Design and Synthesis of Some New Derivatives of Chlorobenzyl-Oxy-Phenyl-Ethyl-Thio-1H-Tetrazole and Study Their Antibacterial and Antifungal Activity

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Design and synthesis of some new derivatives of chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole and study their antibacterial and antifungal activity

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Abstract

In recent years tetrazole scaffolds have been attracted interest in the field of synthetic and medicinal chemistry research. The unique structure of the tetrazole derivatives exhibits widespread applications in biology and technology. The close structural resemblance with carboxylic acid acts as a booster of the latter. Due to this diversified potential utilization, several methods are reported for the synthesis of tetrazole scaffolds.

Here in this chapter, we describe the synthesis of chlorobenzyl-oxy-phenyl-ethyl-thio-1H-tetrazole derivatives (**6a-p**). The newly synthesized derivatives are characterized by spectral characterization and screened for their antifungal activity. Among these, some of the newly synthesized compounds show potent antifungal activity.

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

Introduction:

Escalation of multidrug resistance strain, the evolution of incurable micro-organism, and gain in pathogenic microbes alarm the seriousness of microbial infection [1-3]. To oversight this, researchers are uniformly focused on the synthesis of influential and adequate antimicrobial agents. Numerous antimicrobial scaffolds are disclosed and applied extensively for the treatment, prevention, and control of microbial infection. But still, there are some problems like, adverse

effects, high toxicity, and narrow antimicrobial spectrum which are unresolved. Literature in recent years endorsed that, the total number of publications on the chemistry of N-containing heterocycles consequential boost to discover the new drugs and assuring their biological activity which mainly contains tetrazole moiety.

Tetrazole is an important heterocyclic scaffold containing nitrogen. It is a unique nitrogen-rich compound among the known stable heterocycles and in spite not found in nature [4]. In 1885, for the first time synthesized and characterized compound embedding a tetrazole ring, is 2-phenyl-2H-tetrazole-5-carbonitrile [5]. Tetrazole is a remarkable synthetic scaffold that noticed wide applications in various fields like in medicinal, pharmacological, biochemistry, and in industrial [6-10].

Tetrazole is the lead motif in the field of medicinal chemistry. The uncommon, poly-nitrogen, electron-rich planer structure of the tetrazole ring is answerable to exhibit widespread applications for the treatment of various diseases especially antimicrobial [11-12], antifungal [13-14], antiviral [15-16], antitubercular [17-18], anticancer [19-20], antimalarial [21-22], antiangiogenic [23-24], anti-inflammatory [25-26], anti-HIV [27-28], analgesic [29-30], anti-Alzheimer [31-32], antinociceptive [33-34], and anticonvulsant [35-36]. Weak interactions like hydrogen bonding, van der wals force, or coordination bonding are responsible for binding the tetrazole ring easily with the various enzymes or receptors of microbes and are successfully applied as components for comprehensive pharmaceutical applications [37]. Food and Drug Administration (FDA) approved several drugs possessing tetrazole scaffold as an important pharmaceutical agent [38]. Metabolic stable surrogates for carboxylic acid group and lipophilic spacers of tetrazole derivatives make it important in drug design [39]. It has also relived that the

lipophilic nature of tetrazole ring in a drug revamps its oral bioavailability and cell penetration [37].

Besides this, tetrazole has noticed a broad spectrum of applications in materials like information recording systems [40], rocket propellants [41-42], specialty explosives [43], and agrochemical applications [44-47]. As befitting coordination property, it has also capable to form nitrogen containing ligands and designing stable complex with disparate metal ions and react as precursors to varied nitrogen containing heterocyclic compounds in organic synthesis [48]. Thus, the whispered applications of tetrazole skeleton in numerous fields make interest for the development of novel tetrazole derivatives. Some of them are displayed in **Fig. 1.1**.

Fig 1. Some bioactive molecules bearing tetrazole ring.

By studying the above literature data and as a part of our research to design and synthesis bioactive heterocyclic compounds [49-53], in the present work, our strategy is to synthesize tetrazole embedded novel derivatives and study their antibacterial and antifungal activity.

