Original Article

Design, Synthesis and Molecular Docking Study for New Heterocyclic Derivatives

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Abstract

Due to the evolution of resistance to many traditional antibacterial medications, bacterial infections pose a severe threat to healthcare providers. As a result, there are unmet medical needs for new bacterial targets and antimicrobials. Antimicrobial activity of triazole and tetrazole derivatives is well recognized. The activity spectrum of a variety of novel triazole and tetrazole derivatives against two types of microorganisms Gram-positive bacterial strains and Gram-negative bacterial strains was examined in this work. The most active compounds were 12t and 13t, with minimum inhibitory doses ranging from 1.12 to 1.43 g/mL. According to the results of molecular modeling, heterocyclic derivatives offer a lot of potential. The docking scores for all heterocyclic compounds ranged from 5.48 to 7.02 kcal/mol. The docking scores for the 1,2,3-triazole derivatives (10t and 11t) ligands against (1ecl) protein were 6.51 and 5.45 kcal/mol, respectively, which were less optimum than the binding energies predicted for tetrazole derivatives (12t and 13t) (6.91 and 7.02 kcal/mol). As a result of our findings, these triazole and tetrazole compounds could be antibacterial medication candidates with significant against the two types that was studies.

Keywords: 1,2,3-Triazole, Tetrazole, PDB, MOE, Docking, Antibacterial.

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INTRODUCTION

Bacterial infection treatment has recently become a major therapeutic problem all around the world. In spite of effective antibiotics medications are still found for the best common infections, the advent of multidrug-resistant bacteria and the distribution of novel infectious diseases pose a very danger to the efficacy of the medications presently appropriate for infectious disease treatment [1,2]. Antibacterial compound research against types of bacteria is in a state of flux. While there are still treatment options for drug-resistant of two kinds of microorganism infections tend to be more difficult to control because Gram-negative microorganism have a less penetrable cell wall than Gram-positive kind [3]. The lack of innovation in the development of new antibiotics has increased the difficulty of eradicating and treating assured infections, resulting in a 60 percent drop in the number of new antibiotics medication agreements return to a deficiency of confirmed of new organic molecules with the looked-for activities [4]. Despite the fact that this tendency is shifting, novel antibacterial medicines with divergent and distinct structural properties as well as modes of act that vary from those of existing antibacterial agents are still urgently needed [5]. The organic molecules that contain hetero atoms are frequently employed in medical chemistry and show an essential part in the pharmaceutical industry's hunt for new bioactive molecules. Because they are found in numerous synthetic molecules and natural products with known biological activity, nitrogen-containing heterocycles appear to have a large range of bioactivities [6-9]. In latest years, 1,2,3-triazoles have gotten a lot of interest in drug development because of their exclusive structural properties in terms of hydrogen bonding and metabolic stability, which is a good thing for binding bimolecular targets and can also increase solubility [10,11]. Furthermore, using a 1,3-dipolar cycloaddition Click chemistry technique, these structural motifs were easily synthesized [12-14]. The exclusive structural characteristics of 1,2,3-triazole heterocycles derivatives, as well as their bioactivities value, inspired us to develop a pure 1,4-disubsituted-heterocyclic compounds that contain ring with antibacterial activity via a Cu(I) catalyzed azide-alkyne click chemistry reaction (Cu-AAC) [15-17]. 1D-NMR spectrum and FTIR spectroscopy data, were used to determine the compound structures. The growth inhibition zone was measured in (mm) for antibacterial activity.

Material and Methods

Fluka, Sigma-Aldrich) and commercial source provided all reagents and compounds, which were used without additional purification. The spots were observed under a UV lamp after TLC was done on silica gel (60 F254, Silica gel, Fluka). The melting points were rectified and recorded on a Gallenkamp equipment. The University of Kufa's Faculty of Science used a Bruker ALPHA FT-IR to measure infrared spectra. DMSO-d6 solutions was used to record NMR data with a Bruker spectrometer (300MHz for ^{1}H-NMR and 75MHz for ^{13}C-NMR).

Synthesis of propargyle derivatives (1,2)

2 equivalents of anhydrous potassium carbonate K_2CO_3 were added to (0.01 mole) of (6-methoxybenzothiazol-2-amine, benzothiazol-2-amine) dissolved in acetone, and cooling the reaction below 10ºC, 2.7 equivalent of 3-bromo-1-propyne solution was added in small quantities and in batches, after which the reaction was left under refluxed, and the reaction progress was examined using TLC.. The residue of end product is dissolved in distilled water, then ethyl acetate is added twice for extraction. The organic phase was dried with anhydrous $MgSO_4$. Under lower pressure, the solvent is removed, and the product is purified by colum chromatography (2:3) with a mixture of ethyl acetate and hexane as the eluent [18,19].

5-methoxy-*N*-(prop-2-yn-1-yl)benzothiazol-2-amine (1): m.p. 212–214 ℃; yield, 84%; FTIR, *v* (cm^{-1}): 3412 (NH str.), 3292(-C≡CH str.), 3074 (Ar-H),2965, 2848(C-H, sym., asym.), 2132(C≡C str.), 1627 (C=N ring str.), 1592 (C=C ring str.), 1345 (C–N str.), 1041, 1182 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 7.59-7.23 (m, Ar-H, 3H), 6.63 (s, $NH_{exchangeable,}$ 1H), 4.64 (*t*, *J* = 3.1 Hz, 2H), 3.82 (s, OCH_3, 3H), 2.49 (s, C≡CH, 1H); ^{13}C NMR δ (ppm): 169.85($C_{2\ thiazole\ ring}$), 154.65, 146.57, 128.42, 122.51, 113.28, 106.14(6$C_{aromatic\ ring}$), 79.86(C≡CH), 71.87(≡CH), 55.22(OCH_3),34.33(N-CH_2).

