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Synthesis, Characterization and Antimicrobial Analysis of a Novel Thioether via S-Alkylation of Methyl-4H-1,2,4-Triazole-3-Thiol with Methyl Iodide

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ABSTRACT

The development of structurally novel antimicrobial compounds remains important because of the continuing emergence of antimicrobial resistance and the limited effectiveness of some existing therapeutic agents. Heterocyclic compounds containing the 1,2,4-triazole nucleus have attracted considerable attention because of their diverse chemical and biological properties. In the present study, a novel sulfur-containing 1,2,4-triazole derivative, designated Compound AP, was prepared through S-alkylation of methyl-4H-1,2,4-triazole-3-thiol with methyl iodide under basic conditions. The synthesized compound was characterized by melting-point determination, Fourier-transform infrared spectroscopy (FT-IR), gas chromatography-mass spectrometry (GC-MS), proton nuclear magnetic resonance (1H NMR), and carbon-13 nuclear magnetic resonance (13C NMR) spectroscopy. The Compound exhibited a melting range of 112–114 °C. The FT-IR spectrum showed characteristic absorptions associated with triazole C–H, aliphatic C–H, C=N, ring vibrations, and methyl deformation. GC-MS showed a molecular ion at m/z 129 together with characteristic fragment ions at m/z 114, 85, 72, 70, 59, and 58. The 1H NMR spectrum displayed signals at δ 8.37, 7.79, 3.72, and 2.75 ppm, with the signal at δ 2.75 ppm assigned to the newly introduced S–CH3 group. The 13C NMR spectrum showed resonances at δ 144.997, 141.342, 32.079, and 15.084 ppm, supporting the proposed molecular structure. Antimicrobial screening demonstrated concentration-dependent inhibition against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candida albicans. The largest inhibition zones at 200 µg/mL were observed against B. subtilis (13 mm), followed by S. aureus and C. albicans (12 mm each). The reported MIC values ranged from 64–256 µg/mL, while MBC/MFC values ranged from 128–512 µg/mL. These findings indicate that S-alkylation of a methyl-1,2,4-triazole-3-thiol framework can produce a structurally defined thioether with measurable broad-spectrum antimicrobial activity and provide a basis for further structural optimization and biological investigation.

KEYWORDS: 1,2,4-triazole; thioether; S-alkylation; methyl-4H-1,2,4-triazole-3-thiol; antimicrobial activity; FT-IR; GC-MS; NMR; MIC; MBC.

How to Cite

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1. Introduction

Antimicrobial resistance is a major contemporary public-health challenge that has increased the need for the discovery and development of new antimicrobial chemotypes. The global burden of bacterial

antimicrobial resistance remains substantial, while resistance among clinically important Gram-positive and Gram-negative organisms continues to complicate treatment strategies (GBD 2021 Antimicrobial Resistance Collaborators, 2024; World Health Organization [WHO], 2024a, 2024b). The WHO bacterial priority pathogen framework further emphasizes the importance of developing new antibacterial agents against organisms for which resistance is increasingly problematic (WHO, 2024a).

Heterocyclic chemistry provides an important platform for antimicrobial drug discovery. Among nitrogen-containing heterocycles, the 1,2,4-triazole ring has received considerable attention because its electronic properties and ability to accommodate different substituents permit extensive structural modification. Recent reviews have emphasized the broad biological potential of 1,2,4-triazole derivatives, including antibacterial, antifungal, antitubercular, antiviral, anticancer, and anti-inflammatory activities (Strzelecka & Świątek, 2021; Abdelli *et al.,* 2021; Ohloblina, 2022; Gupta *et al.,* 2023; Salma *et al.,* 2024; Pal, 2025).

The biological importance of 1,2,4-triazoles is also demonstrated by their incorporation into several pharmacologically active molecules. Their antimicrobial properties have stimulated extensive investigations into structure–activity relationships, particularly through the introduction of aromatic, heteroaromatic, sulfur-containing, and other functional groups onto the triazole nucleus (Kazeminejad *et al.,* 2022; Tian *et al.,* 2023; Pal, 2025). The electronic and hydrogen-bonding characteristics of the triazole ring can facilitate interactions with biological macromolecules and may influence lipophilicity, molecular recognition, and biological distribution.

Sulfur-containing heterocycles are particularly interesting in medicinal and agricultural chemistry. The sulfur atom can contribute to molecular polarizability, lipophilicity, conformational properties, and interactions with biological targets. Consequently, thioether-containing derivatives of 1,2,4-triazoles have emerged as useful structures for biological screening. Ding *et al*. (2021), for example, reported a series of 1,2,4-triazole thioethers and demonstrated that selected derivatives possessed significant antibacterial and antifungal activity. Similarly, S-substituted bis-1,2,4-triazole-3-thiones have been reported to exhibit antimicrobial effects against a range of Gram-positive and Gram-negative microorganisms (Karpun *et al.,* 2021).

