrating a wide spectrum of biological activities, thereby underscoring their relevance in pharmacological and medicinal chemistry research. Its structural versatility enables incorporation into diverse therapeutics, enhancing efficacy across multiple diseases [14]. One of the notable biological activities of pyridine compounds is their antimicrobial properties. Certain pyridine derivatives have been identified as effective bactericides and fungicides, which are crucial in agricultural and clinical settings [15]. Additionally, research has shown that pyridine-based compounds can possess significant antioxidant activity, which is beneficial in mitigating oxidative stress-related diseases [16,17].

In the realm of drug design, pyridine derivatives are extensively utilized owing to their capacity to bind to various therapeutic targets. Pyridine-containing compounds have been utilized in the development of therapeutic agents for the treatment of diseases such as tuberculosis, HIV/AIDS, and various forms of cancer. The U.S. FDA has approved numerous pharmaceuticals containing pyridine or dihydropyridine structures, highlighting their therapeutic relevance [18]. The synthesis of novel pyridine derivatives has given rise to the identification of compounds with enhanced biological activity, such as those that inhibit small conductance calcium-activated potassium channels, which are important in

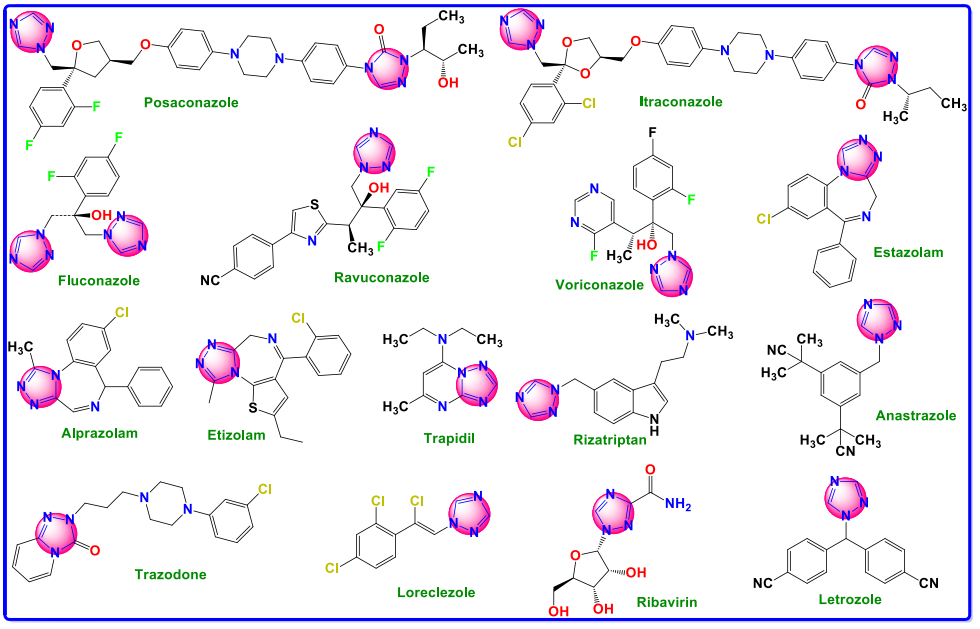


Figure 1. 1,2,4-Triazole containing drugs used in clinical therapy.

regulating neuronal excitability [19]. Additionally, sesquiterpene pyridine alkaloids have been studied for their anti-inflammatory and antitumor properties, further emphasizing the therapeutic potential of pyridine derivatives [20].

Another important azaheterocycle class is the 1,2,4-triazoles, five-membered rings containing three nitrogens, among which a substantial proportion of nitrogen-based heterocycles approved as drugs are azaheterocycles [21]. 1,2,4-Triazoles and their derivatives display diverse pharmacological effects and act as key pharmacophores through strong interactions with biological receptors arising from their dipole character, hydrogen-bonding capacity, rigidity, and solubility [22]. 1,2,4-Triazole is a key component of numerous drugs used in clinical therapy, including antifungals (Posaconazole, Itraconazole, Fluconazole, Ravuconazole, and Voriconazole), hypnotics, anxiolytics, and anticonvulsants (Estazolam and Alprazolam), skeletal muscle relaxants and anxiolytics (Etizolam), antiplatelet drugs (Trapidil), antimigraine agents (Rizatriptan), anticancer agents (Anastrozole), antidepressants (Trazodone), anticonvulsants (Loreclezole), antivirals (Ribavirin), and aromatase inhibitors (Letrozole) [23,24] (Figure 1).

Also, mercapto-1,2,4-triazoles, as well, have garnered considerable interest in medicinal chemistry due to their varied pharmacological properties. These compounds, characterized by the presence of a thiol (-SH) or a thione (C = S) group, possess multifaceted biological properties like antiinflammatory, analgesic, anticancer, anticonvulsant, antiobesity, antiviral, antiurease, antioxidant, antiparasitic, anti-Parkinson's, antineoplastic, antibacterial, antimycobacterial, antifungal, antidepressant, antidiabetic effects ans so forth. Additionally, they are precursors in the synthesis of new sulfur-carrying triazole compounds [10,25].

