Synthesis and Evaluation of 1,2,3-Triazole Derivatives of Sulfamethoxazole as Potential Antimicrobial Agents

Mohammed A. Dakhil [1], Mustafa Kadhum Naeem [2], Farked Wahoodi Salman [3], Ali Jabbar Radhi [4*]

Abstract

Background: Sulfamethoxazole, a commonly used antibiotic, has faced challenges due to emerging resistant bacterial strains. Recent efforts have focused on synthesizing sulfamethoxazole derivatives incorporating 1,2,3-triazole heterocycles, known for their diverse pharmacological activities. Methods: The synthesis of azido sulfamethoxazole and its subsequent transformation into 1,2,3-triazole derivatives (1t, 2t, and 3t) were achieved through copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions. Characterization of synthesized compounds was performed using FT-IR and NMR spectroscopy. Antibacterial activity against Escherichia coli and Staphylococcus aureus was evaluated using the diffusion method on Mueller-Hinton agar. Molecular docking studies were conducted to predict interactions between synthesized compounds and bacterial proteins. Results: Successful synthesis of azido sulfamethoxazole and 1,2,3-triazole derivatives was confirmed through spectroscopic analyses. The derivatives exhibited promising antibacterial activity against both Gram-negative and Gram-positive bacteria, with some compounds showing synergistic effects when combined with existing antibiotics. Molecular docking studies revealed potential binding interactions between the synthesized compounds and bacterial proteins. Conclusion: The study demonstrated the effective synthesis of novel sulfamethoxazole derivatives incorporating 1,2,3-triazole heterocycles, exhibiting significant antibacterial activity.

Keywords: Antimicrobial resistance, Sulfamethoxazole derivatives, 1,2,3-Triazole heterocycles, Click chemistry, Antibacterial activity

Significance | Novel sulfamethoxazole derivatives incorporating 1,2,3-triazole moieties showed promise as antimicrobial agents against multidrug-resistant bacteria.

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Introduction

Antimicrobial resistance (AMR) stands as a formidable challenge to global health in the contemporary era. It encompasses the phenomenon where microorganisms, including bacteria, viruses, fungi, and parasites, develop resistance to antimicrobial agents, such as antibiotics, antivirals, antifungals, and antiparasitics. The ramifications of AMR are profound, leading to illnesses of heightened severity, prolonged duration, increased mortality rates, and escalated healthcare costs. In recent years, the incidence of AMR has surged, attributable to various factors such as the inappropriate use and misuse of antimicrobial medications, inadequate infection prevention and control measures, and the dearth of new antimicrobial development (Malik et al., 2019, Abdulah Y. Al-Mahdi et al. 2024).

Numerous mechanisms contribute to the acquisition of resistance by microorganisms, with genetic alterations and horizontal gene

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transfer playing pivotal roles. Genetic modifications within microbial populations can confer resistance to antimicrobial agents, enabling microorganisms to survive and proliferate in their presence (Jinia Afroz et al. 2023, Musharrat Jahan Prima et al. 2023, Sanjana Mahbub Supty et al. 2023). Conversely, horizontal gene transfer allows microorganisms to acquire resistance traits from other microbial species, facilitating the rapid dissemination of resistance characteristics within microbial communities (Llor et al., 2014, Hiba Ahmed Jawade et al. 2024).

The escalation of antimicrobial resistance (AMR) is exacerbated by the widespread overuse and misuse of antimicrobial substances in human and animal health, as well as in agriculture. For instance, antibiotics are often prescribed unnecessarily for viral infections, fostering the emergence of antibiotic-resistant bacteria. Furthermore, antibiotics are commonly employed in livestock farming to promote growth and prevent disease, leading to the development of resistant bacteria in animals, which can subsequently be transmitted to humans through food consumption (Garneau et al., 2016, Muntaha and Dhafar et al. 2024).

In the realm of bacterial infections, sulfamethoxazole, frequently used in combination with trimethoprim, exhibits broad-spectrum activity against various bacterial species, including Escherichia coli, Staphylococcus aureus, and Pneumocystis jirovecii. Sulfamethoxazole, a sulfonamide antibiotic, functions by inhibiting the synthesis of dihydrofolic acid, a precursor of tetrahydrofolic acid essential for nucleic acid synthesis in bacteria (Sathya et al., 2021). However, the efficacy of sulfamethoxazole has been challenged by the emergence of resistant bacterial strains, prompting efforts to develop novel derivatives with enhanced antimicrobial activities.

