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Synthesis and evaluation of a series of 1-substituted tetrazole derivatives as antimicrobial agents

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Abstract: The screening of compounds is an alternative approach to high-throughput screening for identification of leads for therapeutic targets. A series of novel 1-substituted tetrazole derivatives were synthesized and evaluated for their antibacterial and antifungal activity. All the derivatives were efficiently synthesized by four steps process. In this study, thiazole attached tetrazole derivatives were most active than the piperazine attached tetrazole derivatives. The structure of the newly synthesized compounds was elucidated by their ^{1}H NMR, ^{13}C NMR, LC-MS/MS, IR spectral data and elemental analysis. The detailed synthesis, spectroscopic and biological evaluation data are reported.

Keywords: tetrazole; piperazine; thiazole; antibacterial; antifungal

1. Introduction

Tetrazole and its derivatives have attracted much attention because of their unique structure and applications as antihypertensive, antialergic, antibiotic and anticonvulsant agents.[1-8] Synthesis of tetrazole derivatives is obviously an important task in modern medicinal chemistry. Although a number of synthetic methods are available, there still exists a demand for improved protocol which allows an effective transformation in the presence of a wide range of functional groups. The

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substituted tetrazole derivatives

development of tetrazole chemistry has been largely associated with wide scale of applications of these classes of compounds in medicine, biochemistry, agriculture [1-12]; and also a large number of medicinally important tetrazole heterocyclic incorporated drugs approved by the FDA.[13-14] The tetrazole functionality plays an important role in medicinal chemistry, primarily due to its ability to serve as bioequivalent (bioisostere) of the carboxylic acid group.[3,15] Heterocyclic derivative is the first approved treatment for the partial agonist of dopamine D2 receptors; and also heterocyclic derivatives are widely used as antibacterial agents in human and veterinary medicines.[16-17] Some of tetrazole containing compounds have been used both as anticancer and antimicrobial agents. [3]

1-Substituted tetrazole derivatives are used as antibiotics and optically active tetrazole-containing antifungal preparations of azole type.[18-19] There is always a need for new and effective antifungal and antibacterial agents with broad spectrum antibacterial and antifungal activities. It was decided to exploit this interest by ascertaining the molecules features essential for activity and utilizing them to develop a new class of drugs. Prompted by the various biological activities of tetrazole and its substituted derivatives, we envisioned our approach towards the synthesis of a novel series of 1-substituted tetrazole derivatives and study their biological activities.

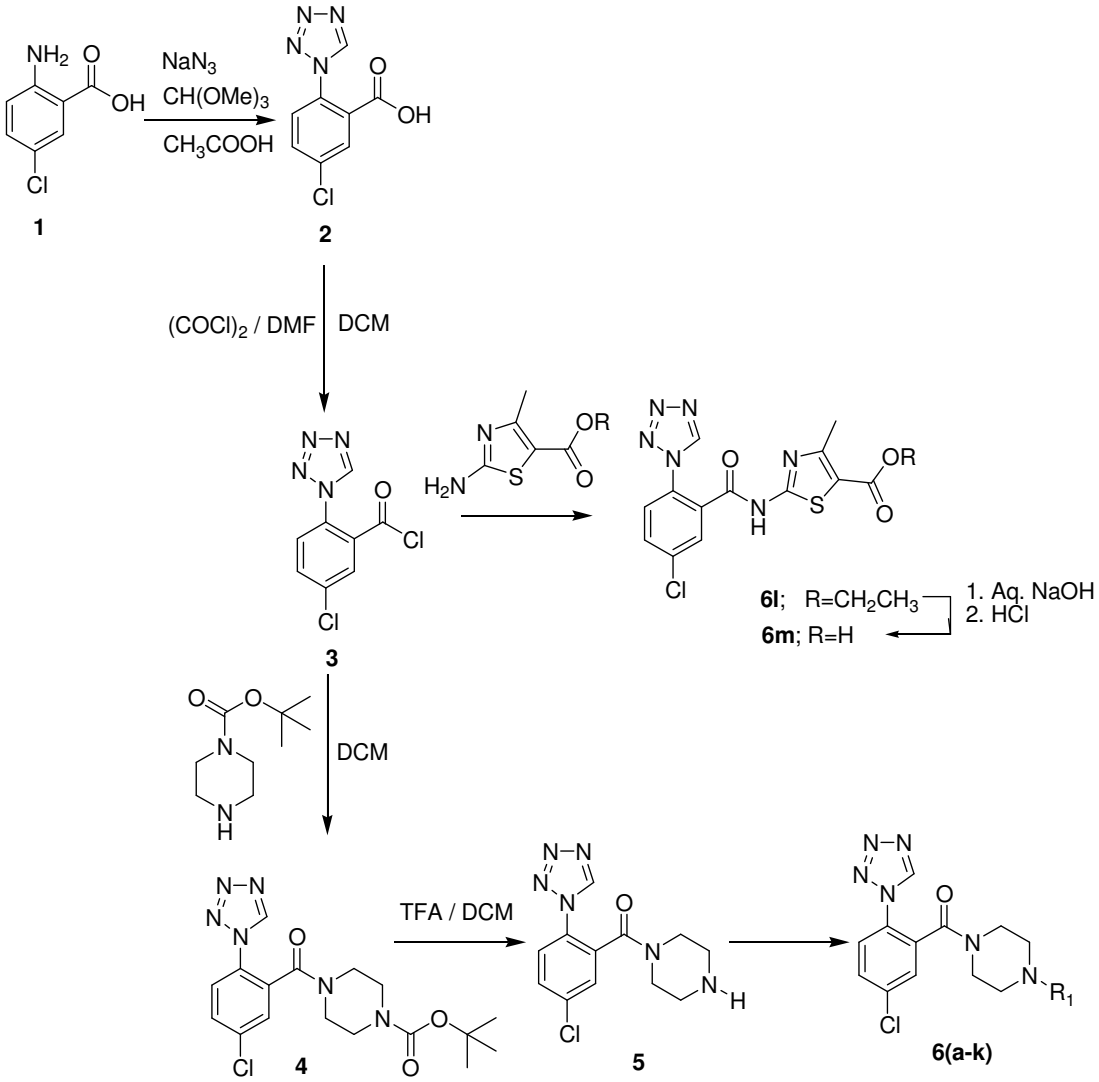
The objective of the present study was to synthesize new substituted tetrazole derivatives and to evaluate their antibacterial and antifungal properties.