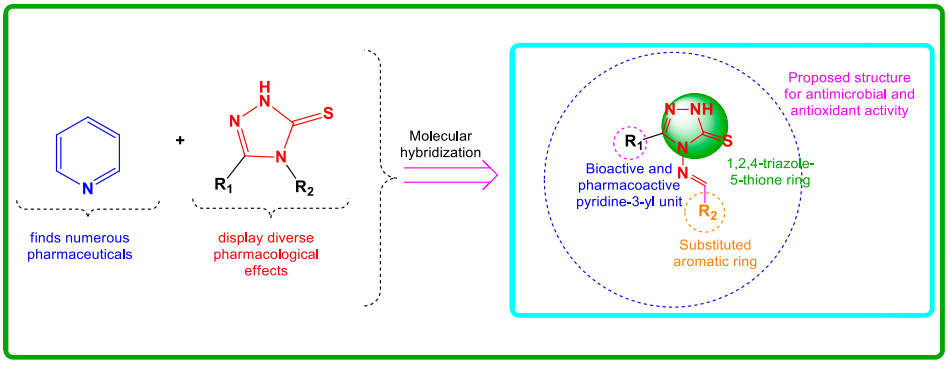


Figure 2. Design of the target compounds by a molecular hybridization approach thought to have potential activity for antimicrobial and antioxidant activity when R_1 is pyridine-3-yl and R_2 is substituted aromatic ring.

Likewise, 4-arylideneamino-1H-1,2,4-triazole-5-thione derivatives exhibit broad activity profiles encompassing antifungal, antibacterial, antiviral, antitubercular, anticonvulsant, antioxidant, anti-inflammatory, anticancer, antityrosinase, antiurease, leishmanicidal, and trypanocidal effects [10,26,27].

Although the antimicrobial properties of pyridin-4-yl-substituted 1,2,4-triazole-5-thiones are well documented [28–33], pyridin-3-yl-substituted analogues have been less extensively explored. Dave et al. synthesized 4-(benzylideneamino)−3-(pyridin-3-yl)−1,2,4-triazole-5-thiones and demonstrated strong inhibition across multiple microbes; under identical conditions, standards produced inhibition zones of 21-25 mm (amoxicillin), 20-24 mm (ampicillin), 15-20 mm (benzylpenicillin), 18-25 mm (norfloxacin), and 18-24 mm (griseofulvin) against *Aspergillus niger* [34]. Similarly, Khalil et al. reported promising antimicrobial activity for 3-(pyridin-3-yl)-1,2,4-triazole Schiff bases relative to standard agents [35].

Despite these advances, the antioxidant properties of pyridine-3-yl-substituted 1,2,4-triazole-5-thione Schiff bases remain under-investigated. Accordingly, this study evaluates both the antimicrobial and antioxidant activities of this compound class. Motivated by these identified gaps, the design strategy of the intended molecules is outlined below. (Figure 2).

2. Materials and methods

2.1. General

The starting materials, solvents, and reagents were obtained from Sigma-Aldrich, Merck, and Alfa-Aesar. All chemicals had a purity of 98% or higher. The reactions were monitored using thin-layer chromatography (TLC) on silica gel HSGF254 plates (0.2 ± 0.03 mm thickness). Ethyl acetate and petroleum ether served as the mobile phase, and detection was performed under UV light.

The melting points were determined on Labart melting point apaparatus. A Shimadzu IR Prestige-21 spectrometer was used to record the infrared spectrum (cm^{-1}). Bruker AVANCE 400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) was used to obtain ^1H

and ^{13}C NMR spectra. Chemical shift values (δ) are reported in parts per million (ppm) relative to solvent signals, with δH around 2.50 ppm (DMSO-d_6) and δC around 40 ppm (DMSO-d_6). Coupling constants (J) are expressed in Hertz (Hz).

2.2. General method for the synthesis of amino-Schiff bases 6a–g

Compound **5** (1.93 g, 10 mmol) was refluxed with the corresponding aldehydes (10 mmol) in acetic acid (10 mL) for 2 h. After the reaction was completed, the mixture was cooled, and the crude products were poured into icy water. The precipitate was then filtered and washed with cold water to remove residual acetic acid. The purified compounds were crystallized from ethanol, yielding compounds **6a–g**, and subsequently dried in a freeze-dryer (Christ ALPHA 1–2 LD plus) for analysis.

2.2.1. (E)-4-((2-hydroxy-3-methylbenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6a)

Light yellow solid (78.32% yield); TLC (ethyl acetate: petroleum ether 1:5); m.p. 216-217°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 3111 (NH), 2916 (Al. CH), 1600 (N = CH), 1259 (C = S), 1186 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** 2.20 (s, 3H, CH_3), Ar-H: [6.91 (t, 1H, J = 8.00 Hz), 7.36 (d, 1H, J = 8.00 Hz), 7.52-7.58 (m, 2H), 8.21 (d, 1H, J = 8.00 Hz), 8.71 (d, 1H, J = 4.00 Hz), 8.98 (s, 1H)], 9.87 (s, 1H, N = CH), 9.97 (s, 1H, OH), 14.36 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 16.3 (CH_3), 117.7, 120.4 (CH), 122.3, 124.2 (CH), 126.5, 128.5, 136.0, 136.4 (CH), 147.0 (triazole C-3), 149,1, 151.8 (CH), 157.3, 163.2 (triazole C-5), 167.8 (N = CH); LC-MS (ESI), m/z (%): 368.64 ($[M + Na + CH_3OH]^+$, 85), 311.79 ($[M + H]^+$, 100).

