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Research Article

Design, Synthesis, Antibacterial Evaluation, and Molecular Docking of Novel Benzotriazole–1,2,3-Triazole Hybrids

Jawad Kadhim Alshams [1], Radhiyah Abdulbaqi Aldujaili[2] and Ali Jabbar Radhi [1,3*]

[1] *College of Pharmacy, University of Alkafeel, Najaf, Iraq.*
[2] *Department of Chemistry, Faculty of Education for Girls, University of Kufa, Iraq.*
[3] *ARCPMS University of AlKafeel, Najaf, Iraq.*
[*] *Corresponding author: alijebar56@gmail.com*

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Abstract

Antimicrobial resistance (AMR) continues to be a major global health concern. This has led to the search for novel chemotherapeutic agents capable of acting on multidrug-resistant pathogens. A new series of benzotriazole–1,2,3-triazole hybrid derivatives (Bt1–Bt6) was synthesized through Cu(I)-catalyzed azide–alkyne cycloaddition using propargylated benzotriazole intermediate (2). Structural elucidation for all the synthesized compounds involved IR, $^{1}H/^{13}C$ NMR spectroscopic analysis which revealed characteristic signals for triazole and functional groups specific to each derivative. Their antibacterial activity was tested against Gram-positive Enterococcus faecalis (ATCC and clinical isolates) and Gram-negative Escherichia coli strains. Two compounds Bt5 and Bt6 showed strongest broad-spectrum activities with MIC values in the range between 0.24–1.24 µg/mL comparable to ciprofloxacin; they also demonstrated very high selectivity together with low cytotoxicity, CC_{50} =120–135 µg/mL on mammalian cells tested [89]. Compound Bt4 exhibited moderate activity while others were less active thus indicating great role played by incorporation sulfonamide heterocycles into molecule towards antimicrobial potency. Molecular docking research at the 7C7N protein expressed essentially binding affinities correlating with biological activity, Bt5 having the best docking score (-7.4426 kcal mol^{-1}) supported through several hydrogen bonds and π interactions with key residues Arg76, Ile78, and Pro79. An RMSD value of 0.1324 Å was obtained on redocking the native ligand to validate further that indeed this is a reliable protocol for use in docking exercises such as reported herein where two compounds labeled Bt5 and Bt6 are proposed as potential leads against bacteria due to their demonstrated strong activities besides exhibiting significant differences structurally while still maintaining good safety profiles thereby making them suitable candidates for further optimization along drug development pipelines.

1. Introduction

Antimicrobial resistance (AMR) develops to a state where present antibiotics can no longer effectively work against infections. AMR has become one of the most severe threats to public health because it accelerates infectious diseases that rapidly evolve into new forms [1]. Multidrug resistance (MDR) bacteria have recently added another massive burden on the healthcare system due to higher mortality associated with complications among MDR infection patients who stay long in hospitals, thus giving enough time for financial strain to set in [2]. Enterococcus faecalis is both a commensal bacterium found within human intestines and an opportunistic pathogen whose major

host comprises immunocompromised or aged individuals [3]. Pathogenesis by E.faecalis relies partly upon its ability first to colonize guts; after disrupting intestinal homeostasis, overgrowths travel through lymphatic systems into bloodstreams crossing supposed barriers [4]. It remains a major health threat and has become multi-drug resistant, therefore posing serious problems in medical therapy of hospital-acquired infections [5], while Escherichia coli (E. coli) happens to be among the most clinically significant Gram-negative bacteria responsible for a broad spectrum of hospital-acquired infections including urinary tract infection and pneumonia associated with ventilators [6]. As per a classification by the World Health Organization (WHO), E. coli is one crucial bacterial species resistant to most commercially available drugs [7]. Escherichia coli (E. coli) bacteria occupy the human gastrointestinal tract as versatile pathogens meanwhile Enterococcus faecalis (E .faecalis) is also described as an inhabitant GI facultative anaerobic Gram-positive coccus [8, 9]. Both, *E.coli*, and, *E.faecalis* are commensal pathogens both colonizing immediately after birth hence good representatives of their corresponding phyla. Technically, both can be easily isolated and cultured or maintained in the laboratory. They are regularly used as proposed human fecal indicators. Therefore, there is a need to develop alternative antimicrobial agents that will reduce the problem of drug resistance in bacteria. 1,2,3-Triazole is a heterocyclic five-membered ring containing three nitrogens and two carbons. 1,2,3-Triazole has provided medicinal chemists with inspiration for many years due to its synthetic ease and varied inhibitory activities [10–12]. It can be prepared by Huisgen 1,3-dipolar cycloaddition between azides and alkynes; copper-catalyzed click reaction [13–16]. Our literature survey found pharmacological effects of 1,2 ,3-triazole very attractive and promising towards designing antibacterial agents . Specifically, wide range moieties conjugated with 1,2,3 -triazoles were reported possessing strong antibacterial properties. In this study, the problem of antibiotic resistance was addressed by designing and synthesizing new benzotriazole–triazole hybrid compounds and assessing their antibacterial activity. Bt5 and Bt6 were found as the best molecules because they showed high activities with low toxicities. Molecular docking on protein 7C7N revealed its strong binding toward important amino acids which supports its biological activity observed experimentally. The study proposes them as potential candidates to fight against resistant bacteria due to chemical synthesis followed by biological evaluation supported through computation.