2. Results and Discussion

In order to prepare a variety of derivatives of tetrazole, the 1-[5-chloro-2-(1*H*-tetrazol-1-yl)benzoyl] piperazine (**5**) was prepared as a pivotal precursor. The synthon **5** is a new compound and reported here for the first time.

Preparation of 1-[5-chloro-2-(1*H*-tetrazol-1-yl)benzoyl]piperazine (**5**) was accomplished by four steps synthesis as given in Scheme1. As depicted in the scheme, the starting 2-amino-5-chlorobenzoic acid (**1**) and sodium azide was suspended in trimethyl orthoformate and glacial acetic acid was added to it, the mixture was stirred at ambient temperature to get 5-chloro-2-(1*H*-tetrazol-1-yl) benzoic acid (**2**).[20] The compound **2** was treated with oxalyl chloride in dichloromethane to get corresponding acid chloride product **3**. The N-Boc piperazine was added slowly to compound **3** at 0 °C to get amide coupled compound **4**. The compound **4** was treated with trifluoroacetic acid to remove N-Boc and to produce novel pivotal precursor **5**. Then the synthon **5** was treated with corresponding isothiocyanate or appropriate halogenated compounds to produce required all new tetrazole derivatives. Reaction completion was monitored by TLC and the product was isolated by column chromatography.

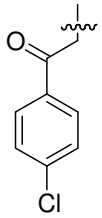
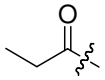
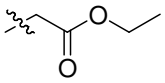
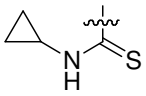
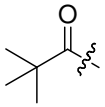
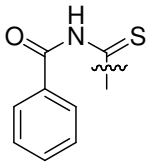
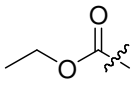
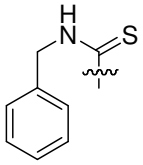
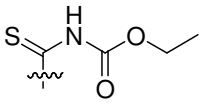
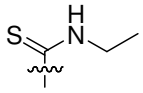
The structure of the newly synthesized compounds was elucidated by their ^{1}H NMR, ^{13}C NMR, LC-MS/MS, IR spectral data analysis. In the IR spectra, the band due to –N=N- and C=N group, which is present in all studies compounds were observed at about 1500 cm^{-1} and 1385 cm^{-1}, respectively. The bands at about 1250 cm^{-1} and 990 cm^{-1} were characteristic for the CN_4 (tetrazole ring). About 1030 cm^{-1} and 1130-1140 cm^{-1} were characteristic for the piperazine ring. In ^{1}H NMR spectra, the tetrazole proton was appeared as singlet at about δ 9.0 ppm in all derivatives. The other aromatic protons were observed at about δ 7.4 to 7.9 ppm. In all the synthesized compounds, the piperazine protons appeared at about δ 3.3 to 4.1 ppm integrated for 8 protons. In ^{13}C NMR spectra of all synthesized compounds, tetrazole carbon and aromatic carbon peaks were observed at about δ 144 and δ 126 to 135 ppm, respectively. Piperazine carbons were observed at about 41 to 47 ppm. In Mass spectra, molecular ion peak or M^{+} or M^{-} peak was obtained from ESI-MS.