***N*-(prop-2-yn-1-yl)benzothiazol-2-amine(2):** m.p. 244–246 ℃; yield, 81%; FTIR, *v* (cm^{-1}): 3387 (NH str.), 3284(-C≡CH str.), 3067 (Ar-H), 2968, 2857(C-H, sym., asym.), 2124(C≡C str.), 1633 (C=N ring str.), 1589 (C=C ring str.), 1357 (C–N str.), 1051, 1177 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 7.49-7.09 (m, Ar-H, 4H), 6.67 (s, $NH_{exchangeable,}$ 1H), 4.59 (*t*, *J* = 3.1 Hz, 2H), 2.47 (s, C≡CH, 1H); ^{13}C NMR δ (ppm): 169.04($C_{2\ thiazole\ ring}$),149.47, 131.22, 127.29, 122.84, 121.27, 120.67, 81.19, 73.11, 33.47(N-CH_2).

Synthesis acetamide derivatives (3,4 and 5)

(0.01 mole) chloroacetylchloride was added dropwise to a cold solution of (0.01 mole) (5-methoxybenzothiazol-2-amine, benzothiazol-2-amine, and morpholine) with (0.01 mole) trietheylamine in dry DMF (20 mL) and refluxed for 7 hours. The organic solvent was removed, and the final product was produced and purified via crystallization from mixture of water and ethanol.

2-chloro-*N*-(5-methoxybenzothiazol-2-yl)acetamide (3): m.p. 174–176 ℃; yield, 78%; FTIR, *v* (cm^{-1}): 3357 (NH str.), 3060 (Ar-H),2947, 2884 (C-H, sym., asym.), 1678 (C=O str.), 1611 (C=N ring str.), 1585 (C=C ring str.), 1352 (C–N str.), 1055, 1191 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 9.98 (s, $NH_{exchangeable,}$ 1H), 7.57-7.27 (m, Ar-H, 3H), 4.39 (s, 2H, -CH_2), 3.82 (s, OCH_3, 3H), ^{13}C NMR δ (ppm): 170.44 (C=O),161.52($C_{2\ thiazole\ ring}$),151.85, 147.47, 131.47, 126.21, 116.23, 110.48 (6$C_{aromatic\ ring}$), 54.76(OCH_3),44.82(-CH_2).

***N*-(benzothiazol-2-yl)-2-chloroacetamide (4):** m.p.189–191 ℃; yield, 83%; FTIR, *v* (cm^{-1}): 3374 (NH str.), 3088 (Ar-H), 2984, 2864(C-H, sym., asym.), 1685 (C=O), 1618 (C=N ring str.), 1584 (C=C ring str.), 1347 (C–N str.), 1048, 1175 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 9.95 (s, $NH_{exchangeable}$, 1H), 7.45-7.14 (m, Ar-H, 4H), 4.39 (s, 2H, -CH_2); ^{13}C NMR δ (ppm): 169.98(C=O), ($C_{2\ thiazole\ ring}$),154.38, 147.87,131.87, 126.79, 122.24, 121.08, 119.27, (6$C_{aromatic\ ring}$), 43.41(-CH_2).

2-chloro-1-morpholinoethan-1-one (5): yield, 78%; FTIR, *v* (cm^{-1}): 2989, 2882(C-H, sym., asym.), 1671 (C=O), 1331 (C–N str.); ^{1}H NMR δ (ppm): 4.39 (s, 2H, -CH_2), 3.72 (s, 4H, 2$CH_{2\ morpholin}$), 3.38 (s, 4H, 2$CH_{2\ morpholin}$)**.**

Synthesis of nitrile derivatives (6)

The (2-chloro-1-morpholinoethan-1-one (5) was dissolved in 10 mL of DMSO (14.5mmol), and then 1.2 equivalents of NaCN was added to the reaction mixture of the derivative and the solvent. After completion of the reaction (observed by TLC, n-hexane:ethylacetate 3:1.5), work-up was done by extraction using petroleum ether, the organic phase was removed, dried with (MgSO4), and the solvent was removed with a rotary evaporator [20].

3-morpholino-3-oxopropanenitrile (6): yield, 78%; FTIR, *v* (cm^{-1}): 2965, 2870(C-H, sym., asym.), 2196(C≡N), 1668 (C=O), 1341 (C–N str.); ^{1}H NMR (300 MHz, DMSO-d*6*) δ (ppm): 3.89 (s, 2H, -CH_2), 3.72 (s, 4H, 2CH_2), 3.41 (s, 4H, 2CH_2)**.**

Synthesis of azide derivatives (7,8 and 9)

[2-chloro-N-(5-methoxybenzothiazol-2-yl) acetamide (3), N-(benzothiazol-2-yl)-2-chloroacetamide (4), and 2-chloro-1-morpholinoethan-1-one (5)] were added to 15 mL of DMSO (12 mmole) and then 1.2 equivalent of NaN_3 was added to the reaction mixture of the derivative and the solvent. After end of the reaction was determined by TLC, n-hexane:ethylacetate 3:1.5), the organic solvent phase was removed, dried with (MgSO4), and the solvent was evaporated using a rotary evaporator.

2- azido-*N*-(5-methoxybenzothiazol-2-yl)acetamide (7): m.p. 207–209 ℃; yield, 71%; FTIR, *v* (cm^{-1}): 3360 (NH str.), 3051 (Ar-H),2945, 2862 (C-H, sym., asym.),2115(N_3), 1674 (C=O str.), 1615 (C=N ring str.), 1580 (C=C ring str.), 1338 (C–N str.), 1046, 1188 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 10.02 (s, $NH_{exchangeable,}$ 1H), 7.54-7.23 (m, Ar-H, 3H),

3.85 (s, OCH_3, 3H), 3.27(s, 2H, -CH_2); ^{13}C NMR δ (ppm): 169.24 (C=O),160.80($C_{2\ thiazole\ ring}$),156.47, 148.28, 127.78, 122.79, 113.52, 101.26($6C_{aromatic\ ring}$), 55.22(OCH_3), 47.89(-CH_2).

2-azido-*N*-(benzothiazol-2-yl)acetamide (8): m.p.176–178 ℃; yield, 74%; FTIR, *ν* (cm^{-1}): 3392 (NH str.), 3065 (Ar-H), 2957, 2867(C-H, sym., asym.), 2118(N_3), 1675 (C=O), 1625 (C=N ring str.), 1590 (C=C ring str.), 1351 (C–N str.), sym., asym.); ^{1}H NMR δ (ppm): 10.03 (s, $NH_{exchangeable}$, 1H), 7.44-7.21 (m, Ar-H, 4H), 3.21 (s, 2H, -CH_2); ^{13}C NMR δ (ppm): 169.57(C=O), ($C_{2\ thiazole\ ring}$),156.18, 147.75,132.57, 127.77, 124.76, 122.14, 118.48 ($6C_{aromatic\ ring}$), 48.12(-CH_2).