2. Experimental Section

Commercial suppliers (Sigma-Aldrich, Merck) provided all reagents and solvents used without further purification unless otherwise specified. The progress of the reaction was monitored by thin-layer chromatography on silica gel plates (60 F254) visualized under UV light at 254 nm. Purification was performed using silica gel (60–120 mesh) column chromatography. The melting points were determined in a digital apparatus and are uncorrected. A Bruker spectrophotometer recorded the FT-IR spectra using KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument operating at 400 MHz using DMSO-d_6. Chemical shifts (δ) are reported in ppm downfield from TMS, internal standard. Mass spectra (ESI-MS) were recorded on a Thermo Scientific mass spectrometer.

2.1. General Procedure for the Synthesis of Compound (2)

A solution of 1-(hydroxymethyl)-1H-benzo[d][1,2,3]triazole (1.0 mmol) in 15 mL dry acetone was prepared to which anhydrous K_2CO_3 (2.0-2.5 mmol) was added and the mixture was stirred for 15 min at room temperature. Propargyl bromide (1.2 mmol) was then added dropwise and the reaction mixture refluxed for 6 hr with constant stirring while monitoring by TLC until completion after which it is allowed to cool; filtered inorganic salts removed by filtration evaporated under reduced pressure until dryness gives a crude product that is purified over silica gel column chromatography using hexane/ethyl acetate (3:1) as eluent giving compound (2).

1-((prop-2-yn-1-yloxy)methyl)-1H-benzo[d][1,2,3]triazole (2): Yield: 79% ; pale-yellow solid, mp 98-99 °C; IR (cm^{-1}), 3274 ($\equiv C$–H stretch of terminal alkyne ($C \equiv C$–H), 3141 (C-H of triazole ring, 3088 (aromatic C–H stretching (benzotriazole/aromatic rings), 2122 ($-C \equiv C$ stretch, alkyne), 1587 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81-7.43(m, 4H, Ar-H), 5.89 (s, 2H, CH_2 attached to triazole), 4.21 (s, 2H, CH_2 linked to benzotriazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.74, 135.03, 127.63, 124.24, 119.72, 110.33, (6C, of aromatic carbons), 80.36 (1C, $C \equiv$), 75.47 (1C, $\equiv C$–H),73.91 (1C, CH_2 linked to benzotriazole), 57.59 (1C, CH_2 attached to terminal alkyne).

2.2. General procedure for the synthesis of 1,2,3-triazole derivatives (Bt1–Bt6)

A solution of compound 2 (1.00 mmol) and the corresponding organic azide (R–N_3, 1.10–1.50 mmol) in a 3:1 mixture of ethanol and H_2O (15 mL) was prepared in a round-bottom flask fitted with a magnetic stir bar. Copper (II) sulfate pentahydrate ($CuSO_4$ $5H_2O$, 0.05 mmol, 5 mol%) and sodium ascorbate (0.20 mmol, 20 mol%) were added to the reaction mixture which was then heated at 70–75°C under vigorous stirring. The course of the reaction was followed by TLC (hexane/EtOAc). After 5–6 h or on complete consumption of alkyne whichever is earlier, it is allowed to attain room temperature naturally and diluted with water (20 mL). It is extracted using ethyl acetate (3×20 mL). The combined organic layers are washed with brine followed by drying over anhydrous Na_2SO_4 and filtered. The crude residue was either passed through a short pad of SiO_2 or washed with 0.05 M EDTA solution to remove residual copper before evaporation of the solvent under reduced pressure. The crude product was then purified by column chromatography on silica gel (hexane/ethyl acetate) to afford the desired triazole (Bt1–Bt6).

1-(((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-1H-benzo[d][1,2,3]triazole (Bt1):71% ; pale-yellow solid, mp 187-189 °C; IR (cm^{-1}), 3134 (C-H of triazole ring), 3043 (aromatic C–H stretching, aromatic rings), 1547 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H, triazole-H), 7.78-7.24 (m, 8H, Ar-H), 5.85 (s, 2H, CH_2 attached to triazole), 4.70 (s, 2H, CH_2 linked to benzotriazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.14,118.89 (2C, C4 and C5 of triazole ring), 144.82, 135.11, 134.31, 133.58, 127.51, 124.32, 122.68, 121.61, 117.61, 110.14 (12C, of aromatic carbons), 73.35 (1C, CH_2 attached to triazole), 58.47 (1C, CH_2 linked to benzotriazole).

4-(4-(((1H-benzo[d](1,2,3-triazol-1-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid (Bt2): 74% ; White solid, mp 145-147 °C; IR (cm^{-1}), 3378 (COOH), 3154 (C-H of triazole ring), 3081 (aromatic C–H stretching, aromatic rings), 1734 (carbonyl of carboxylic group), 1591 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H, COOH), (8.28 (s, 1H, triazole-H), 7.87-7.29 (m, 8H, Ar-H), 5.88 (s,

2H, CH_2 attached to triazole), 4.73 (s, 2H, CH_2 linked to benzotriazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.14 (1C, (1C, COOH), 149.49, 118.54 (2C, C4 and C5 of triazole ring), 144.81, 137.97, 135.01, 130.47, 127.49, 125.23, 124.25, 120.83, 116.93, 110.19, (12C, of aromatic carbons), 73.65 (1C, CH_2 attached to triazole), 58.97 (1C, CH_2 linked to benzotriazole).