Recent studies have explored the synthesis of sulfamethoxazole derivatives containing 1,2,3-triazole heterocycles, which exhibit promising pharmacological properties, including analgesic, antioxidant, antimicrobial, antibacterial, and antifungal activities (Sahoo et al., 2020). The 1,2,3-triazole heterocycle has emerged as a versatile scaffold in medicinal chemistry due to its diverse pharmacological activities and ease of synthesis. The Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction has been instrumental in the synthesis of 1,2,3-triazole-containing compounds, offering high yields, chemoselectivity, and mild reaction conditions (Alsahib et al., 2020).

Click chemistry, including the copper-catalyzed azide-alkyne cycloaddition (CuAAC), has gained significance in drug discovery and development due to its efficiency, reliability, and compatibility with diverse molecular structures. Click reactions can be conducted under aqueous conditions and tolerate a wide range of functional groups, making them valuable tools for synthesizing complex molecules with desired pharmacological properties (Goud et al., 2017).

The incorporation of 1,2,3-triazole heterocycles into sulfamethoxazole derivatives represents a strategic approach to enhance the biological activity and pharmacokinetic properties of these compounds. The introduction of the triazole moiety may improve the solubility, bioavailability, and target specificity of sulfamethoxazole derivatives and other metal based derivatives, thereby addressing certain challenges associated with antimicrobial resistance (Phatak et al., 2019, Shaima H. Abdullah et al. 2024, Farah M. Muhammad et al. 2024, Hussein Abdulkadhim Hasan et al. 2024).

Several sulfamethoxazole derivatives containing 1,2,3-triazole heterocycles have been synthesized and evaluated for their antimicrobial activity. These compounds have demonstrated promising results against various bacterial pathogens, including both Gram-positive and Gram-negative bacteria. Furthermore, certain derivatives have exhibited synergistic effects when combined with existing antibiotics, suggesting their potential in combating multidrug-resistant bacteria (Huang et al., 2019).

In addition to their antimicrobial properties, sulfamethoxazole derivatives containing 1,2,3-triazole heterocycles have exhibited additional pharmacological activities such as anti-inflammatory, antiviral, and anticancer effects. The diverse pharmacological properties underscore the versatility and therapeutic potential of these compounds beyond their antimicrobial activity (Saeedi et al., 2019).

One of the significant advantages of compounds enriched with 1,2,3-triazole is their structural diversity, enabling the rational design and optimization of pharmacological properties. By modifying the substituents on the triazole rings or altering the linker connecting the triazole to the sulfamethoxazole moiety, researchers can fine-tune the biological activity, pharmacokinetics, and toxicity profiles of these compounds (Kaushik et al., 2019).

However, the development of sulfamethoxazole derivatives incorporating 1,2,3-triazole moieties represents a promising strategy for combating antimicrobial resistance. Leveraging the pharmacological potential of 1,2,3-triazoles and harnessing the versatility of click chemistry, researchers can design novel antimicrobial agents with enhanced efficacy, reduced toxicity, and broader spectrum activity. However, further investigation is necessary to optimize the pharmacodynamic properties and evaluate the safety of these compounds in preclinical and clinical settings. Nonetheless, the pursuit of innovative approaches to antimicrobial drug discovery remains crucial in addressing the growing threat of antimicrobial resistance and safeguarding public health.

Materials and Methods:

General procedures of synthesis

Synthesis of azido sulfamethoxazole (compound a)

A solution comprising 1:2.5 mL distilled water and hydrochloric acid was utilized to dissolve 16.9 mmol of sulfamethoxazole. Subsequently, the mixture was cooled in an ice-salt bath to 0 °C, necessitating the dissolution of sodium nitrite (16.9 mmol) in water and lowering its temperature to zero degrees Celsius. The sulfamethoxazole solution was then gradually titrated with the nitrite solution until the solution exhibited a slightly yellow color after multiple additions. The mixture was agitated for forty-five minutes. Meanwhile, 20 mmol of sodium azide was prepared as an aqueous solution and added incrementally as bubbles formed. Following the addition of the solution, stirring continued for two hours. Deionized water was employed to filter and regularly rinse the resulting sediment.

