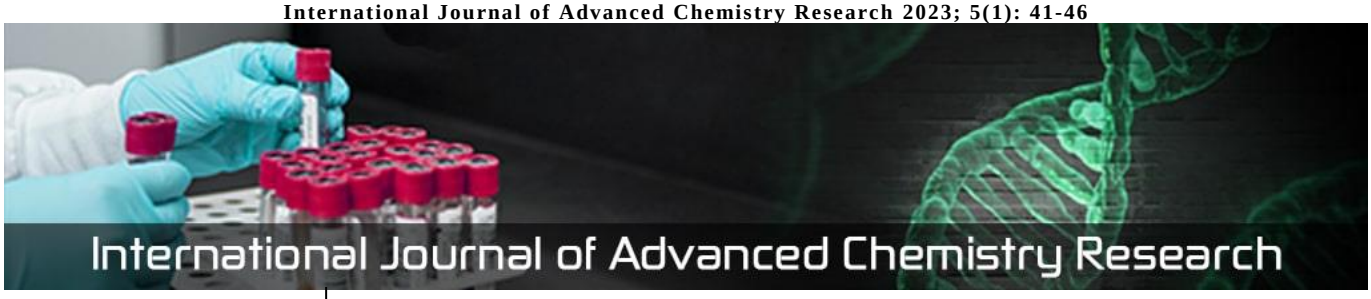

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Synthesis, characterization and biological evaluation of tetrazole derivatives

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Abstract
Heptanitrile was used in preparation azide β-lactam derivatives to produce a number of new derivatives contain tetrazole ring connected to the β-lactam moiety, which were then determined the chemical structure of prepared end products by using NMR and IR spectroscopy. The biological test against selected microorganisms showed that the majority of the end derivatives had good antifungal and antibacterial activity. The antifungal and antibacterial activity of the (t2) derivative in particular was equivalent to or even superior to that of the reference drugs Amoxicillin and Fluconazole.

Keywords: β-Lactam ring, tetrazole compounds, antifungal activity, anti-bacterial activity, imine derivatives

Introduction
The rapid rise in multidrug-resistant strains, uncontrollable pathogenic fungi and bacteria, and novel emerging pathogens have raised concerns about microbiological illnesses [1-4]. To treat a wide range of community- and hospital-acquired microbial diseases, numerous synthetic antimicrobial medicines and semi-synthetic have been identified and are widely employed in clinical settings. Particularly, the FDA-approved medicine auranofin, which has received a lot of attention recently, has a remarkable impact on Gram-positive and multidrug-resistant bacteria [5, 6]. Narrow antibacterial spectrum, negative effects, high toxicity, and other issues with clinical medicines remain unresolved, nevertheless. One of the key methods to increase effectiveness and bioavailability while treating many diseases in the clinic is combination therapy, which involves the use of two or more drugs. Combination therapy can typically overcome multi-drug resistance [7, 8]. However, it is very desirable to find and create structurally new antimicrobial drugs that have outstanding action against resistant bacteria and favorable pharmacological proles [9–11]. Tetrazole is a significant five-membered aromatic heterocyclic molecule with planar structural characteristics rich in polynitrogen electrons. Tetrazole compounds can readily bind with different receptors or enzymes in organisms thanks to their special structure, which enables them to do so through weak interactions like van der Waals forces, cation-, anion-, hydrogen bonds, and coordination bonds, among others. As a result, they display a variety of biological functions and are important to the pharmaceutical sector [12, 13]. Tetrazole rings serve as enticing linkers that can be used to stabilize or combine various pharmacophore fragments to create unique functionalized compounds [14, 15]. In addition, the tetrazole moiety functions as an amide [16], isostere of carboxyl [17, 18], and a few heterocycles (benzotriazole, [19] 1,2,3-triazole, 1,2,4-triazole [20, 21], imidazole [22], carbazole [23], benzimidazole,) [24]. Molecules that contain tetrazole derivatives have been effectively produced and widely used as clinical medications to treat a variety of disorders, including antibiotics Flomoxef [25, 26], antihypertensives Lorsartan and Valsartan [27, 28], and antinociceptive Alfentanil [29]. According to certain research, tetrazole derivatives can successfully stop the manufacture of microorganism's proteins [30, 31] to suppress the growth of a variety of bacteria. This shows that tetrazole compounds have a great deal of potential as a new class of antimicrobial medications. We are very interested in researching tetrazole derivatives as a new derivatives of possible

antibacterial medicines in light of the aforementioned factors and as a continuation of our ongoing study. Here, we'd like to report on the creation of molecules of β-lactam connected with tetrazole ring. The antibacterial and antifungal capabilities of each novel molecule were examined *in vitro*, and the interaction between the most potent tetrazole molecule and prescription medications was also examined.