4-(4-(((1H-benzo[d](1,2,3-triazol-1-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)phenol (Bt3): 70% ; pale-yellow solid, mp 166-168 °C; IR (cm^{-1}), 3324 (O–H stretch of phenol), 3152 (C-H of triazole ring), 3049 (aromatic C–H stretching, aromatic rings), 1578 (aromatic C=C); ^1H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H, phenol-H), 8.33 (s, 1H, triazole-H), 7.71-7.20 (m, 8H, Ar-H), 5.82 (s, 2H, CH_2 attached to triazole), 4.75 (s, 2H, CH_2 linked to benzotriazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.87, 118.97 (2C, C4 and C5 of triazole ring), 157.55, 144.99, 135.02, 128.34, 127.53, 124.34, 120.67, 119.58, 117.00, 110.23, (12C, of aromatic carbons), 73.41 (1C, CH_2 attached to triazole), 58.69 (1C, CH_2 linked to benzotriazole).

4-(4-(((1H-benzo[d](1,2,3-triazol-1-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)benzaldehyde (Bt4): 73% ; White solid, mp 174-176 °C; IR (cm^{-1}), 3135 (C–H stretch of terminal aldehyde), 3166 (C-H of triazole ring), 3064 (aromatic C–H stretching (aromatic rings), 1715 (carbonyl of aldehyde), 1577 (aromatic C=C); ^1H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H, benzaldehyde-H), 8.22 (s, 1H, triazole-H), 7.79-7.25 (m, 8H, Ar-H), 5.84 (s, 2H, CH_2 attached to triazole), 4.76 (s, 2H, CH_2 linked to benzotriazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.57 (1C, benzaldehyde-C), 149.68, 118.87 (2C, C4 and C5 of triazole ring), 144.94, 138.09, 135.02, 134.49, 129.92, 127.49, 124.34, 121.74, 119.44, 110.23, (12C, of aromatic carbons), 73.46 (1C, CH_2 attached to triazole), 58.98 (1C, CH_2 linked to benzotriazole).

4-(4-(((1H-benzo[d](1,2,3-triazol-1-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (Bt5): 67%; Off-white solid, mp 241-243 °C; IR (cm^{-1}), 3274 (N–H stretch of sulfonamide), 3165 (C-H of triazole ring), 3095 (aromatic C–H stretching, aromatic rings), 1595 (aromatic C=C); ^1H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H, N-H), 8.31 (s, 1H, triazole-H), 7.97-7.04 (m, 11H, Ar-H), 5.87 (s, 2H, CH_2 attached to triazole), 4.73 (s, 2H, CH_2 linked to benzotriazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 143.47, 118.97 (2C, C4 and C5 of triazole ring), 157.54, 156.49, 144.74, 139.24, 137.45, 135.27, 129.51, 127.47, 124.23, 121.75, 117.69, 112.76, 110.74, (15 C, of aromatic carbons), 73.46 (1C, CH_2 attached to triazole), 58.98 (1C, CH_2 linked to benzotriazole).

4-(4-(((1H-benzo[d](1,2,3-triazol-1-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (Bt6): 69%; pale-white solid, mp 241-243 °C; IR (cm^{-1}), 3263 (N–H stretch of sulfonamide), 3114 (C-H of triazole ring), 3072 (aromatic C–H stretching, aromatic rings), 1579 (aromatic C=C); ^1H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H, N-H), 8.36 (s, 1H, triazole-H), 7.91-7.18 (m, 13H, Ar-H), 6.11 (s, 1H, CH, isoxazole), 5.83 (s, 2H, CH_2 attached to triazole), 4.70 (s, 2H, CH_2 linked to benzotriazole), 2.31 (s, 3H, CH_3 attached to isoxazole),; ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.24, 156.54, 96.87 (3C, C5, C3 and C4 of isoxazole ring), 142.98, 118.91 (2C, C4 and C5 of triazole ring), 144.87, 139.68, 137.79, 135.28, 129.65, 127.87, 124.29, 121.75, 120.55, 112.27, (10C, of aromatic carbons), 73.78 (1C, CH_2 attached to triazole), 58.61 (1C, CH_2 linked to benzotriazole), 13.14 (1C, CH_3 linked to isoxazole).

2.3. Biological assays

The strains of bacteria used for the in vitro antibacterial studies included E. faecalis (ATCC 29212), a clinical isolate of E. faecalis, E. coli (ATCC 25922), and a clinical isolate of E. coli. The stock cultures were maintained at the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. All ATCC and clinical strains were obtained from American Type Culture Collection (ATCC). Before screening, fresh microbial broth cultures were prepared in normal saline. Ciprofloxacin was used as reference drug to determine antibacterial activity. Minimum inhibitory concentration (MIC) was determined by micro-dilution method using serial dilutions (10fold) of each compound38. In a microtiter plate, the different concentrations of compounds were serially diluted. In every tube/well of the microtiter plate10μL standardized inoculums (1-2$\times$107cfu/mL) was added. The plates were incubated for 24 h at 37 °C under aerobic conditions. The minimum inhibitory concentration (MIC) was defined as the lowest concentrations of the compounds at which they showed no visible sign of bacterial growth compared to the wells containing the control and no turbidity in the solution.