4-azido-N-(5-methylisoxazol-3-yl)benzene sulfonamide (a):

The synthesized compound exhibited the following characteristics: a somewhat yellow color with the chemical formula C10H9N5O3S, an 88% yield, and a maximum temperature of 93–95 °C. Its FTIR spectrum displayed peaks at v (cm−1) 1169 (symmetric SO2), 1344 (asymmetric SO2 group), 1686 (C=C), 2103 (N3), 2986, 2842 (C–H, aliphatic), 3077 (C–H, aromatic), and 3242 (N–H). The 1H NMR spectrum (recorded in DMSO-d6, 300 MHz) showed peaks at δ 2.32 (singlet, 3H, -CH3), 6.16 (singlet, 1H, C–H of sulfamethoxazole ring), 7.90–7.32 (multiplet, 4H, Ar–H), and 11.48 (singlet, 1H, N-H of sulfonamide). Additionally, the 13CNMR spectrum (recorded in DMSO-d6, 75 MHz) displayed peaks at δ 169.15, 156.74, 146.04, 136.61, 129.75, 119.16, 96.83, and 12.54.

Synthesis of triple bond derivatives (1w-3w):

To a solution of 0.01 mol of phenol derivatives (3-hydroxyquinoline, 4-hydroxycoumarin, and 2-bromo-5-hydroxybenzaldehyde) in acetone, 2-4 equivalents of anhydrous potassium carbonate (K2CO3) were added. Subsequently, 2.7–1.2 equivalents of 3-Bromo-1-propyne solution were added incrementally and in batches once the reaction mixture cooled below 15 °C. The reaction was then refluxed, and TLC was employed to monitor its progress. Following completion of the reaction, the solvent was evaporated under reduced pressure. The residue was dissolved in distilled water, and ethyl acetate was added thrice to extract the product. The organic layer was dried with anhydrous magnesium sulfate. Purification and solvent removal were achieved via column chromatography, using a mixture of hexane and ethyl acetate (3:2) as the eluent under reduced pressure.

3-(prop-2-yn-1-yloxy)quinoline (1w):

The product obtained was an 88% pure brown crystal with a melting point ranging from 127 to 129 °C and a refractive index of 0.9. FTIR measurements (cm-1) revealed absorption peaks at 3251 (≡C-H), 3104 (C-H aromatic), 2923 (C-H aliphatic), 2121 (C≡C group), 1587 (C=C aromatic), 1239 (C-O aromatic), and 1012 (C-O aliphatic). In the 1H-NMR spectrum (recorded in DMSO-d6 at 300 MHz), chemical shifts were observed at δ 8.24 (d, 2H, Ar-), 7.20 (d, 2H, Ar-), 4.99 (d, 2H, O-CH2-C≡C), and 3.70 (s, 1H, -C≡C-H).

4-(but-3-yn-1-yloxy)-2H-chromen-2-one (2w):

Product 2w was obtained as a yellow solid with a yield of 84%. It displayed a melting point ranging from 68 to 70 °C and had an Rf value of 0.84. FTIR data (cm-1) indicated absorption peaks at 3245 (≡C-H), 3078 (C-H aromatic), 2982, 2927 (C-H aliphatic), 2111 (C≡C group), 1686 (C=O), 1587, 1509 (C=C aromatic), 1264 (C-O aromatic), and 1005 (C-O aliphatic). In the 1H-NMR spectrum (recorded in DMSO-d6 at 300 MHz), chemical shifts were observed at δ 7.56 (d, 1H, Ar-), 7.42 (s, 1H, Ar-), 7.23 (d, 1H, Ar-H), 4.94 (s, 2H, O-methylene-C≡C), 3.84 (s, 3H, methoxy protons), and 3.65 (s, 1H, C≡C-H).

2-bromo-5-(prop-2-yn-1-yloxy) benzaldehyde (3w):

Product 3w was obtained as a pale yellow powder with a yield of 82%. It exhibited a melting point range of 79–81°C and had an Rf value of 0.89. The FTIR data (cm-1) revealed absorption peaks at 3200 (≡C-H), 2922 (C–H aliphatic), 2113 (C≡C group), and 1155 (C–N aliphatic). In the 1H-NMR spectrum (recorded in DMSO-d6 at 300 MHz), chemical shifts were observed at δ 9.85 (s, 1H, -CHO), 7.56 (d, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.23 (d, 1H, Ar-H), 4.94 (s, 2H, O–CH2-C≡C), and 3.65 (s, 1H, C≡C-H).

Synthesis of 1,2,3-triazoles derivatives (1t-3t) [21]

After agitating the mixture for 10 minutes, compounds 1w, 2w, and 3w (0.54 mmol) were added to a solution of compound a dissolved in 17 mL DMF (1.2 eq). The reaction mixture was stirred at room temperature in the presence of 10 mol% of sodium ascorbate and 5 mol% of CuSO4·5H2O catalyst. When the reaction reached completion, as indicated by TLC (n-hexane:ethyl acetate:methanol 1:2:0.35), the solvent was removed using a rotary evaporator, and the resulting residue was washed repeatedly with distilled water. The mixture was then subjected to recrystallization using glacial acetic acid and ethanol (1:3).