Experimental Techniques and Materials

The chemical supply came from Merck, Fluka, and Acros Chemicals. NMR spectra from Bruker's DMSO-d6 were taken (operating at 300 MHz for 1H-NMR and 75 MHz for 13C-NMR). The TMS revealed ppm-level chemical alterations downfield. It is stated in Hertz for each coupling constant (J). An FT-IR 8300 spectrophotometer from Shimadzu was used to generate FTIR spectra. An electronic melting point instrument has researched melting points. The reactions with different polarity solvent solutions were observed using TLC.

Synthesis of Schiff base (s1-s3) [32]

A solution (25 mmol) of 5-methoxybenzothiazol-2-amine was dissolved in 40 mL ethanol with presence 2-3 drops of glacial acetic acid and then (25 mmol) benzaldehyde derivatives was added. The mixture of reaction was stirred for 6 hrs at 80 ℃ continued stirring until the reaction wascomplete. TLC ethylacetate: hexane (1: 1) was used as the eluent after the reaction course to produce the desired product.Under reduced pressure, the solvent was wit hdrawn, and the residue was dissolved in 80 mL of DCM an d 150 mL of water. The organic layer was dried by using $MgSO_4$, filtered and concentrated by evaporated solvent.

Compound (s1)

Yield, 84%; m.p. 187–189 ℃; FTIR, *v* (cm^{-1}): (imine group, N=CH)3125, (Phenyl-H)3074, (aliphatic sym., C-H) 2957, (aliphatic asym., C-H) 2840, (imine group, C=N)1627, (C=C phenyl ring)1592, (C–N)1345, (methoxy sym., OCH_3)1041, (methoxy asym., OCH_3) 1182; ^{1}H NMR δ: 8.89 ppm (s, 1H, N=CH, imine proton), 7.61-7.23 ppm (m, 8H, Ar-H, aromatics protons), 3.82 ppm (s, 3H, OCH_3, methoxy protons); ^{13}C NMR δ: 158.85 ppm (C_2 in thiazole ring), 153.27 ppm (N=CH, imine carbon), 156.88 ppm, 146.86 ppm, 133.81 ppm, 131.74 ppm, 130.50 ppm, 128.40 ppm, 123.78 ppm, 122.36 ppm, 114.97 ppm, 100.65 ppm (12C, carbons of aromatic ring), 55.22 ppm (OCH_3, metoxy carbon).

Compound (s2)

Yield, 78%; M.P. 168–170 ℃; FTIR, *v* (cm^{-1}): (imine group, N=CH) 3135, (Phenyl -H) 3065, (aliphatic sym., C-H) 2970, (aliphatic asym., C-H), (C=N, imine group) 1625, (C=C phenyl ring)1585, 1340 (C–N str.), (OCH_3, sym.) 1045, (OCH_3, asym.) 1175; ^{1}H NMR δ: 8.85 ppm (s, 1H, N=CH, imine proton), 7.59-7.25 ppm (m, 8H, Ar-H, aromatics protons), 3.84 ppm (s, 3H, OCH_3, methoxy protons); ^{13}C NMR δ (ppm): 158.74 ppm (C_2 in thiazole ring), 153.35 ppm (N=CH, imine carbon), 156.45 ppm, 147.28 ppm, 132.74 ppm, 130.74 ppm, 129.68 ppm, 128.25 ppm, 123.17 ppm, 122.07 ppm, 114.45 ppm, 102.60 ppm (12 C, carbons of aromatic ring), 55.87 ppm (OCH_3, metoxy carbon).

Compound (s3)

Yield, 80%; m.p. 195–197 ℃; FTIR, *v* (cm^{-1}): (imine group, N=CH) 3145, (Phenyl-H) 3078, (aliphatic sym., C-H) 2985, (aliphatic asym., C-H) 2855, (C=C phenyl ring)1580, (C–N)1345, (OCH_3, sym.)1039, (OCH_3, asym.) 1172; ^{1}H NMR) δ: 8.82 ppm (s, 1H, N=CH, imine proton), 7.52-7.21 ppm (m, 8H, Ar-H, aromatics protons), 3.82 ppm (s, 3H, OCH_3, methoxy protons) [13]; C NMR δ: 158.74 ppm ppm (C_2 in thiazole ring), 154.35 ppm (N=CH, nimine carbon), 155.74 ppm, 148.78 ppm, 131.78 ppm, 130.07 ppm, 129.18 ppm, 128.02 ppm, 123.87 ppm, 122.33 ppm, 115.45 ppm, 103.68 ppm (12 C, carbons of aromatic ring),55.68 ppm (OCH_3, metoxy carbon).