3. Results Discussion

Compound (2), 1-((prop-2-yn-1-yloxy)methyl)-1H-benzo[d][1,2,3]triazole was synthesized via a nucleophilic substitution reaction between 1-(hydroxymethyl)-1H-benzo[d][1,2,3]triazole and propargyl bromide in the presence of anhydrous potassium carbonate. Dry acetone and mild basic conditions were chosen for the easy deprotonation of the hydroxyl group to form alkoxide which would undergo an SN2 reaction with propargyl bromide to give the desired propargyl ether Figure 1. The IR spectrum showed that the etherification was successful together with alkyne moiety present as shown by sharp bands at 3274 cm^{-1} ($\equiv C$–H stretching) and 2122 cm^{-1} ($C \equiv C$ stretch). Aromatic, triazole C–H stretching bands appeared between 3088–3141 cm^{-1}. The formation of compound (2) is further evidenced by its ^1H NMR spectrum showing singlet peaks due to two methylene groups attached respectively on triazole ring and benzotriazole core observed at δ values 5 .89 and 4 .21ppm, The ^{13}C NMR spectrum appears in good agreement with the proposed structure, especially signals at δ 80.36 and 75.47 ppm which are characteristic of terminal alkyne carbons [14]. The synthesized alkyne intermediate (2) was a key precursor for Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction to give a series of 1,4-disubstituted triazole derivatives (Bt1–Bt6). Click reaction in ethanol/water medium under $CuSO_4$ $5H_2O$/sodium ascorbate catalytic system efficiently generates Cu(I) species in situ allowed the regioselective [15, 16] formation of 1,4-substituted 1,2,3-triazoles. TLC monitoring revealed very efficient cycloaddition giving yields in the range 67–74% with different azide substrates showing suitability of reaction conditions toward a wide range of azide substrates.

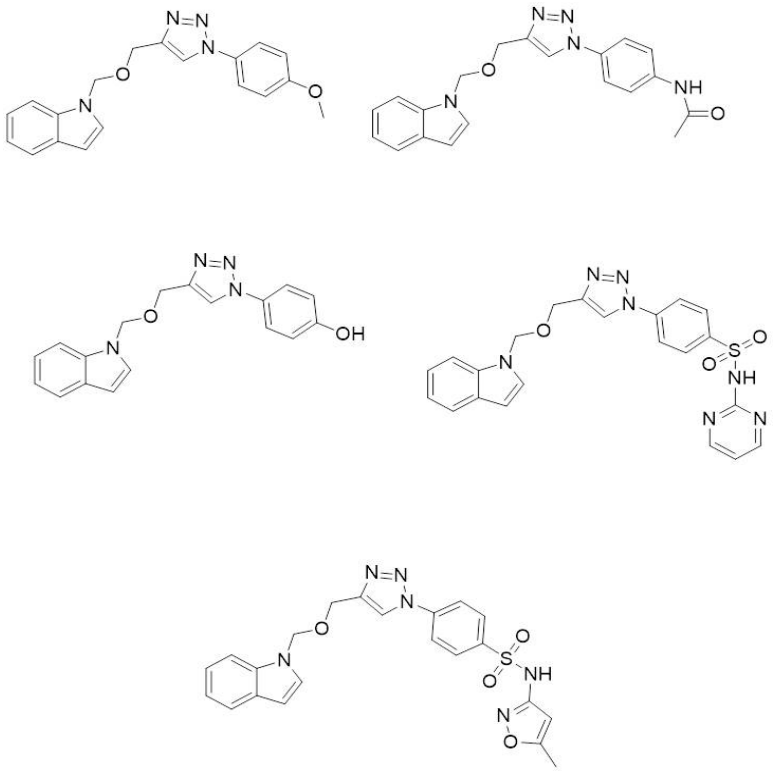

Figure 1: Synthesis of 1,2,3-triazole derivatives (Bt1-Bt6)

The spectral data of the derivatives confirmed the cycloaddition. A triazole C-H singlet was observed at δ 8.2-8.3 ppm in the ^{1}H NMR spectra of all the compounds along with aromatic multiplets due to benzotriazole and aryl/heteroaryl substituents. The methylene bridges between benzo-triazole and triazole rings appeared as singlets at about δ 5.82-5.88 ppm and 4.70-4.76 ppm, respectively. In their IR spectra, disappearance of alkyne stretches (3274 and 2122 cm^{-1}) together with presence both triazole as well aromatic C-H bands proved full cycloaddition. Each derivative showed also diagnostic functional-group absorptions coherent its structure: COOH stretch Bt2 (3378 & 1734), phenolic O–H band Bt3(3324), aldehydic C=O Bt4(1715). For sulfonamide containing derivatives (Bt5 & Bt6) strong N–H stretches around 3263–3274 together additional aromatic signals confirm successful incorporation sulfonamide moiety. The NMR spectra further revealed differences in the structures of the various derivatives. For example, Bt6 displayed extra signals at δ 6.11 and 2.31 ppm attributable respectively to the isoxazole proton and its methyl substituent consistent with heterocyclic substitution. In all cases, the ^{13}C NMR spectra showed triazole carbons around δ 118–149 ppm together with aromatic carbon distributions agreeing with proposed structures. Unique carbonyl, heteroaryl or substituent specific signals provided additional evidence for each compound's identity. IR and NMR data clearly indicated successful synthesis not only of propargyl benzotriazole intermediate but also its subsequent conversion into a diverse library of 1,4-disubstituted 1,2,3-triazole derivatives via CuAAC as reflected by high yields accompanied by clean spectral profiles plus stability observed for obtained compounds thereby demonstrating this synthetic approach to be highly efficient besides being versatile in generating multifunctional benzotriazole–triazole hybrid molecules.