2.2.2. (E)-4-((2-hydroxy-3-methoxybenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6b)

Light gray solid (81.43% yield); TLC (ethyl acetate: petroleum ether 1:6); m.p. 293-294°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 3365 (OH), 3109 (NH), 2960 (Al. CH), 1598 (N = CH), 1257 (C = S), 1188 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** 3.83 (s, 3H, OCH_3), Ar-H: [6.87 (t, 1H, J = 8.00 Hz), 7.15 (d, 1H, J = 8.00 Hz), 7.39 (d, 1H, J = 8.00 Hz), 7.54-7.57 (m, 1H), 8.23 (d, 1H, J = 8.00 Hz), 8.69 (s, 1H), 9.01 (s, 1H)], 9.80 (s, 1H, N = CH), 10.10 (s, 1H, OH), 14.24 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 56.5 (OCH_3), 116.0, 118.5 (CH), 119.0, 119.9 (CH), 122.5, 122.9, 124.1, 136.4 (CH), 147.2 (triazole C-3), 148.7, 148.8, 149,2, 151.6 (CH), 162.8 (triazole C-5), 162.8 (N = CH); LC-MS (ESI), m/z (%): 349.74 ($[M + Na]^+$, 100), 327.75 ($[M + H]^+$, 50).

2.2.3. (E)-4-((3-ethoxy-2-hydroxybenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6c)

White solid (86.39% yield); TLC (ethyl acetate: petroleum ether 1:5); m.p. 202-203°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 2927 (Al. CH), 1598 (N = CH), 1263 (C = S), 1074 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** 1.34 (t, 3H, CH_3, J = 6.00 Hz), 4.06-4.11 (m, 2H), Ar-H: [6.86 (t, 1H, J = 8.00 Hz), 7.14 (d, 1H, J = 8.00 Hz), 7.37 (d, 1H, J = 8.00 Hz), 7.54-7.57 (q, 1H), 8.23 (d, 1H, J = 8.00 Hz), 8.70 (d, 1H, J = 4.00 Hz), 9.01 (s, 1H)], 9.61 (s, 1H, OH), 10.08 (s, 1H, N = CH), 14.29 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 15.0 (CH_3), 64.8 (OCH_2), 117.2 (CH), 118.6 (CH), 119.0, 120.0 (CH), 122.5, 124.1 (CH), 136.4 (CH),

147.2 (triazole C-3), 147.8, 149.1, 149,2, 151.6 (CH), 162.8 (triazole C-5), 163.1 (N = CH); LC-MS (ESI), m/z (%): 363.88 ($[M + Na]^+$, 100), 341.83 ($[M + H]^+$, 77).

2.2.4. (E)-4-((2-hydroxy-5-methoxybenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6d)

White solid (79.27% yield); TLC (ethyl acetate: petroleum ether 1:6); m.p. 209-210°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 3109 (NH), 2937 (Al. CH), 1600 (N = CH), 1269 (C = S), 1161 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** 3.68 (s, 3H, OCH_3), Ar-H: [6.92 (dd, 1H, $J = 8.00$ Hz), 7.28 (d, 1H, $J = 4.00$ Hz), 7.54-7.58 (m, 1H), 8.25 (d, 1H, $J = 8.00$ Hz), 8.70 (d, 1H, $J = 4.00$ Hz), 9.02 (s, 1H)], 10.03 and 10.04 (s, 2H, OH + N = CH), 14.28 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 55.9 (OCH_3), 118.4 (CH), 118.7, 122.4 (CH), 122.5, 124.0 (CH), 136.4 (CH), 147.3 (triazole C-3), 149.2, 151.6 (CH), 152.7, 153.4, 162.3 (N = CH) 162.8 (triazole C-5); LC-MS (ESI), m/z (%): 349.81 ($[M + Na]^+$, 36), 327.82 ($[M + H]^+$, 100).

2.2.5. (E)-4-((5-chloro-2-hydroxybenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6e)

Bright yellow solid (76.23% yield); TLC (ethyl acetate: petroleum ether 1:3); m.p. 294-295°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 3107 (NH), 1597 (N = CH), 1267 (C = S), 1172 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** Ar-H: [7.01 (d, 1H, $J = 12.00$ Hz), 7.44-7.46 (q, 1H), 7.55-7.58 (q, 1H), 7.73 (s, 1H), 8.23 (d, 1H, $J = 8.00$ Hz), 8.70 (s, 1H), 9.00 (s, 1H)], 10.11 (s, 1H, N = CH), 10.80 (s, 1H, OH), 14.32 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 119.1 (CH), 120.3, 122.4, 123.8 124.1, 125.9, 134.3, 136.5 (CH), 147.4 (triazole C-3), 149,3, 151.7 (CH), 157.8, 160.7 (N = CH), 162.8 (triazole C-5); LC-MS (ESI), m/z (%): 368.78 ($[M + K]^+$, 29), 352.89 ($[M + Na]^+$, 30), 333.78 ($[M + H]^+$ (^{37}Cl), 42), 332.03 ($[M + H]^+$ (^{35}Cl), 100).

2.2.6. (E)-4-((5-bromo-2-hydroxybenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6f)

Bright yellow solid (84.72% yield); TLC (ethyl acetate: petroleum ether 1:4); m.p. 236-237°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 3109 (NH), 1595 (N = CH), 1267 (C = S), 1172 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** Ar-H: [6.95 (d, 1H, $J = 8.00$ Hz), 7.54-7.58 (m, 2H), 8.22 (d, 1H, $J = 8.00$ Hz), 8.71 (d, 1H, $J = 4.00$ Hz), 9.01 (s, 1H)], 10.10 (s, 1H, N = CH), 10.82 (s, 1H, OH), 14.32 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 111.3, 119.5 (CH), 120.9, 122.4, 124.1 (CH), 129.0 (CH), 136.5 (CH), 137.0 (CH), 147.4 (triazole C-3), 149,3 (CH), 150.5 (CH), 151.7 (CH), 158.2, 153.7 (CH), 160.7 (N = CH), 162.8 (triazole C-5); LC-MS (ESI), m/z (%): 412.81 ($[M + K]^+$ (^{81}Br), 71), 376.06 ($[M + H]^+$, 58), 246.81 (100).