Scheme 1. Synthesis of 1-substituted tetrazole derivatives

substituted tetrazole derivatives

Table 1. Structures of substituent R_1 in compounds **6(a-m)**

Compound	R_1	Compound	R_1
6a		**6h**	
6b		**6i**	
6c		**6j**	
6d		**6k**	
6e		**6l**	$-CH_2-CH_3$
6f		**6m**	-H
6g		**-----**	-----

2.1. Antibacterial activity

The newly synthesized tetrazole derivatives were investigated for their inhibition growth against *Staphylococcus aureus* (ATTC-25923), *Escherichia coli* (ATTC-25922), *Pseudomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion method.[21-26] Batches of 100 discs were dispensed to each screw capped bottle and sterilized by dry heat at 140 °C for one hour. The test compounds were prepared with different concentration using dimethylformamide (DMF). 1 mL containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The disc of each concentration was placed in triplicate in a nutrient agar medium separately seeded with fresh bacteria. The incubation was carried out at 37 °C for 24 h. Solvent and growth controls were kept separately, the zones of inhibition and minimum inhibitory concentration (MIC) were measured. Results of these studies are given in table 2 and compared with standard Ampicillin drug. Interestingly, it is observed that out of fourteen compounds, eleven compounds were found to have good antibacterial activity. Among these compounds **6e**, **6g**, **6l** and **6m** were most active against the four bacterial strains. Compound **5**, **6b**, **6h**, **6i**, **6j** and **6k** showed

good growth inhibition towards four bacterial strains, and **6h** and **6j** showed the pronounced growth inhibition for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The remaining compounds **6a**, **6d** and **6f** were found to have less active against four bacterial strains**.** The **6c** was found to have very less active against four bacterial strains.

2.2. Antifungal activity

We have also investigated newly synthesized compounds for their antifungal activity against four fungal strains namely, *Aspergillus flavus* (NCIM No.524), *Aspergillus fumigatus* (NCIM No.902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured). Sabouraud's agar media was prepared by dissolving peptone (1.0 g), D-Glucose (4.0 g) and agar (2.0 g) in sterile water (100 mL) and the pH was adjusted to 5.7. Normal saline was use to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL of saline to get suspension of corresponding species. Agar media of 20 mL was poured in to each petri dish. Excess of suspension was decanted and the plates were dried by placing them in an incubator at 37 °C for 1 h. Using an agar, punch wells were made on these seeded agar plates, from 6.25 µg/mL to 100 µg/mL of the test compounds in DMSO was added in to each well labeled disc. Controls were run using DMSO at the same concentration as used with the test compounds. The petri dishes were prepared in triplicate and maintained at 37 °C for 3 to 4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone.[27-28] The results of these studies was given in table 3 and compared with the standard Itraconazole drug. It was observed that most of the compounds were exhibited good antifungal activity. Compounds **5**, **6a** and **6f** were less active against all the above organisms. On the other hand, compounds **6g**, **6l** and **6m** showed most antifungal activity against all the four fungal strains namely, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes*. The **6c** was found to have very less active against four fungal organisms**.** Compounds **6b**, **6d**, **6e**, **6h**, **6i**, **6j** and **6k** were showed good antifungal activity against *Penicillium marneffei* and *Trichophyton mentagrophytes*, and pronounced antifungal activity towards other two fungal organisms.

3. Conclusion

In conclusion, the antimicrobial screening suggests that all the newly synthesized compounds showed moderate to good activity against the tested organisms. Among the newly synthesized compounds, **6e**, **6g**, **6l** and **6m** showed the most promising antibacterial and antifungal activity. Hence the fact that the compounds prepared in this study are chemically unrelated to the current medication, suggests that further work with similar analogues is clearly warranted

4. Experimental

4.1. General

All the reagents were purchased from Aldrich and used as received. Glacial acetic acid and dry solvents were supplied by Spectrochem, India. All the chemical reactions were performed under nitrogen atmosphere using standard techniques**.** The ^{1}H NMR and ^{13}C NMR experiments were performed using either Oxford AS 400 NMR (Varian, City, USA) with dual broad band or Bruker AMX 400. ^{1}H NMR chemical shift values were reported on the δ scale in ppm relative to TMS (δ = 0.0 ppm), and ^{13}C NMR chemical shift values were reported relative to DMSO (δ = 39.5 ppm). IR spectra were recorded on Perkin Elmer spectrum 100 FT-IR model. Column chromatography was

performed with silica gel 60-120 mesh (Merck, Mumbai, India.). All the compounds were routinely checked for their reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposing them to UV lamp or iodine vapor or $KMnO_4$ reagents. LC part of the LC/MS/MS system consisted of an Agilent-1100 series quaternary gradient pump with a degasser, an auto sampler and a column oven. MS/MS part of the system contained API-2000 system (Sciex, Applied Bio-Systems, Canada). Yield reported is the isolated yield after purification of the compounds.

4.2. Synthesis

Procedure for the preparation of 5-chloro-2-(1H-tetrazol-1-yl) benzoic acid (2): 2-Amino-5-chlorobenzoic acid (10.0 g, 58.5 mmol) and sodium azide (12.0 g, 184.6 mmol) was suspended in trimethyl orthoformate (20 mL) and cooled to 0 °C. Glacial acetic acid (200 mL) was added, and the mixture was initially stirred at 0 °C for 3 h and then at ambient temperature for 16 h. The slurry was concentrated in vacuum, and residue was partitioned between ethyl acetate (550 mL) and 3N HCl (300 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under vacuum to get the compound as off-white powder. The yield of the product **2**[20] was 89%.

