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DESIGN AND SYNTHESIS OF NOVEL DERIVATIVES OF CHLOROBENZYL-TETRAZOLE AND STUDYING THEM FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract:
A novel series of substituted chlorobenzyl-tetrazole derivatives were synthesized and screened for their antibacterial and antifungal activities. Pharmaceutical investigation studies revealed that the most of newly synthesized compounds show significant antibacterial and antifungal activities against the tested strains. Among the synthesized compounds, some compound displayed significant antibacterial activity equipotent to ciprofloxacin against S. aureus. In addition, some compound shows potent antifungal activity against both the three strains. Most of the target compounds exhibits good antibacterial and antifungal activities against all the tested strains regarding the reference drug.

Keywords: Tetrazole, Bleaching Earth Clay (BEC), PEG-400, Antibacterial, Antifungal.

Introduction:

Escalation of multidrug resistance strains, evolution of incurable micro-organisms, and gain in pathogenic microbes alarm the seriousness of microbial infection[i-iii]. To oversight this, researchers uniformly focused on the synthesis of influential and adequate antimicrobial agents. Many antimicrobial scaffolds are disclosed and applied extensively for the treatment, prevention, and control of microbial infection. Despite that, there are some hurdles like adverse effects, high toxicity, and narrow antimicrobial spectrum, which are unresolved. Literature in recent years has highlighted the significant contribution of publications on the chemistry of N-containing heterocycles in the discovery of new drugs with biological activity, particularly those with tetrazole moiety.

Tetrazole is an important heterocyclic scaffold containing nitrogen. It is a unique nitrogen-rich compound among the known stable heterocycles, even so, not found in nature[iv]. In 1885, 2-phenyl-2H-tetrazole-5-carbonitrile became the first synthesized compound to embed a tetrazole ring[v]. Tetrazole is a remarkable synthetic scaffold that noticed wide applications in various fields like medicinal, pharmacological, biochemistry, and material science[vi-x]. Tetrazole is the lead motif in medicinal chemistry where the uncommon, poly-nitrogen,

electron-rich planer ring structure is answerable to exhibit widespread applications to treat various diseases especially antimicrobial[xi-xii], antifungal[xiii-xiv], antiviral[xv-xvi], antitubercular[xvii-xviii], anticancer[xix-xx], antimalarial[xxi-xxii], antiangiogenic[xxiii-xxiv], anti-inflammatory[xxv-xxvi], anti-HIV[xxvii-xxviii], analgesic[xxix-xxx], anti-Alzheimer[xxxi-xxxii], antinociceptive[xxxiii-xxxiv], and anticonvulsant[xxxv-xxxvi]. Weak interactions like hydrogen bonding, van der wals force, or coordination bonding bound the tetrazole ring easily with the various enzymes or receptors of microbes and successfully applied as components for comprehensive pharmaceutical applications[xxxvii]. Food and Drug Administration (FDA) approved several drugs possessing tetrazole scaffold as an important pharmaceutical agent[xxxviii]. Metabolic stable surrogates for carboxylic acid group and lipophilic spacers of tetrazole derivatives make it important in drug design[xxxix]. It has also revealed that the lipophilic nature of the tetrazole ring in a drug revamps its oral bioavailability and cell penetration[xxxvii]. Besides this, tetrazole has noticed a broad spectrum of applications in materials like information recording systems[xl], rocket propellants[xli-xlii], specialty explosives[xliii], and agrochemical applications[xliv-xlvii]. As a befitting coordination property, it is also capable of forming nitrogen containing ligands and designing stable complex with disparate metal ions and reacting as precursors to varied nitrogen containing heterocyclic compounds in organic synthesis[xlviii]. Thus, the versatile applications of tetrazole skeleton in many fields make interest in the development of novel tetrazole derivatives. We display some of them in **Fig. 1.**

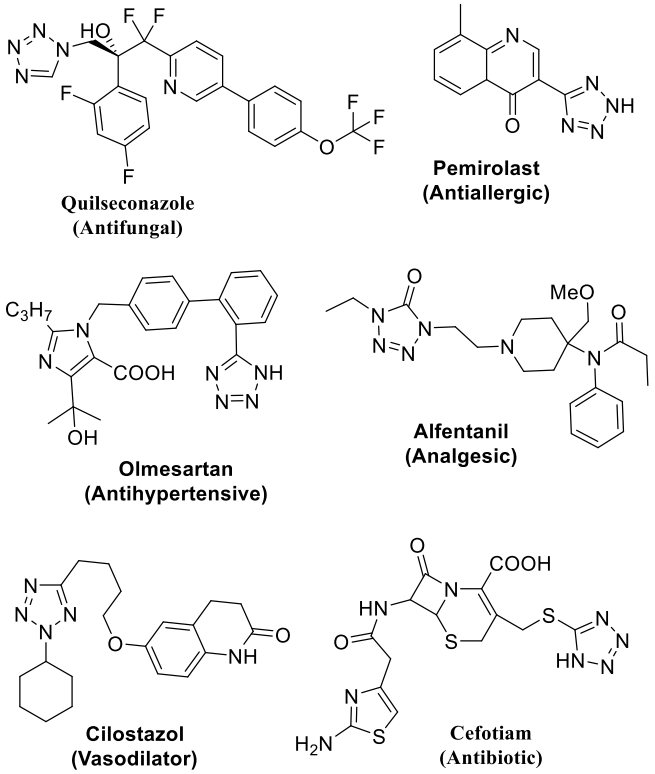

Fig 1. FDA Approved some selected drugs containing tetrazole moiety.

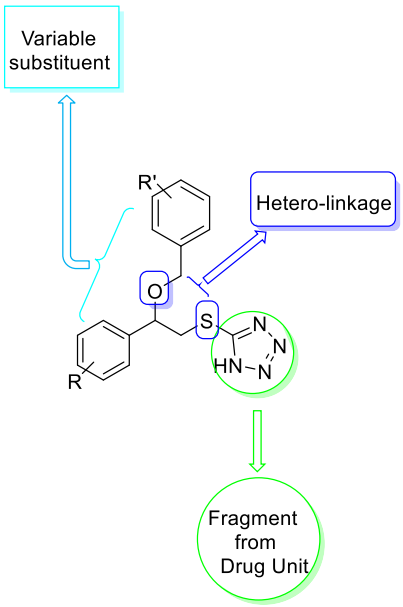

Fig 2. Molecular structure of chlorobenzyl-oxy-phenyl-ethyl-thio1H-tetrazole derivatives used in present study

Fig 1.FDA Approved Some Selected Drugs Containing Tetrazole Moiety.

As we analyze the literature mentioned above and conduct our research on creating and producing bioactive heterocyclic compounds[xlix-liii], our current approach is to develop unique derivatives containing tetrazole and evaluate their effectiveness against bacteria and fungi.