3.1. Antibacterial Activity

Antibacterial results of benzotriazole–triazole hybrids (Bt1–Bt6) on both Gram-positive and Gram-negative strains showed different activity spectra which were strongly correlated to the nature of substituent attached to triazole moiety, as shown in Table 1. Most compounds displayed broad-spectrum antibacterial potential with minimum inhibitory concentration (MIC) values obtained against some strains comparable with or even lower than the reference drug ciprofloxacin.

Table 1: Antibacterial activity (MIC µg mL^{-1}) and cytotoxicity (mg mL^{-1}) of compounds (Bt1-Bt6)

Com. no.	Gram + ve strains		Gram - ve strains		CC_{50} value (µg mL^{-1})
	E. faecalis (ATCC29212)	E. faecalis (Clinical isolate)	E. coli (ATCC25922)	E. coli (Clinical isolate)	
Bt1	12.25	51	18.24	5.87	45
Bt2	18.57	50	31.24	15.58	70
Bt3	8.98	28.36	1.85	8.57	95
Bt4	3.14	5.57	2.35	3.24	60
Bt5	0.49	7.24	0.24	1.04	120
Bt6	0.57	11.20	0.47	1.24	135
Ciprofloxacin	0.781	12.5	0.12	0.781	nd
Chloroquine	nd	nd	nd	nd	>100

Bt5 and Bt6 exhibited the most potent antibacterial activity among the series, particularly against Gram-negative E. coli (ATCC 25922 and clinical isolate). Bt5 was highly potent with MIC values of 0.24 µg/mL and 1.04 µg/mL, while Bt6 recorded similarly low MIC values of 0.47 µg/mL and 1.24 µg/mL, respectively. These are very close to ciprofloxacin (0.12–0.78 µg/mL) thus indicating that the introduction of sulfonamide heterocycles (pyrimidine in Bt5 and isoxazole in Bt6) has a major effect on antimicrobial potency [165]. Their activities against Gram-positive *E. faecalis* strains followed the same trend with Bt5 (0.49 µg /m L) being the most active derivative [166]. The results show that sulfonamide functionality leads to better interaction with bacterial enzymatic targets presumably by way of hydrogen bonding or due to increased polarity. Bt4 showed moderate activity with MIC values ranging from 2.35 to 5.57 µg/mL, thus emphasizing that the presence of an aldehyde group increases its electron-withdrawing character and makes the antibacterial action better than Bt1-Bt3. Compounds Bt1, Bt2, and Bt3 manifested much weaker antimicrobial effects, particularly against the E. faecalis clinical isolate wherein MIC values were recorded at 28-50 µg/mL; this clearly indicates that very simple phenyl or carboxylic acid or phenolic substituents provide insufficient enhancement to antibacterial activity.

3.2. Cytotoxicity Studies

CC_{50} values indicated that most of the compounds were not significantly toxic, mainly Bt5 (120 µg/mL) and Bt6 (135 µg/mL), with maximum CC_{50} values recorded for the series Figure. 2. This result is quite indicative of a wide safety margin and good selectivity toward bacterial cells relative to mammalian cells. Bt3 also exhibited a high CC_{50} (95 µg/mL); Bt1, Bt2, and Bt4 displayed moderate cytotoxicity (45-70 µg/mL). Interestingly, the two most active antibacterial compounds were also found to be least toxic thus exhibiting an excellent therapeutic index. On comparing synthesized derivatives with reference antibiotics; ciprofloxacin still remained more potent but only slightly so when compared with either Bt5 or Bt6. Chloroquine showed very weak antibacterial activity hence was used primarily as a reference for cytotoxicity. A clear SAR tendency is revealed by the combined results: heterocyclic sulfonamide substituents markedly enhance antibacterial activity while maintaining low toxicity, whereas simpler electron-donating or neutral substituents result in weaker activity. Bt5 and Bt6 can be considered the best antibacterial candidates of this series because they are not only highly effective-and their effects cover a broad spectrum-but also safe to a high degree. Further development and fine-tuning studies may thus follow based on these two compounds.

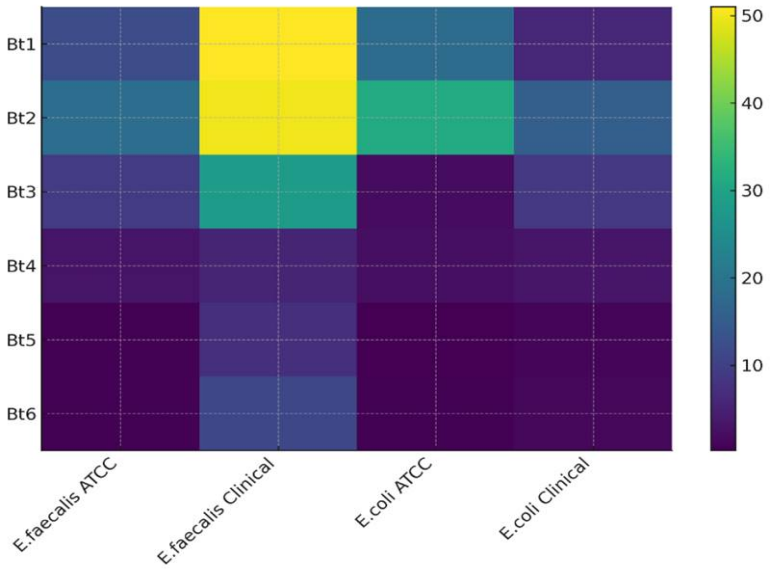

Figure 2: Heatmap of Bt1–Bt6 Bioactivity Against Clinical and ATCC Strains of E. faecalis and E. coli