Procedure for the preparation of 5-chloro-2-(1H-tetrazol-1-yl) benzoyl chloride (3) and tert-butyl 4-[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]piperazine-1-carboxylate (4): 5-Chloro-2-(1*H*-tetrazol-1-yl)benzoic acid (**2**) (5.0 g, 22.3 mmol) and dichloromethane (40 mL) and oxalyl chloride (3.75 g, 29.5 mmol) was added at ambient temperature, then catalytic amount of dimethylformamide (0.5 mL) was added and stirred at ambient temperature for 30 minutes under nitrogen. Reaction mixture was concentrated directly to remove dichloromethane and excess of oxalyl chloride; and the resulting acid chloride residue **3** (5.0 g, 20.6 mmol) was dissolved in fresh dry dichloromethane (100 mL), and trimethylamine (5 mL) was added. N-Boc piperazine (3.83 g, 20.6 mmol) was added slowly at 0 °C to the above reaction mixture and stirred at ambient temperature for overnight. Reaction mixture was quenched with water (100 mL) and extracted with dichloromethane (2 x100 mL) and the combined organic layer was washed with water (100 mL) and brine solution (50 mL), dried over Na_2SO_4, filtered and concentrated. The solid crude product **4** (7.3 g; 90%) was used without further purification in the next step.

Procedure for the preparation of 1-[5-chloro-2-(1H-tetrazol-1yl)benzoyl]piperazine(5): *tert*-Butyl 4-[5-chloro-2-(1*H*-tetrazol-1-yl)benzoyl]piperazine-1-carboxylate(**4**) (5.0 g, 12.7 mmol) in dry dichloromethane (40 mL) and trifluoroacetic acid (2.9 g, 25.5 mmol) was added slowly at 0 °C then stirred at ambient temperature for 2 h. Reaction was monitored by TLC; after the completion of reaction, the reaction mixture was quenched with water and basified with $NaHCO_3$ solution (pH~ 8.0), and extracted with dichloromethane (2 x 150 mL). The combined organic layer was washed with water (50 mL) and brine solution (50 mL), dried over Na_2SO_4 and evaporated solvents under vacuum to get **5** as brown colored solid (3.5 g; 94 %). Mp:184-186.5 °C; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 2.12 (s, 1H), 2.38 - 3.45 (m, 8H), 7.75 - 7.86 (m, 3H, ArH), 9.80 (s, 1H, tetrazole) ; ^{13}C NMR (100 MHz, DMSO) δ (ppm): 42.5, 44.8, 45.1, 47.9, 127.5, 127.7, 128.9, 130.2, 133.9, 135.2, 144.1, 163.3; IR (KBr) cm^{-1}: 3316 (NH), 3067 (CH), 1613 (C=O), 1504 (N=N), 1444 (C=C), 1397 (C=N); MS calculated for $C_{12}H_{13}ClN_6O$: 292.72, Found: M^++1(293.2); MS/MS (m/z) : 265.1, 248.3, 222.1, 205.1, 179.2, 151.0 and 124.1; Elemental analysis: Calculated: C, 49.19; H, 4.44; N, 28.70. Found: C, 49.12; H, 4.46; N, 28.75.

General procedure for the preparation of derivatives 6a-b: To a solution of 1-[5-chloro-2(1*H*-tetrazol-1-yl)benzoyl]piperazine (200 mg, 0.68 mmol), potassium carbonate (190 mg, 1.4 mmol) in dimethylformamide (2 mL) and 2-bromo-1-(4-chlorophenyl)ethanone (190 mg, 0.71 mmol) or ethyl 2-bromo acetate (136 mg, 0.81 mmol) was added and stirred at ambient temperature for 1 h. Reaction was monitored by TLC, after the completion of reaction, it was diluted with water (10 mL) and

extracted with ethyl acetate (50 mL) and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure to give crude product. The pure product was isolated by using column chromatography. The column was started at 10% ethyl acetate in hexane and slowly increased to 70% ethyl acetate.

General procedure for the preparation of derivatives 6c-g: 1-[5-Chloro-2(1*H*-tetrazol-1-yl)benzoyl]piperazine (200 mg, 0.68 mmol) and tetrahydrofuran (10 mL) and isothiocyanato cyclopropane (74 mg, 0.75 mmol) or benzoyl isothiocyanate (122 mg, 0.75 mmol) or (isothiocyanate methyl) benzene (111 mg, 0.75 mmol) or ethyl isothiocyanate carbonate (98 mg, 0.75 mmol) or isothiocyanato ethane (65 mg, 0.75 mmol) was added and stirred at ambient temperature for 1.5 h. Reaction was monitored by TLC, after the completion of reaction; reaction mixture was concentrated directly to remove the solvent. The crude product was recrystallised with diethylether to get pure product.

General procedure for the preparation of derivatives 6h-k: 1-[5-Chloro-2(1*H*-tetrazol-1-yl)benzoyl]piperazine (200 mg, 0.68 mmol) and triethylamine (0.3 mL) in methylene chloride (10 mL) was added to propionyl chloride (76 mg, 0.82 mmol) or acetyl chloride (64 mg, 0.82 mmol) or 2,2-dimethyl propionyl chloride (98 mg, 0.82 mmol) or ethyl chlorocarbonate (88 mg, 0.82 mmol) at 0 °C and then stirred at ambient temperature for 2 h. Reaction mixture was quenched with water and extracted with dichloromethane (100 mL) and organic layer was washed with water (80 mL), and then brine solution (20 mL), dried over Na_2SO_4 and evaporated to dryness.