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 bromides (**1a-h**) with potassium thiocyanate in PEG-400 to provide the thiocyanated intermediate (**2a-h**). In turn compound (**2a-h**) was treated with sodium azide in presence of TBAB (Tetra Butyl Ammonium Bromide) as a phase transfer catalyst and PEG-400 to generate cyclized intermediate (**3a-h**). Further, the C=O bond of the compound (**3a-h**) undergoes reduction by Sodium borohydride (NaBH₄) in PEG-400 at room temperature to yield the next intermediate products (**4a-h**). Then

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-1*H*-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 accomplishes 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, ^1H NMR, ^{13}C NMR, ESI-MS and elemental analyses data. The IR spectra of compound **3a**, formation of tetrazole ring was confirmed by the disappearance of nitrile peak ($-\text{C}\equiv\text{N}$) of compound **2a** at 2155 cm^{-1} and formation of expected $-\text{NH}$ stretching abortion at 3249 cm^{-1} . Absorption band for $\text{C}=\text{O}$ group was absorbed at 1739 cm^{-1} . Aliphatic and aromatic $-\text{C}-\text{H}$ stretching frequency was located at 2968 cm^{-1} and 3028 cm^{-1} respectively. In ^1H NMR spectral analysis, compound **3a** displayed a singlet of one proton present on tetrazole ring at δ 8.21 ppm. Another singlet for two protons of methylene group exhibits at δ 3.94 ppm. Whereas, remaining protons appeared at their corresponding aromatic region. The IR spectra of compound **4a** showed a strong absorption band at 3423 cm^{-1} for $-\text{OH}$ group which is evident for the reduction of $>\text{C}=\text{O}$ group. It is also confirmed by the absence of carbonyl absorption band in the region $1730\text{--}1680\text{ cm}^{-1}$. In ^1H NMR spectra of compound **4a** observed a singlet at δ 13.74 ppm for one proton of $-\text{OH}$. Another singlet is displayed at δ 7.98 ppm for one proton of tetrazole ring. Similarly a triplet is detected for one proton of aliphatic $-\text{CH}$ at 6.23 ppm and a doublet for two methylene protons at 5.29 ppm. The remaining protons were observed in their predictable aromatic region. The IR spectra of final compound **6a** displayed the expected absorption band for $-\text{NH}$ of 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 $-\text{NH}$ proton of tetrazole ring. A triplet for $-\text{CH}$ and a doublet for $-\text{CH}_2$ proton appeared at δ 6.35 and δ 5.15 ppm respectively. A sharp singlet at δ 5.86 ppm is attributed to other methylene protons. Whereas, remaining all other aromatic protons were displayed at their

corresponding aromatic region. Similarly in ^{13}C NMR spectra, $>\text{C}=\text{N}$ group of tetrazole ring appeared at 160 ppm, while asymmetric carbon appeared at 104 ppm. Moreover, methylene carbons are displayed at 79 ppm and 46 ppm. Whereas the aromatic carbons were appear in the range of 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 100 $\mu\text{g/mL}$ concentration against *S. aureus*, *B. subtilis* as Gram-positive bacteria and *E.coli*, *P. aeruginosa* and *K. pneumoniae* as Gram-negative bacteria. They were also screened for their in vitro antifungal potential against *A. niger*, *A. flaus*, and *C. albican*. Ciprofloxacin and Fluconazol were used as standard drugs for biological screening. The results of biological screening against the standard strains are recorded in **Fig 2** and **Fig 3** which manifestly depicted the distinct sense of antibacterial and fungal strains toward 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 to ciprofloxacin (MIC 3.12 $\mu\text{g/mL}$) against *S.aureus*, whereas the analogs **6e**, and **6m** (MIC 6.25 $\mu\text{g/mL}$) were 50% less active than ciprofloxacin. Moreover, compound **6k** (MIC 12.5 $\mu\text{g/mL}$) showed 25% of the activity of ciprofloxacin against the same organism. Concerning the activity against *B. subtilis*, the best activity was displayed by compound **6l** (MIC 6.25 $\mu\text{g/mL}$), which represented half the potency of ciprofloxacin. On the other

side, analog **6d**, **6e**, **6k**, and **6m** (MIC 12.25g/mL) exhibited 25% of the potency then ciprofloxacin against the same species. On the other hand, investigation of antibacterial activity of the active compounds against the three tested Gram negative strains revealed that two analogs namely **6d** and **6l** were able to 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 organism. Meanwhile, the activity against *P. aeruginosa*, compound **6d**, **6e**, **6l**, and **6m** (MIC 12.5g/mL) exhibited 50% potency as compared to ciprofloxacin. The reaming synthesized compounds were proved to be weakly sensitive against the examined strains. The screening result was exhibited in **Fig 2**.