EXPERIMENTAL SECTION

CHEMISTRY

All the melting points of newly synthesized compounds were determined in an open capillary tube and were uncorrected. Used reagents and solvents are laboratory grade and purified. IR spectra were recorded on an FTIR Perkin Elmer/Schimadzu/Bruker spectrophotometer as KBr pellets. ^{1}HNMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on an Avance spectrometer (Bruker, Germany) at a 400-MHz frequency using DMSO-d_6 as a solvent, with TMS as an internal standard. Mass spectra were recorded on an EI-Shimadzu QP 2010 PLUS GC-MS system (Shimadzu, Japan). Elemental analysis was performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer (Perkin-Elmer, USA). Thin layer chromatography was performed on precoated sheets of silica gel-G (Merck, Germany) with UV lamp.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 1-(SUBSTITUTED PHENYL)-2-THIOCYNATOETHANONE (2a-h)

A mixture of potassium thiocyanate (1.00 mm) and substituted phenacyl bromide (1.00 mm) was stirred continuously in PEG-400 for 1-2 hrs at 60-70°C. TLC monitored the progress of the reaction and poured the crude reaction mixture into ice-cold water. The separated solid was filtered simply, dried, and recrystallized in aq. acetic acid to afford the pure product.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 2-((1H-TETRAZOL-5-YL)THIO)-1-(SUBSTITUTED PHENYL)ETHANONE (3a-h)

Compounds **2a-h** (1.00 mm) and sodium azide (1.00 mm) were taken in PEG-400 with a catalytic amount of TBAB. Reflux this reaction mixture at 70-80°C for 1-2 hrs. The reaction was monitored by TLC. After completion of the reaction, it was poured into ice-cold water to isolate the solid product. A simple filtration method collected the solid product, dried and recrystallized it in aq. acetic acid.

2-((1H-tetrazol-5-yl)thio)-1-(4-chlorophenyl)ethanone (3a): IR (KBr, cm^{-1}): 3249 (-NH), 3028 (Ar, C-H), 1739 (>C=O), 1591 (>C=N), 1091 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.21 (s, 1H, -NH of tetrazole), 7.95-7.47 (m, 4H, Ar-H), 3.94 (s, 2H, -CH_2); EIMS: 254 [M+]; $C_9H_7ClN_4OS$.

2-((1H-tetrazol-5-yl)thio)-1-(4-bromophenyl)ethanone (3b): IR (KBr, cm^{-1}): 3432 (-NH), 3012 (Ar, C-H), 1721 (>C=O), 1612 (>C=N); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.48 (s, 1H, -NH of tetrazole), 8.10-7.52 (m, 4H, Ar-H), 4.24 (s, 2H, -CH_2); EIMS: 299 [M+]; $C_9H_7BrN_4OS$.

2-((1H-tetrazol-5-yl)thio)-1-(3-nitrophenyl)ethanone (3d): IR (KBr, cm-1): 3440 (-NH), 3011 (Ar, C-H), 1735 (>C=O), 1605 (>C=N), 1030 (C-S-C); 1H NMR (400 MHz, DMSO-d6, TMS, δ, ppm): 8.67 (s, 1H, -NH of tetrazole), 8.31-7.87 (m, 4H, Ar-H), 4.05 (s, 2H, -CH2); EIMS: 265 [M+]; C9H7N5O3S.

2-((1H-tetrazol-5-yl)thio)-1-(4-fluorophenyl)ethanone (3f):IR (KBr, cm^{-1}): 3418 (-NH), 3022 (Ar, C-H), 1730 (>C=O), 1624 (>C=N), 1051 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.32 (s, 1H, -NH of tetrazole), 8.05-7.72 (m, 4H, Ar-H), 3.88 (s, 2H, -CH_2); EIMS: 238 [M+]; $C_9H_7FN_4OS$.

2-((1H-tetrazol-5-yl)thio)-1-(3,4-dichlorophenyl)ethanone (3h):IR (KBr, cm^{-1}): 3449 (-NH), 3032 (Ar, C-H), 1732 (>C=O), 1611 (>C=N), 1014 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.82 (s, 1H, -NH of tetrazole), 8.18-7.87 (m, 3H, Ar-H), 4.57 (s, 2H, -CH_2); EIMS: 289 [M+]; $C_9H_6Cl_2N_4OS$.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 2-((1H-TETRAZOL-5-YL)THIO)-1-SUBSTITUTEDPHENYLETHANOL (4a-h)

Compounds **3a-h** (1.00 mm) stirred in PEG-400 at room temperature in the presence of a catalytic amount of sodium borohydrate (0.25 mm) for 30 min. After completion of the reaction (monitored by TLC), the reaction mixture stands for 1hr at room temperature and is poured in ice- cold water. The separated product was filtered, dried, washed with water, and recrystallized in aq. acetic acid

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2-((1H-tetrazol-5-yl)thio)-1-(4-chlorophenyl)ethanol (4a): IR (KBr, cm^{-1}): 3423 (-OH), 3217 (-NH), 1615 (>C=N), 1091 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 13.74 (s, 1H, -OH), 7.98 (s, 1H, –NH of tetrazole), 7.86 -7.46 (m, 4H, Ar-H), 6.23 (t, 1H, -CH), 5.29 (d, 2H, -CH_2).

2-((1H-tetrazol-5-yl)thio)-1-(4-bromophenyl)ethanol (4b): IR (KBr, cm^{-1}): 3457 (-OH), 3192 (-NH), 1622 (>C=N), 1108 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 13.88 (s, 1H, -OH), 8.32 (s, 1H, –NH of tetrazole), 8.11 -7.87 (m, 4H, Ar-H), 6.37 (t, 1H, -CH), 5.12 (d, 2H, -CH_2).

2-((1H-tetrazol-5-yl)thio)-1-(3-nitrophenyl)ethanol (4d): IR (KBr, cm^{-1}): 3528 (-OH), 3196 (-NH), 1620 (>C=N), 1128 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 13.87 (s, 1H, -OH), 8.56 (s, 1H, –NH of tetrazole), 8.28 -7.89 (m, 4H, Ar-H), 6.42 (t, 1H, -CH), 5.14 (d, 2H, -CH_2).

2-((1H-tetrazol-5-yl)thio)-1-(4-fluorophenyl)ethanol (4f): IR (KBr, cm^{-1}): 3487 (-OH), 3174 (-NH), 1631 (>C=N), 1132 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 13.17 (s, 1H, -OH), 8.38 (s, 1H, –NH of tetrazole), 8.17 -7.81 (m, 4H, Ar-H), 6.46 (t, 1H, -CH), 5.30 (d, 2H, -CH_2).

2-((1H-tetrazol-5-yl)thio)-1-(3,4-dichlorophenyl)ethanol (4h): IR (KBr, cm^{-1}): 3413 (-OH), 3237 (-NH), 1625 (>C=N), 1128 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 13.87 (s, 1H, -OH), 8.66 (s, 1H, –NH of tetrazole), 8.43 -7.96 (m, 4H, Ar-H), 6.22 (t, 1H, -CH), 5.30 (d, 2H, -CH_2).

GENERAL PROCEDURE FOR THE SYNTHESIS OF 5-((2-((4-CHLOROBENZYL)OXY)-2-(4-CHLOROPHENYL)ETHYL)THIO)-1H-TETRAZOLE (6a-p)

An equimolar mixture of compounds **4a-h** (1.00 mmole) and substituted chlobenzylchloride (1.00 mmole) stirred continuously in the presence of a catalytic amount of Bleaching Earth Clay (pH-12.5, 10 wt%) as a heterogeneous catalyst to attain basic media in PEG-400 for 1 hr at 70-80ºC. After completion of the reaction monitored by TLC, the reaction mixture cooled at room temperature and worked up with ice-cold water. After filtration, the separated product was washed with hot water, dried, and recrystallized from aq. acetic acid to get a pure final product.