2.2.7. (E)-4-((2-hydroxy-5-nitrobenzylidene)amino)-3-(pyridin-3-yl)-1H-1,2,4-triazole-5(4H)-thione (6 g)

Yellow solid (90.06% yield); TLC (ethyl acetate: petroleum ether 1:5); m.p. 234-235°C; IR (FTIR-ATR, υ_{max} in cm^{-1}): 3095 (NH), 1604 (N = CH), 1531 (Asymmetrical N-O), 1342 (Symmetrical N-O), 1273 (C = S), 1174 (C–O); **^{1}H-NMR (400 MHz, DMSO-d_6):** Ar-H: [7.15 (d, 1H, $J = 8.00$ Hz), 7.55-7.58 (q, 1H), 8.23-8.28 (m, 2H), 8.58 (d, 1H, $J = 4.00$ Hz), 8.72 (d, 1H, $J = 4.00$ Hz), 9.02 (s, 1H)], 10.30 (s, 1H, N = CH), 12.12 (bs, 1H, OH),

14.36 (s, 1H, SH); **^{13}C-NMR (100 MHz, DMSO-d_6):** 117.9 (CH), 119.4, 122.4, 122.9 (CH), 124.1 (CH), 129.6 (CH), 136.6 (CH), 140.4, 147.5 (triazole C-3), 149,3, 151.7 (CH), 159.3 (N = CH), 162.8 (triazole C-5), 164.1; LC-MS (ESI), m/z (%): 343.08 ($[M + H]^+$, 100).

2.3. Statistical analysis

The experimental results were calculated as the mean and standard deviation of three independent experiments (arithmetic mean ± standard deviation). The normality of the results was checked using the Shapiro–Wilk test, and after confirming the normal distribution, One-way ANOVA was applied for antioxidant analyses. For post-hoc group comparisons, the Tukey test was used ($p < 0.05$).

2.4. Amtimicrobial activities

2.4.1. Determination of antimicrobial activities

Antimicrobial efficacy of the synthesized compounds was assessed via the disk diffusion assay with appropriate modifications, following the protocol outlined by Balouiri et al. [36]. In this study, *Staphylococcus pyogenes* ATCC 25923 and *Staphylococcus aureus* ATCC 25923 were selected to represent Gram-positive bacteria, while *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were chosen to represent Gram-negative bacteria. Additionally, *Candida albicans* ATCC 18804 served as a model organism representing yeast and mold species.

After activation, the passaged test organisms were incubated overnight to prepare fresh cultures. The pathogens with freshly prepared cultures were then diluted in sterile distilled water to a turbidity of 0.5 McFarland standard (1.5×10^8 CFU/mL) using a McFarland densitometer. In the next step, each microorganism was inoculated onto Mueller-Hinton Agar (MHA) plates using the spread plate technique with a sterile swab. Sterile blank disks with a diameter of 6 mm were placed on the plates inoculated with the test microorganisms. Onto these sterile disks, 15 µL of the samples prepared at concentrations of 24 and 12 mg/mL were pipetted. The plates were kept at 4 °C for 2 h to allow diffusion of the samples into the agar. Commercially prepared imipenem disks at a concentration of 10 µg/disk were used as the positive control. Dimethyl sulfoxide (DMSO), the solvent used for the samples, served as the negative control. Each sample was pipetted onto sterile blank disks in a volume of 15 µL. Bacterial cultures were incubated at 37 °C for 24 h, whereas yeast cultures were maintained at 28 °C for 48 h to allow optimal growth. Following the incubation period, the diameters of the inhibition zones surrounding the disks were measured using a standard ruler.

2.4.2. Determination of Minimum Inhibitory Concentration (MIC) values

The MIC values of the compounds were determined using the broth dilution method with necessary modifications according to CLSI [37]. Sterile, lidded 96-well microplates were used for the assay. Mueller-Hinton Broth (MHB) was chosen as the culture medium. During the experiment, 100 µL of double-strength broth was added to the first wells of the microplates, while 100 µL of standard-strength broth was added to the remaining wells. In the next step, the initial concentration of the samples (20 mg/mL) was prepared using DMSO. From these solutions, 100 µL was pipetted into the first wells of the microplates. Ten

different concentrations of each sample and the selected antibiotic were obtained through a two-fold serial dilution. Suspensions of the selected pathogens (10 µL, adjusted to 0.5 McFarland turbidity) were then added to the wells. After inoculation, bacterial plates were incubated at 37 °C for 24 h, while yeast cultures were incubated at 28 °C for 48 h. Minimum inhibitory concentrations (MICs) of the tested samples and Imipenem were then determined as the lowest concentrations that prevented visible growth of the respective pathogenic microorganisms.

2.5. Antioxidant activities

2.5.1. Determination of Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power of the synthesized compounds was expressed as mg Trolox equivalent per gram of compound. A modified version of the method developed by Oyaizu was used. In this method, 40 µL of the compound was mixed with 100 µL of 0.2 M phosphate buffer (pH 6.6) and 100 µL of potassium ferricyanide, and the mixture was incubated at 50 °C in the dark for 20 min. After incubation, the samples were cooled under water. Then, 100 µL of 10% trichloroacetic acid (TCA) was added to the mixture, which was subsequently centrifuged at 3000×g for 10 min. From the upper phase of the centrifuged samples, 100 µL was transferred into wells of a 96-well microplate. To each well, 100 µL of distilled water and 20 µL of ferric (III) chloride were added. The final mixture was incubated at room temperature in the dark for 5 min. Following incubation, absorbance was measured at 700 nm using a microplate reader. All analyses were performed in triplicate [38].