2-azido-1-morpholinoethan-1-one (9): yield, 72%; FTIR, *ν* (cm^{-1}): 2974, 2845 (C-H, sym., asym.), 2122(N_3), 1674 (C=O), 1344 (C–N str.); ^{1}H NMR δ (ppm): 3.67 (s, 4H, 2CH_2), 3.39 (s, 4H, 2CH_2)**,** 3.21 (s, 2H, -CH_2).

Synthesis of 1,2,3-triazoles derivatives (10t and 11t) [17]

(1.2eq) propargyl derivatives (1 and 2) to (0.5mmol) 2-azido-1-morpholinoethan-1-one (9) dissolved in 17 mL DMF, after 10 minutes of stirring, added (5mol percent) CuSO4.5H2O and (10mol percent) sodium ascorbate, after which the reaction mixture was left to stir at laboratory temperature, the end of reaction was determined using TLC, ethylacetate:n-hexane (1:3).

2-(4-(((5-methoxybenzo[d]thiazol-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)-1-morpholinoethan-1-one (10t): m.p. 159–161 ℃; yield, 82%; FTIR, *ν* (cm^{-1}): 3344 (NH str.), 3122($CH_{triazole}$), 3082 (Ar-H),2945, 2865(C-H, sym., asym.), 1661 (C=O ring str.), 1633 (C=N ring str.), 1585 (C=C ring str.), 1355 (C–N str.), 1038, 1178 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 7.81(s, triazole-H, 1H), 7.51-7.21 (m, Ar-H, 3H), 5.32 (s,CO-CH_2, 2H), 4.89 (s,NH-CH_2, 2H), 3.82 (s, OCH_3, 3H), 3.61 (s, 4H, 2CH_2), 3.48 (s, 4H, 2CH_2)**.** ^{13}C NMR δ (ppm): 171.24 (C=O),166.07($C_{2\ thiazole\ ring}$), 157.84, 149.85, 128.35, 122.54, 113.51, 101.37($6C_{aromatic\ ring}$),144.40, 121.67($2C_{triazole\ ring}$), 66.35, 46.23($4C_{morhpoline\ ring}$), 55.21(OCH_3), 52.17(CO-CH_2), 37.96(NH-CH_2).

2-(4-((benzo[d]thiazol-2-ylamino)methyl)-1H-1,2,3-triazol-1-yl)-1-morpholinoethan-1-one(11t): m.p. 128–130 ℃; yield, 75%; FTIR, *ν* (cm^{-1}): 3324 (NH str.), 3146($CH_{triazole}$), 3074 (Ar-H),2947, 2861(C-H, sym., asym.), 1672 (C=O ring str.), 1620 (C=N ring str.), 1581 (C=C ring str.), 1342(C–N str.); ^{1}H NMR δ (ppm): 7.81(s, triazole-H, 1H), 7.46-7.19 (m, Ar-H, 3H), 5.39 (s,CO-CH_2, 2H), 4.81 (s,NH-CH_2, 2H), 3.58 (s, 4H, 2CH_2), 3.47 (s, 4H, 2CH_2)**.** ^{13}C NMR δ (ppm): 169.78 (C=O),166.28($C_{2\ thiazole\ ring}$), 150.60, 130.71, 125.61, 121.85, 120.37, 119.79, ($6C_{aromatic\ ring}$),143.75, 121.75($2C_{triazole\ ring}$), 66.87, 46.57($4C_{morhpoline\ ring}$), 52.78(CO-CH_2), 38.24(NH-CH_2).

Synthesis of tetrazole derivatives (12t and 13t) [21]

2-azido-N-(benzothiazol-2-yl)acetamide (8), 2-azido-1-morpholinoethan -1-one (9) (1mmol) in DMF (20mL) are added slowly to a solution of 2-azido-N-(benzothiazol-2-yl)acetamide (8), 2-azido-1-morpholinoethan -1-one (9) (1mmol) in DMF (20m The temperature of the reaction is increased to 120°C. The TLC is used to track the course of the reaction (ethylacetate :n-hexane:methanol 2:1:0.3). A rotary evaporator is used to evaporate the solvent after it has been completed. The crude product, ethyl acetate, is recrystallized from hot glacial acetic acid (3:1).

***N*-(5-methoxybenzothiazol-2-yl)-2-(5-(morpholinomethyl)-1H-tetrazol-1-yl)acetamide (12t):** m.p. 212–214℃; yield, 70%; FTIR, *ν* (cm^{-1}): 3278 (NH str.), 3077 (Ar-H),2937, 2874(C-H, sym., asym.), 1667 (C=O ring str.), 1624 (C=N ring str.), 1580 (C=C ring str.), 1348 (C–N str.), 1041, 1174 (OCH_3, sym., asym.); ^{1}H NMR δ (ppm): 10.02 (s, NH,1H), 7.47-7.22 (m, Ar-H, 3H), 5.21 (s,CO-CH_2, 2H), 3.89 (s,N-CH_2, 2H), 3.78 (s, OCH_3, 3H), 3.61 (s, 4H, 2CH_2), 3.04 (s, 4H, 2CH_2)**.** ^{13}C NMR δ (ppm): 170.47 (C=O),161.44($C_{2\ thiazole\ ring}$), 152.93($C_{tetrazole\ ring}$), 157.76, 150.20, 127.87, 122.68, 113.51, 101.36, ($6C_{aromatic\ ring}$), 66.55, 51.14($4C_{morhpoline\ ring}$), 54.74(OCH_3), 52.07(CO-CH_2), 49.96(N-CH_2).