5-((2-((4-chlorobenzyl)oxy)-2-(4-chlorophenyl)ethyl)thio)-1H-tetrazole (6b): M.P. 167-169°C; Yield: 86%; IR (KBr, cm^{-1}): 3215 (-NH), 3068 (aromatic-C-H), 1617 (>C=N), 1073 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.23 (s, 1H, -NH of tetrazole), 7.86-7.46 (m, 8H, Ar-H), 6.35 (d, 1H, -CH), 5.86 (s, 2H, -CH_2), 5.15 (t, 2H, -CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm); 160.41, 143.12, 138.16, 136.86, 135.19, 132.51, 131.30,

130.16, 129.81, 129.31, 128.92, 121.46, 104.43, 79.19, 46.86; EIMS: 380 [M+]; Elemental Analysis: Caculated (found) for $C_{16}H_{14}Cl_2N_4OS$: % C, 50.40 (50.42); H, 3.70 (3.73); N, 14.69 (14.67); S, 8.41 (8.43).

5-((2-((4-chlorobenzyl)oxy)-2-(4-nitrophenyl)ethyl)thio)-1H-tetrazole (6d): M.P. 182-184°C; Yield: 92%; IR (KBr, cm^{-1}): 3262 (-NH), 3120 (aromatic-C-H), 1622 (>C=N), 1112 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.73 (s, 1H, -NH of tetrazole), 8.37-7.35 (m, 8H, Ar-H), 6.43 (t, 1H, -CH), 5.72 (s, 2H, -CH_2), 5.22 (d, 2H, -CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm); 164.62, 146.89, 140.84, 138.18, 136.37, 135.42, 134.98, 133.72, 131.46, 130.61, 129.28, 126.55, 100.49, 71.76, 43.68; EIMS: 391 [M+]; Elemental Analysis: Calculated (found) for $C_{16}H_{14}ClN_5O_3S$: % C, 49.04 (49.01); H, 3.60 (3.62); N, 17.87 (17.85); S, 8.18 (8.20).

5-((2-((4-chlorobenzyl)oxy)-2-(3-nitrophenyl)ethyl)thio)-1H-tetrazole (6e): M.P. 177-179°C; Yield: 94%; IR (KBr, cm^{-1}): 3289 (-NH), 2977 (aromatic-C-H), 1639 (>C=N), 1127 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.47 (s, 1H, -NH of tetrazole), 8.20-7.24 (m, 8H, Ar-H), 6.93 (t, 1H, -CH), 5.98 (s, 2H, -CH_2), 5.37 (d, 2H, -CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm); 155.45, 147.75, 138.47, 137.64 132.45, 129.94, 129.86, 129.71, 128.24, 127.69, 124.04, 123.95, 94.32, 80.24, 47.86; EIMS: 391 [M+]; Elemental Analysis: Calculated (found) for $C_{16}H_{14}ClN_5O_3S$: % C, 49.04 (49.01); H, 3.60 (3.62); N, 17.87 (17.90); S, 8.18 (8.20).

5-((2-((4-chlorobenzyl)oxy)-2-(p-tolyl)ethyl)thio)-1H-tetrazole (6g): M.P. 163-165°C; Yield: 91%; IR (KBr, cm^{-1}): 3187 (-NH), 3066 (Aromatic-C-H), 1608 (>C=N), 1124 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.67 (s, 1H, -NH of tetrazole), 8.78-7.29 (m, 8H, Ar-H), 6.13 (t, 2H, -CH_2), 5.69 (s, 1H, -CH), 4.67 (d, 2H, -CH_2), 2.29 (s, 3H, -CH3); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm); 161.83, 141.73, 138.57, 136.31, 135.28, 130.91, 130.33, 129.42, 122.89, 128.01, 126.94, 126.13, 98.36, 78.53, 46.29, 22.78; EIMS: 360 [M+]; Elemental Analysis: Calculated (found) for $C_{17}H_{17}ClN_4OS$: % C, 56.58 (56.56); H, 4.75 (4.78); N, 15.53 (15.52); S, 8.89 (8.87).

5-((2-((2,4-dichlorobenzyl)oxy)-2-(4-nitrophenyl)ethyl)thio)-1H-tetrazole (6l): M.P. 187-189°C; Yield: 90%; IR (KBr, cm^{-1}): 3171 (-NH), 3129 (Aromatic-C-H), 1597 (>C=N), 1079 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.29 (s, 1H, -NH of tetrazole), 8.16-7.42 (m, 8H, Ar-H), 6.54 (t, 1H, -CH), 5.71 (s, 2H, -CH_2), 5.29 (d, 2H, -CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm); 158.73, 147.62, 136.81, 135.19, 134.57, 133.66, 132.95, 129.36, 128.47, 128.92, 126.63, 124.52, 96.39, 71.98, 43.74; EIMS: 425 [M+]; Elemental Analysis: Calculated (found) for $C_{16}H_{13}Cl_2N_5OS$: % C, 45.08 (45.05); H, 3.07 (3.09); N, 16.43 (16.45); S, 7.52 (7.54).

5-((2-((2,4-dichlorobenzyl)oxy)-2-phenylethyl)thio)-1H-tetrazole (6p): M.P. 165-167°C; Yield: 85%; IR (KBr, cm^{-1}): 3159 (-NH), 3143 (Aromatic-C-H), 1588 (>C=N), 1069 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ, ppm): 8.67 (s, 1H, -NH of tetrazole), 8.47-7.72 (m, 9H, Ar-H), 6.58 (t, 1H, -CH), 6.08 (s, 2H, -CH_2), 5.78 (d, 2H, -CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm); 159.82, 142.53, 137.86, 135.28, 134.93, 131.47, 130.61, 129.77, 129.03, 128.94, 127.84, 127.06, 120.24, 102.33, 82.47, 44.80; EIMS: 380 [M+]; Elemental Analysis: Calculated (found) for $C_{16}H_{14}Cl_2N_4OS$: % C, 50.40 (50.37); H, 3.70 (3.67); N, 14.69 (14.66); S, 8.41 (8.39).