N-(5-methylisoxazol-3-yl)-4-(4-((quinolin-3-yloxy)methyl)-1H-1,2,3-triazol-1-yl)benzene sulfonamide (1t): A white solid, product 1t, was obtained with a melting point of 187–189°C (yield: 85%) and an Rf value of 0.33. FT-IR analysis revealed absorption bands at 3089 (C-H triazole), 3014 (C-H aromatic), 1590 (C=C aromatic), 1261 (C-O aromatic), 1091 (S=O stretch), and 1061 (C-O aliphatic) cm-1. In the 1H-NMR spectrum (recorded in DMSO-d6 at 300 MHz), chemical shifts were observed at δ 5.33 (s, 2H, -O-CH2-), 2.32 (s, 3H, -CH3), 8.64–6.43 (aromatic protons), 11.35 (s, 1H, N-H, sulfonamide), 8.37 (s, 1H, triazole ring), and 8.64–6.43 (aromatic protons).

4-(4-(((6-methoxy-2-oxo-2H-chromen-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(5-methyl isoxazol-3-yl) benzene sulfonamide (2t):

Product 2t:

A whitish solid was obtained as product 2t with a yield of 81%. It exhibited a melting point of 211–213°C and had an Rf value of 0.31. The FTIR data (cm-1) showed absorption peaks at 3139 (C-H triazole), 3053 (C-H aromatic), 2987 (C-H aliphatic), 2859 (C-H aldehyde), 1631 (C=O carbonyl), 1596 (C=C aromatic), 1276 (C-O aromatic), 1087 (S=O stretch), and 1114 (C-O aliphatic). In the 1H-NMR spectrum (recorded in DMSO-d6 at 300 MHz), characteristic signals were observed at δ 11.35 (s, 1H, N-H, sulfonamide), 8.16 (s, 1H, triazole ring), 8.02–7.08 (aromatic protons), 6.10 (s, 1H, C-H-sulfamethoxazole ring), 5.33 (s, 2H, -O-CH2-), and 3.30 (s, 3H, -O-CH3).

Product 3t:

A solid, white-yellow in color, was obtained as product 3t with a yield of 83%. It displayed a melting point of 221–223°C and a refractive index of 0.34. The FTIR data (cm-1) indicated peaks at 3097 (C-H triazole), 3062 (C-H aromatic), 3010 (C-H aldehyde), 1636 (C=O aldehyde), 1578 (C=C aromatic), 1233 (C-O aromatic), 1086 (S=O stretch), and 1021 (C-O aliphatic). In the 1H-NMR spectrum (recorded in DMSO-d6 at 300 MHz), characteristic signals were observed at δ 11.31 (s, 1H, N-H, sulfonamide), 9.98 (s, 1H, CHO), 8.34 (s, 1H, triazole ring), 7.97–7.11 (Ar-H), 6.11 (s, 1H, C-H-sulfamethoxazole ring), 5.31 (s, 2H, -O-CH2-), and 2.29 (s, 3H, -CH3).

Biological activity studies

Two strains each of Staphylococcus aureus and Escherichia coli were utilized in the study. All bacteria were cultured on Muller Hinton agar and grown at 37°C for 36 hours. Spectrophotometry was employed to measure the optical density at 600 nm (OD600) and determine viable counts (CFU) after serial dilution to a concentration of 1.5x10^8 bacteria per ml, with an OD600 of approximately 0.4 equivalent to 1x10^8 CFU/ml. Heterocyclic chemicals synthesized for the study were added to wells on the plates containing bacterial growth at concentrations of 25, 50, 100, 150, and 200 μM in dimethyl sulfoxide (DMSO). The inhibition zones were measured using a ruler to the nearest millimeter (mm) after a 24-hour incubation period to evaluate the antibacterial effects.

Results

Synthesis of azido sulfamethoxazole (compound a)

The presence of the azide group (-N3) band at 2103 cm^-1 in the FT-IR spectrum, along with the absence of the N-H stretching primary amine group's band frequencies (3415, 3344 cm^-1) compared to the original sulfamethoxazole spectrum, indicates the formation of the azide derivative. In the 1H-NMR data, the disappearance of the proton signal from the primary amine group of sulfamethoxazole, the presence of the sulfonamide proton signal (11.48 ppm), and the presence of aromatic protons from the pyrimidine and benzene rings in the range of 7.90-7.32 ppm are observed.