***N*-(benzothiazol-2-yl)-2-(5-(morpholinomethyl)-1H-tetrazol-1-yl)acetamide(13t):** m.p. 198 –200 ℃; yield, 70%; FTIR, *ν* (cm^{-1}): 3279 (NH str.), 3058 (Ar-H),2977, 2867(C-H, sym., asym.), 1666 (C=O ring str.), 1633(C=N ring str.), 1584 (C=C ring str.), 1335(C–N str.); ^{1}H NMR δ (ppm): 10.02 (s, NH,1H), 7.41-7.20 (m, Ar-H, 4H), 5.32 (s,CO-CH_2, 2H), 3.87 (s,N-CH_2, 2H), 3.66 (s, 4H, 2CH_2), 3.01 (s, 4H, 2CH_2)**;** ^{13}C NMR δ (ppm): 169.17 (C=O),163.27($C_{2\ thiazole\ ring}$), 153.08($C_{tetrazole\ ring}$), 150.95, 131.03, 125.24, 124.25, 120.74, 119.48, ($6C_{aromatic\ ring}$),143.75, 121.75($2C_{triazole\ ring}$), 66.47, 49.74 ($4C_{morhpoline\ ring}$), 54.74(CO-CH_2), 48.97(NH-CH_2).

Antibacterial Activity

Assay Conditions and Medium

For all substances, the bactericidal assay was continue using the diffusion method. (38.0 g in 1000 mL distilled water) Mueller Hinton agar was autoclaved, solidified at 25 ℃. Pipettes were used to transfer (106 cells/mL) the bacteria to the plates, which were then dispersed on the agar with L-shaped spreaders. Wells were created in the agar for the well diffusion experiment, and each chemical was administered to individual wells (50 L/well). For 24 hours, plates were incubated at 37°C. Using a transparent scale, the diameter of the inhibition zone (mm) was measured to determine bacterial inhibition. Amoxicillin (5.0 mg/mL). was used positive controls.

Test Microorganisms

The two types of bacteria were used *(ATCC 25923)* Gram-positive bacteria, *Staphylococcus aureus* and *(ATCC 25922)* Gram-negative bacteria, *Escherichia coli* were utilized to evaluated antibacterial activity.

Minimum Inhibitory Concentration (MIC) [22]

The synthesized heterocyclic compounds (1,2,3-trazole and tetrazole) were diluted in sdistilled water for the MIC assay. The culture tubes were inoculated with of suspensions of bacterial containing 105 cells/mL and incubated at 37 ℃ for 24 hours. The test dish with no evident bacterial growth was designated as the MIC value of the test chemical in g/mL for MIC determination after 24 hours. Each substance was tested three times, with the average of the data used to calculate the MIC.

Docking Simulation Studies

The crystal structure data for E. coli and Staphylococcus aureus was collected from the protein data bank (PDB) 1ecl and 8hce [23,24]. All ligand molecules and water molecules were removed from the protein structure using the soft wear (MOE) Molecular Operating Environment.

Results and Discussion

Heterocyclic compounds have long been known to have antibacterial properties. Many studies [13,15,17] have reported the design and manufacture of antibacterial medicines including 1,2,3-triazole and tetrazole. In the framework of developing new drug-like molecules, *N*-(benzothiazol-2-yl)-2-(5-(morpholinomethyl)-1H-tetrazol-1-yl)acetamide derivatives and *N*-(benzothiazol-2-yl)-2-(5-(morpholinomethyl)-1H-tetrazol-1-yl)acetamide derivatives have been used. We concentrated our research on the synthesis of novel heterocyclic derivatives since (thiazole, 1,2,3-triazole, and tetrazole ring) scaffold seem to be related with antimicrobial activity. As a result, we in employment a modest and simple design as a template (Figure 3) for the preparation of a novel heterocyclic molecules with good significant antibacterial activity. Initially, 2-amino benzothiazole compounds with a triple bond moiety were produced (1 and 2) as shown in figure 1.

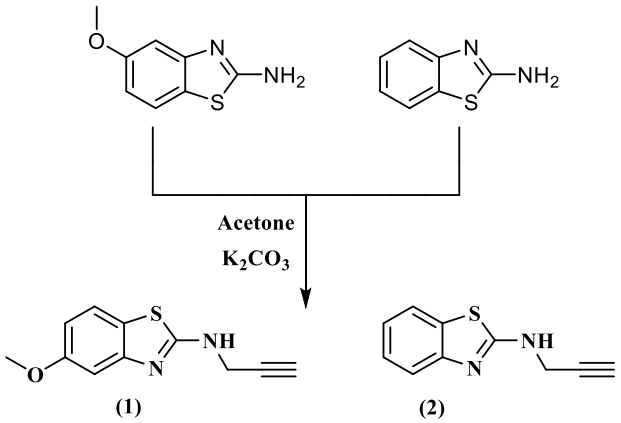

Figure 1. Synthesis of propargylic derivatives (1 and 2)

The nucleophilic substitution reaction involving terminal 2-aminobenzothiazole derivatives and propargyl bromide was used to make compounds 1 and 2. The reaction took place with potassium carbonate as a catalyst and acetone as a solvent. Analytical approaches such as NMR and FTIR spectroscopy were used to confirm the structures of the produced compounds. Compounds (1 and 2) revealed typical absorption bands in the area 3292,3284 cm^{-1} due to the -CCH moiety, as well as the band of the carbon-carbon triple bond 2132,2124 cm^{-1} in the FTIR spectrum. Above 3412, 3387 cm^{-1} and 2965, 2968 cm^{-1}, additional bands formed, which were assigned to N-H and aliphatic stretching of C-H. The proton (-CCH) of an acetylene group resonating at = 2.49, 2.47 ppm was found in the ^{1}H-NMR spectrum. Whereas the signal emerged at = 6.63, 6.67 ppm in our analysis, and it was exchanged with D2O, which belongs to the secondary amine group. At = 4.61, 4.59 ppm, the singlet corresponding to NH-CH2 emerged and was integrated into two protons. The acetylenic carbons were found at = 79.86, 81.19 and 71.87, 73.11 ppm in the 13C-NMR investigation. The aromatic ring carbons appeared between = 106-154 ppm, whereas the thiazole C2 resonated at = 169.85, 169.04 ppm. Azide and nitrile derivatives, on the other hand, are made by reacting chloroacetylchloride with (5-methoxybenzothiazol-2-amine, benzothiazol-2-amine, and morpholine) in the presence of trimethylamine as a catalyst. Then, using the strong nucleophile sodium azide and the obtained chemicals, azide derivatives were produced (3,4 and 5). figure 2.