3.3. Validation of Docking Protocol

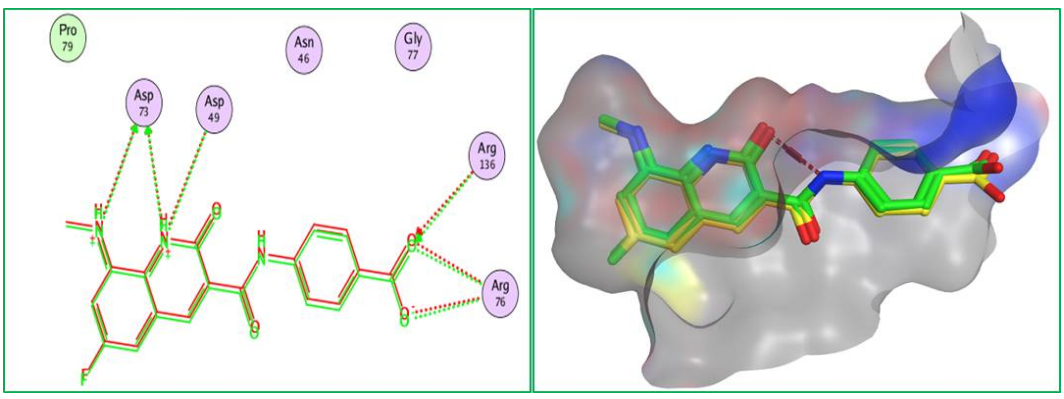

Figure 3: Validation of the Docking Protocol for Protein 7C7N Using the Native Ligand (RMSD = 0.1324 Å

Figure 3 shows the result from redocking its native ligand that achieved an RMSD of only 0.1324 Å to validate the docking protocol for protein 7C7N. This small value between crystallographic pose(green) and redocked pose(red) means binding mode observed experimentally can be reproduced by docking procedure accurately as shown in Figure 3. The conserved interactions with important active-site residues such as Asp49, Asp73, Pro79, Asn46, Gly77, Arg76 and Arg136 suggest reliability on setup used for this work because they are hydrogen bonds plus electrostatic contacts which scoring function seems physiochemically well defined inside 7C7N active site so low RMSD fully preserving critical interactions makes methodology robust hence validated support subsequent designed compound dockings.

3.4. Molecular Docking Studies

Binding affinities and interactions of Bt1–Bt6 ligands show different behaviors because their structures are somewhat different, as seen from results scores on the 7C7N protein. Bt5 recorded the best or lowest docking score among all ligands at –7.4426 kcal mol^{-1}; hence, it can be said to have the highest binding affinity with a high number of hydrogen bond donor-acceptor interactions involving Arg76 together with π–H contacts involving Ile78 and Pro79 that stabilize its orientation within the binding pocket. The other two compounds which also gave good docking scores were Bt2 (–7.3436) & Bt1 (–7.1984); both formed important stabilizing hydrogen bonds Asp73/Val71 (involved in π–H interaction)/Ile78. Bt3, Bt4, and Bt6 recorded slightly weaker binding energies relative to the best ligands but they made relevant interactions with important residues in the active site. Bt3 and Bt4 made more contacts with Asp49 and Asp73 which means that acidic residues are very instrumental in holding these ligands as shown in Figures 6 and 7. With a moderate docking score of -6.8270 kcal mol^{-1}, Bt6 formed the most varied interaction profile comprising hydrogen bond acceptor and donor interactions with Arg76, Arg136, Met95 together with a strong π–cation interaction with Arg76 Figure 9.

Table 2: Molecular docking scores and binding interactions of ligands (Bt1-Bt6) against (PDB: 7C7N)

Ligand	RMSD (Å)	Dock Score (E, kcal·mol^{-1})	Interaction type	ΔE (kcal·mol^{-1}) per interaction	Distance (Å)	Residue / Partner
Bt1	1.9964	-7.1984	H-B-D	-1.7	3.40	VAL 71
			pi-H	-0.7	3.70	ILE 78
			pi-H	-0.6	4.52	ILE 78
Bt2	0.9776	-7.3436	H-B-D	-1.0	3.25	ASP 73
			H-B-D	-0.7	3.20	VAL 71
Bt3	1.4186	-6.8305	H-B-D	3.26	-0.8	ASP 49
Bt4	1.2893	-6.9847	H-B-D	3.18	-0.7	ASP 73
			pi-H	4.28	-0.9	ILE 78
			pi-H	3.68	-1.5	PRO 79
Bt5	1.9269	-7.4426	H-B-D	2.96	-2.4	ARG 76
			H-B-A	3.09	-2.9	ARG 76
			pi-H	4.20	-1.4	ILE 78
			pi-H	3.58	-0.7	PRO 79
Bt6	1.9124	-6.827	H-B-D	4.07	-0.3	MET 95
			H-B-A	3.31	-1.4	ARG 136
			H-B-A	3.34	-0.9	ARG 136
			H-B-A	3.01	-1.1	ARG 76
			pi-cation	3.63	-1.8	ARG 76
			pi-H	3.78	-1.3	ILE 78

Note: Legend: H-B-D = hydrogen bond donor; H-B-A = hydrogen bond acceptor

The interactions show a wider binding footprint that could be responsible for its moderate yet stable docking score. The key binding residues highlighted in the ligand interaction profiles are Arg76, Ile78, Pro79 and also Asp73 plus Val71. Hydrogen bonds as well as hydrophobic or π interactions seem important for strong binding of a ligand to this protein wherein Bt5 is indicated by its network synergy effect among interactions formed within it into being most likely candidate binder towards 7C7N protein target used here. Ligands occupy about same region inside active site but oriented differently according structural features (see two/ three-dimensional interaction diagrams; Figures 4-9) thus Bt-series ligands could bind effectively against seven proteins meanwhile guiding future optimization based on structure towards drug design.