Synthesis new β-Lactam rings (b1-b3) [33]

In 50 mL of dioxane, Schiff base compounds (s1, s2, s3) (15 mmol) were dissolved. Trimethylamine (8 mmol) was then added slowly, and the mixture of reaction was chilled in an ice-salt bath. After stirring the mixture for 10 minutes, 16 mmol of chloroacetyl chloride was gradually added over the course of an hour. The mixture was continuously stirred for a whole day at room temperature until the reaction was finished. TLC ethylacetate: hexane (1:1) was used as an eluent to afford the product after the reaction course. Under reduced pressure, the solvent was withdrawn, and the residue of product was dissolved in organic solvent about 50 mL of DCM and 50 mL of water. The organic layer was dried using MgSO4, filtered, and removed organic solvent that had evaporated.

Compound (b1)

Yield, 72%; M.P. 158–160 ℃; FTIR, *v* (cm^{-1}): (phenyl-H) 3088, (aliphatic, C-H, asym.) 2941, (aliphatic, C-H, asym.) 2854, (C=O group of β-lactam ring)1721, (C=C in phenyl ring) 1587, (C–N)1341, (OCH_3, methoxy sym.) 1047, (OCH_3, methoxy asym.) 1171; ^{1}H NMR δ: 7.66-7.21 ppm (m, 3H, phenyl-H, protons of aromatic ring),5. 35 (d, J = 7.4 Hz, 1H, N-CH-Cl of β-lactam ring), 4.81ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam ring), 3.83 ppm (s, 3H, OCH_3, methyl protons in methoxy group), ^{13}C NMR δ: 169.74 ppm (C=O of β-lactam ring), 157.24 ppm (C_2 in thiazole ring), 156.53 ppm, 150.77 ppm, 132.41 ppm, 131.38 ppm, 128.48 ppm, 127.46 ppm, 121.66 ppm, 121.57 ppm, 113.60 ppm, 101.83 ppm(12C carbons of phenyl rings), 71.04 ppm (1C, CH-Cl carbon in -β-lactam ring), 62.97 ppm (N-CH- carbon in -β-lactam ring), 55.76 ppm (1C, carbon of methoxy group, OCH_3).

Compound (b2)

Yield, 75%; M.P. 188–190 ℃; FTIR, *v* (cm^{-1}): (phenyl-H) 3080, (aliphatic, C-H, asym.)2959, (aliphatic, C-H, asym.) 2850, (C=O group of β-lactam ring) 1737, (C=C in phenyl ring) 1581, (C–N) 1344, (OCH_3, methoxy sym.) 1040, (OCH_3, methoxy asym.) 1155; ^{1}H NMR δ: 7.63-7.24 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 41 ppm (d, J = 7.4 Hz, 1H, N-CH-Cl of β-lactam ring), 4.87 ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam ring), 3.82 ppm (s, 3H, OCH_3, methyl protons in methoxy group), ^{13}C NMR δ: 164.57 ppm (C=O of β-lactam ring), 156.74 ppm (C_2 in thiazole ring), 155.53 ppm, 149.72 ppm, 133.47 ppm, 131.38 ppm, 127.25 ppm, 126.87 ppm, 121.68 ppm, 119.57 ppm, 113.58 ppm, 102.47 ppm (12C carbons of phenyl rings), 69.78 ppm (1C, CH-Cl carbon in -β-lactam ring), 61.89 ppm (N-CH- carbon in -β-lactam ring), 55.87 ppm (1 C, carbon of methoxy group, OCH_3).