Procedure for the preparation of ethyl 2-{[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]amino}-4-methyl-1, 3-thiazole-5-carboxylate (6l): The resulting residue **3** (2.0 g, 8.2 mmol) was dissolved in fresh dry dichloromethane (50 mL), and trimethylamine (2 mL) was added to it. Ethyl 2-amino-4-methyl-1,3-thiazole-5-carboxylate (1.52 g, 8.2 mmol) was added slowly at 0 °C to the above reaction mixture and stirred at ambient temperature for overnight. Reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (2 x50 mL) and the combined organic layers was washed with water (50 mL) and brine solution (30 mL), dried over Na_2SO_4 and concentrated. The crude product was crystallized using diethyl ether to give **6l** (2.9 g; 90%).

Procedure for the preparation of ethyl 2-{[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]amino}-4-methyl-1,3-thiazole-5-carboxylic acid (6m): To the solution of **6l** (1.0 g, 2.55 mmol) in methanol, water and tetrahydrofuran [(1:1:2), 20 mL] and sodium hydroxide (153 mg, 3.8 mmol) was added and stirred overnight at ambient temperature. Reaction mixture was directly concentrated to remove organic volatiles. The residue was dissolved in water (20 mL), acidified with 1.5M HCl and extracted with ethyl acetate (2 x 30 mL) and the combined organic layer was washed with water (30mL), brine solution (20 mL), dried over Na_2SO_4 and evaporated to dryness to get the crude product. The crude product was recrytallized with diethyl ether and dried under vacuum (0.69 g; 74 %).

The representative analytical data for:
1-(4-Chlorophenyl)-2{4-[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]piperazin-1-yl}ethanone (6a): Off-white powder. The yield of the product **6a** was 69 %. Mp:178-181°C.; ^{1}H NMR (400 MHz, MeOH) δ (ppm): 2.85 - 3.31 (m, 8H), 3.93 (s, 2H), 7.48 - 8.00 (m, 7H, ArH), 9.80 (s, 1H, tetrazole) ; IR (KBr) υ_{max} cm^{-1}: 3073 (CH), 1666, 1639 (C=O), 1505 (N=N), 1439 (C=C), 1397 (C=N); MS calculated for $C_{20}H_{18}Cl_2N_6O_2$: 445.30, Found: M^+ +1(444.9); MS/MS (m/z): 418.3, 400.0, 249.3, 240.0, 222.9, 196.2, 178.8; Elemental Analysis: Calculated: C, 53.90; H, 4.04; N, 18.86. Found: C, 53.84; H, 4.02; N, 18.89.

substituted tetrazole derivatives

Ethyl{4-[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]piperazin-1-yl}acetate (6b): Off-white powder. The yield of the product **6b** was 89 %. Mp:180-183 °C.; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.26 - 1.28 (t, 3H, *J*=7.2 Hz), 4.15 - 4.21 (q, 2H, *J*=6.8 Hz), 1.95 - 3.90 (m, 8H), 3.28 (s, 2H), 7.48 - 7.63 (m , 3H, ArH), 8.99 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 14.5, 61.0, 46.3, 42.8, 42.6, 41.1, 127.4, 127.8, 129.1, 130.5, 133.2, 135.2, 144.1, 154.5, 163.8; IR (KBr) υ/cm^{-1}: 3072 (CH), 1745, 1634 (C=O), 1504 (N=N), 1473 (C=C), 1400 (C=N); MS calculated for $C_{16}H_{19}ClN_6O_3$: 378.81, Found: M^++1(379.6); MS/MS (m/z): 351.1, 276.9, 248.1, 221.8, 178.8, 173.1, 155.9, 130.3; Elemental Analysis: Calculated: C, 50.69; H, 5.02; N, 22.17. Found: C, 50.74; H, 5.01; N, 22.21.

4-[5-Chloro-2(1H-tetrazol-1-yl)benzoyl]-N-cyclopropylpiperazine-1-carbothioamide (6c):
Off-white powder. The yield of the product **6c** was 78 %. Mp: 205-208 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 0.56 - 0.69 (m,4H), 2.95 - 2.99 (m,1H), 3.29 - 3.83 (m, 8H), 7.74 - 7.90 (m, 3H, ArH), 9.86 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 6.7, 28.3, 41.0, 45.9, 46.1, 46.8, 127.4, 127.9, 129.1, 130.5, 133.2, 135.2, 144.1, 163.8, 183.3; IR (KBr) υ/cm^{-1}: 3290 (NH), 3072 (CH), 1635 (C=O), 1506 (N=N), 1478 (C=C), 1366 (C=N); MS calculated for $C_{16}H_{18}ClN_7OS$: 391.88, Found: M^++1(392.4); MS/MS (m/z): 334.9, 307.3, 248.3, 222.0, 194.9, 179.2, 129.2, 87.0, 70.2; Elemental Analysis: Calculated: C, 48.99; H, 4.59; N, 25.01. Found: C, 48.96; H, 4.57; N, 25.05.