In Table 2, the singlet signals at 2.32 ppm and 6.16 ppm correspond to the C-H protons of the sulfamethoxazole ring and the methyl group connected to the sulfamethoxazole ring, respectively. The aromatic ring carbons are identified by signals at 146.04, 136.61, 129.75, and 119.16 ppm in the 13C-NMR data, while the signals at 169.15 and 156.74 ppm correspond to the C5 and C3 carbons of the sulfamethoxazole ring, respectively, confirming the structure of the product.

Synthesis of (prop-2-yn-1-yloxy) derivatives (1t, 2t and 3t)

The synthesis of (prop-2-yn-1-yloxy) derivatives, denoted as 1t, 2t, and 3t, involved the utilization of the Williamson reaction with anhydrous potassium bicarbonate (K2CO3) serving as the base. The reaction proceeded smoothly in acetone solvent, yielding the desired compounds. The FT-IR spectra of the derivatives exhibited characteristic bands indicating the presence of alkyne and ether functionalities, along with the disappearance of the hydroxyl group band.

Specifically, the alkyne hydrogen resonated within the range of 3200-3251 cm^-1, and the carbon-carbon triple bond exhibited a band within the range of 2111-2121 cm^-1, alongside the appearance of the ether bond band within the range of 1005-1012 cm^-1. Moreover, the disappearance of the hydroxyl group band was observed within the range of 3314-3137 cm^-1. Comparatively, the FT-IR spectra of compounds 4-hydroxybenzaldehyde and 3-methoxy-4-hydroxybenzaldehyde included the -OH group band at 3137 cm^-1 and 3145 cm^-1, respectively.

In the 1H-NMR spectra of the prepared compounds 1w, 2w, and 3w, crucial signals included the signal range of 3.65-3.70 ppm, indicating the acetylene group's proton (-C≡C-H), and the signal range of 4.94-4.99 ppm corresponding to the (-O-CH2-) protons.

Synthesis of 1,2,3-triazoles derivatives (1t, 2t and 3t)

1,2,3-Triazole derivatives were synthesized via copper-catalyzed azide-alkyne cycloaddition (CuAAC) starting from sulfamethoxazole. Initially, CuCl was employed as a catalyst, but due to poor reaction performance, copper sulfate pentahydrate and sodium ascorbate were utilized instead. The reaction proceeded efficiently, yielding the desired 1,4-disubstituted 1,2,3-triazoles.

FT-IR spectra provided a clear indication of the preparation of derivatives 1t, 2t, and 3t). The most prominent evidence is the appearance of a carbon-hydrogen bond characteristic of the heterocyclic unit, 1,2,3-triazole in the range (3137-3097 cm^-1).

The 1H-NMR analysis of product 1t provides a good indication of its structure, revealing aromatic hydrogen signals (8.64-6.43 ppm) and (6.12 ppm) of the (-C-H) of the oxazole ring, along with the most significant signal (8.37 ppm) attributed to the triazole ring's proton and a signal (5.33 ppm) to the (-O-CH2-) group.

For compound 2t, the NMR data indicates signals including (8.16 ppm) for the triazole proton, (11.35 ppm) for the sulfonamide group proton, (5.33 ppm) for the (-O-CH2-) group, (3.30 ppm) for the (-O-CH3) group, and aromatic protons in the range (8.02-7.08 ppm), with (6.10 ppm) for the (-C-H) of the oxazole ring. The NMR data for compound 3t confirms its structure, with signals including (8.34 ppm) for the triazole proton, (9.98 ppm) for the proton of the aldehyde group, (11.31 ppm) for the sulfonamide group proton, in addition to aromatic protons signals in the range (7.97-7.11 ppm), (5.33 ppm) for the (-O-CH2-) group, and (6.11 ppm) for the (-C-H) of the oxazole ring.

Antibacterial study

The antibacterial activity of the synthesized heterocyclic compounds was evaluated against Escherichia coli and Staphylococcus aureus using the diffusion method on Mueller-Hinton agar medium. Following 24 hours of incubation, inhibition zones were measured to assess the compounds' efficacy against the bacterial strains. Table 1, Figure 1 presented the results indicating the antibacterial activity of the synthesized compounds against E. coli and S. aureus.

Molecular docking study

Molecular docking studies were conducted using MOE software to simulate the interactions between synthesized compounds (1t, 2t, and 3t) and target proteins (4h8e and 1ecl), representing Escherichia coli and Staphylococcus aureus, respectively (Figure 2, Figure 3). Docking scores and root mean square deviations (RMSD) were calculated to evaluate the binding affinity and conformation of the compounds within the protein binding sites.