2.5.2. DPPH radical scavenging activity

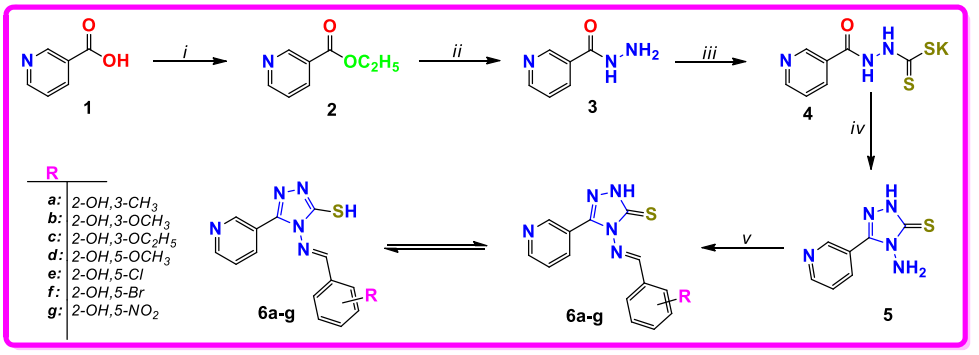
The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the synthesized compounds was determined using a modified method developed by Yu et al. [39]. To 125 µL of the compound, 125 µL of a 0.1 mM DPPH radical solution was added, and the mixture was incubated in the dark at room temperature for 30 min. After incubation, the absorbance values were measured at 517 nm using a microplate reader. The results were expressed as IC50 values. A calibration curve was constructed based on the absorbance values of the samples at 5 different concentrations, and the IC50 value was defined as the concentration corresponding to 50% inhibition [40]. The percentage inhibition value was calculated using the formula in Equation (1).

$$\%\text{Inhibition} = ((\text{Abs\_control} - \text{Abs\_sample})/\text{Abs\_control}) \times 100 \quad (1)$$

3. Results and discussion

3.1. Chemistry

The current study presents the synthesis, antimicrobial, and antioxidant activity evaluations of 1,2,4-triazole-5-thione–pyridine hybrid molecules **6(a-g)**. A series of novel 1,2,4-triazole-5-thione–pyridine hybrid molecules **6(a-g)** were synthesized in five steps, as outlined in Scheme 1. The structures of the synthesized triazole-pyridine hybrid compounds were characterized using Fourier transform infrared-attenuated total reflectance (FTIR-ATR), ^{1}H nuclear magnetic resonance (NMR), and ^{13}C NMR techniques.



Scheme 1. Synthesis of the target compounds **6a-g**. Reagents and conditions: (i) H_2SO_4/absolute C_2H_5OH, reflux; (ii) $NH_2NH_2.H_2O$/absolute C_2H_5OH, reflux; (iii) CS_2/KOH, absolute C_2H_5OH; (iv) $NH_2NH_2.H_2O$/water, reflux; (v) aromatic aldehydes, glacial CH_3COOH, reflux.

The compounds (**1**-**5**) were synthesized according to the methods described in the literature [25,41,42]. The carboxylic acid (**1**) was obtained from Merck company and used without further purification. A solution of compound **1** in absolute ethanol was heated at reflux for 4 h in the presence of catalytic sulfuric acid (H_2SO_4), with reaction progress monitored by TLC. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and water; the organic phase was washed with saturated aqueous sodium carbonate (to neutralize residual acid), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The hydrazide (**3**) was obtained by treating ester (**2**) with hydrazine hydrate in ethanol. The mixture was heated at reflux for **4 h.** After cooling to 0–5 °C, the precipitated solid was collected by filtration. The potassium salt (**4**) was obtained by treating compound **3** with CS_2 in ethanol in the presence of KOH. A yellow precipitate of the potassium dithiocarbazate salt (**4**) formed. The solid was filtered, washed quickly with cold absolute EtOH and Et_2O, and used directly in the next step to minimize decomposition. Crude salt (**4**) was suspended in deionized water. Hydrazine monohydrate was added, and the mixture was heated at reflux for 8 h to effect cyclization to the 1,2,4-triazole-5-thione (**5**). After cooling to rt, the reaction was acidified dropwise with 10% HCl to pH 5–6, causing precipitation. The solid was collected, washed thoroughly with cold water until neutral, and dried under vacuum to give **5** as an off-white solid. The (E)−4-(substitutedbenzylideneamino)−3-(pyridine-3-yl)−1H-1,2,4-triazole-5(4H)-thione molecules **6(a-g)** were prepared by reacting 4-amino-3-(pyridine-3-yl)−1H-1,2,4-triazole-5(4H)-thione (**5**) with suitable aromatic aldehydes in acetic acid.

Both thiol (C-SH) and thione (C = S) tautomeric forms of the triazole-pyridine hybrid compounds **6(a-g)** are possible [43,44]. The infrared spectra of all mercapto triazoles **6(a-g)** showed absorption bands for the C = S group around 1260 cm^{-1} and N-H stretching at 3100 cm^{-1}. Meanwhile, the N = CH absorption bands appeared near 1600 cm^{-1}, and the C–O absorption bands were observed around 1170 cm^{-1}. The absence of NH_2 absorption (3226–3165 cm^{-1}) in the IR spectra of the triazole-pyridine hybrid compounds **6(a-g)**, in contrast to the corresponding 3-pyridine-5-mercapto-1,2,4-triazoles (**5**), confirmed the occurrence of the Schiff base reaction. The lack of SH band in IR, combined with the

Table 1. MIC values (μg/mL) of the pyridine-substituted 1,2,4-triazole-5-thione Schiff bases against tested microorganisms.