BIOLOGY

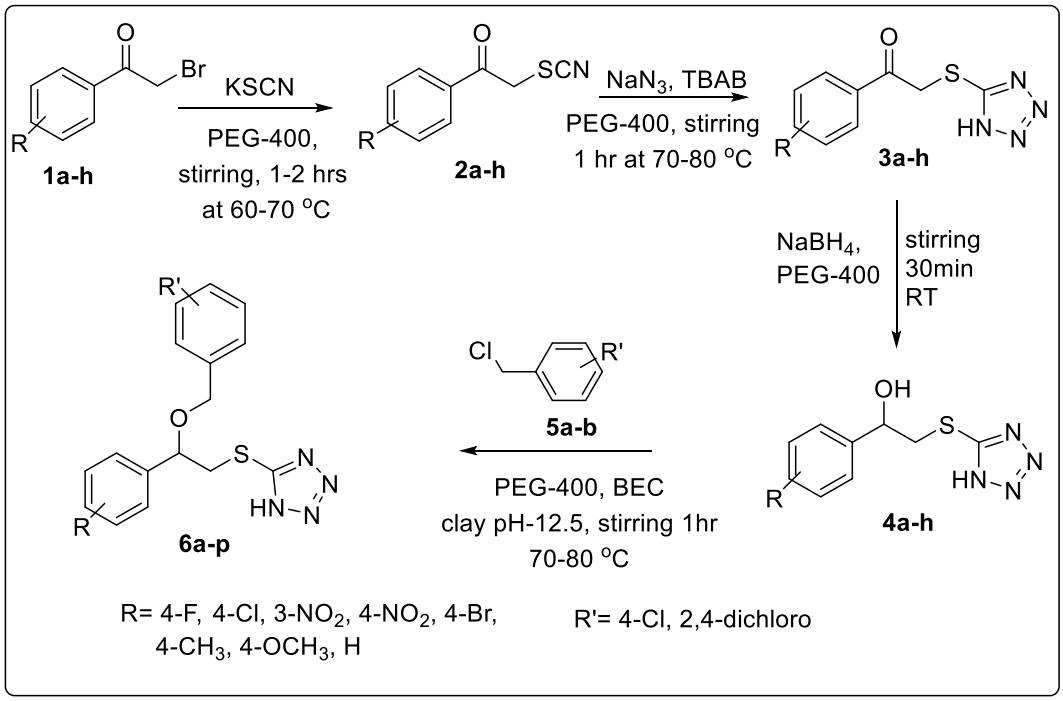
All the newly synthesized target compounds (**6a-p**) were evaluated for their in vitro antibacterial and antifungal activities at 100µg/mL concentration against Staphylococcus aureus (MTCC), Bacillis subtilis (MTCC) as examples of Gram-positive bacteria and Esherichia coli (MTCC), and Klebsiella pneumoniae (MTCC) as examples of Gram-negative

bacteria. We also screened them for their in vitro antifungal potential against Aspergillus niger (MTCC282),Aspergillus flaus (MTCC 3008), and Candida albican (MTCC 227). The evolution of preliminary antibacterial and antifungal activities was determined by agar-diffusion method[liv] using a 1 cm micro-plate well. Ciprofloxacin and Fluconazol were used as standard drugs for biological screening. The results of biological screening against the standard strains recorded in **Fig. 1** and **Fig. 2** which manifestly depicted the distinct sense of antibacterial and fungal strains toward the tested compounds.

RESULT AND DISCUSSION:

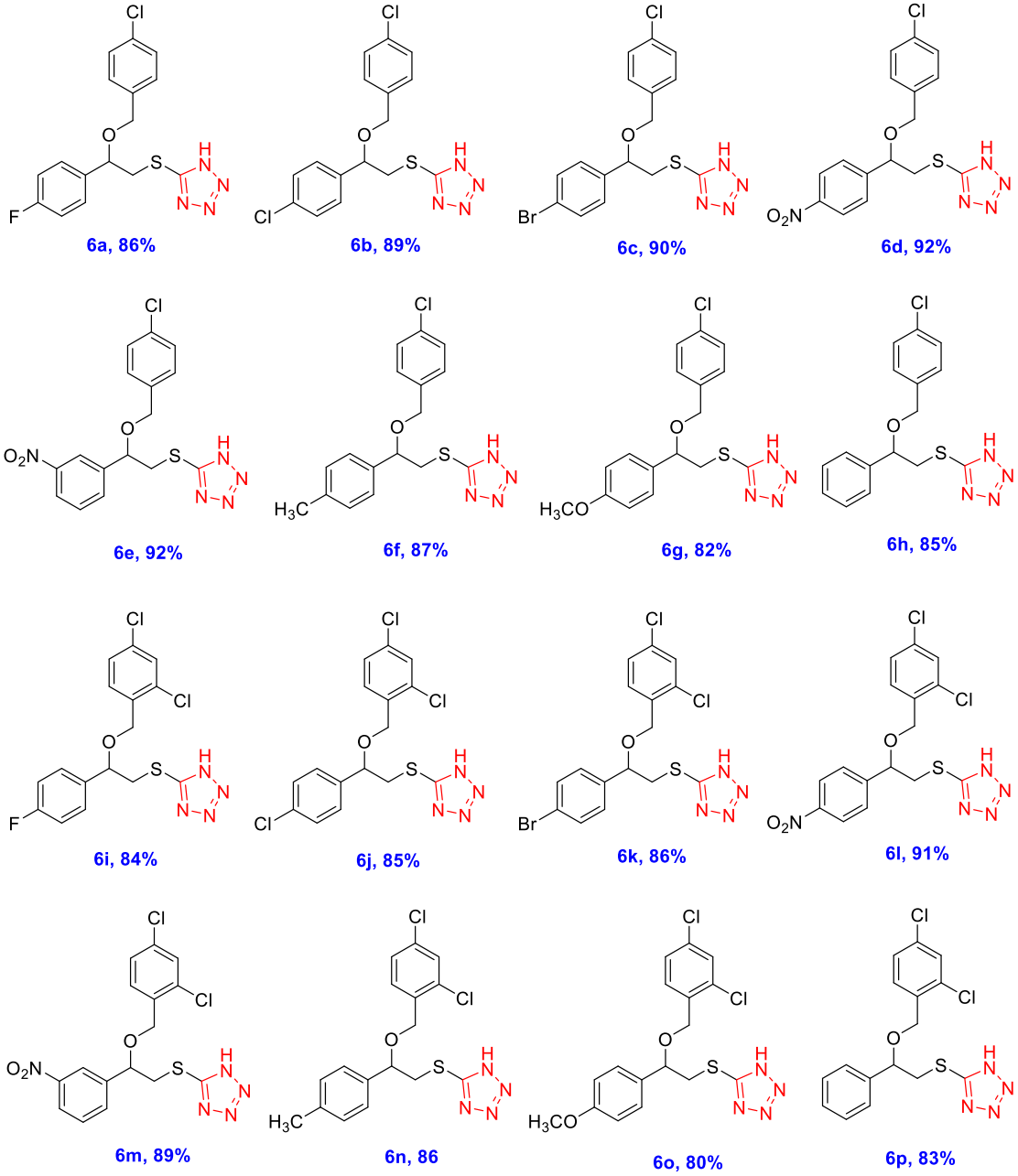
CHEMISTRY

The synthetic pathway of the target compound chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole derivatives **(6a-p)** is summarized in **Scheme 1.**



Scheme 1. Synthetic pathway of *chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole* derivatives **(6a-p)**

This scheme involves a reaction between substituted phenacyl bromide (**1a-h**) with potassium thiocyanate in PEG-400 to provide the thiocyanated intermediate (**2a-h**). Compound (**2a-h**) treated with sodium azide in the presence of TBAB (Tetra Butyl Ammonium Bromide) as a phase transfer catalyst and PEG-400 to create a cyclized intermediate. (**3a-h**). Further, the C=O bond of the compound (**3a-h**) undergoes reduction by sodium borohydride ($NaBH_4$) in PEG-400 at room temperature to yield the next intermediate products (**4a-h**). Then the compounds (**4a-h**) were to couple with chlorobenzyl chloride (**5a-b**) by using catalytic amount BEC in PEG-400 to give the targeted compound chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole derivatives (**6a-p**) in admirable yields (**Scheme 2**).