S-alkylation represents an important method for functionalizing thiol-containing heterocycles. Conversion of an S–H group into an S–alkyl substituent modifies the electronic environment and intermolecular interactions of the parent molecule while producing a thioether linkage. Recent studies involving S-functionalized 1,2,4-triazoles have demonstrated the versatility of sulfur substitution in generating compounds with measurable antimicrobial activity (Chen *et al.,* 2021; Ding *et al.,* 2021; Karpun *et al.,* 2021; Jiang *et al.,* 2025).

The antimicrobial potential of sulfur-containing triazoles has been investigated against both bacteria and fungi. Chen *et al.* (2021) reported S-substituted 1,2,4-triazole derivatives with activity against several phytopathogenic fungi and bacteria, whereas Karpun *et al.* (2021) observed antimicrobial activity for S-substituted bis-triazole derivatives. More recently, Jiang *et al.* (2025) synthesized novel 1,2,4-triazole thioethers incorporating a 1,3,4-thiadiazole moiety and reported antibacterial and antifungal activities. These findings demonstrate that sulfur-containing 1,2,4-triazole frameworks remain an active area of contemporary antimicrobial research.

The present study therefore focused on the preparation and antimicrobial evaluation of a methyl-substituted 1,2,4-triazole thioether obtained by S-alkylation of methyl-4H-1,2,4-triazole-3-thiol. The compound was characterized using complementary spectroscopic and physicochemical techniques, and its activity was evaluated against selected Gram-positive bacteria, Gram-negative bacteria, and a fungal organism.

2. Materials and Methods

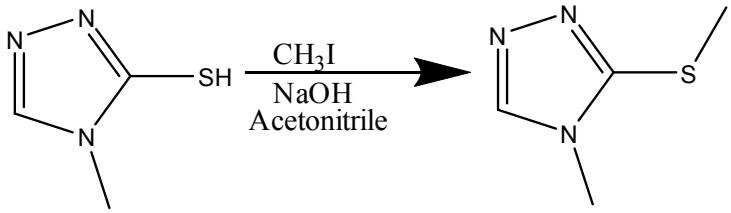
2.1 Chemicals and reagents

Methyl-4H-1,2,4-triazole-3-thiol, sodium hydroxide, methyl iodide, acetonitrile, dichloromethane, sodium sulfate, ethyl acetate, hexane, and other analytical reagents were used as supplied or according to the laboratory's established analytical procedures. All experimental work involving volatile, toxic, corrosive or otherwise hazardous reagents was conducted using appropriate institutional chemical-safety procedures and personal protective equipment.

2.2 Synthesis of the target Compound

The Target Compound was prepared by S-alkylation of methyl-4H-1,2,4-triazole-3-thiol with methyl iodide under basic conditions. The thiol substrate was treated with sodium hydroxide in acetonitrile to generate the corresponding sulfur-centered nucleophile. Methyl iodide was subsequently introduced and the reaction mixture was maintained under controlled laboratory conditions until chromatographic monitoring indicated disappearance of the starting thiol.

The reaction proceeds through nucleophilic substitution at the methyl carbon of the alkylating reagent. Deprotonation of the thiol increases the nucleophilicity of sulfur, allowing formation of the S–CH_3 bond and conversion of the parent thiol into the corresponding thioether. This type of sulfur functionalization is consistent with contemporary approaches used for preparing S-substituted 1,2,4-triazole derivatives (Abdelli *et al.,* 2021; Ding *et al.,* 2021; Karpun *et al.,* 2021).
Following completion, the reaction mixture was subjected to aqueous work-up and organic extraction. The organic fraction was dried and concentrated under reduced pressure. The resulting pale solid was designated Compound **AP**.



2.3 Melting-point determination
The melting point of the Compound was determined using a standard melting-point apparatus. The observed melting range was 112–114 °C. Melting-point determination was used as an initial physicochemical criterion for assessing the identity and purity of the isolated compound.

2.5 Antimicrobial evaluation
The Synthesised Compound was evaluated against six test organisms comprising three Gram-positive/Gram-negative bacterial groups and one fungal species: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*. Antimicrobial activity was evaluated at 25, 50, 100, and 200 µg/mL.
The inhibition-zone results were expressed in millimetres. MIC and MBC/MFC values were reported in µg/mL. Interpretation of MIC testing should be aligned with a recognized standardized methodology such as CLSI M07 for aerobic bacteria and appropriate current yeast susceptibility guidance, while EUCAST also provides standardized MIC methodology and quality-control guidance (Clinical and Laboratory Standards Institute [CLSI], 2024a; EUCAST, 2024, 2026).