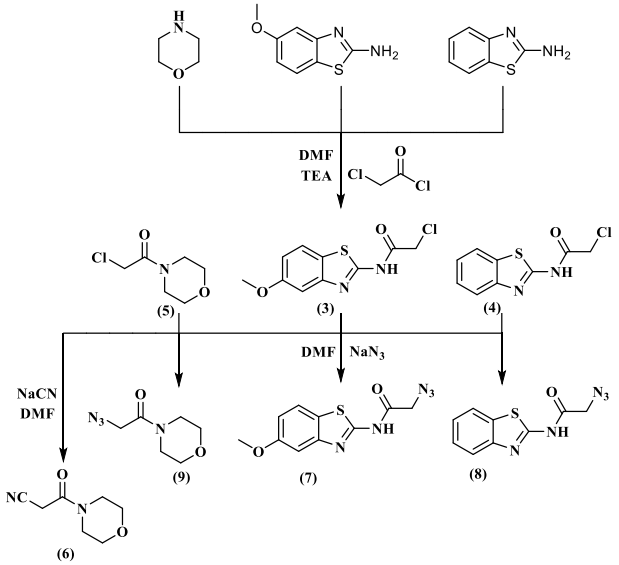

Figure 2. Synthesis of morpholine derivatives (3-9)

FT-IR and NMR spectroscopy techniques were used to analyze the structures of novel azide compounds (7,8, and 9). For example, FT-IR spectroscopy revealed distinct absorption bands for the N_3 groups at 2115, 2118, and 2122 cm^{-1}, while the NH stretches in compounds 7 and 8 were found in the region 3360-3392 cm^{-1}. In addition, the (C=O) carbonyl group has bands at 1674, 1674, and 1675 cm^{-1}. While the new bands at 2196, 1668 cm^{-1} in compound is attributed to the CN and carbonyl groups (6). In DMSO-d6, the ^{1}H NMR spectra of azide compounds (7,8, and 9) were obtained. The methylene protons in compounds (7 and 8)

appeared as a singlet set at=3.27, 3.21 ppm, whereas the OCH_3 in compound (7) showed singlet signals at=3.85 ppm, and the amide protons in compounds (7 and 8) reverberated as a singlet set at=10.02,10.03 ppm, as expected. The remaining aromatic protons resonate in the range of =7.54-7.2 ppm further downfield. The morpholine derivatives (6 and 9) have methylene protons of =3.72, 3.67 ppm and =3.41, 3.39 ppm and=3.67-3.06 ppm, respectively. The methylene protons that bonded to the CN group at 3.89 ppm in compound, on the other hand (6). The carbon atoms connected to the methylene protons resonate at =47.89, 48.12 ppm, according to the novel compounds' 13C NMR spectra. Carbonyl group carbon is represented by the signal at =169.24,169.57 ppm, while aromatics carbon is represented by the signal at =101-148 ppm. In compounds 7 and 8, the C-2carbon of the thiazole ring linked to the amine group showed in the range of =156.47, 156.18 ppm. All of the compounds 10-13 were created using the techniques outlined in Figure 3. Click chemistry between terminal alkyne and nitrile derivatives (1,2 and 6) and azide derivatives yielded heterocyclic derivatives based on the morhpoline ring (7,8, and 9). The reaction was continuing in the presence of sodium ascorbate and $CuSO_4.5H_2O$ as a catalyst.

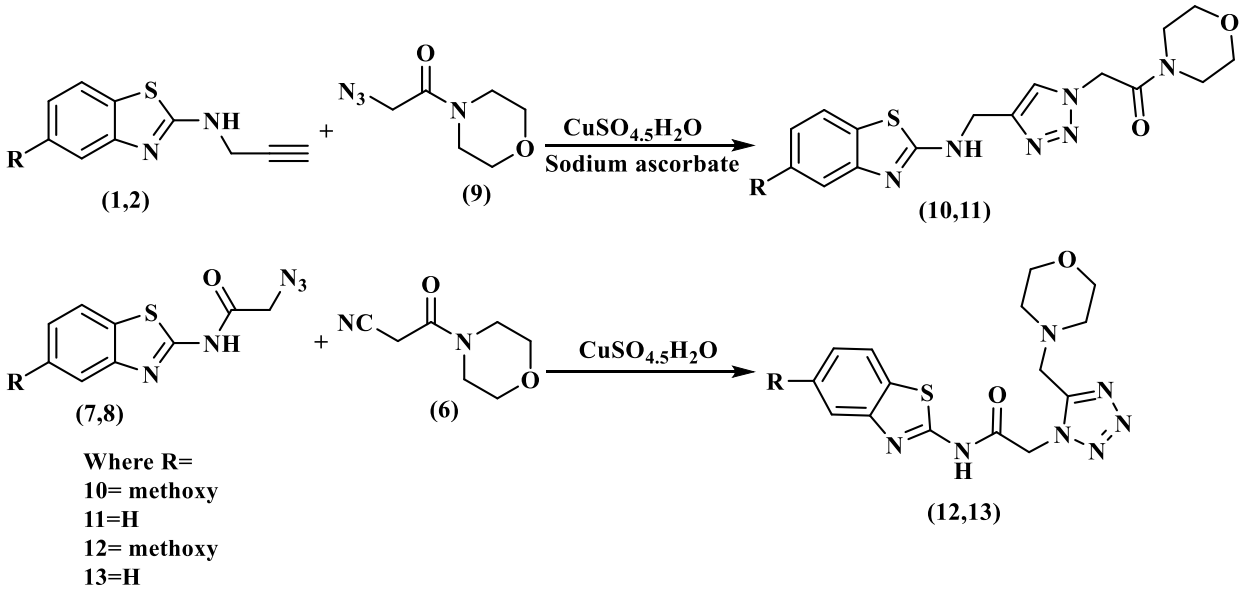

Figure 3. Synthesis of heterocyclic derivatives (10-13)