Scheme 2. Variation of substituent on tetrazole (**6a-p**)

To achieve the target compounds (**6a-p**), here we reported an eco-friendly synthetic route which accomplished by using BEC (pH 12.5) as a heterogeneous catalyst to attain the basic media in PEG-600 as a green reaction solvent.

Finally, the structures of the synthesized products were determined by IR, ^{1}H NMR, ^{13}C NMR, ESI-MS, and elemental analysis data. From the IR spectra of compound **3a**, the disappearance of the nitrile peak (-C≡N) of compound **2a** at 2155 cm^{-1} and new -NH stretching absorption at 3249 cm^{-1} confirms the formation of the tetrazole ring. The absorption band for C=O group appears at 1739 cm^{-1}. Aliphatic and aromatic -C-H stretching frequency was at 2968 cm^{-1} and 3028 cm^{-1}, respectively. In 1H NMR spectral analysis, compound **3a** displayed a singlet of one proton present in the tetrazole ring at δ 8.21 ppm. Another singlet for two protons of the methylene group exhibits at δ 3.94 ppm. The remaining protons appeared in their corresponding aromatic region. The IR spectra of compound **4a** showed a strong absorption

band at 3423 cm^{-1} for -OH group which is clear for the reduction of >C=O group. Absence of a carbonyl absorption band in the region 1730-1680 cm^{-1} also confirmed the reduction of carbonyl group. The ^1H NMR spectra of compound **4a** observed a singlet at δ 13.74 ppm for –OH proton. Another singlet displayed at δ 7.98 ppm for tetrazole ring proton. Similarly, a triplet was detected for one proton of aliphatic –CH at δ 6.23 ppm and a doublet for two methylene protons at δ 5.29 ppm. The remaining protons were observed in their corresponding aromatic region. The IR spectra of final compound **6a** displayed the expected absorption band for –NH of the tetrazole ring at 3223 cm^{-1}. In the 1H NMR spectrum analysis, compound **6a** displayed a singlet resonating at δ 8.32 ppm attributed to –NH proton of the tetrazole ring. A triplet for –CH and a doublet for $–CH_2$ proton appeared at δ 6.35 and δ 5.15 ppm, respectively. A sharp singlet at δ 5.86 ppm attributed to another methylene protons. Remaining aromatic protons are displayed at their corresponding region. Similarly, in 13C NMR spectra, >C=N group of tetrazole ring appeared at 160 ppm, while asymmetric carbon appeared at 104 ppm. Methylene carbons are displayed at 79 ppm and 46 ppm. The aromatic carbons appeared between 143-121 ppm. In addition, ESI-MS confirmed the identity of compound **6b** at m /z 381.

BIOLOGY

All the newly synthesized target compounds (**6a-p**) were evaluated for their in vitro antibacterial and antifungal activities at 100µg/mL concentration against S. aureus, B. subtilis as Gram-positive bacteria and E. coli, P. aeruginosa and K. pneumoniae as Gram-negative bacteria. We also screened them for their in vitro antifungal potential against A. niger, A. flaus, and C. albican. Ciprofloxacin and Fluconazole are used as standard drugs for biological screening. We have recorded the results of biological screening against the standard strains depicted in **Fig 2 and Fig 3**. The figures demonstrate the different reactions of antibacterial and antifungal strains to the tested compounds.

ANTIBACTERIAL ACTIVITY:

Regarding the antibacterial activity, the results revealed that the newly synthesized compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. Some of the synthesized compounds showed relatively high sensitivity against Gram positive bacterial strains, namely S. aureus and B. subtilis. In this view, compound **6l** was equipotent for ciprofloxacin (MIC 3.12g/mL) against S.aureus, whereas the analogs **6e**, and **6m** (MIC 6.25g/mL) were 50% less active than ciprofloxacin. Compound **6k** (MIC 12.5g/mL) showed 25% of the activity of ciprofloxacin against the same species. Concerning the activity against B. subtilis, compound **6l** (MIC 6.25g/mL) displayed the best activity, which represented half the potency of ciprofloxacin. On the other side, analogs **6d**, **6e**, **6k**, and **6m** (MIC 12.25g/mL) exhibited 25% of the potency then ciprofloxacin against the same species. Investigation of antibacterial activity of the active compounds against the three tested Gram negative strains revealed that two analogs namely **6d** and **6l** could produce moderate growth inhibitory activity against E. coli (MIC 6.25g/mL) which was 25% of the activity of ciprofloxacin. Whereas, compounds **6e** and **6m**, (MIC 12.5g/mL), exhibited moderate activity against the same species. Meanwhile, the activity against P. aeruginosa, compounds **6d**, **6e**, **6l**, and **6m** (MIC 12.5g/mL) exhibited 50% potency as compared to ciprofloxacin. Reaming synthesized compounds also proves to be weakly sensitive to the examined strains. Screening results are exhibited in **Fig 2**.

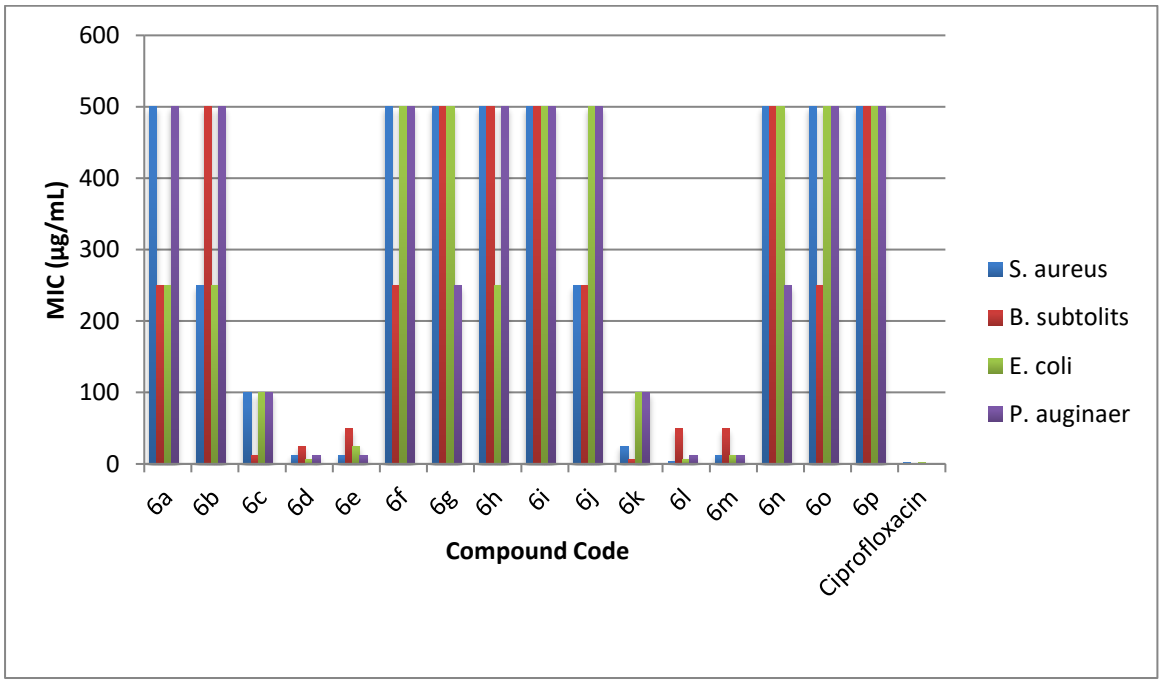


Fig 2. Graphical representation of antibacterial screening of chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole derivatives (**6a-p**)