N-({4-[5-Chloro-2-(1H-tetrazol-1-yl)benzoyl]piperazin-1-yl}carbonothioyl)benzamide (6d): Cream coloured powder. The yield of the product **6d** was 84 %. Mp:178 - 180.5 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 3.59 - 4.11(m, 8H), 7.51 - 7.95 (m, 8H, ArH), 9.88 (s, 1H, tetrazole), 10.91 (s, NH) ; ^{13}C NMR (100 MHz, DMSO) δ (ppm): 46.4, 45.8, 41.5, 41.0, 127.8, 128.4, 128.6, 128.8, 129.6, 131.0, 132.9, 132.9, 133.4, 135.6, 144.5, 164.4, 164.5, 180.7; IR (KBr) υ/cm^{-1}: 3135 (NH), 1659, 1641 (C=O), 1520 (N=N), 1430 (C=C), 1365 (C=N); MS calculated for $C_{20}H_{18}ClN_7O_2S$: 455.92, Found: M^++1(456.3); MS/MS (m/z): 335.1, 307.2, 292.8, 265.2, 248.1, 222.1, 178.9, 154.0, 129.0, 105.2; Elemental Analysis: Calculated: C, 52.64; H, 3.95; N, 21.49. Found: C, 52.68; H, 3.94; N, 21.55.

N-Benzyl-4-[5-chloro-2(1H-tetrazol-1-yl)benzoyl]piperazine-1-carbothioamide (6e): Off-white powder. The yield of the product **6e** was 75 %. Mp: 206 - 208.5 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 4.79 - 4.81 (s, 2H), 3.37 - 3.91(m, 8H), 7.21 - 7.33 (m, 5H, ArH), 7.83 - 7.91 (m, 3H, ArH), 9.88 (s, 1H, tetrazole), 8.33 - 8.35 (s, NH) ; ^{13}C NMR (100 MHz, DMSO) δ (ppm): 41.0, 45.9, 46.3, 46.9, 48.4, 126.6, 127.1, 127.4, 127.9, 128.0, 129.1, 130.5, 133.2, 135.2, 139.4, 144.1, 163.9, 182.1; IR (KBr) υ/cm^{-1}: 3302 (NH), 3076 (CH), 1639 (C=O), 1504 (N=N), 1473 (C=C), 1396 (C=N); MS calculated for $C_{20}H_{20}ClN_7OS$: 441.94, Found: M^++1(442.5) ; MS/MS (m/z): 335.2, 307.0, 293.0, 248.4, 221.9, 179.3, 153.9, 122.8, 90.9, 87.0, 70.0; Elemental Analysis: Calculated: C, 54.31; H, 4.53; N, 22.17. Found: C, 54.38; H, 4.50; N, 22.20.

Ethyl({4-[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]piperazin-1-yl}carbonothioyl)carbamate (6f): Off-white powder. The yield of the product **6f** was 94 %. Mp:138.5 - 140.5 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.16 - 1.21 (t, 3H, *J*=7.3 Hz), 4.06 - 4.08 (q, 2H, *J*=7.3 Hz), 3.32 - 3.56 (m, 8H), 7.84 - 7.87 (m, 3H, ArH), 9.87 (s, 1H, tetrazole), 10.24 (s, NH); ^{13}C NMR (100 MHz, DMSO) δ (ppm):14.3, 41.0, 45.9, 49.0, 49.5, 61.3,127.3, 127.9, 129.2, 130.6, 133.0, 135.2, 144.1, 151.4, 163.9, 180.2; IR (KBr) υ/cm^{-1}: 3073 (NH), 2980 (CH), 1756, 1638 (C=O), 1504 (N=N), 1470 (C=C), 1356 (C=N); MS calculated for $C_{16}H_{18}ClN_7O_3S$: 423.88, Found: M^+ +1(422.4); MS/MS (m/z): 347.8, 288.2, 262.9, 219.9, 193.9, 179.2, 57.9; Elemental Analysis: Calculated: C, 45.30; H, 4.25; N, 23.12. Found: C, 45.23; H, 4.23; N, 23.15.

4-[5-Chloro-2-(1H-tetrazol-1-yl)benzoyl]-N-ethylpiperazine-1-carboxamide (6g): Off-white powder. The yield of the product **6g** was 90 %. Mp:154 – 156 °C.; ^{1}H NMR (400 MHz, MeOH) δ (ppm): 1.09 - 1.13 (t, 3H, *J*=7.1 Hz), 3.16 - 3.22 (q, 2H, *J*=7.1 Hz), 3.30 - 3.50 (m, 8H), 7.73 - 7.77 (m, 3H, ArH), 9.57 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm):15.4, 34.8, 41.2, 42.7, 42.9, 46.4, 127.5,

127.8, 129.1, 130.4, 133.4, 135.2, 144.1, 157.2, 163.7; IR (KBr) υ/cm^{-1}: 3343 (NH), 3069 (CH), 1633, 1588 (C=O), 1506 (N=N), 1480 (C=C), 1403 (C=N); MS calculated for $C_{15}H_{18}ClN_7O_2$: 363.80, Found: M^++1(364.2); MS/MS (m/z): 293.3, 291.2, 265.3, 248.2, 221.9, 179.2, 112.9, 87.2, 70.3; Elemental Analysis: Calculated: C, 49.48; H, 4.95; N, 26.94. Found: C, 49.43; H, 4.93; N, 26.90.