On the other hand, the FT-IR, and NMR were used to confirm the newly synthesized derivatives. For the NH group, FT-IR spectroscopy revealed distinctive starching vibration bands at 3344,3324 cm^{-1} and 3278, 3279 cm^{-1}. In addition, the (C=O) carbonyl group had bands at 1661,1672 cm^{-1} and 1667, 1666 cm^{-1}, as well as disappearing azide and nitrile group absorption in the 2100-2196 cm^{-1} range. The protons triazole ring signal appeared as a singlet about 7.81 ppm in the 1H NMR spectra of manufactured derivatives (1,2,3-triazole, 10t, and 11t) produced in DMSO-d6. The aromatic protons' resonances were found to be at 7.51-7.19. While morpholine derivative methylene protons showed at 3.67-3.58 ppm and 3.48-3.01 ppm, respectively, OCH3 emerged as a singlet signal at 3.82, 3.78 ppm in compound (10 and13). The novel compounds' 13C NMR spectra (1,2,3-triazole, 10t and 11t) revealed signals at = 144.20,143.75 ppm and = 121.27,121.75 ppm, which correspond to the triazole ring's C-4 and C-5, respectively, and signals at = 152.93, 153.08 ppm, which correspond to the tetrazole ring's carbon (12t and 13t). The aromatic ring carbons (= 157-101 ppm) and the morpholine ring methylene carbons (= 66.35, 66.87,66.55, 66.47 ppm and 46.23, 46.57, 51.14, 49.74 ppm) resonate at 66.35, 66.87,66.55, 66.47 ppm and 46.23, 46.57, 51.14, 49.74 pp Furthermore, the signal for the carbon C=O group was 171.24,169.96,170.49, 169.17 ppm, with an extra signal for the C2 of the thiazole ring at 166.07,166.28,161.44,163.27 ppm. The signal of the carbon methoxy group (compound, 10t and 12t) was exhibited at = 52.17, 54.74ppm.

Biological Studies

Antimicrobial Actions Using amoxicillin as control positive antibiotic and the well diffusion method, heterocyclic derivatives (1,2,3-tiazole and tetrazole) were tested for *in vitro* antimicrobial activity against *S. aureus (ATCC 25923*) and *E. coli (ATCC 25922).* Table 3 summarizes the MIC results of screening compounds (1,2,3-tiazole and tetrazole). The compounds with the thiazole moiety linked to the triazole or tetrazole ring showed the best broad-spectrum action. Compounds 12t and 13t from the 1,2,3-triazole and tetrazole family showed strong antibacterial activity against selected bacteria, with MICs of 1.14 and 1.43 g/mL, respectively. Both derivatives 12t (1.12 g/mL against *E. coli* and 1.78 g/mL against *S. aureus*) and 13t (1.12 µg/mL against *E. coli* and 1.43 µg/mL against *S. aureus*). The two chemicals were also

effective against Gram-negative bacteria. Both derivatives 10t and 11t had MICs of 2.85 and 3.65 g/mL against *E. coli*, respectively. Compounds 10t and 13t had modest antibacterial activity against Gram-positive *S. aureus* strains (MICs of 4.51 and 4.85 g/mL, respectively). In vitro testing of the novel heterocyclic analogs (10t,11t,12t, and 13t) (1,2,3-triazole and tetrazole) found promising antibacterial activity profiles against selected bacteria for this study (Table 1, Figure 1).

Table 1: The produced compounds 10t, 11t, 12t, and 13t each have a minimum inhibitory concentration (MIC) of g/mL.

Compound	**Gram-positive bacteria**	**Gram-negative bacteria**
	S. aureus	***E. coli***
10t	4.51	2.85
11t	5.45	3.65
12t	1.78	1.43
13t	4.85	1.14

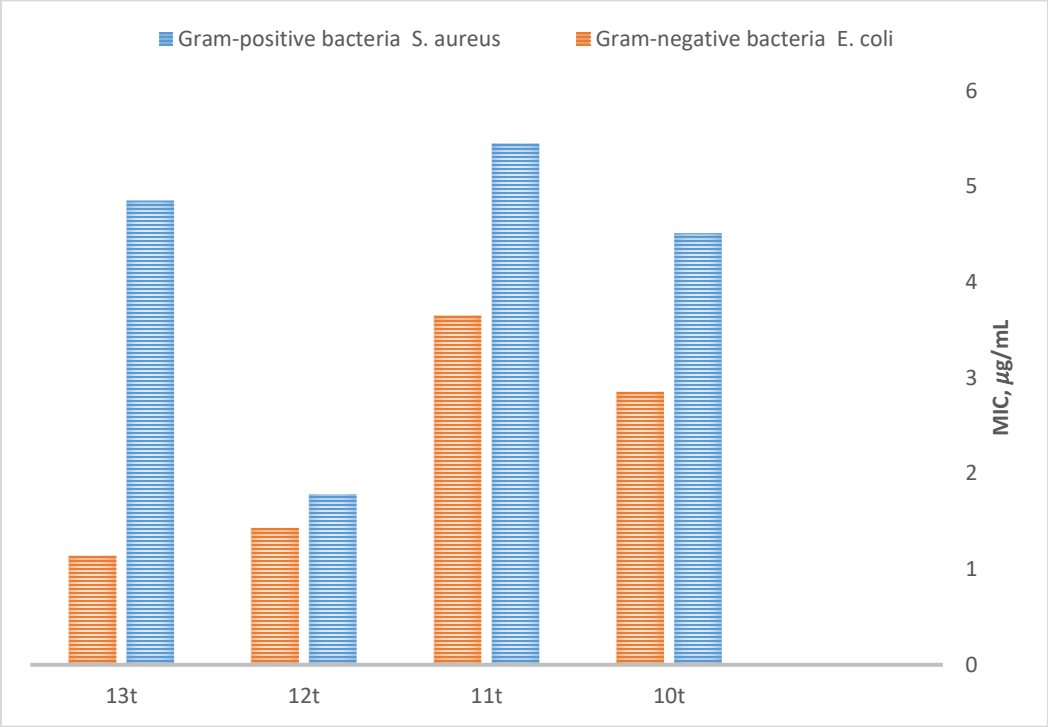

Figure 1 Biological activity of the synthesized heterocyclic 10t, 11t, 12 and 13t

Table 3.1 docking interaction parameters for effective synthesizes 10t,11t,12t & 13t ligands against 4h8e proteins