Compound (b3)
Yield, 70%; M.P. 127–129 °C; FTIR, *v* (cm^{-1}): (phenyl-H) 3068, (aliphatic, C-H, asym.)2954, (aliphatic, C-H, asym.) 2874, (C=O group of β-lactam ring) 1733, (C=C in phenyl ring) 1585, (C–N) 1336, (OCH_3, methoxy sym.) 1058, (OCH_3, methoxy asym.) 1165; ^{1}H NMR δ: 7.58-7.23 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 47 ppm (d, J = 7.4 Hz, 1H, N-CH-Cl of β-lactam ring), 4.79 ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam ring), 3.81 ppm (s, 3H, OCH_3, methyl protons in methoxy group); ^{13}C NMR δ: 165.47 ppm (C=O of β-lactam ring), 155.89 ppm (C_2 in thiazole ring), 154.74 ppm, 149.71 ppm, 132.27 ppm, 129.78 ppm, 128.07 ppm, 126.86 ppm, 121.87 ppm, 120.54 ppm, 114.78 ppm, 102.85 ppm (12C carbons of phenyl rings), 72.14 ppm (1C, CH-Cl carbon in -β-lactam ring), 61.89 ppm (N-CH- carbon in -β-lactam ring), 56.04 ppm (1C, carbon of methoxy group, OCH_3).

Synthesis of β-lactam azide derivatives (z1-z3)
(22 mmol) of sodium azide was added to a solution of β-lactam derivatives (b1-b3) in (15 mL) DMF, and the mixture was agitated for 8 hours at 85 °C. TLC ethyl acetate: hexane (1:1) was used as the eluent in the reaction to produce the product. A 50 mL quantity of petroleum ether was used for extraction, the layer of organic solvent was separated, the residue was washed several time with petroleum ether, and the solvent was then evaporated using a rotary evaporator.

Compound (z1)
M.P. 184–186 °C; yield, 82%; FTIR, *v* (cm^{-1}): (phenyl-H) 3058, (aliphatic, C-H, asym.)2984, (aliphatic, C-H, asym.) 2850, (azide group, -N_3)2133, (C=O group of β-lactam ring) 1735, (C=C in phenyl ring) 1595, (C–N) 1350, (OCH_3, methoxy sym.) 1065, (OCH_3, methoxy asym.) 1162; ^{1}H NMR δ: 7.61-7.18 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 15 ppm (d, J = 7.4 Hz, 1H, N-CH-azide of β-lactam ring), 4.75 ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam ring), 3.85 ppm (s, 3H, OCH_3, methyl protons in methoxy group); ^{13}C NMR δ: 168.14 ppm (C=O of β-lactam ring), 157.77 ppm (C_2 in thiazole ring), 157.47 ppm, 149.97 ppm, 134.65 ppm, 133.21 ppm, 128.98 ppm, 128.33 ppm, 127.01 ppm, 121.18 ppm, 113.85 ppm, 101.55 ppm (12C carbons of phenyl rings), 63.22 (CH-N_3 carbon in -β-lactam ring), 61.19 ppm (N-CH-carbon in -β-lactam ring), 56.04 ppm (1C, carbon of methoxy group, OCH_3).

Compound (z2)
Yield, 75%; M.P. 202–204 °C; FTIR, *v* (cm^{-1}): (phenyl-H) 3112, (aliphatic, C-H, asym.) 2985, (aliphatic, C-H, asym.) 2874, (azide group, -N_3) 2124, (C=O group of β-lactam ring) 1736, (C=C in phenyl ring) 1588, (C–N) 1354, (OCH_3, methoxy sym.) 1045, (OCH_3, methoxy asym.) 1160; ^{1}H NMR δ: 7.60-7.24 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 18 ppm (d, J = 7.4 Hz, 1H, N-CH-azide of β-lactam ring), 4.73 ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam ring), 3.85 ppm (s, 3H, OCH_3, methyl protons in methoxy group); ^{13}C NMR δ: 167.59 ppm (C=O of β-lactam ring), 158.37 ppm (C_2 in thiazole ring), 157.99 ppm, 150.97 ppm, 132.50 ppm, 132.44 ppm, 128.62 ppm, 128.21 ppm, 127.37 ppm, 121.27 ppm, 113.68 ppm, 101.90 ppm (12C carbons of phenyl rings), 63.47 ppm (CH-N_3 carbon in -β-lactam ring), 60.78 ppm (N-CH-carbon in -β-lactam ring), 55.14 ppm (1C, carbon of methoxy group, OCH_3).