3.0 Results and Discussion
Compound AP was obtained as a pale solid following S-alkylation of methyl-4H-1,2,4-triazole-3-thiol. The transformation is chemically consistent with nucleophilic substitution in which the sulfur atom of the deprotonated thiol attacks the electrophilic carbon of the methylating reagent.
The conversion of the parent thiol into the S-methyl thioether is particularly significant because sulfur substitution changes the hydrogen-bonding characteristics, steric environment, polarity, and molecular packing of the heterocycle. S-functionalization has been extensively investigated in contemporary 1,2,4-triazole chemistry and has produced compounds with diverse biological profiles (Chen *et al.,* 2021; Ding *et al.,* 2021; Karpun *et al.,* 2021; Salma *et al.,* 2024).
The spectroscopic data discussed below provide complementary evidence supporting the proposed structure.

3.1 Melting-point analysis
Compound AP exhibited a melting range of **112–114 °C**. The relatively narrow melting interval suggests the isolation of a reasonably homogeneous crystalline material. The observed melting behavior differs from that expected for the parent thiol and can reasonably be associated with structural modification following S-alkylation.
Replacement of the thiol hydrogen with a methyl group removes an S–H donor and introduces a nonpolar substituent. This change can influence intermolecular hydrogen bonding and crystal packing, potentially producing a lower melting transition. However, melting-point differences alone should not be considered definitive proof of molecular identity and should be interpreted together with spectroscopic and mass-spectral data.
Similar approaches combining melting-point measurements with FT-IR, NMR, and mass spectrometry are widely employed for structural characterization of newly synthesized 1,2,4-triazole derivatives (Chen *et al.,* 2021; Karpun *et al.,* 2021; Sabzi & Al-Mudhafar, 2023; Mosleh & Dakhel, 2024).

3.2 FT-IR characterization

The FT-IR spectrum of Compound **Ap** exhibited characteristic absorption bands that supported the formation of the methyl-substituted 1,2,4-triazole thioether. A broad absorption band observed at 3371.9 cm^{-1} was assigned to the N–H stretching vibration of the triazole ring. The absorption at 3134.1 cm^{-1} was attributed to C–H stretching of the heteroaromatic triazole ring, while the bands observed around 3015.5, 2969.4, and 2950.3 cm^{-1} were assigned to aliphatic and methyl C–H stretching vibrations. A band at 1636.8 cm^{-1} was attributed mainly to C=N stretching within the 1,2,4-triazole ring, while the absorption at 1520.9 cm^{-1} was associated with characteristic stretching vibrations of the conjugated triazole ring system. The bands observed at 1434.5 and 1380.2 cm^{-1} were assigned to C–H bending/deformation vibrations of the methyl groups. The absorption at 1061.8 cm^{-1} can be attributed to C–N/C–C vibrations within the triazole ring, while the bands at 954.6 and 772.8 cm^{-1} were associated with heteroaromatic ring deformation and out-of-plane vibrations. Importantly, the spectrum did not show a clear strong S–H stretching absorption in the expected thiol region (approximately 2550–2600 cm^{-1}). The absence of the S–H band is consistent with the conversion of the thiol functionality into an S-methyl thioether during the reaction with methyl iodide. The appearance of additional aliphatic C–H stretching bands around 2950–3020 cm^{-1} is also consistent with the presence of methyl substituents in Compound **Ap**. FT-IR spectroscopy is commonly used alongside NMR and mass spectrometry for confirming functional-group transformations in newly synthesized 1,2,4-triazole derivatives (Amin *et al.,* 2021; Sabzi & Al-Mudhafar, 2023; Mosleh & Dakhel, 2024).

3.3 GC-MS characterization

GC–MS analysis further supported the formation of Compound **Ap**. The mass spectrum exhibited a prominent molecular-ion peak at m/z 129, which corresponded to the calculated molecular mass of 129.18 g mol^{-1} for the proposed molecular formula $C_4H_7N_3S$. The molecular ion appeared as the base peak, indicating appreciable stability of the compound under the electron-ionization conditions. A significant fragment ion was observed at m/z 114, corresponding to the loss of 15 mass units from the molecular ion and tentatively assigned to the loss of a methyl radical. Other characteristic fragment ions were observed at m/z 85, 72, 70, 59, 58, 57, 56, and 55, which were attributed to successive fragmentation of the methyl-substituted triazole–sulfur framework. The agreement between the observed molecular ion (*m/z* 129) and the expected molecular mass, together with the characteristic fragmentation pattern, provided further evidence for the proposed structure of Compound Ap.

The GC-MS findings are consistent with the use of mass spectrometry as a complementary structural tool for S-substituted triazoles. Previous investigations of sulfur-containing 1,2,4-triazoles have similarly used mass spectrometry together with NMR and other spectroscopic methods to establish molecular structures (Karpun *et al.,* 2021).