Compound docked	Receptor	Interaction	Distance(Å)	E (Kcal/mol)	S (energy score)	rmsd_refine (Å)
10t	GLU115, ASP192, GLU115, GLU115, ASP192.	H-donor, Ionic, Ionic, Ionic, Ionic, Ionic.	3.39, 3.01, 3.39, 3.52, 3.41	-0.7, -4.4, -2.4,-1.8,-2.3	-5.7526	1.2725
11t	MET189, LYS191.	H-donor, H-acceptor.	3.50, 2.85	-0.4, -7.4	-5.4745	0.9485
12t	ARG84, LYS40, ARG46.	H-acceptor, H-acceptor, pi-cation.	3.15, 3.16, 3.47	-5.1, -6.4, -0.9	-6.0852	1.5885
13t	HIS160, LYS163, LYS163.	H-acceptor, H-acceptor, pi-cation.	3.07, 3.42, 4.33	-2.8, -2.0, -0.8	-5.6601	1.1711

Table 3. docking interaction parameters for effective synthesizes 10t,11t,12t & 13t ligands against 1ecl proteins

Compound docked	Receptor	Interaction	Distance(Å)	E (Kcal/mol)	S (energy score)	rmsd_refine (Å)
10t	ASP323, ASP323, ASN555, ASP323, ASP323, ASP323, ASP323.	H-donor, H-donor, H-acceptor, Ionic, Ionic, Ionic, Ionic.	2.94, 2.85, 3.13, 3.10, 2.94, 3.59, 2.85.	-5.9, -2.6, -2.5,-3.9, -4.9, -1.6, -5.6.	-6.5100	1.475
11t	GLN241, GLN241, PHE246, ARG247.	H-donor, H-donor, Pi-H, Pi-H.	2.91,2.96, 4.23, 3.52.	-4.2, -1.1, -1.3, -2.3.	-5.4590	1.2409
12t	ASP323, ARG161, ARG114, ARG493, ASP323.	H-donor, H-acceptor, H-acceptor, H-acceptor, H-acceptor.	3.04, 3.12, 2.47, 2.45, 2.80.	-5.8, -6.1, -2.9, -1.1, -2.5.	-6.9188	1.8022
13t	ARG493, ARG168.	H-acceptor, H-acceptor.	3.13, 2.91	-1.8, -3.0	-7.0207	1.4361

Docking Study

The PBP is one of the greatest common microorganism marks for irreversible inhibitors. PBP could be a possible target for our newly prepared heterocyclic molecules, we hypothesized. The predicted docking interaction parameters for effective synthesizes 10t,11t,12t & 13t ligands against 4h8e, 1ecl proteins are listed in Table 2 and Table 3. The docking scores for all heterocyclic compounds ranged from 5.48 to 7.02 kcal/mol. The docking scores for the 1,2,3-triazole derivatives (10t and 11t) ligands against (1ecl) protein were 6.51 and 5.45 kcal/mol, respectively, which were less optimum than the binding energies predicted for tetrazole derivatives (12t and 13t) (6.91 and 7.02 kcal/mol). The docking scores for the 1,2,3-triazole derivatives (10t and 11t) against (4h8e) protein were 5.75 and 5.47 kcal/mol, respectively, which are the binding energies expected for tetrazole derivatives (12t and 13t) (6.08 and 5.66 kcal/mol). Among all docked compounds, the tetrazole ring-containing derivatives (12t and 13t) had the best docking scores against (1ecl) protein, which was validated by the MIC testing results (Table 1). The docked poses of compounds 13t (Figure 3) (1ecl) protein revealed that the hetero atom in the morpholine ring and the carbonyl group were well-inserted in a hydrogen accepter made up of ARG493 and ARG168 side chains. The hetero atom in the morpholine and thiazole rings, as well as the carbonyl and amine groups, were well-inserted in a hydrogen accepter and hydrogen donor comprising the side chains of ASP323, ARG161, ARG114, and ARG493, which could explain the superiority of this moiety over all the other tested types, according to ligand 12t (Figure 3) (1ecl) protein. The heterocyclic derivatives of the other ligands, on the other hand, obtained good docking scores (Figure 2- Figure 5). Thus, heterocyclic derivatives (1,2,3-triazole and tetrazole) appear to be able to enter the target's active site and become oriented in a favorable position for nucleophilic assault by PBP residues (through specific interactions).

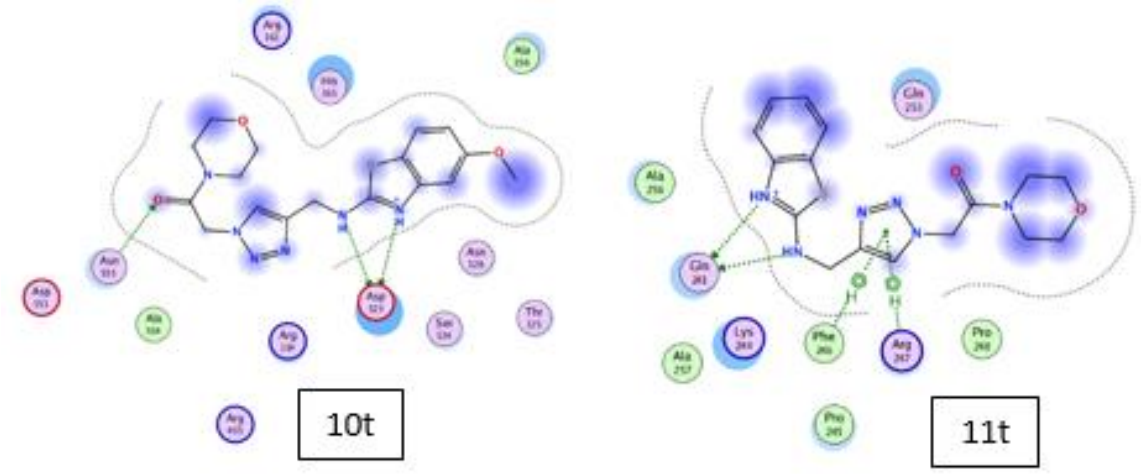

Figure 2. 2D interaction diagrams representing the docked conformation of ligand 10t and 11t against 1ecl