ANTIFUNGAL ACTIVITY:

We carried out the antifungal activity of recently synthesized compounds on A. niger, A. flavus, and C. albicans. The screening result reveals that the compound **6g** shows potent antifungal activity against both the three strains. Compounds **6h** and **6o** displayed very good potency, whereas a compound **6f**, **6n**, and **6p** show pleasurable activity against A. niger, A. flavus and C. albicans. However, the remaining compounds exhibited moderate potency as compared to Fluconazol. We display the antifungal screening results in **Fig. 3**

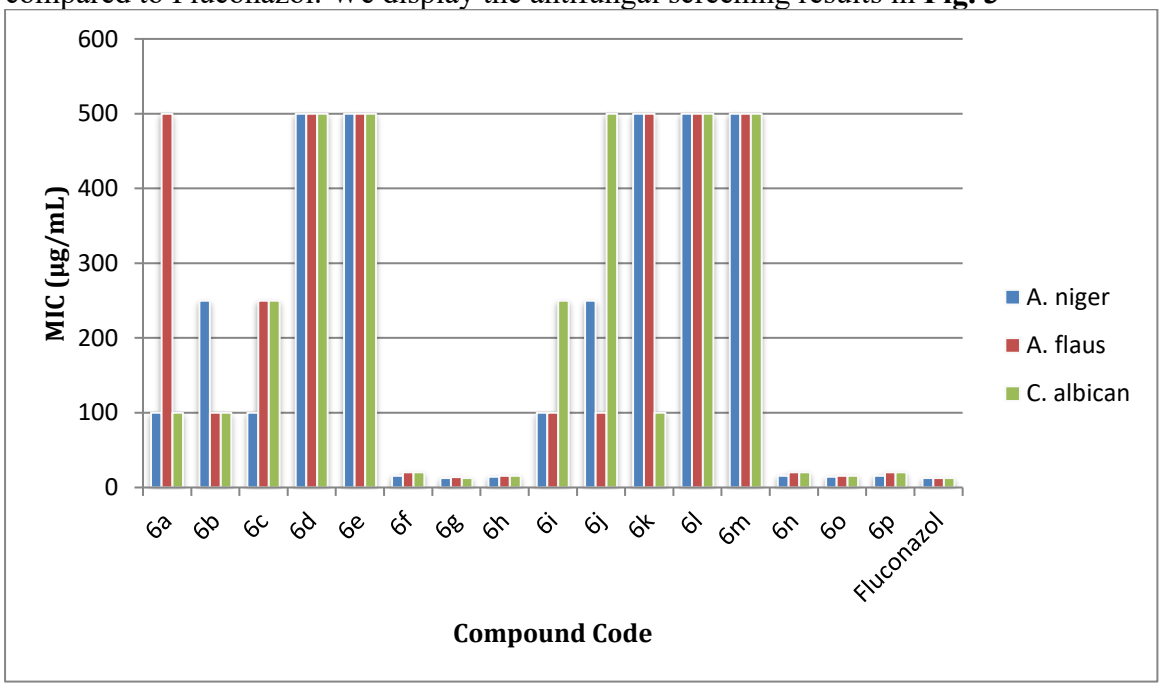


Fig 3. Graphical representation of antifungal screening of chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole (**6a-p**)

CONCLUSIONS:

The research study focused on the designing and synthesis of novel potentially active antibacterial and antifungal analogs on the tetrazole system. The result of in vitro pharmacological screening shows that the analogs containing electron-withdrawing substituent at the para position exhibit the highest antibacterial activity as compared to the standard drug. Whereas, compounds that have electron donating groups at para position showed promising antifungal activities. Overall, the pharmacological result of all the synthesized novel derivatives of the tetrazole system can point out the active leads which provide a powerful incentive for further research area.

REFERENCES:

i. Zhang L.; Peng X.M.; Damu G.L.V.; Geng R.X.; Zhou C.H; Comprehensive Review in Current Developments of Imidazole-Based Medicinal Chemistry; Med. Res. Rev.; 2014, **34** (2), 340-437.

ii. Peng X.M.; Damu G.L.V.; Zhou C.H.; Current Developments of Coumarin Compounds in Medicinal Chemistry; Curr. Pharm. Des.; 2013, **19** (21), 3884-3930.

iii. Scheffler R.J.; Colmer S.; Tynan H.; Demain A.L.; Gullo V.P; Antimicrobials, drug discovery, and genome mining; Appl. Microbiol. Biotechnol.; 2013. **97** (3), 969-978.

iv. Constantinos N.; Ting Z.; Alexander D.; Tetrazoles via Multicomponent Reactions; Chem. Rev.; 2019. **119** (3), 1970-2042.

v. Jaroslav R.; Katerina V.; Alexandr H.; Synthesis and Functionalization of 5-Substituted Tetrazoles; Eur. J. Org. Chem.; 2012, 2012 (31), 6101-6118.

vi. Wei C.X.; Bian M.; Gong G.H.; Tetrazolium Compounds: Synthesis and Applications in Medicine; Molecules.; 2015. **20** (4), 5528-5553.

vii. Frija L.M.T.; Ismael A.; Cristiano M.L.S.; Photochemical Transformations of Tetrazole Derivatives: Applications in Organic Synthesis; Molecules.; 2010, **15** (5), 3757-3774.

viii. Shmatova O.I.; Nenajdenko V.G.; Synthesis of Tetrazole Derived Organocatalysts via Azido-Ugi Reaction with Cyclic Ketimines; J. Org. Chem.; 2013, **78** (18), 9214-9222.

ix. Ostrovskii V.A.; Trifonov R.E.; Popova E.A.; Medicinal chemistry of tetrazoles; Russ. Chem. Bull.; 2012, **61** (4), 768-780.

x. Rostom S.A.F.; Ashour H.M.A.; Razik H.A.E.; Fattah A.E.H.; Fattah A.E.; Nagwa N.E.D.; Azole antimicrobial pharmacophore-based tetrazoles: Synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents; Bio. Org. Med. Chem.; 2009, **17**(6), 2410-2422.

xi. Feng G.; Jiaqi X.; Gang H.; Current scenario of tetrazole hybrids for antibacterial activity; Eur. J. Med. Chem.; 2019; **184**, 111744.

xii. Daniel S.; Dobrowolski M.A.; Roszkowski P.; Bielenica A.; Stefanska J.; Kolinski M.; Kmiccik S.; Jozwiak M.; Wrzosek M.; Olejarz W.; Struga M.; Design and synthesis of novel 1H-tetrazol-5-amine based potent antimicrobial agents: DNA topoisomerase IV and gyrase affinity evaluation supported by molecular docking studies; Eur. J. Med. Chem.; 2018, **156** (5), 631-640.