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

Antifungal Activity:

The antifungal activity of the recently synthesized compounds was carried out 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 good activity against *A. niger*, *A. flavus* and *C. albicans*. However, the remaining compounds exhibited moderate potency as compared to Fluconazol. All the antifungal screen results was displayed in **Fig 3**

Fig 2. 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 new potentially active antibacterial and antifungal analogs on the tetrazole system. The result of in vitro pharmacological screening demonstrates that, the analogs containing electron withdrawing substituent at 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 result pharmacological result of all the synthesized novel derivatives of the tetrazole system can point out the active leads which provide a powerful incentive for the further research area.

Material and Methods

Chemistry

All melting points of newly synthesized compounds are determined in an open capillary tube and are uncorrected. Used reagents and solvents are laboratory grade and purified. IR spectra were recorded on an FTIR Perkin Elmer/Schimidzu/Bruker spectrophotometer as KBr pellets. ^1H NMR (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 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) in PEG-400 was stirred continuously for 1-2 hrs at 60-70°C. Progress of the reaction was monitored by TLC and the product was separated from the crude reaction mixture in 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. This reaction mixture was stirred at 70-80°C for 1-2 hrs. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured in ice cold water to isolate the solid product. The solid products were collected by simple filtration method, dried and recrystallized 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 ($>\text{C}=\text{O}$), 1591 ($>\text{C}=\text{N}$), 1091 (C-S-C); ^1H 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, $-\text{CH}_2$); EIMS: 254 $[\text{M}^+]$; $\text{C}_9\text{H}_7\text{ClN}_4\text{OS}$.

2-((1H-tetrazol-5-yl)thio)-1-(4-bromophenyl)ethanone (3b)

IR (KBr, cm^{-1}): 3432 (-NH), 3012 (Ar, C-H), 1721 ($>\text{C}=\text{O}$), 1612 ($>\text{C}=\text{N}$); ^1H 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, $-\text{CH}_2$); EIMS: 299 $[\text{M}^+]$; $\text{C}_9\text{H}_7\text{BrN}_4\text{OS}$.

2-((1H-tetrazol-5-yl)thio)-1-(3-nitrophenyl)ethanone (3d)

IR (KBr, cm^{-1}): 3440 (-NH), 3011 (Ar, C-H), 1735 ($>\text{C}=\text{O}$), 1605 ($>\text{C}=\text{N}$), 1030 (C-S-C); ^1H NMR (400 MHz, DMSO- d_6 , TMS, δ , ppm): 8.67 (s, 1H, -NH of tetrazole), 8.31-7.87 (m, 4H, Ar-H), 4.05 (s, 2H, $-\text{CH}_2$); EIMS: 265 $[\text{M}^+]$; $\text{C}_9\text{H}_7\text{N}_5\text{O}_3\text{S}$.

2-((1H-tetrazol-5-yl)thio)-1-(4-fluorophenyl)ethanone (3f)

IR (KBr, cm^{-1}): 3418 (-NH), 3022 (Ar, C-H), 1730 ($>\text{C}=\text{O}$), 1624 ($>\text{C}=\text{N}$), 1051 (C-S-C); ^1H 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, $-\text{CH}_2$); EIMS: 238 $[\text{M}^+]$; $\text{C}_9\text{H}_7\text{FN}_4\text{OS}$.

2-((1H-tetrazol-5-yl)thio)-1-(3,4-dichlorophenyl)ethanone (3h)

IR (KBr, cm^{-1}): 3449 (-NH), 3032 (Ar, C-H), 1732 ($>\text{C}=\text{O}$), 1611 ($>\text{C}=\text{N}$), 1014 (C-S-C); ^1H 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, $-\text{CH}_2$); EIMS: 289 $[\text{M}^+]$; $\text{C}_9\text{H}_6\text{Cl}_2\text{N}_4\text{OS}$.

General procedure for the synthesis of 2-((1H-tetrazol-5-yl)thio)-1-substitutedphenylethanol (4a-h)

Compounds **3a-h** (1.00 mm) in PEG-400 was stirred at room temperature in 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 wash with water and recrystallized from aq. acetic acid.