	MIC values of the synthesized compounds (μg/mL)				
	Gram (−)		Gram (+)		Yeast
Compound	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923	*S. pyogenes* ATCC 19615	*C. albicans* ATCC 10231
6a	625	2500	–	156.25	1250
6b	156.25	1250	1250	19.531	2500
6c	312.5	2500	2500	78.125	2500
6d	625	2500	312.5	19.531	–
6e	156.25	5000	5000	39.063	10000
6f	2500	2500	5000	39.063	2500
6g	312.5	2500	156.25	19.531	625
P.C.	0.25	1	0.25	0.50	1
N.C.	–	–	–	–	–

P.C. = Positive Control (Imipenem), N.C. = Negative Control (DMSO), – = Not Detected

presence of NH and C = S bands, suggested that the synthesized triazole-pyridine hybrid Schiff bases primarily adopt their thione form rather than the thiol form. These findings align with previous studies. [25,43,44].

The ^{1}H-NMR spectra displayed singlet peaks for N = CH protons in the range of 9.61–10.30 ppm. The SH protons showed resonance at 14.24–14.36 ppm, while the OH protons resonated between 9.97 and 12.12 ppm. Aromatic signals were detected between 6.86 and 9.02 ppm. In the ^{13}C-NMR spectra, the triazole C-3 carbons resonated at approximately 147 ppm, while the C-5 carbons of the triazole appeared around 162 ppm. The N = CH carbons were observed between 159.26 and 167.82 ppm, with the shift attributed to the position of the benzene ring substituents. The NMR and IR spectroscopy confirm that the structures exist in the NH form in the solid state and in the SH form in the liquid state.

Furthermore, another piece of evidence supporting the formation of triazole-pyridine hybrid compounds **6(a-g)** is that the molecular ion peaks ($[M+H]^+$ and/or $[M+Na]^+$) corresponded with the expected values based on the respective chemical structures.

The newly synthesized compounds are stable in air, exist as powders, and are non-hygroscopic solids. These compounds are insoluble in water but soluble in common organic solvents such as ethanol, methanol, dimethyl sulfoxide, acetone, dimethylformamide, dichloromethane, and chloroform.

3.2. Antimicrobial activities

Synthesized pyridine-1,2,4-triazole hybrid Schiff base derivatives (**6a-g**) were evaluated for their ability to inhibit the growth of bacteria and fungi *in vitro*. The efficacy of the antibacterial activity was evaluated against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, and *S. pyogenes* ATCC 19615. Antifungal activity was assessed against *C. albicans* ATCC 10231. The minimal inhibitory concentration (MIC) values, as shown in Table 1, were used to evaluate the potency of both antibacterial and antifungal activities.

In general, the compounds exhibited relatively low antibacterial activity against Gram-negative bacteria. The MIC values were predominantly high, particularly against *P. aeruginosa*, for which most compounds showed MICs $\geq$ 2500 µg/mL. The only exception was compound **6b** (bearing 3-OCH_3 group), which displayed a moderate level of activity with an MIC of 1250 µg/mL. On the other hand, *E. coli* was moderately susceptible to compounds **6b** and **6e** (containing 5-Cl group), both of which demonstrated notable antibacterial activity with MIC values of 156.25 µg/mL. This suggests that crossing the outer membrane of the Gram-negative bacteria is optimized by moderately polar/compact substituents (meta-OCH_3 or a small halogen), while the heavier halogen is lipophilic yet bulky and thus less effective.

Against Gram-positive bacteria, several compounds showed markedly improved activity. Notably, compounds **6b**, **6d**, and **6 g**, which bear 3-OCH_3, 5-OCH_3, and 5-NO_2 substituents, respectively, exhibited the lowest MIC values (19.531 µg/mL) against *S. pyogenes*, indicating their strong antibacterial potential. In Gram-positive bacteria, the pattern '5-NO_2 (and partly 5-OMe) strong, halogens weak' can be explained by the absence of an outer membrane, favorable electronic effects, and possible bioreduction. Because there is no outer membrane, the pK_a-lowering effect of 5-NO_2 does not hinder diffusion; moreover, the resonance donation of 5-OMe and the −I/−M effects of 5-NO_2 enhance hydrogen bonding, $\pi - \pi$ stacking, and metal-center interactions at the targets. In addition, compound **6 g** also exhibited antimicrobial activity against *S. aureus*, demonstrating a minimum inhibitory concentration (MIC) of 156.25 µg/mL. The main reasons halogenated derivatives (especially 5-Cl/5-Br) perform poorly against Gram-positive bacteria are as follows: in the absence of an outer membrane, 'lipophilicity alone' confers little advantage; halogens are poor hydrogen-bond partners (weak donors/acceptors) and, by generally rendering the ring more electron-deficient, reduce the chelation and multipoint binding capacity of the o-phenolate/C = S/imine triad; bulky substituents like Br can disrupt planarity/conformation and weaken fit within binding pockets; increased lipophilicity can diminish local aqueous distribution within the thick peptidoglycan matrix; and they offer limited electrostatic/multipoint engagement with the anionic surface of teichoic acids. By contrast, 5-NO_2 (which lowers pK_a, stabilizes the phenolate, and may generate reactive intermediates via bioreduction) and 5-OMe (resonance donation) can establish richer interaction networks with the target, thereby showing clear superiority in Gram-positive contexts.