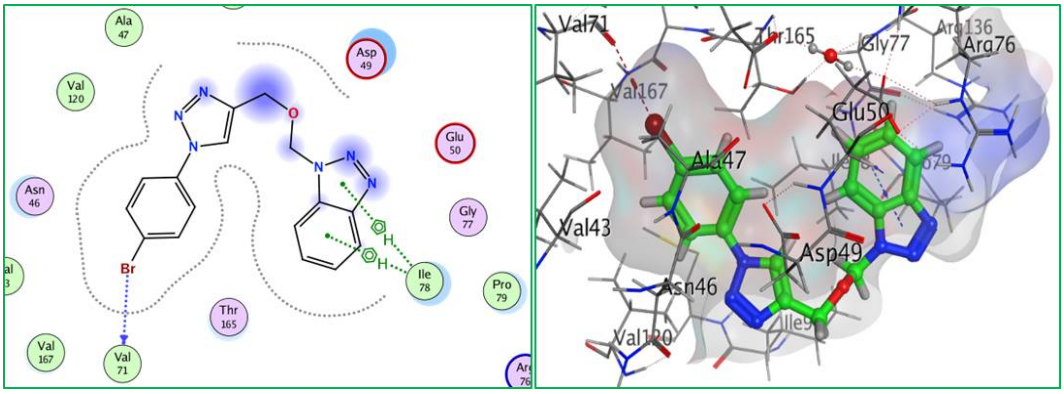

Figure 4: 2D and 3D interaction ligand (Bt1) with active sit of (7C7N)

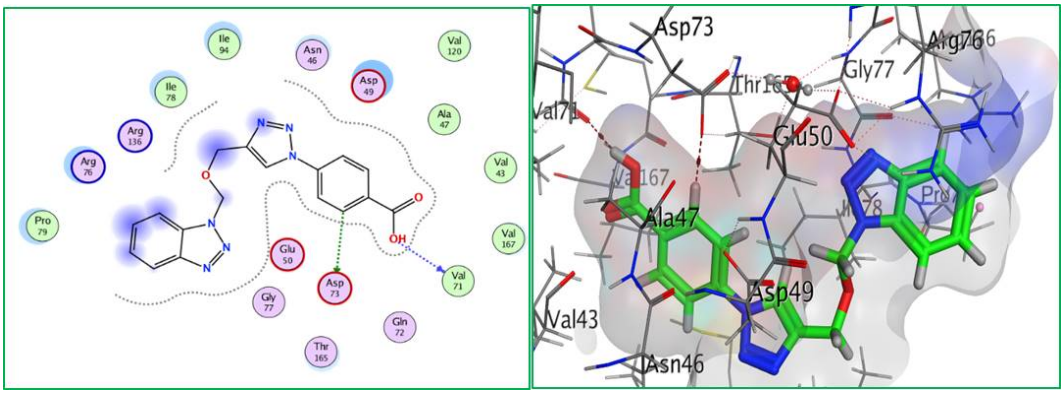

Figure 5: 2D and 3D interaction ligand (Bt2) with active sit of (7C7N)

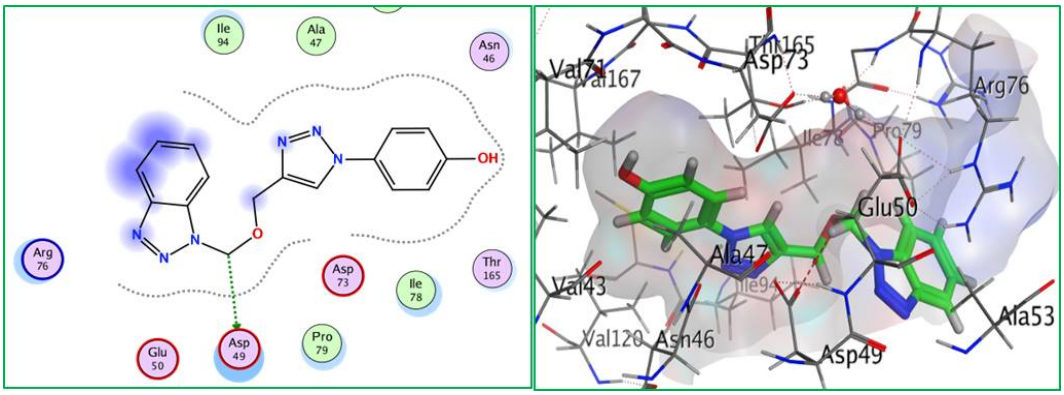

Figure 6: 2D and 3D interaction ligand (Bt3) with active sit of (7C7N)