3.4 ^{1}H NMR characterization

The ^{1}H NMR spectrum further supported the proposed structure of Compound **Ap,** Signals were observed at δ 8.37, 7.79, 7.27, 3.72, and 2.75 ppm. The resonance at δ 3.72 ppm was assigned to the N-methyl (N–CH_3) protons, whereas the signal at δ 2.75 ppm was attributed to the methyl group attached to sulfur (S–CH_3), confirming the incorporation of the methyl group through S-alkylation of the starting triazole thiol. The downfield resonance at δ 8.37 ppm was attributed to the proton of the electron-deficient 1,2,4-triazole ring. The signal at δ 7.27 ppm was assigned to residual $CHCl_3$ from the deuterated chloroform solvent. The additional resonance at δ 7.79 ppm should be considered alongside its integration and multiplicity to establish whether it represents a second compound proton or a minor impurity. Overall, the appearance of the characteristic N–CH_3 and S–CH_3 resonances, together with the heteroaromatic signal, was consistent with the proposed S-methylated triazole structure.

NMR characterization is widely used in recent studies of sulfur-substituted 1,2,4-triazoles and related heterocyclic systems (Chen *et al.,* 2021; Ding *et al.,* 2021; Sabzi & Al-Mudhafar, 2023; Mosleh & Dakhel, 2024).

3.5 ^{13}C NMR characterization

The ^{13}C NMR spectrum further supported the proposed structure of Compound **Ap**. The spectrum exhibited characteristic resonances at δ 144.997 and 141.342 ppm, which were assigned to the two carbon atoms of the 1,2,4-triazole ring. The relatively downfield positions of these resonances were consistent with the electron-deficient nature of the heteroaromatic ring and the influence of the adjacent nitrogen atoms. A resonance at δ 32.079 ppm was attributed to the N–CH_3 carbon, while the signal at δ 15.084 ppm was

assigned to the S–CH_3 carbon introduced during the S-alkylation reaction. The appearance of the S–CH_3 resonance provided further evidence for conversion of the thiol group into the corresponding thioether. The intense resonance at approximately δ 77 ppm was attributed to the $CDCl_3$ solvent. An additional resonance at δ 30.949 ppm was observed and may represent a minor impurity or residual component; therefore, it was not assigned definitively to the proposed structure.
Overall, the combined spectroscopic evidence confirmed the successful conversion of 4-methyl-4H-1,2,4-triazole-3-thiol into its corresponding S-methyl thioether derivative. This observation is consistent with recent reports that 1,2,4-triazole-3-thiol derivatives readily undergo S-alkylation reactions to produce structurally diverse compounds with potential pharmaceutical, agricultural, and material applications. (Kumar et al., 2026; Rodrigues et al., 2025; kalchenko and Shcherbyna, 2026).

4. Antimicrobial Activity

4.1 Preliminary inhibition-zone assay

The antimicrobial activity of Compound **AP** was evaluated against six microorganisms using Disc diffusion methods comprising four bacterial species and one yeast species at concentrations of 25, 50, 100, and 200 µg/mL. The inhibition-zone results are summarized in Table 1. Compound AP demonstrated measurable antimicrobial activity against all tested organisms, with inhibition zones increasing progressively with increasing concentration.
At 25 µg/mL, inhibition zones ranged from 6 to 7 mm, whereas at 200 µg/mL, the inhibition zones increased to 10–13 mm. The greatest inhibition was observed against *Bacillus subtilis*, which produced a zone of 13 mm at 200 µg/mL. *Staphylococcus aureus* and *Candida albicans* exhibited maximum inhibition zones of 12 mm, while *Escherichia coli* and *Klebsiella pneumoniae* showed maximum zones of 11 mm. *Pseudomonas aeruginosa* exhibited the lowest response at the highest concentration, with an inhibition zone of 10 mm.
The progressive increase in inhibition-zone diameter with concentration indicates a concentration-dependent antimicrobial response under the experimental conditions. However, inhibition-zone diameter should not be interpreted as a direct measure of antimicrobial potency because diffusion through agar is influenced by factors such as molecular size, solubility, compound stability, medium composition, and growth characteristics of the test organism. Consequently, quantitative susceptibility parameters such as the minimum inhibitory concentration (MIC) provide a more appropriate basis for comparing antimicrobial activity. Standardized broth-dilution procedures are recommended for MIC determination (CLSI, 2024).
The activity of Compound **AP** against both Gram-positive and Gram-negative bacteria indicates that the compound possesses a broad preliminary antibacterial spectrum. Its activity against *C. albicans* also suggests potential antifungal activity. These findings are consistent with the recognized biological versatility of the 1,2,4-triazole scaffold. Recent reviews have highlighted the antibacterial and antifungal potential of structurally diverse 1,2,4-triazole derivatives and their hybrids, with activity influenced strongly by the nature and position of substituents attached to the heterocyclic ring (Gupta *et al.,* 2023; Tian *et al.,* 2023; Khomenko, 2024).

Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal Concentration

The MIC and MBC/MFC values obtained for Compound AP are presented in Table 2. *B. subtilis* exhibited the greatest susceptibility, with an MIC of 64–128 µg/mL and an MBC of 128–256 µg/mL. This result agrees with the agar-diffusion assay, in which *B. subtilis* produced the largest inhibition zone (13 mm at 200 µg/mL). *Staphylococcus aureus* and *C. albicans* exhibited MIC values of 128 µg/mL and MBC/MFC values of 256 µg/mL. The MIC values for *E. coli* and *K. pneumoniae* ranged from 128 to 256 µg/mL, with corresponding MBC values of 256–512 µg/mL. *P. aeruginosa* showed the lowest susceptibility, with an MIC of 256 µg/mL and an MBC of 512 µg/mL.
The generally higher MBC/MFC values relative to MIC values indicate that concentrations required to produce complete bactericidal or fungicidal activity were greater than those required to inhibit visible microbial growth. The relatively greater susceptibility of *B. subtilis* compared with *P. aeruginosa* may reflect differences in cell-envelope structure, permeability, efflux activity, and other organism-specific resistance mechanisms. In particular, *P. aeruginosa* is characterized by low outer-membrane permeability and multiple intrinsic and acquired resistance mechanisms, which can reduce susceptibility to structurally diverse antimicrobial agents.
The observed activity is noteworthy in the context of current research on triazole-based antimicrobial agents. Recent studies continue to demonstrate that modification of the 1,2,4-triazole nucleus can produce compounds with activity against both bacterial and fungal pathogens, while structure–activity relationships remain highly dependent on the substituents introduced around the heterocyclic core (Sucman *et al.,* 2024).

Overall, Compound **AP** demonstrated concentration-dependent antimicrobial activity against all six tested microorganisms, with *B. subtilis* being the most susceptible and *P. aeruginosa* the least susceptible organism. The MIC/MBC data further support the antimicrobial potential of the compound, although its activity was moderate rather than exceptionally potent at the concentrations examined. Further investigation using standardized CLSI/EUCAST procedures, appropriate reference antimicrobial agents, replicate measurements, and statistical analysis would be necessary to establish the reproducibility and comparative potency of Compound **AP**.

Table 1. Antimicrobial activity of Compound AP expressed as inhibition-zone diameter (mm)

Microorganism	Type	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
Staphylococcus aureus	Gram-positive	6	8	10	12
Bacillus subtilis	Gram-positive	7	9	11	13
Escherichia coli	Gram-negative	6	7	9	11
Pseudomonas aeruginosa	Gram-negative	6	7	8	10
Klebsiella pneumoniae	Gram-negative	6	8	9	11
Candida albicans	Yeast	6	8	10	12

Table 2. MIC and MBC/MFC values of Compound AP

Microorganism	MIC (µg/mL)	MBC/MFC (µg/mL)
S. aureus	128	256
B. subtilis	64–128	128–256
E. coli	128–256	256–512
P. aeruginosa	256	512
K. pneumoniae	128–256	256–512
C. albicans	128	256

5. Structure–Activity Considerations

The antimicrobial findings suggest that the S-methyl group may contribute to the biological properties of the Synthesised Compound. S-alkylation converts the parent thiol into a thioether and consequently changes the electronic distribution, hydrogen-bonding capacity, and physicochemical characteristics of the molecule. The introduction of a methyl group at sulfur removes the thiol hydrogen and may increase hydrophobic character. Such a modification can influence the interaction of a small molecule with microbial membranes or intracellular targets. However, the present data do not establish a specific molecular mechanism of antimicrobial action.

The relationship between structural modification and biological activity is a major theme in current triazole research. Recent structure–activity relationship studies have demonstrated that substitution patterns on the triazole nucleus can substantially influence antimicrobial potency (Tian *et al.,* 2023; Hao *et al.,* 2024; Meng *et al.,* 2024; Pal, 2025).

Sulfur-containing 1,2,4-triazoles are particularly attractive because sulfur substitution offers a relatively straightforward route for modifying molecular structure. Ding *et al.* (2021) demonstrated that 1,2,4-triazole thioether derivatives can exhibit substantial antibacterial and antifungal effects, while Jiang *et al.* (2025) reported additional evidence supporting thioether-containing triazoles as promising antimicrobial scaffolds. Compound AP is considerably less structurally elaborate than many recently reported triazole hybrids. This may be advantageous from a synthetic-design perspective because a relatively small molecular framework provides a useful starting point for subsequent structure–activity relationship studies. Future work could investigate systematic variation of the sulfur substituent and assess whether increasing molecular complexity improves antimicrobial potency.

6. Comparison with Recent Literature

The results obtained in the present study are consistent with the continuing interest in 1,2,4-triazole chemistry as a source of biologically active molecules. Strzelecka and Świątek (2021) reviewed the antibacterial potential of 1,2,4-triazoles and highlighted the ability of this scaffold to accommodate structural modifications associated with antibacterial activity.

Ding *et al.* (2021) synthesized 52 novel 1,2,4-triazole thioether and related derivatives and demonstrated that selected compounds had significant antibacterial and antifungal activity. Their work is particularly relevant to the present study because it directly establishes thioether-functionalized 1,2,4-triazoles as an important class of antimicrobial compounds.