Compound (z3)
Yield, 80%;**)**: M.P. 133–135 °C; FTIR, *v* (cm^{-1}): (phenyl-H)3102, (aliphatic, C-H, asym.)2965, (aliphatic, C-H, asym.) 2851, (azide group, -N_3) 2125(N_3), (C=O group of β-lactam ring)1728, (C=C in phenyl ring) 1579, (C–N) 1358, (OCH_3, methoxy sym.) 1050, (OCH_3, methoxy asym.) 1164: ^{1}H NMR δ: 7.58-7.23 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 14 ppm (d, J = 7.4 Hz, 1H, N-CH-azide of β-lactam ring), 4.78 ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam ring), 3.84 ppm (s, 3H, OCH_3, methyl protons in methoxy group); ^{13}C NMR δ: 169.14 ppm (C=O of β-lactam ring), 158.37 ppm (C_2 in thiazole ring), 157.99 ppm, 150.97 ppm, 147.06 ppm, 137.62 ppm, 128.21 ppm, 126.83 ppm, 123.52 ppm, 121.32 ppm, 113.68 ppm, 101.91 ppm (12C carbons of phenyl rings), ppm (CH-N_3 carbon in -β-lactam ring), 61.14 ppm (N-CH-carbon in -β-lactam ring), 54.87 ppm (1C, carbon of methoxy group, OCH_3).

Synthesis of tetrazole derivatives (t1-t3) [34]
(12 mmol) of the -lactam azide derivatives (z1-z3) were added to a solution (10 mmol) of the chemical heptanenitrile in (15 mL) of DMF. Ammonium chloride (20 mmol) was added once the mixture had achieved homogeneity, and the mixture was agitated at 120 °C for 8 hrs. TLC ethyl acetate: hexane (1:1) was used as the eluent after the reaction course. The solvent was eliminated at reduced pressure, and the leftover substance was absorbed by ethanol and glacial acetic acid to cause recrystallization.

Compound (t1)
Yield, 79%; M.P. 201–203 °C; FTIR, *v* (cm^{-1}): (phenyl-H) 3089, (aliphatic, C-H, asym.), 2971, (aliphatic, C-H, asym.) 2841, (C=O group of β-lactam ring) 1729, (C=C in phenyl ring) 1588, (C–N) 1358, (OCH_3, methoxy sym.) 1039, (OCH_3, methoxy asym.) 1168; ^{1}H NMR δ: 7.52-7.24 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 87 ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam connected tetrazole ring), 5.14 ppm (d, J = 7.4 Hz, 1H, N-CH-Cl of β-lactam ring),3.83ppm (s, 3H, OCH_3, methyl protons in methoxy group), 2.86 ppm (t, J = 6.0 Hz, 2H, protons of methylene group connected directly tetrazole ring-CH_2-), 1.94 – 1.86 ppm(m, 2H, protons of methylene group connected tetrazole ring-CH_2-CH_2-), 1.49-1.29 ppm(m, 6H, -CH_2-)$_3$, protons of alkyl chain), 0.86 ppm (t, J = 6.0 Hz, 3H, -CH_3, methyl protons in the end alkyl chain); ^{13}C NMR δ: 163.98 ppm (C=O of β-lactam ring), 158.33 ppm (C_2 in thiazole ring), 157.14 ppm, 151.24 ppm, 133.54 ppm, 130.14 ppm, 129.96 ppm, 128.11 ppm, 122.11 ppm, 114.13 ppm, 109.19 ppm (12C carbons of phenyl rings), 154.61 ppm (1C carbon of tetrazole ring), 64.02 ppm (1C, CH- methylene carbon connected tetrazole-β-lactam), 60.36 ppm (1C, N-CH-methylene carbon connected tetrazole ring), 56.19 ppm (1C, carbon of methoxy group, OCH_3), 31.67 ppm, 29.98 ppm, 26.87 ppm, 25.69 ppm, 22.80 ppm (5C, methylene carbons in alkyl chain, (-CH_2-)$_5$), 14.27 ppm (1C,carbon methyl in the end alkyl chain, -CH_3).

Compound (t2)
Yield, 75%;): M.P. 191-193 °C; FTIR, *v* (cm^{-1}): (phenyl-H)3097, (aliphatic, C-H, asym.), 2974, (aliphatic, C-H, asym.) 2861, (C=O group of β-lactam ring) 1728, (C=C in phenyl ring) 1592, (C–N) 1362, (OCH_3, methoxy sym.) 1031, (OCH_3, methoxy asym.)1159. ^{1}H NMR δ: 7.58-7.28 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 47 ppm