xiii. Kritchenkov A.S.; Lipkan N.A.; Kurliuk A.V.; Shakola T.V.; Egorov A.R.; Volkova O.V.; Meledina T.V.; Suchkova E.P.; Zabodalova L.A.; Dysin A.P.; Synthesis and Antibacterial Activity of Chitin Tetrazole Derivatives; Pharm. Chem. J**.;** 2020, **54** (2), 138-141.

xiv. Edyta L.C.; Anna K.; Malgorzata G.; Miroslawa K.; Monika S.; Synthesis of tetrazole derivatives bearing pyrrolidine scaffold and evaluation of their antifungal activity against Candida albicans. Eur. J. Med. Chem.; 2019, **164** (15), 106-120.

xv. Shaikh S.K.; Kamble R.R.; Somagond S.M.; Devarajegowda H.C.; Dixit S.R.; Joshi S.D.; Tetrazolylmethylquinolines: Design, docking studies, synthesis, anticancer and antifungal analyses; Eur. J. Med. Chem.; 2017, **128** (10), 258-273.

xvi. Mikolaichuk O.V.; Zarubaev V.V.; Muryleva A.A.; Esaulkova Y.L.; Spasibenko D.V.; Batyrenko A.A.; Kornyakov I.V.; Trifonov R.E.; Synthesis, structure, and antiviral properties of novel 2-adamantyl-5-aryl-2H-tetrazoles; Chem. Het. Comp.; 2021, **57** (4)**,** 442-447.

xvii. Gao C.; Chang L.; Xu Z.; Yan X.F.; Ding C.; Zhao F.; Wu X.; Feng L.S.; Recent advances of tetrazole derivatives as potential anti-tubercular and anti-malarial agents; Eur. J. Med. Chem.; 2019, **163** (1), 404-412.

xviii. Zhang S.; Xu Z.; Gao C.; Ren Q.C.; Le C.; Lv Z.S.; Feng L.S.; Triazole derivatives and their anti-tubercular activity; Eur. J. Med. Chem.; 2017, **138** (29), 501-513.

xix. Elena A.P.; Aleksandra V.P.; Rostislav E.T.; Tetrazole Derivatives as Promising Anticancer Agents; Anticancer Agents Med. Chem.; 2018, **17** (14), 1856-1868.

xx. Kohler S.C.; Vahdati S.; Scholz M.S.; Wiese M.; Structure activity relationships, multidrug resistance reversal and selectivity of heteroarylphenyl ABCG2 inhibitors; Eur. J. Med. Chem.; 2018, **146** (25), 483-495.

xxi. Kalaria P.N.; Karad S.C.; Raval D.K.; A review on diverse heterocyclic compounds as the privileged scaffolds in antimalarial drug discovery; Eur. J. Med. Chem.; 2018, **158** (5), 917-927.

xxii. Pandey S.; Agarwal P.; Srivastava K.; Rajkumar S.; Puri S.K.; Verma P.; Saxena J.K.; Sharma A.; Lal J.; Chauhan P.M.S.; Synthesis and bioevaluation of novel 4-aminoquinoline-tetrazole derivatives as potent antimalarial agents; Eur. J. Med. Chem.; 2013. **66** (8), 69-81.

xxiii. Li Y.; Pasunooti K.K.; Li R.J.; Liu W.; Head S.A.; Shi W.Q.; Liu J.O.; Novel Tetrazole-Containing Analogues of Itraconazole as Potent Antiangiogenic Agents with Reduced Cytochrome P450 3A4 Inhibition; J. Med, Chem.; 2018, **61**(24), 11158-11168.

xxiv. Li Y.; Pasunooti K.K.; Peng H.; Li R.J.; Shi W.Q.; Liu W.; Cheng Z.; Head S.A.; Liu J.O.; Design and Synthesis of Tetrazole- and Pyridine-Containing Itraconazole Analogs as Potent Angiogenesis Inhibitors; Med. Chem. Lett.; 2020, **11**(6), 1111-1117.

xxv. Esirden I.; Tanc M.; Supuran C.T.; Kaya M.; Microwave assisted synthesis of novel tetrazole/sulfonamide derivatives based on octahydroacridine, xanthene and chromene skeletons as inhibitors of the carbonic anhydrases isoforms I, II, IV and VII; Bioorg. Med. Chem. Lett.; 2017, **27** (1), 86-89.

xxvi. Lamie P.F.; Philoppes J.N.; Azouz A.A.; Safwatc N.M.; Novel tetrazole and cyanamide derivatives as inhibitors of cyclooxygenase-2 enzyme: design, synthesis, anti-inflammatory evaluation, ulcerogenic liability and docking study; J. Enzyme. Inhib. Med. Chem.; 2017, **32** (1), 805-820.

xxvii. Zhan P.; Li Z.; Liu X.; Clercq E.D.; Sulfanyltriazole/tetrazoles: a promising class of HIV-1 NNRTIs; Mini. Rev. Med. Chem.; 2009. **9** (8),1014-1023.

xxviii. Zhan P.; Liu H.; Liu X.; Wang Y.; Pannecouque C.; Witvrouw M.; Clercq E.D.; Synthesis and anti-HIV activity evaluation of novel N′-arylidene-2-[1-(naphthalen-1-yl)-1H-tetrazol-5-ylthio]acetohydrazides; Med. Chem. Res.; 2010, **19** (7), 652-663.

xxix. Rajasekaran A.; Thampi P.P.; Synthesis and analgesic evaluation of some 5-[β-(10-phenothiazinyl)ethyl]-1-(acyl)-1,2,3,4-tetrazoles; Eur. J. Med. Chem.; 2004. **39** (3), 273-279.

xxx. Kharb R.; Sharma P.C.; Yar M.S.; Pharmacological significance of triazole scaffold; J. Enzy. Inhib. Med. Chem.; 2011; **26** (1), 1-21.

xxxi. Kushwaha O.; Fatima S.; Upadhyay A.; Gupta S.; Bhagwati S.; Baghel T.; Siddiqi M.I.; Nazir A.; Sashidhara K.V.; Synthesis, biological evaluation and molecular dynamic simulations of novel Benzofuran-tetrazole derivatives as potential agents against Alzheimer's disease; Bioorg. Med. Chem. Lett.; 2019, **29** (1), 66-72.

xxxii. Hassan A.E.A.; Abou-Elkhair R.A.I.; Parker W.B.; Allan P.W.; Secrist J.A.; 6-Methylpurine derived sugar modified nucleosides: Synthesis and evaluation of their substrate activity with purine nucleoside phosphorylases; Bioorg. Chem.; 2016, **65** (4), 9-16.

xxxiii. Cardoso C.S.; Silva D.P.B.; Silva D.M.; Florentino I.F.; Fajemiroye J.O.; Moreira L.K.S.; Vasconcelos J.P.; Sanz G.; Vaz B.G.; Liao L.M.; Lima D.S.; Santos F.C.A.; Menegatti R.; Costa E.A.; Mechanisms involved in the antinociceptive and anti-inflammatory effects of a new triazole derivative: 5-[1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl]-1H-tetrazole (LQFM-096); Inflammopharmacol. 2020, **28** (4), 877-892.