Karpun *et al.* (2021) similarly reported S-substituted bis-1,2,4-triazole-3-thiones with antimicrobial effects against several bacterial strains. Chen *et al.* (2021) also demonstrated that S-substitution of 1,2,4-triazole derivatives can produce compounds with biological activity against both fungal and bacterial pathogens.
More recent investigations have continued to support this trend. Tian *et al.* (2023) reviewed the structure–activity relationships of 1,2,3- and 1,2,4-triazole antimicrobial hybrids, emphasizing the importance of structural optimization in developing more effective antibacterial agents. Salma *et al.* (2024) further highlighted the broad biological relevance of triazole derivatives, while Pal (2025) reviewed recent synthetic approaches and antibacterial applications of 1,2,4-triazole derivatives.
The results of the Synthesised Compound therefore contribute to an established but still developing research area by demonstrating that a relatively simple S-methyl derivative possesses measurable activity against representative Gram-positive bacteria, Gram-negative bacteria, and *C. albicans*.

7. Conclusion

A novel methyl-substituted 1,2,4-triazole thioether, was prepared through S-alkylation of methyl-4H-1,2,4-triazole-3-thiol. The isolated compound exhibited a melting range of 112–114 °C and was characterized using FT-IR, GC-MS, ^{1}H NMR and ^{13}C NMR spectroscopy. These spectroscopic data were consistent with the proposed S-methyl structure. In particular, the ^{1}H NMR signal at δ 2.75 ppm and ^{13}C NMR resonance at δ 15.084 ppm provided evidence for the presence of the S–CH_3 group formed during alkylation. GC-MS further supported the proposed molecular structure through the molecular ion at m/z 129 and characteristic fragment ions.
the Synthesised Compound demonstrated concentration-dependent antimicrobial activity against all six tested microorganisms. *B. subtilis* was the most susceptible organism based on both inhibition-zone and MIC results, whereas *P. aeruginosa* showed the lowest susceptibility. The compound also exhibited activity against *C. albicans*, indicating potential antifungal activity.
Overall, the findings demonstrate that S-alkylation provides an effective structural modification of the 1,2,4-triazole-3-thiol framework and that the resulting thioether warrants further investigation. Future studies should include replicate experiments with statistical analysis, appropriate reference antimicrobial agents, standardized susceptibility-testing procedures, toxicity/cytotoxicity assessment, expanded microbial panels, and systematic modification of the sulfur substituent to establish a more reliable structure–activity relationship.

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References

Abdelli, A., Azzouni, S., Plais, R., Gaucher, A., Efrit, M. L., & Prim, D. (2021). Recent advances in the chemistry of 1,2,4-triazoles: Synthesis, reactivity and biological activities. *Tetrahedron Letters, 86*, 153518. https://doi.org/10.1016/j.tetlet.2021.153518

Amin, N. H., et al. (2021). Design, synthesis and mechanistic study of new 1,2,4-triazole derivatives as antimicrobial agents. *Bioorganic Chemistry, 111*, 104841. https://doi.org/10.1016/j.bioorg.2021.104841

Borne, A. L., Brulet, J. W., Yuan, K., & Hsu, K.-L. (2021). Development and biological applications of sulfur–triazole exchange (SuTEx) chemistry. *RSC Chemical Biology, 2*, 322–337. https://doi.org/10.1039/D0CB00180E

Chen, M., Lu, D., Zhang, X., Chen, M., Dong, C., Wang, X., Wu, W., Zhang, G., & Luo, H. (2021). Synthesis and biological activities of novel S-β-D-glucopyranoside derivatives of 1,2,4-triazole. *Phosphorus, Sulfur, and Silicon and the Related Elements, 196*(7), 679–684. https://doi.org/10.1080/10426507.2021.1901704

Clinical and Laboratory Standards Institute. (2024). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (12th ed.; CLSI standard M07). CLSI. (CLSI)

Clinical and Laboratory Standards Institute. (2024a). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (12th ed.; CLSI standard M07). CLSI.

Clinical and Laboratory Standards Institute. (2026). *Performance standards for antimicrobial susceptibility testing* (36th ed.; CLSI supplement M100). CLSI.

Deng, C., Yan, H., Wang, J., Liu, K., Liu, B.-S., & Shi, Y.-M. (2022). 1,2,3-Triazole-containing hybrids with potential antibacterial activity against ESKAPE pathogens. *European Journal of Medicinal*

Chemistry, 244, 114888. https://doi.org/10.1016/j.ejmech.2022.114888

Ding, M., Wan, S., Wu, N., Yan, Y., Li, J., & Bao, X. (2021). Synthesis, structural characterization, and antibacterial and antifungal activities of novel 1,2,4-triazole thioether and thiazolo[3,2-b]-1,2,4-triazole derivatives bearing the 6-fluoroquinazolinyl moiety. *Journal of Agricultural and Food Chemistry, 69*(50), 15084–15096. https://doi.org/10.1021/acs.jafc.1c02144

El-Hazek, R. M., Elkenawy, N. M., Zaher, N. H., & El-Gazzar, M. G. (2022). Green synthesis of novel antifungal 1,2,4-triazoles effective against γ-irradiated *Candida parapsilosis*. *Archiv der Pharmazie, 355*(1), e2100287. https://doi.org/10.1002/ardp.202100287

EUCAST. (2024). *MIC determination of non-fastidious and fastidious organisms*. European Committee on Antimicrobial Susceptibility Testing.