In the case of *C. albicans*, compound **6 g** emerged as the most effective antifungal agent, showing an MIC value of 625 µg/mL. The remaining compounds generally exhibited lower antifungal activity, with MIC values ranging from 1250 µg/mL to above.

The antimicrobial activity of the pyridine-substituted 1,2,4-triazole-5-thione Schiff bases (**6a – g)** was systematically assessed using the agar diffusion method against a panel of clinically relevant microorganisms, including Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853), Gram-positive bacteria (*S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615), and yeast (*C. albicans* ATCC 10231). The compounds were tested at concentrations of 24 and 12 mg/mL, and the inhibition zone diameters were recorded in millimeters (mm), as presented in Table 2. Imipenem and DMSO were used as positive and negative controls, respectively.

Overall, the tested compounds exhibited limited antimicrobial effects. Compound **6 g** demonstrated the most promising results, displaying significant inhibitory activity

Table 2. Disk diffusion diameters of the pyridine-substituted 1,2,4-triazole-5-thione Schiff bases against tested microorganisms (mm).

	Gram (−)				Gram (+)				Yeast	
	E. coli ATCC 25922		*P. aeruginosa* ATCC 27853		*S. aureus* ATCC 25923		*S. pyogenes* ATCC 19615		*C. albicans* ATCC 10231	
Compound	24 mg/mL	12 mg/mL	24 mg/mL	12 mg/mL	24 mg/mL	12 mg/mL	24 mg/mL	12 mg/mL	24 mg/mL	12 mg/mL
6a	–	–	–	–	–	–	–	–	–	–
6b	–	–	–	–	–	–	–	9.33±0.47	10±0.81	8.66±0.47
6c	10±0.82	9.33±0.47	–	9±0	–	–	–	–	–	–
6d	–	–	–	–	–	–	–		9.66±0.47	9±0
6e	8.33±0.47	9±0	–	–	–	–	–	–	–	–
6f	–	–	–	–	–	–	–	–	–	–
6g	–	–	–	–	10.66±1.88		10±0	9±0	9.66±0.47	10±0
P.C.	32±0.81		28±0.81		47.66±0.47		34±0.81		–	
N.C.	–	–	–	–	–	–	–	–	–	–

P.C. = Positive Control (Imipenem), N.C. = Negative Control (DMSO), – = Not Detected

against Gram-positive bacteria (*S. aureus*, 10.66 mm; *S. pyogenes*, 10 mm) and *C. albicans* (9–10 mm). Notably, **6 g** was the only compound active against all tested strains, indicating its potential as a broad-spectrum antimicrobial agent.

Compounds **6c** and **6e**, bearing 3-OC_2H_5 and 5-Cl groups, showed moderate activity, with **6c** producing inhibition zones between 9 and 10 mm against *E. coli*, *P. aeruginosa*, and *S. pyogenes*, while **6e** exhibited weak activity against certain Gram-negative bacteria (8.33–9 mm). Compound **6d** demonstrated mild antifungal effects exclusively against *C. albicans* (9.66 mm). In contrast, compounds **6a**, **6b**, and **6f** did not display any significant antimicrobial activity under the tested conditions.

As anticipated, the positive control imipenem exhibited strong antibacterial activity against all bacterial strains, with inhibition zones ranging from 28 to 47.66 mm. The negative control (DMSO) showed no inhibitory effect.

These findings suggest that among the evaluated derivatives, compound **6 g,** which bears 5-NO_2 group, holds the greatest promise for further development as an antimicrobial agent, particularly against Gram-positive pathogens and fungi.

The data obtained from both the MIC (Minimum Inhibitory Concentration) and disc diffusion methods revealed that the antimicrobial activity of the compounds varied depending on the type of microorganism. The reduced permeability of the outer membrane in Gram-negative bacteria may hinder the penetration of these compounds, thereby restricting their antimicrobial effectiveness [45,46]. In contrast, compounds **6b**, **6d**, and **6 g** showed notable activity against Gram-positive bacteria, especially *S. pyogenes*, indicating their potential as therapeutic candidates. In terms of antifungal activity, compound **6 g** emerged as the most effective agent against *C. albicans*.

The results indicate that the antimicrobial activity of the tested Schiff bases varies depending on the structural features of the compounds. In particular, compound **6 g** exhibited a broad-spectrum activity profile, demonstrating efficacy against both Gram-positive bacteria and yeast cells. This suggests that the compound may possess structural characteristics that enable it to target cell wall and membrane structures or act through broad-spectrum biochemical mechanisms.

3.3. Antioxidant capacities

Although numerous natural antioxidants exist, synthetic antioxidants are prevalently employed across diverse areas such as food, medicine, agriculture, and biotechnology [47]. In this study, the antioxidant capacities of the newly synthesized compounds were evaluated using two distinct methods: FRAP and DPPH.

Based on the DPPH assay (predominantly H-atom transfer; sensitive to solvent and lipophilic access), compound **6f**, bearing 5-bromine group, exhibited the strongest antioxidant activity, while compound **6d** showed the weakest activity among the tested compounds. The inductive effect of halogens likely increases the acidity of the phenolic O-H, easing H-atom donation, while enhanced lipophilicity may improve contact with the radical. The remaining compounds displayed moderate antioxidant activity; however, all tested compounds were less effective compared to the standard antioxidant, Trolox (Figure 3).