xxxiv. Rajasekaran A.; Thampi P.P.; Synthesis and antinociceptive activity of some substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones; Eur. J. Med. Chem.; 2005, **40** (12),1359-1364.

xxxv. Bian M.; Deng X.Q.; Gona G.H.; Wei C.X.; Quan Z.S.; Synthesis and anticonvulsant activity evaluation of 6-substituted-[1,2,4]triazolo[3,4-a](tetrazolo[5,1-a])phthalazine derivatives; J. Enzy. Inhib. Med. Chem.; 2013, **28** (4), 792-800.

xxxvi. Rajasekaran A.; Murugesan S.; AnandaRajagopal K.; Antibacterial, antifungal and anticonvulsant evaluation of novel newly synthesized 1-[2-(1H-tetrazol-5-yl)ethyl]-1H-benzo[d][1,2,3]triazoles; Arch. Pharm. Res.; 2006, **29** (7), 535-540.

xxxvii. Liljebris C.; Larsen S.D.; Ogg D.; Palazuk B.J.; Bleasdale J.E.; Investigation of Potential Bioisosteric Replacements for the Carboxyl Groups of Peptidomimetic Inhibitors of Protein Tyrosine Phosphatase 1B: Identification of a Tetrazole-Containing Inhibitor with Cellular Activity; J. Med. Chem.; 2002, **45** (9), 1785-1798.

xxxviii. Myznikov L.V.; Hrabalek A.; Koldobskii G.I.; Drugs in the tetrazole series. (Review); Chem. Heterocycl. Compd.; 2007, **43** (1), 1-9.

xxxix. Kozikowski A.P.; Zhang J.; Nan F.; Petukhov P.A.; Grajkowska E.; Wroblewski J.T.; Yamamoto T.; Bzdega T.; Wroblewska B.; Neale J.; Synthesis of Urea-Based Inhibitors as Active Site Probes of Glutamate Carboxypeptidase II: Efficacy as Analgesic Agents; J. Med. Chem.; 2004, **47** (7), 1729-1738.

xl. Koldobskii G.I.; Ostrovskii V.A.; Tetrazoles; UspKhim.; 1994, **63** (10), 847-865.

xli. Fischer D.; Klapotke T.M.; Stierstorfer J.; Angew.; 1,5-Di(nitramino)tetrazole: High Sensitivity and Superior Explosive Performance; ChemInt. Ed.; 2015, **54** (35), 10299-10302.

xlii. Klapotke T.M.; Stierstorfer J.; Green Energetic Materials, John Wiley & Sons, Ltd, 2014.

xliii. Dippold A.A.; Izsak D.; Klapötke T.M.; Pflüger C.; Combining the Advantages of Tetrazoles and 1,2,3-Triazoles: 4,5-Bis(tetrazol-5-yl)-1,2,3-triazole, 4,5-Bis(1-hydroxytetrazol-5-yl)-1,2,3-triazole, and their Energetic Derivatives; Chem. Eur. J.; 2016, **22** (5), 1768-1778.

xliv. Yu Z.; Ho L.Y.; Wang Z.; Lin Q.; Discovery of new photoactivatablediaryltetrazoles for photoclick chemistry via 'scaffold hopping'; Bioorg. Med. Chem. Lett.; 2011, **21** (17), 5033-5036.

xlv. Wang Y.; Lin Q.; Synthesis and Evaluation of PhotoreactiveTetrazole Amino Acids; Org. Lett. 2009, **11** (16), 3570-3573.

xlvi. Wang Y.; Hu W.J.; Song W.; Lim R.K.V.; Lin Q.; Org. Lett.; 2008, **10** (17), 3725-3728.

xlvii. Wurzenberger M.H.H.; Gruhne M.S.; Lommel M.; Szimhardt N.; Klapotke T.M.; Stierstorfer J.; Comparison of 1-Ethyl-5H-tetrazole and 1-Azidoethyl-5H-tetrazole as Ligands in Energetic Transition Metal Complexes; Chem. Asian. J.; 2019, **14** (11), 2018-2028.

xlviii. Yang L.; Shen Y.; Chen Y.; Pan X.; Wang X.; A novel octamolybdate-based metal-organic complex constructed from a bis(tetrazole)-functionalized thioether ligand and an Anderson-type polyoxometalate; Inorg. Chem. Comm.; 2019, **108** (10), 107493.

xlix. Ambhore A.N.; Kamble S.S.; Kadam S.N.; Kamble R.D.; Hebade M.J.; Hese S.V.; Gaikwad M.V.; Mesharam R.J.; Gacche R.N.; Dawane B.S.; Design, synthesis and in silico study of pyridine based 1, 3, 4-oxadiazole embedded hydrazinecarbothioamide derivatives as potent anti-tubercular agent; Comp. Bio. Chem.; 2019, **80** (6), 54-65.

l. Ambhore A.N.; Surywanshi V.D.; Kamble R.D.; Hese S.V.; Mogale P.P.; Kadam S.S.; Dawane B.S.; Synthesis and biological investigation of isoxazolo [4, 5-e][1, 2, 4] triazine derivatives; Der. Phar. Chem.; 2015, **7** (4), 278-283.

li. Kadam S.N.; Ambhore A.N.; Kamble R.D.; Wakhradkar M.G.; Gavhane P.D.; Gaikwad M.V.; Gunturu K.C.; Dawane B.S.; Metal-free efficient thiolation of C(sp2) functionalization via in situ-generated NHTS for the synthesis of novel sulfenylated 2-aminothiazole and imidazothiazole; N. J. Chem.; 2021, **45** (12), 4632-4637.

lii. Gavhane P.D.; Kadam S.N.; Ambhore A.N.; Dawane B.S.; Silica-supported sodium carbonate: an efficient heterogeneous catalyst for the synthesis of new thiazolopyrimidine derivatives; Res. Chem. Inter.; 2021, **47**, 3999-4011.

liii. Kadam S.N.; Ambhore A.N.; Hebade M.J.; Kamble R.D.; Hese S.V.; Gaikwad M.V.; Gavhane P.D.; Dawane B.S.; Metal-Free One-Pot Chemoselective thiocyanation of imidazothiazoles and 2-aminothiazoles with in situ generated N-thiocyanatosuccinimide; Synlett.; 2018, **29** (14), 1902-1908.

liv. Hese S.V.; Kamble R.D.; Mogale P.P.; Kadam S.S.; Hebade M.J.; Ambhore A.N.; Dawane B.S.; Green synthesis and antimicrobial evaluation of pyrido [1, 2-a] pyrimidine-3-carbonitrile derivatives; Der. Pharma. Chem.; 2015, **7** (4), 249-256.

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Design and synthesis of novel derivatives of chlorobenzyl-tetrazole and studying them for antibacterial and antifungal activities

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Synthesis of a novel series of substituted chlorobenzyl-tetrazole derivatives and screened for their antibacterial and antifungal activities. Pharmaceutical investigation studies revealed that the most of newly synthesized compounds exhibits good antibacterial and antifungal activities against all the tested strains regarding the reference drug.