EUCAST. (2026). *Disk diffusion and quality control: Method manual and quality-control guidance*. European Committee on Antimicrobial Susceptibility Testing.

Ezeh, C. K., Eze, C. N., Dibua, M. E. U., & Emencheta, S. C. (2023). A meta-analysis on the prevalence of resistance of *Staphylococcus aureus* to different antibiotics in Nigeria. *Antimicrobial Resistance & Infection Control, 12*, 40. https://doi.org/10.1186/s13756-023-01243-x

GBD 2021 Antimicrobial Resistance Collaborators. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *The Lancet, 404*(10459), 1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1

Gupta, O., Pradhan, T., & Chawla, G. (2023). An updated review on diverse range of biological activities of 1,2,4-triazole derivatives: Insight into structure activity relationship. *Journal of Molecular Structure, 1274*, 134487. https://doi.org/10.1016/j.molstruc.2022.134487

Hao, Y., Feng, Y., Dong, Y., Ren, Y., Huang, J., Ma, H., Wang, C., Jin, K., Shang, D., & Zhang, X. (2024). Synthesis and antifungal properties of 1,2,4-triazole Schiff base agents based on a 3D-QSAR model. *Chemistry & Biodiversity, 21*(5), e202302064. https://doi.org/10.1002/cbdv.202302064

Jiang, Y., An, J., Xia, Y., Fei, Q., Wang, D., Lu, Y., & Wu, W. (2025). Design, synthesis, and antimicrobial evaluation of novel 1,2,4-triazole thioether derivatives with a 1,3,4-thiadiazole skeleton. *RSC Advances, 15*, 28084–28092. https://doi.org/10.1039/D5RA04574F

Kalchenko, V. V., & Shcherbyna, R. O. (2026). Modern approaches to the synthesis and biological screening of 1,2,4-triazole-3-thiol derivatives: Literature review. *Current Issues in Pharmacy and Medicine: Science and Practice, 19*(2), 184–194. https://doi.org/10.14739/2409-2932.2026.2.357016

Karpun, Y., Parchenko, V., Fotina, T., Demianenko, D., Fotin, A., Nahornyi, V., & Nahorna, N. (2021). The investigation of antimicrobial activity of some S-substituted bis-1,2,4-triazole-3-thiones. *Pharmacia, 68*(4), 797–804. https://doi.org/10.3897/pharmacia.68.e65761

Kazeminejad, Z., Marzi, M., Shiroudi, A., Kouhpayeh, S. A., Farjam, M., & Zarenezhad, E. (2022). Novel 1,2,4-triazoles as antifungal agents. *BioMed Research International, 2022*, 4584846. https://doi.org/10.1155/2022/4584846

Khomenko, K. V. (2024). Some issues of antimicrobial and antifungal activity of 1,2,4-triazole derivatives: Literature review. *Ukrainian Journal of Military Medicine, 1*(5), 124–. https://doi.org/10.46847/ujmm.2024.1(5)-124 (ujmm.org.ua)

Kumar, S., Misra, S. K., Kashaw, S. K., Kashaw, V., & Kumar, A. (2026). A comprehensive review on 1,2,4-triazole: Synthesis, properties and therapeutic potential. *Talanta, 305*, 129543. https://doi.org/10.1016/j.talanta.2026.129543

Kumari, M., Tahlan, S., Narasimhan, B., Ramasamy, K., Lim, S. M., Shah, S. A. A., Mani, V., & Kakkar, S. (2021). Synthesis and biological evaluation of heterocyclic 1,2,4-triazole scaffolds as promising pharmacological agents. *BMC Chemistry, 15*, 5. https://doi.org/10.1186/s13065-020-00717-y

Meng, K., Deng, T., Liu, M., Pu, H., Zhang, Y., Zou, H., Xing, Y., & Xue, W. (2024). Novel flavonoid derivatives containing 1,2,4-triazole Schiff bases as potential antifungal agents: Design, synthesis, and biological evaluation. *Bioorganic Chemistry, 153*, 107965. https://doi.org/10.1016/j.bioorg.2024.107965

Mosleh, A. A., & Dakhel, Z. A. (2024). Molecular docking, microwave-assisted synthesis, characterization, and preliminary evaluation of the anti-microbial activity of new sulfonamide derivatives of the 1,2,4-triazole-3-thiol ring system. *Pharmacia, 71*, 1–9. https://doi.org/10.3897/pharmacia.71.e132720

Ohloblina, M. V. (2022). Biological features of new 1,2,4-triazole derivatives: A literature review. *Current Issues in Pharmacy and Medicine: Science and Practice*. https://doi.org/10.14739/2409-2932.2022.1.252305