The values are presented as arithmetic mean ± standard deviation. *p*-values were obtained using One-way ANOVA, and group comparisons were made using the Tukey

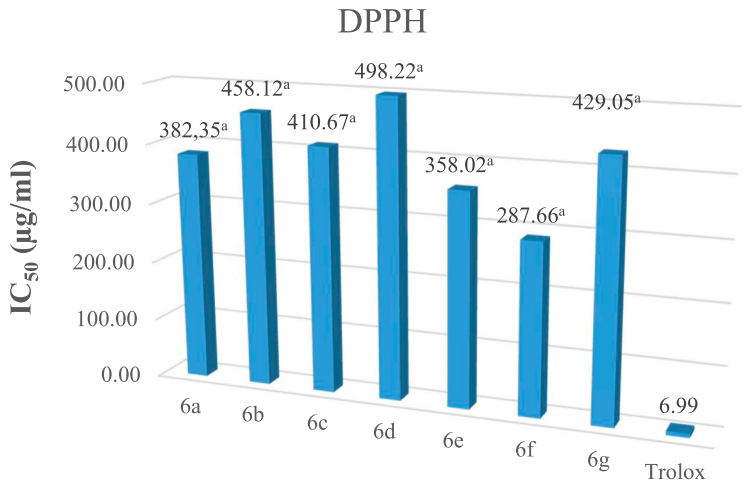


Figure 3. The DPPH assay results for the synthesized compounds.

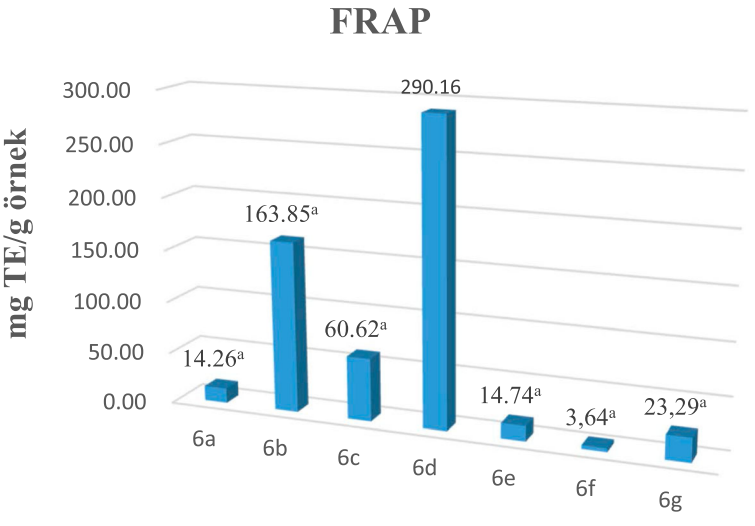


Figure 4. The FRAP assay results for the synthesized compounds.

test. Comparisons with the Trolox group were conducted, and a p-value of < 0.05 was considered statistically significant.

According to the FRAP method (electron-donation/reducing power), the most active compound was **6d**, which contains a 5-methoxy group, with a value of 290.16 mg TE/g, while the least active compound was **6f**, containing a 5-bromo group, with a value of 3.64 mg TE/g. This trend follows the classical model in which electron-donating groups at the para position stabilize the phenoxyl radical via resonance and facilitate electron transfer. Compounds **6a** and **6e** exhibited nearly identical values, with 14.26 and 14.74 mg TE/g, respectively. The values for compounds **6b**, **6c**, and **6g** were 163.85, 60.62, and 23.39 mg TE/g, respectively (Figure 4).

The values are presented as arithmetic mean ± standard deviation. *p*-values were calculated using the One-way ANOVA test, and intragroup comparisons were evaluated using the Tukey test. Comparisons were made with sample **6d**, and a *p*-value of < 0.05 was considered statistically significant.

The strongly electron-withdrawing **6 g** (5-NO_2) remains limited in both assays, suggesting that excessive withdrawal depresses overall reducing capacity.

4. Conclusion

In this study, the synthesized pyridine-containing mercapto-1,2,4-triazole-Schiff bases exhibited significant antimicrobial activity, particularly against Gram-positive bacteria. According to the MIC analyses and disk diffusion results, compounds **6b**, **6d**, and **6 g** stood out with their low MIC values against *S. pyogenes,* each reaching 19.531 µg/mL. Notably, **6 g** emerged as a potential broad-spectrum antimicrobial agent, showing activity against S. aureus (156.25 µg/mL; zones 10.66 ± 1.88 mm at 12–24 mg/mL), S. pyogenes (zones 10 ± 0 mm at 24 mg/mL and 9 ± 0 mm at 12 mg/mL), and C. albicans (625 µg/mL; zones 9.66 ± 0.47 mm at 24 mg/mL and 10 ± 0 mm at 12 mg/mL). However, structural optimization is required to enhance its antifungal activity. Additionally, the marked antioxidant activity exhibited by compound **6d,** with the highest FRAP value in the series (290.16 mg TE/g) and DPPH performance still inferior to Trolox, suggests that this derivative may possess multifunctional pharmacological potential. In light of these findings, further advanced biological and pharmacokinetic studies of compounds **6d** and **6 g** could represent a significant step toward the development of new therapeutic strategies.

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Authorship contribution statement

E. Gultekin: Conceptualization, Methodology, Investigation, Material, Data Curation, Original Draft Writing, Review and Editing, Visualization, Observation.

AI declaration

I hereby declare that I have used artificial intelligence to improve the English language quality of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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