(d, J = 7.4 Hz, 1H, CH- proton of β-lactam connected tetrazole ring), 4.89 ppm (d, J = 7.4 Hz, 1H, N-CH-Cl of β-lactam ring),3.84 ppm (s, 3H, OCH_3, methyl protons in methoxy group), 2.89 ppm (t, J = 6.0 Hz, 2H, protons of methylene group connected directly tetrazole ring-CH_2-),1.91– 1.83 ppm (m, 2H, protons of methylene group connected tetrazole ring-CH_2-CH_2-), 1.48– 1.27 ppm (m, 6H, -CH_2-)$_3$, protons of alkyl chain), 0.84ppm (t, J = 6.0 Hz, 3H, -CH_3, methyl protons in the end alkyl chain); ^{13}C NMR δ: 162.86 ppm (C=O of β-lactam ring), 158.78 ppm (C_2 in thiazole ring), 158.38 ppm, 151.17 ppm, 132.45 ppm, 130.28 ppm, 129.38 ppm, 128.24 ppm,124.87 ppm, 121.98 ppm,116.84 ppm, 108.78 ppm (12C carbons of phenyl rings), 154.09 ppm (1C carbon of tetrazole ring), 63.78 ppm (1C, CH- methylene carbon connected tetrazole-β-lactam), 60.98(ppm (1C, N-CH- methylene carbon connected tetrazole ring), 56.81 ppm (1C, carbon of methoxy group, OCH_3), 31.98ppm, 29.85ppm,27.12 ppm, 25.19 ppm, 22.48 ppm(5C, methylene carbons in alkyl chain, (-CH_2-)$_5$), 14.38 ppm (1C,carbon methyl in the end alkyl chain, -CH_3).

Compound (t3)

Yield, 74%; M.P. 215–217 ℃; FTIR, *ν* (cm^{-1}): (phenyl-H) 3061, (aliphatic, C-H, asym.), 2981, (aliphatic, C-H, asym.) 2884, (C=O group of β-lactam ring) 1741, (C=C in phenyl ring) 1587, (C–N) 1355, (OCH_3, methoxy sym.) 1041, (OCH_3, methoxy asym.)1171.

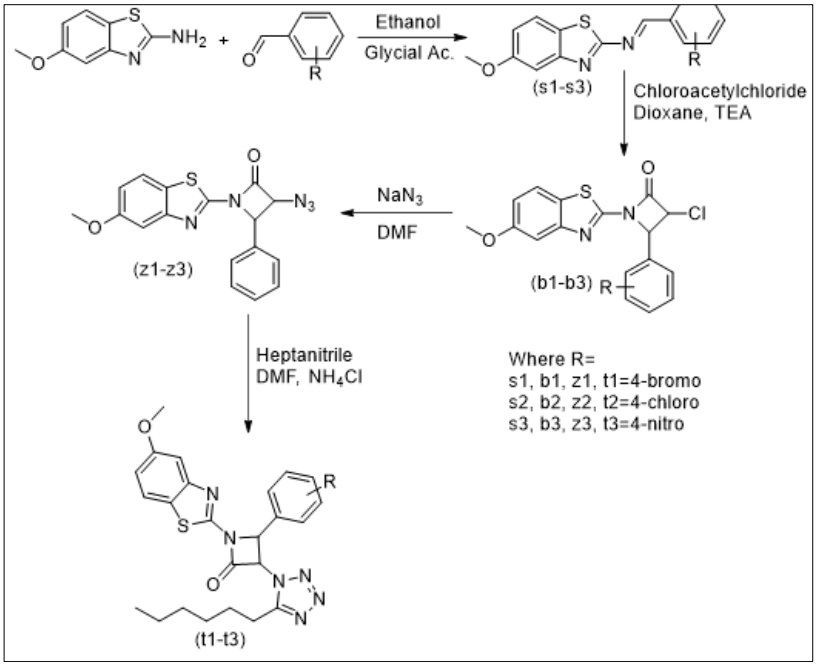
^{1}H NMR δ: 7.59-7.27 ppm (m, 3H, phenyl-H, protons of aromatic ring), 5. 74ppm (d, J = 7.4 Hz, 1H, CH- proton of β-lactam connected tetrazole ring), 4.88 ppm (d, J = 7.4 Hz, 1H, N-CH-Cl of β-lactam ring), 3.82 ppm (s, 3H, OCH_3, methyl protons in methoxy group), 2.90 (t, J = 6.0 Hz, 2H, protons of methylene group connected directly tetrazole ring-CH_2-),1.94 – 1.86 ppm (m, 2H, protons of methylene group connected tetrazole ring-CH_2-CH_2-), 1.47-1.27 ppm (m, 6H, -CH_2-)$_3$, protons of alkyl chain), 0.88 ppm (t, J = 6.0 Hz, 3H, -CH_3, methyl protons in the end alkyl chain); ^{13}C NMR δ: 163.91 ppm (C=O of β-lactam ring), 158.74 ppm (C_2 in thiazole ring), 158.17 ppm, 148.99 ppm, 147.07 ppm, 137.47 ppm, 129.17 ppm, 128.57 ppm, 123.49 ppm, 122.47 ppm, 115.47 ppm, 109.89 ppm (12C carbons of phenyl rings), 154.27 ppm (1C carbon of tetrazole ring), 63.79 ppm (1C, CH- methylene carbon connected tetrazole-β-lactam), 60.92 ppm (1C, N-CH- methylene carbon connected tetrazole ring), 55.84 ppm (1C, carbon of methoxy group, OCH_3), 31.48 ppm, 29.71 ppm,27.37 ppm, 25.47 ppm, 22.378 ppm(5C, methylene carbons in alkyl chain, (-CH_2-)$_5$), 14.17 ppm (1C,carbon methyl in the end alkyl chain, -CH_3).