1-[5-Chloro-2-(1H-tetrazol-1-yl)benzoyl]-4-propionylpiperazine (6h): Light yellow coloured powder. The yield of the product **6h** was 84 %. Mp: 143 – 146 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 0.96 - 0.99 (t, 3H, *J*=7.4 Hz), 2.28 - 2.35 (q, 2H, *J*=7.4 Hz), 3.32 - 3.46 (m, 8H), 7.81 - 7.89 (m, 3H, ArH), 9.86 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 9.6, 25.9, 41.9, 44.6, 46.8, 47.2, 127.9, 128.2, 129.6, 130.9, 133.8, 135.6, 144.5, 164.2, 172.0; IR (KBr) υ/cm^{-1}: 3073 (CH), 1636 (C=O), 1502 (N=N), 1472 (C=C), 1400 (C=N); MS calculated for $C_{15}H_{17}ClN_6O_2$: 348.78, Found: M^++1(349.3); MS/MS (m/z): 321.2, 293.2, 278.3, 265.3, 222.1, 179.2, 143.2, 113.0, 87.1, 70.1; Elemental Analysis: Calculated: C, 51.61; H, 4.87; N, 24.08. Found: C, 51.67; H, 4.89; N, 24.09.

1-Acetyl-4-[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]piperazine (6i): Brown coloured solid. The yield of the product **6i** was 79 %. Mp:128 – 130 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 2.03 (s, 3H), 3.35 - 3.71 (m, 8H), 7.82 - 7.88 (m, 3H, ArH), 9.86 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 21.1, 41.5, 45.1, 46.3, 46.7, 127.4, 127.8, 129.1, 130.5, 133.3, 135.2, 144.1, 163.8, 168.5; IR (KBr) υ/cm^{-1}: IR (KBr) υ/cm^{-1}: 3073 (CH), 1633 (C=O), 1501 (N=N), 1473 (C=C), 1385 (C=N); MS calculated for $C_{14}H_{15}ClN_6O_2$: 334.76, Found: M^+ +1(335.2); MS/MS (m/z): 307.0, 288.9, 265.1, 222.1, 178.9, 129.1, 113.3, 87.3, 70.2; Elemental Analysis; Calculated: C, 50.19; H, 4.48; N, 25.09. Found: C, 50.13; H, 4.47; N, 25.05.

1-[5-Chloro-2-(1H-tetrazol-1-yl)benzoyl]-4-(2,2-dimethylpropanoyl)piperazine (6j): Off-white powder. The yield of the product **6j** was 78 %. Mp:185 – 188 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.12 (s, 9H), 3.32 - 3.42 (m, 8H), 7.82 - 7.89 (m, 3H, ArH), 9.86 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 8.8, 38.5, 41.5, 44.3, 44.6, 47.5, 127.9, 128.2, 129.6, 130.9, 133.7, 135.6, 144.6, 164.1, 175.8; IR (KBr) υ/cm^{-1}: 3077 (CH), 1635 (C=O), 1502 (N=N), 1474 (C=C), 1398 (C=N); MS calculated for $C_{17}H_{21}ClN_6O_2$: 376.84, Found: M^++1(377.6); MS/MS (m/z): 349.3, 264.8, 221.9, 161.0; Elemental Analysis: Calculated: C, 54.13; H, 5.57; N, 22.29. Found: C, 54.17; H, 5.56; N, 22.30.

Ethyl 4-[5-chloro-2-(1H-tetrazol-1yl)benzoyl]piperazine-1-carboxylate (6k): Off-white powder. The yield of the product **6k** was 83 %. Mp:179 – 182 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.16 - 1.17 (t, 3H, *J*=7.3 Hz), 4.02 - 4.07 (q, 2H, *J*=7.3 Hz), 3.16 - 3.47 (m, 8H), 7.80 -7.89 (m, 3H, ArH), 9.85 (s, 1H, tetrazole); ^{13}C NMR (100 MHz, DMSO) δ (ppm):14.5, 61.0, 41.1, 42.6, 42.8, 46.2, 127.4, 127.8, 129.1, 130.5, 133.2, 135.2, 144.1, 154.5, 163.8; IR (KBr) υ/cm^{-1}: 3073 (CH), 1706, 1636 (C=O), 1503 (N=N), 1470 (C=C), 1400 (C=N); MS calculated for $C_{15}H_{17}ClN_6O_3$: 364.78, Found: M^+ +1(365.3); MS/MS (m/z): 337.3, 309.1, 290.9, 265.2, 247.9, 222.1, 179.3, 159.3, 113.0, 86.9; Elemental Analysis: Calculated: C, 49.34; H, 4.66; N, 23.03. Found: C, 49.38; H, 4.65; N, 23.06.