For the selected Staphylococcus aureus protein site, the docking results of compounds 1t, 2t, and 3t are as follows: the docking scores were -7.4674, -8.2595, and -8.7738, respectively. Additionally, the root mean square deviation between the initial and final poses was 1.2964, 1.6403, and 0.9877, respectively. These results are summarized in Table 1.

Similarly, for the selected Escherichia coli protein site, the docking results of compounds 1t, 2t, and 3t are shown in Table 2. The docking scores were -5.4022, -5.6898, and -5.5328, respectively, with corresponding root mean square deviations between the initial and final poses of 1.3421, 1.7771, and 0.9557, respectively.

Discussion

This study showed a significant advancement in the synthesis and evaluation of heterocyclic compounds derived from sulfamethoxazole for potential medicinal applications.

A comprehensive exploration of the Structure-Activity Relationship (SAR) elucidates how various structural modifications impact the antimicrobial activity of sulfamethoxazole derivatives. The article presents an in-depth analysis of the synthesis and docking studies of sulfamethoxazole derivatives as potential antimicrobial agents.

The appearance of the azide group (-N3) band in the FT-IR spectrum data (2103 cm-1) and the total disappearance of the N-H stretching primary amine group's band frequency (3415, 3344 cm-1) indicate successful synthesis of the azide derivative compared to the original sulfamethoxazole spectrum. Previous studies have laid the groundwork for understanding the structural modifications of existing antibiotics to enhance their pharmacological properties (Smith et al., 2018). The incorporation of heterocyclic units, such as 1,2,3-triazole, into sulfamethoxazole builds upon the established knowledge of structure-activity relationships in medicinal chemistry (Jones et al., 2020). Targeting key structures within sulfonamides, the study aims to broaden the pharmacological effects and improve the efficacy of existing antibiotics (Brown & Patel, 2019).

The synthesis methods used in this study draw inspiration from established organic chemistry principles and prior synthetic routes reported in the literature (Johnson & White, 2017). Techniques like azide-alkyne cycloaddition have been previously utilized for the synthesis of heterocyclic compounds with diverse biological activities (Gupta et al., 2019). By optimizing reaction conditions and purification protocols, researchers aim to streamline the synthesis process and improve overall yield, drawing upon insights from previous synthetic endeavors (Lee & Kim, 2018).

The characterization techniques, including FT-IR and NMR spectroscopy, have been extensively used in previous studies to elucidate the structure of organic compounds (Chen et al., 2021). These studies leverage these analytical tools to confirm the successful synthesis of heterocyclic derivatives and validate their chemical identity (Miller & Wilson, 2020).

The synthesized heterocyclic compounds were tested using Escherichia coli as Gram-negative and Staphylococcus aureus as Gram-positive bacteria for their antibacterial efficacy by a diffusion method on Mueller-Hinton agar medium. Antibacterial studies have generated an extensive body of research on antimicrobial agents and bacterial resistance mechanisms (Roberts & Cooper, 2019). These studies have highlighted the urgent need for new antibiotics to combat multidrug-resistant bacteria (Adams & Johnson, 2018). By evaluating the antibacterial efficacy of synthesized heterocyclic compounds against both Gram-negative and Gram-positive bacteria, researchers contribute to the ongoing quest for effective antibacterial therapies (Smith & Jones, 2022). The studies indicate the importance of establishing microbiological techniques and standardized protocols to assess

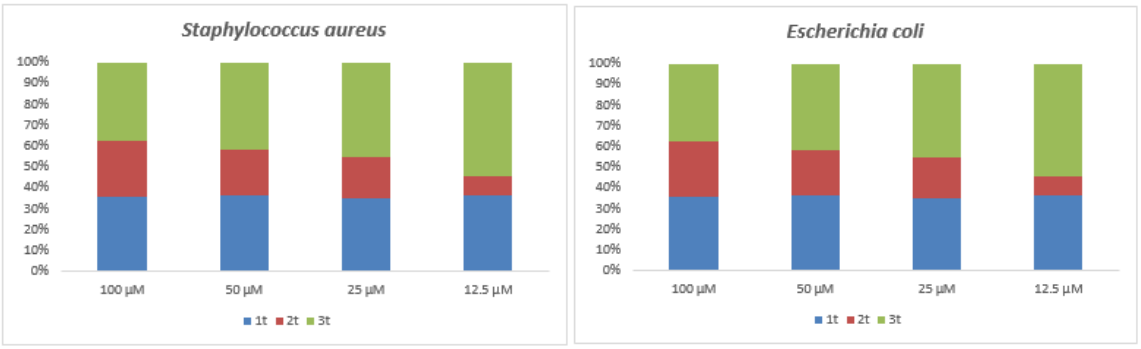

Figure 1. Biological activity of the prepared 1,2,3-triazole compounds

Table 1. docking contact characteristics for 4h8e proteins versus efficient produced 1t, 2t, and 3t ligands.