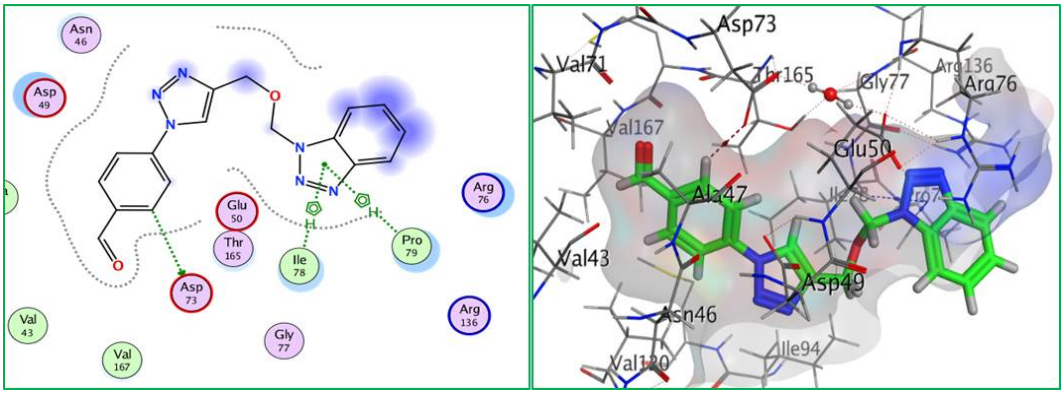

Figure 7: 2D and 3D interaction ligand (Bt4) with active sit of (7C7N)

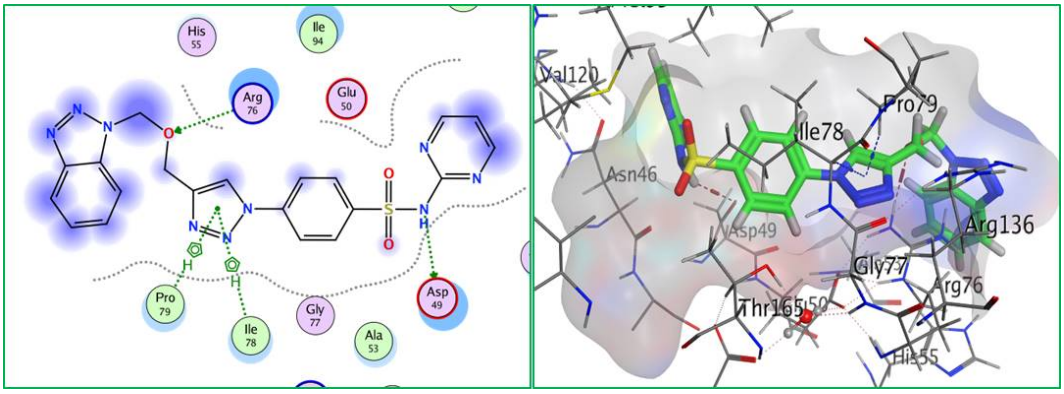

Figure 8: 2D and 3D interaction ligand (Bt5) with active sit of (7C7N)

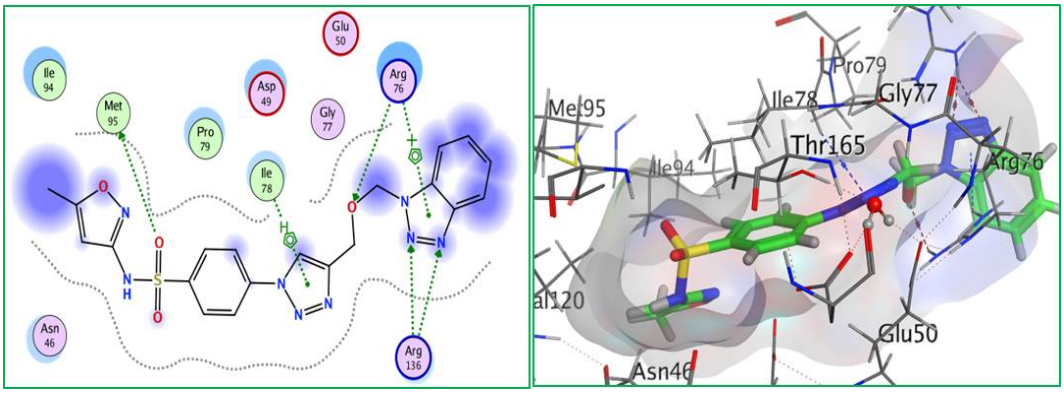

Figure 9: 2D and 3D interaction ligand (Bt6) with active sit of (7C7N)

4. Conclusion

A novel series of benzotriazole–1,2,3-triazole hybrid derivatives (Bt1–Bt6) was synthesized by Cu(I)-catalyzed azide-alkyne cycloaddition reaction. The structures were elucidated using different spectroscopic techniques. Antibacterial results showed that molecular modifications at the triazole moiety assumed an important role in its biological activity; Bt5 and Bt6 exhibited minimum and broadest antibacterial activities among the synthesized compounds with MIC values against most bacteria comparable to ciprofloxacin, a standard drug, besides excellent selectivity manifested through low cytotoxicity hence indicating high antimicrobial effect brought about by sulfonamide linked heterocycles. Molecular docking study supported antibacterial experimental results since two compounds Bt5 & Bt6 had good binding scores against 7C7N protein target where compound Bt5 was found having best docking score forming many stable interactions with important active site residues. The docking protocol was validated by redocking of the native ligand, giving a very low RMSD value (0.1324 Å). This supports the reliability of this computational approach. In summary, benzotriazole–triazole hybrid compounds Bt5 and Bt6 can be considered promising lead scaffolds for developing new antibacterial agents to act against multidrug-resistant pathogens discovered through studies reported herein. Further optimization on these compounds as well as detailed mechanism study is warranted before pushing them towards preclinical evaluation.

Article Information

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

Competing Interests: Authors have declared that no competing interests exist.

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