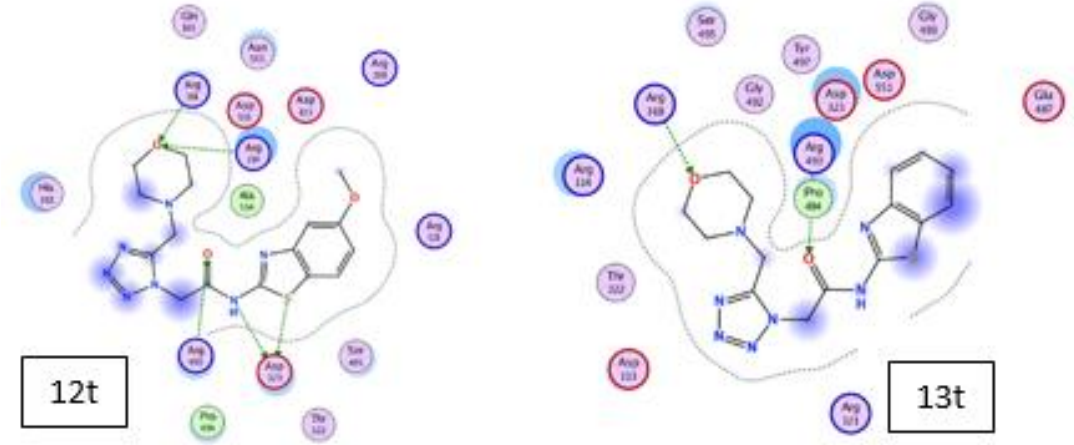

Figure 3. 2D interaction diagrams representing the docked conformation of ligand 12t and 13t against 1ecl

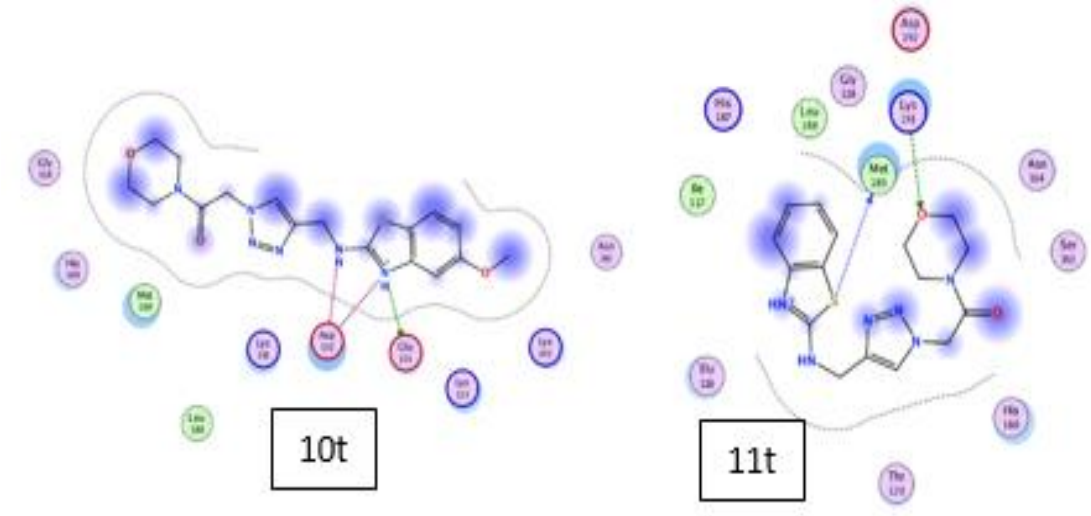

Figure 4. 2D interaction diagrams representing the docked conformation of ligand 10t and 11t against 4h8e

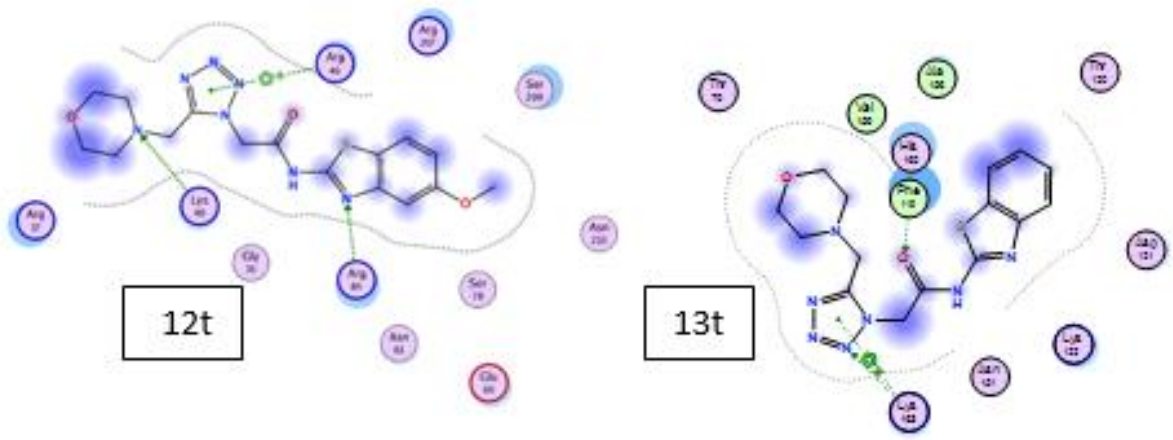

Figure 5. 2D interaction diagrams representing the docked conformation of ligand 12t and 13t against 4h8e

CONCLUSION

(1,2,3-triazolr 10t and 11t) and (tetrazole12t and 13t) are two new kind of heterocyclic derivatives that have been designed and produced. Spectroscopic techniques such as NMR and FTIR were used to confirm the structures of the synthesized compounds. Antibacterial activity of the new prepared molecules was tested against two types harmful microorganisms. Some of the new synthesized heterocyclic compounds showed considerable pharmacological effects in vitro, according to the data. Compounds 12t and 13t, which contain a tetrazole moiety, showed the broadest antibacterial activity. The MIC values for these substances ranged from 1.12 to 1.43 g/mL. Interestingly. As a result, this study added to the growing body of data suggesting the new synthetic heterocyclic chemicals (1,2,3-triazolr and tetrazole) have the potential to be evaluated as novel antibiotics for bacterial infections.

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