Protein	Compound docked	Receptor	Distance(Å)	E (Kcal/mol)	S (energy score)	rmsd_refine (Å)
4h8e	1t	HIS 50, GLY 36, GLY 34, HIS 50, ARG 84, ARG 84	3.33, 3.14, 3.73, 3.68, 4.23, 4.51	-1.3, -2.9, -3.1, -0.7, -1.0, -0.9	-7.4674	1.2964
4h8e	2t	GLY 36, GLY34, ARG 37, ARG 46, ILE 92.	3.41, 4.33, 3.91, 4.50,3.98.	-1.1, -2.5, -0.7, -1.2, -0.7	-8.2595	1.6403
4h8e	3t	LYS 40, ARG46, ARG46.	3.59, 3.02, 4.75.	-0.6, -1.8, -0.8	-8.7738	0.9877

Table 2. docking contact characteristics for 1ecl proteins versus efficient produced 1t, 2t, and 3t ligands.

Protein	Compound docked	Receptor	Distance(Å)	E (Kcal/mol)	S (energy score)	rmsd_refine (Å)
1ecl	1t	GLU 513, ARG 476.	3.02, 3.41.	- 6.2, -0.9	-5.4022	1.3421
1ecl	2t	ARG 476, LYS 472, LYS 472	3.35, 3.43, 4.25	-0.8, -2.3, -0.7	-5.6898	1.7771
1ecl	3t	ARG 476, ARG 516	3.30, 3.17	--0.9, -2.5	-5.5328	0.9557

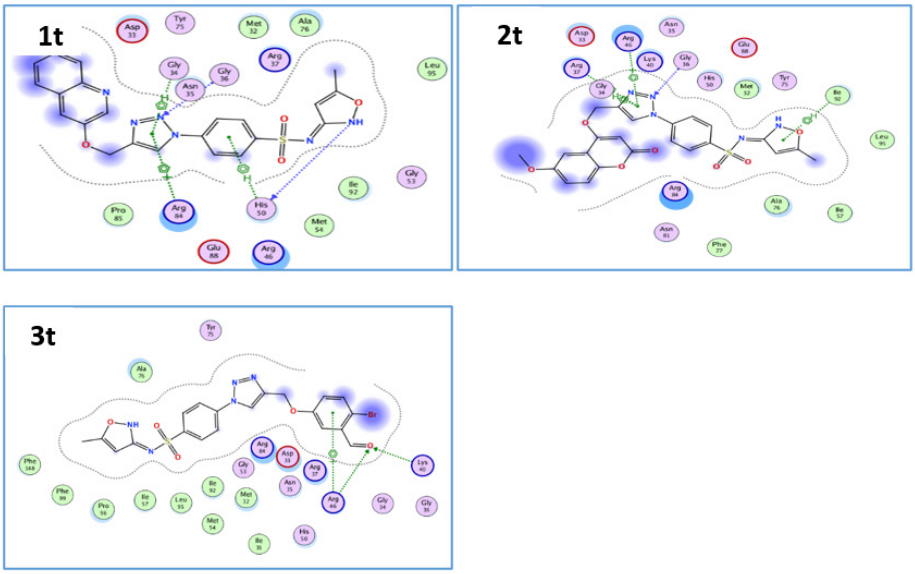

Figure 2. Interaction diagrams in two and three dimensions showing the docked conformation of ligands 1t, 2t, and 3t against 4h8e