Anti-microbial screening

Escherichia coli ATCC No. 25922 and Staphylococcus aureus, ATCC No. 29213, were two Gram positive bacteria that the target derivatives were tested against *in vitro*. They were tested for their antifungal effectiveness against selected fungi [35]. In this study, the method was used Mueller Hinton Agar to determine the inhibition efficacy against the bacteria selected for the study. New vaccines have been produced from these bacteria and diluted with sterile saline. The turbidity of these cultures was corrected using 0.5 McFarland. A consistent bacterial herb was made using sterile cotton swabs. Using a 6 mm drill bit, boreholes were drilled by drilling out diseased plates. Dimethyl sulfoxide (DMSO), which served as a negative control for the bioassay, was used to dilute each sample. DMSO was utilized at a concentration of 100 g/mL. These plates underwent a 24-hour incubation period at 37 °C. DMSO was utilized as a negative control, and ampicillin and fluconazole were employed as positive controls, at doses ranging from 100 g/mL. The diameter of the inhibition zone, measured in millimeters, was used to gauge the antibacterial activity of the produced derivatives (t1-t3) after taking the activity of the negative control into account.

Results and discussion

The synthesized tetrazole compounds t1, t2 and t3 were prepared by refluxing heptanenitrile with prepared β-lactam azide derivatives (z1-z3) in DMF as solvent and NH_4Cl as catalyst at 120 ℃ (Scheme 1). Firstly, Schiff base derivatives (s1-s3) were synthesized by stirring aminobenzothiazole derivative and selected benzaldehyde derivatives in presence an acid and absolute ethanol. The benzothazole amine (NH_2) absorption band vanished, in addition to, the other IR spectra of prepared compounds showed the presence of new bands at 3145-3125 cm^{-1} (-N=CH-) and 1727-1717 cm^{-1} (-N=C-) due to imine group. The ^{1}H NMR spectra showed the signal of the (N=CH) in imine group at rang 8.89-8.82 ppm. On the other hand, the ^{13}C NMR spectra showed a new signal at the range 154.35–153.27 ppm due to the imine carbon group. β-lactam derivatives (b1-b3) was prepared by reaction prepared Schiff base(s1-s3) and triethyamine, an organic base, and chloroacetylchloride in dioxane as the solvent. The IR spectra of β-lactam compounds revealed the presence of new bands that corresponded to the carbonyl groups connected β-lactam ring in the range 1737-1721 cm^{-1}. The ^{1}H NMR spectra revealed doublet signals at 4.87 and 4.79 ppm for (-N-CH-) in the -lactam ring, doublet signals for (-CH-Cl) in the -lactam ring at 5.47 and 5.35 ppm, and multiplet signals for aromatic proton at 7.66 and 7.21 ppm. ^{13}C NMR spectra give good data such as new signals in the region of 169.74–164.57 ppm assigned to carbonyl moiety, 72.14–69.78 ppm attributed to (CH-Cl that connected in 3 position β-lactam), and 62.97–61.89 ppm due to (N-CH- that connected in 4 position β-lactam). By re-flushing beta-lactam derivatives (b1-b3) with sodium azide in DMF at 60–65 °C, beta-lactam azide derivatives (z1-z3) were generated. The infrared spectra of the synthesized azide-lactam compounds appearance new absorption bands at 2133-2124 cm-1 and 1735-1727 cm^{-1}, which due to the azide group (N_3) and carbonyl group that connected in 2 position β-lactam, respectively. The ^{1}H NMR data give signals in aromatic region at 7.61–7.18 ppm, a doublet at 4.78–4.74 ppm for (-N-CH- that connected in 4 position β-lactam), and a double at 5.18–5.14 ppm for (-CH-N_3). In the β-lactam ring. ^{13}C NMR spectra revealed new signals in regions 64.09–63.22 ppm assigned to (CH-N_3 that connected in 3 position β-lactam) and 61.19–60.78 ppm due to (N-CH-lactam) in the β-lactam ring, all attributed to the carbonyl.