2-((1H-tetrazol-5-yl)thio)-1-(4-chlorophenyl)ethanol (4a)

IR (KBr, cm^{-1}): 3423 (-OH), 3217 (-NH), 1615 ($>\text{C}=\text{N}$), 1091 (C-O-C); ^1H 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, $-\text{CH}_2$).

2-((1H-tetrazol-5-yl)thio)-1-(4-bromophenyl)ethanol (4b)

IR (KBr, cm^{-1}): 3457 (-OH), 3192 (-NH), 1622 ($>\text{C}=\text{N}$), 1108 (C-O-C); ^1H 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, $-\text{CH}_2$).

2-((1H-tetrazol-5-yl)thio)-1-(3-nitrophenyl)ethanol (4d)

IR (KBr, cm^{-1}): 3528 (-OH), 3196 (-NH), 1620 ($>\text{C}=\text{N}$), 1128 (C-O-C); ^1H 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, -CH₂).

2-((1H-tetrazol-5-yl)thio)-1-(4-fluorophenyl)ethanol (4f)

IR (KBr, cm^{-1}): 3487 (-OH), 3174 (-NH), 1631 ($>\text{C}=\text{N}$), 1132 (C-O-C); ^1H 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, -CH₂).

2-((1H-tetrazol-5-yl)thio)-1-(3,4-dichlorophenyl)ethanol (4h)

IR (KBr, cm^{-1}): 3413 (-OH), 3237 (-NH), 1625 ($>\text{C}=\text{N}$), 1128 (C-O-C); ^1H 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, -CH₂).

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 chlorobenzylchloride (1.00 mmole) were stirred continuously in 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 70-80°C. After completion of reaction monitored by TLC the reaction mixture was 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 obtain a pure final product.

5-((2-((4-chlorobenzyl)oxy)-2-(4-chlorophenyl)ethyl)thio)-1H-tetrazole (6a)

M.P. 167-169°C; Yield: 86%; IR (KBr, cm^{-1}): 3215 (-NH), 3068 (aromatic-C-H), 1617 ($>\text{C}=\text{N}$), 1073 (C-O-C); ^1H 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, -CH₂), 5.15 (t, 2H, -CH₂); ^{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: Calculated (found) for C₁₆H₁₄Cl₂N₄OS: % 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 (6c)

M.P. 182-184°C; Yield: 92%; IR (KBr, cm^{-1}): 3262 (-NH), 3120 (aromatic-C-H), 1622 ($>\text{C}=\text{N}$), 1112 (C-O-C); ^1H 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, -CH₂), 5.22 (d, 2H, -CH₂); ^{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 C₁₆H₁₄ClN₅O₃S: % 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 (6d)

M.P. 177-179°C; Yield: 94%; IR (KBr, cm^{-1}): 3289 (-NH), 2977 (aromatic-C-H), 1639 ($>\text{C}=\text{N}$), 1127 (C-O-C); ^1H 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, -CH₂), 5.37 (d, 2H, -CH₂); ^{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); 1H 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, -CH₂), 5.69 (s, 1H, -CH), 4.67 (d, 2H, -CH₂), 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 (6j)

M.P. 187-189°C; Yield: 90%; IR (KBr, cm^{-1}): 3171 (-NH), 3129 (Aromatic-C-H), 1597 ($>C=N$), 1079 (C-O-C); 1H 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, -CH₂), 5.29 (d, 2H, -CH₂); ^{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 (6m)

M.P. 165-167°C; Yield: 85%; IR (KBr, cm^{-1}): 3159 (-NH), 3143 (Aromatic-C-H), 1588 ($>C=N$), 1069 (C-O-C); 1H 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, -CH₂), 5.78 (d, 2H, -CH₂); ^{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 C₁₆H₁₄Cl₂N₄OS: % 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), *Bacillus subtilis* (MTCC) as examples of Gram-positive bacteria and *Escherichia coli* (MTCC), and *Klebsiella pneumoniae* (MTCC) as examples of Gram-negative bacteria. They were also screened 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 [54] using a 1 cm microplate well. Ciprofloxacin and Fluconazol were used as standard drugs for biological screening. The results of biological screening against the standard strains are recorded in **Fig. 1** and **Fig. 2** which manifestly depicted the distinct sense of antibacterial and fungal strains toward the tested compounds.

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