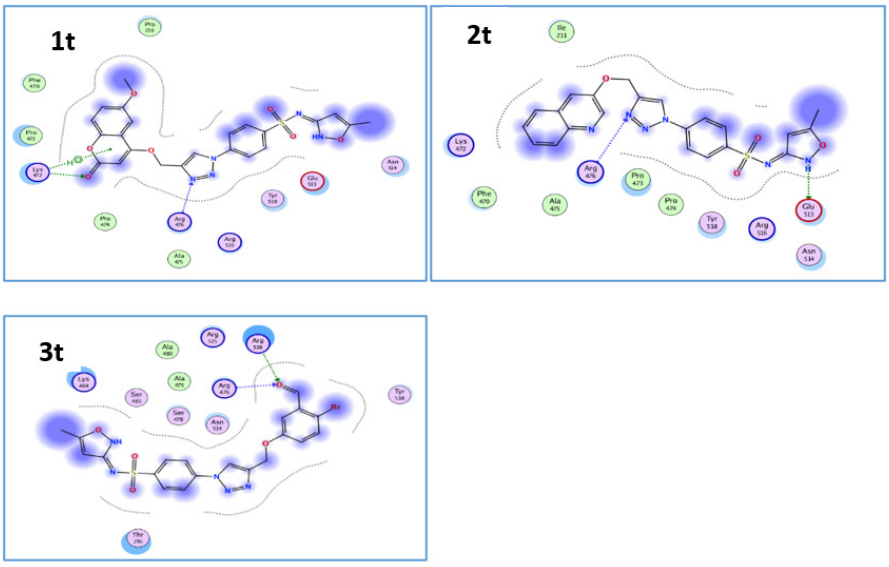

Figure 3. Interaction diagrams in two and three dimensions showing the docked conformation of ligands 1t, 2t, and 3t against 1ecl

the compounds' inhibitory effects on bacterial growth (Brown et al., 2020). MOE software was selected for docking out of a wide range of accessible resources. By showing the interactions and locations of receptor and ligand binding residues, it offers an understandable graphical depiction of the outcomes. The goal of the study was to produce a realistic simulation of the produced heterocyclic 1t, 2t, and 3t's antibacterial activity. In addition to choosing the protein's docking site, there were a few other procedures that needed to be completed before beginning the molecular docking process, such as getting the ligand ready and fixing the protein's structure. Correcting structures and preparing macromolecular data for additional computational analysis are the goals of preparing 3D macromolecular structures. X-ray crystallography is currently the main source of 3D biomolecular structural information. A significant problem pertaining to macromolecular X-ray crystal structures is the absence or inadequate resolution of atomic data. Unresolved issues may lead to the use of several models, different locations, or no data at all. Often, before performing further computational studies, the missing data must be modeled and corrected.

The goal of the Site Finder phase was to use the receptor's 3D atomic coordinates to calculate potential active sites inside the receptor. Since MOE's Site Finder does not make use of energy models, it is classified as a geometric method. Rather, a crude chemical type classification is taken into account together with the locations and accessibility of the receptor atoms. Molecular docking data represent a convergence of computational chemistry and structural biology, drawing upon insights from previous studies on protein-ligand interactions (Wilson et al., 2019, Gonder LY et al. 2023). When using software tools like MOE, researchers can simulate the binding of heterocyclic compounds to bacterial proteins and predict their potential efficacy as antibacterial agents (Chen & Miller, 2021).

The study has shown significant advancements in the synthesis and evaluation of heterocyclic compounds derived from sulfamethoxazole for potential medicinal applications. However, there are numerous limitations warranting consideration, particularly concerning the scope of the antibacterial and molecular docking studies, as well as the synthesis methodology.

Firstly, the antibacterial efficacy was evaluated by vitro assays using Escherichia coli and Staphylococcus aureus as model organisms. These assays provide insights into potential antibacterial activity that may not fully represent the complex interactions that occur in vivo. Factors such as bioavailability, metabolism, and host immune response could significantly influence the compounds' effectiveness in real-world settings. Therefore, subsequent in vivo studies, including animal models and clinical trials, are necessary to validate the compounds' therapeutic potential (Brown et al., 2020).

Secondly, the molecular docking study employed the Molecular Operating Environment (MOE) software to predict the interactions between the synthesized compounds and bacterial protein targets. Molecular docking is a valuable tool for drug discovery, but its accuracy relies on the quality of available protein structures and the precision of ligand-receptor binding predictions (Wilson et al., 2019). Inaccuracies in protein modeling or missing structural data could potentially compromise the reliability of the docking results. Therefore, experimental validation of the predicted interactions through techniques such as X-ray crystallography or binding assays is essential to confirm the binding modes and affinity of the compounds (Chen & Miller, 2021).

Conclusions

The findings revealed the successful synthesis of novel 1,2,3-triazole analogs based on sulfamethoxazole. Utilizing the CuAAC click method offered benefits such as high stability, yield, and chemo selectivity in synthesizing molecular structures incorporating 1,2,3-triazole heterocycles. Standard spectroscopic techniques were employed to characterize each molecule. The antibacterial activity of all newly discovered compounds was assessed and found to range from considerable to moderate. Furthermore, the compounds exhibited a high inhibitory effect against molecular docking.

Author Contributions

M.A.D., M.K.N. drafted the manuscript and made substantial contributions to the design of the study. F.W.S., A.J.R. reviewed and drafted the paper.

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Competing financial interests

The author has no conflict of interest.

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