Scheme 1: Synthesis new tetrazole derivatives

When β-lactam azide derivatives (z1-z3) were stirred with heptanitrile and ammonium chloride in DMF to produce new tetrazole derivatives (t1-t3). The absorption band of CN and N3, present in starting materials heptanitrile and prepared azide derivatives, can no longer be detected in the IR spectra. They also showed the presence of absorption bands for carbonyl that connected in position 2 in β-lactam ring between 1741 and 1728 cm-1. Through aromatic Ar-H substitution, some additional peaks appear, with group-induced (C–N) absorption bands at 3089 and 3067 cm-1 and 1362 and 1355 cm-1, respectively. double at 7.59-7.24 ppm, which assigned to protons of aromatic rings, double at 5.14-4.89 ppm, which due to (-N-CH- β-lactam ring), and double at 5.87-5.47 ppm, which return to the -CH β-lactam ring attached to the tetrazole ring are common signals in ^{1}HNMR spectra. A triple signal at 2.91–2.86 ppm for the methylene protons that attach to the tetrazole ring, and a single signal at 3.82–3.84 ppm for OCH3. New signals were detected in the ^{13} C NMR spectra in the carbon-carbon resonance band of 163.98–162.86 ppm and the four-ring carbon resonance band of 154.61–154.09 ppm. On the other hand, the earlier signals at 56.81-55.84 ppm were attributed to the methoxy carbon, while the new ones in the region 64.02.-63.78 ppm were attributed to (CH-) linked to the tetrazole ring, as well as 60.98-60.36 ppm due to (N -CH-) in the β-lactam ring.

Antimicrobial activity

The antibacterial activity of the synthetic derivatives (t1, t2, and t3) was tested against a variety of pathogenic bacterial strains, including Gram-negative *Escherichia coli* (ATTC-25922) and Gram-positive *Staphylococcus aureus* (ATTC-25923). These chemicals (t1, t2, and t3) were tested against the fungus *Candida albicans* for their antifungal properties (MTCC 227). Serial plate dilution was used to conduct the antibacterial activities, as described in [35, 36]. In Table 1, you can see the minimum inhibitory concentrations (MICs) of the medications that were researched. When determining fluconazole's antifungal and antibacterial effects, reference drugs fluconazole and ampicillin were employed.

Antibacterial activity

Table 1 lists the outcomes of the produced compounds' antibacterial screening. Most of the investigated chemicals show modest antibacterial activity. With MIC values between 75 and 125 µg/mL, the synthesized compounds (t1, t2, and t3) demonstrated good to moderate activity. Particularly, substances (t2 and t3) demonstrated strong action against *E. coli* (MIC values 75–90 µg/mL) while (t1) demonstrated good activity (MIC value 125 µg/mL). Comparatively to the reference medication ampicillin, compounds (t2 and t3) had good activity (MIC values 125–140 µg/mL) while (t2) demonstrated moderate activity (MIC values 200 µg/mL) against *S. aureus.*

Antifungal activity

The results of the generated compounds' antifungal screening are shown in Table 1. The synthetic drugs (t1, t2, and t3) showed better activity against *C. albicans* than Fluconazole (MIC value: 150–250 µg/mL).

Table 1: Compounds t1, t2, and t3's biological profiles

Compounds	(MIC) µg/Ml		
	Tested bacteria		Tested fungi
	Gram-negative	Gram-positive	
	E. coli	*S. aureus*	*C. albicans*
t1	125	200	250
t2	75	125	150
t3	90	140	175
Ampicillin	100	100	-
Fluconazole	-	-	250

Conclusions

The first successful synthesis of a novel type of terazole from commercially available 5-methoxybenzothiazol-2-amine was accomplished employing a simple, instructive, and practical synthesis method. A complete analysis of the antibacterial activity of the produced compounds showed that the -Cl and -NO2 containing compounds performed better than the other compounds. Fluconazole was used in the test strains (t1, t2, and t3) instead of a placebo.

Fluconazole was discovered to be less effective than compound (t2) when administered just to treat C. albicans (MIC value: µg/mL). Comparing the generated compounds (t1, t2, and t3) to the reference antibiotic ampicillin revealed good to moderate efficacy against Escherichia coli and S. aureus, with MIC values in the range of 75-125 µg/mL.

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