Ethyl-2-{[5-chloro-2-(1H-tetrazol-1-yl)benzoyl]amino}-4-methyl-1,3-thiazole-5-carboxylate (6l): Off-white powder. The yield of the product **6l** was 90 %. Mp: 287.5 – 290 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 1.26 - 1.29 (t, 3H, *J*=5.3 Hz), 4.22 - 4.27 (q, 2H, *J*=5.3 Hz), 2.56 (s, 3H), 7.88 - 8.11 (m, 3H, ArH), 9.93 (s, 1H, tetrazole), 13.26 (s, NH); IR (KBr) υ/cm^{-1}: 3274 (NH), 3010 (CH), 1688 (C=O), 1505 (N=N), 1378 (C=N); MS calculated for $C_{15}H_{13}ClN_6O_3S$: 392.82, Found: M^+ +1(393.8); MS/MS (m/z): 366.1, 350.8, 338.1, 321.4, 214.4, 178.9, 158.8; Elemental Analysis: Calculated: C, 45.82; H, 3.31; N, 21.38. Found: C, 45.88; H, 3.30; N, 21.39.

substituted tetrazole derivatives

2-{[5-Chloro-2-(1H-tetrazol-1-yl)benzoyl]amino}-4-methyl-1,3-thiazole-5- carboxylic acid (6m): Light brown coloured solid. The yield of the product **6m** was 74 %. Mp: 268 – 271 °C.; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 2.54 (s, 3H), 7.84 - 8.10 (m, 3H, ArH), 9.91 (s, 1H, tetrazole), 13.08 (brs, 2H, NH & COOH); IR (KBr) υ/cm^{-1}: 3154 (OH-broad), 2952 (CH), 1679 (C=O), 1532 (NH-bending), 1507 (N=N), 1378 (C=N), 1318 (C-O); MS calculated for $C_{13}H_9ClN_6O_3S$: 364.77, Found: M^+ +1(365.3); MS/MS (m/z): 291.3, 275.9, 224.2, 219.2, 192.1, 178.9, 71.1; Elemental Analysis: Calculated: C, 42.77; H, 2.47; N, 23.03. Found: C, 42.71; H, 2.48; N, 23.06.

Table 2. Antibacterial activities of the synthesized compounds (zone of inhibition in mm, #MIC in mg/mL given in parenthesis).

Compounds	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
5	16 (12.5)	17 (12.5)	16 (12.5)	16 (12.5)
6a	<10 (50)	<10 (50)	<10 (50)	<10 (50)
6b	17 (12.5)	16 (12.5)	16 (12.5)	17 (12.5)
6c	<10 (100)	<10 (100)	<10 (100)	<10 (100)
6d	<10 (50)	<10 (50)	<10 (50)	<10 (50)
6e	19 (6.25)	21 (6.25)	20 (6.25)	20 (6.25)
6f	<10 (50)	<10 (50)	<10 (50)	<10 (50)
6g	20 (6.25)	21 (6.25)	19 (6.25)	20 (6.25)
6h	16 (12.5)	17 (12.5)	10 (12.5)	11 (12.5)
6i	15 (12.5)	16 (12.5)	15 (12.5)	14 (12.5)
6j	14 (12.5)	15 (12.5)	11 (12.5)	10 (12.5)
6k	15 (12.5)	15 (12.5)	16 (12.5)	15 (12.5)
6l	19 (12.5)	18 (12.5)	19 (12.5)	18 (12.5)
6m	20 (6.25)	21 (6.25)	20 (6.25)	22 (6.25)
Ampicillin	22 (6.25)	22 (6.25)	22 (6.25)	23 (6.25)

MIC: Minimum inhibitory concentration.

Table 3. Antifungal activities of the synthesized compounds (zone of inhibition in mm, #MIC in mg/mL given in parenthesis).

Compounds	*Trichophyton mentagrophytes*	*Penicillium marneffei*	*Aspergillus flavus*	*Aspergillus fumigatus*
5	<10 (50)	<10 (50)	<10 (50)	<10 (50)
6a	<10 (50)	<10 (50)	<10 (50)	<10 (50)
6b	16 (12.5)	16 (12.5)	13 (12.5)	12 (12.5)
6c	<10 (100)	<10 (100)	<10 (100)	<10 (100)
6d	14 (12.5)	15 (12.5)	13 (12.5)	13 (12.5)
6e	17 (12.5)	16 (12.5)	15 (12.5)	14 (12.5)
6f	<10 (50)	<10 (50)	<10 (50)	<10 (50)
6g	21 (6.25)	20 (6.25)	21 (6.25)	20 (6.25)
6h	16 (12.5)	17 (12.5)	15 (12.5)	13 (12.5)
6i	15 (12.5)	16 (12.5)	14 (12.5)	14 (12.5)
6j	16 (12.5)	14 (12.5)	13 (12.5)	14 (12.5)
6k	14 (12.5)	16 (12.5)	15 (12.5)	13 (12.5)
6l	22 (6.25)	21 (6.25)	20 (6.25)	20 (6.25)
6m	21 (6.25)	23 (6.25)	21 (6.25)	22 (6.25)
Itraconazole	23 (6.25)	24 (6.25)	21 (6.25)	22 (6.25)

MIC: Minimum inhibitory concentration.

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