Pal, P. (2025). A comprehensive review on synthetic approaches and antibacterial activity studies of 1,2,4-

triazole derivatives over the past sixteen years (2009–present). *Current Pharmaceutical Design, 22*(7), 829–881. https://doi.org/10.2174/0115701794362320250418081311

Prytula, R. L., Shmatenko, O. P., Bushueva, I. V., Parchenko, V. V., Yerenko, O. K., & Drozdov, D. V. (2024). New 1,2,4-triazole derivatives as antifungal agents: Literature review. *Ukrainian Journal of Military Medicine*. https://doi.org/10.46847/ujmm.2024.1(5)-103

Rodrigues, S. C., Moraes, R. S. M., Pinto, G. T. A., Martins, M. T. M., Nascimento, P. A., Soares, D. L. A., Botelho, A. B. M., Cruz, C. C., & Cunha, A. C. (2025). A review on chemistry and methods of synthesis of 1,2,4-triazole derivatives. *The Chemical Record, 25*(1), e202400190. https://doi.org/10.1002/tcr.202400190

Sabzi, N. A. H., & Al-Mudhafar, M. M. J. (2023). Synthesis, characterization, and antimicrobial evaluation of new Schiff bases derived from vanillic acid conjugated to heterocyclic 4H-1,2,4-triazole-3-thiol. *Pharmacia, 70*(3), 657–663. https://doi.org/10.3897/pharmacia.70.e104579

Sadeghian, S., Emami, L., Mojaddami, A., Khabnadideh, S., Faghih, Z., Zomorodian, K., Rashidi, M., & Rezaei, Z. (2022). 1,2,4-Triazole derivatives as novel and potent antifungal agents: Design, synthesis and biological evaluation. *Journal of Molecular Structure, 1259*, 132039. https://doi.org/10.1016/j.molstruc.2022.134039

Salma, U., Ahmad, S., Alam, M. Z., & Khan, S. A. (2024). A review: Synthetic approaches and biological applications of triazole derivatives. *Journal of Molecular Structure, 1301*, 137240. https://doi.org/10.1016/j.molstruc.2023.137240

Sendra, E., Fernández-Muñoz, A., Zamorano, L., Oliver, A., Horcajada, J. P., Juan, C., & Gómez-Zorrilla, S. (2024). Impact of multidrug resistance on the virulence and fitness of *Pseudomonas aeruginosa*: A microbiological and clinical perspective. *Infection, 52*, 1235–1268. https://doi.org/10.1007/s15010-024-02313-x

Singh, A., Singh, K., Sharma, A., Joshi, K., Singh, B., Sharma, S., Batra, K., Kaur, K., Singh, D., Chadha, R., & Bedi, P. M. S. (2023). 1,2,3-Triazole derivatives as an emerging scaffold for antifungal drug development against *Candida albicans*: A comprehensive review. *Chemistry & Biodiversity, 20*(5), e202300024. https://doi.org/10.1002/cbdv.202300024

Strzelecka, M., & Świątek, P. (2021). 1,2,4-Triazoles as important antibacterial agents. *Pharmaceuticals, 14*(3), 224. https://doi.org/10.3390/ph14030224

Sucman, N., Stingaci, E., Lupascu, L., Smetanscaia, A., Valica, V., Uncu, L., Shova, S., Petrou, A., Glamočlija, J., Soković, M., Geronikaki, A., & Macaev, F. (2024). New 1H-1,2,4-triazolyl derivatives as antimicrobial agents. *Chemistry & Biodiversity, 21*, e202400316. https://doi.org/10.1002/cbdv.202400316 (Wiley Online Library)

Tian, G., Song, Q., Liu, Z., Guo, J., Cao, S., & Long, S. (2023). Recent advances in 1,2,3- and 1,2,4-triazole hybrids as antimicrobials and their SAR: A critical review. *European Journal of Medicinal Chemistry, 259*, 115603. https://doi.org/10.1016/j.ejmech.2023.115603

Wang, J., Shi, H., & Lu, A. (2024). Design, synthesis, and antifungal/anti-oomycete activities of novel 1,2,4-triazole derivatives containing carboxamide fragments. *Journal of Fungi, 10*(2), 160. https://doi.org/10.3390/jof10020160

World Health Organization. (2024a). *WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance*. WHO.

World Health Organization. (2024b). *Antimicrobial resistance*. WHO.

Yang, S., et al. (2025). Novel 1,2,4-triazole derivatives containing a benzenesulfonamide moiety as potential antifungal agents for controlling postharvest soft rot in kiwifruit. *Journal of Agricultural and Food Chemistry, 73*(39), 24549–24560. https://doi.org/10.1021/acs.jafc.5c02767

Zoidis, G., et al. (2021). The triazole ring as a privileged scaffold for putative antifungals: Synthesis and evaluation of a series of new analogues. *ChemMedChem, 16*(1), 134–144. https://doi.org/10.1002/cmdc.202000312