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Synthesis, Molecular Docking and Evaluation of Library of 3-Mercapto-1,2,4-Triazole Derivatives as Antimicrobial Agents

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Due to the increasing microbial resistance to antibacterial and antifungal drugs, the development of new antimicrobial agents is an urgent priority. In search of newer antimicrobial agents, a series of 4,5-disubstituted-3-mercapto-1,2,4-triazole derivatives were synthesized from aromatic acids and substituted isothiocyanates. The *in silico* study was performed to study the binding interactions of the synthesized compounds with the active pocket of CYP51. Among the synthesized 3-mercapto-triazole derivatives, compounds **6r, 6s** and **6u** exhibited promising antimicrobial activity comparable to standard drugs. The results suggested that the structural modification to 3-mercapto-1,2,4-triazole derivatives could lead to promising antimicrobial scaffolds.

Keywords: 3-Mercapto-1,2,4-triazoles, Intramolecular cyclization, Molecular docking, Antibacterial activity, Antifungal activity.

INTRODUCTION

Antibacterial and antifungal infections and associated mortality have been increasing rapidly in recent years due to a large number of immune-deficient individuals and a limited number of drugs. The appearance of new antibiotics alarming problem towards human health. To combat these situation new drugs are emerging to cure bacterial and fungal infections [1]. Triazole, an important class of heterocyclic moiety, due to its excellent selectivity, low toxicity and exhibit various biological properties such as anticonvulsant [2-4], antitubercular [5,6], antioxidant [7,8], antimicrobial [9-11], anticancer [12]. Some of the triazole based drugs are cefatrizine (antibiotic), tazobactum (antibacterial), carboxyamido-triazole (anticancer) and TSAO (anti-HIV) [13]. 1,2,4-Triazole moieties exhibited enhanced biological activities due to its dipolar nature, rigidity, hydrogen bonding capability and stability under *in vivo* conditions [14,15]. Triazole moieties having antifungal property are widely used as first-line antifungal therapy for the treatment of antifungal infections [16].

1,2,4-Triazoles are the most potent anti-TB agent and unveiled effective antibacterial activity against Gram-positive as well as Gram-negative strains. Triazoles inhibit lipid bio-synthesis to prevent the growth of bacteria [17-19]. Also, triazole ring having a mercapto group used as an intermediate in organic chemistry for the synthesis of different bioactive compounds. The mercapto group act as a nucleophilic centre in the synthesis of condensed heterocyclic rings [19,20]. Mercapto substituted 1,2,4-triazole moiety has attracted the attention of chemists who are in search of new drug molecules due to its chemo-preventive and chemotherapeutic effects on cancer [20-22]. Encouraged by the diverse pharmacological applications of 1,2,4-triazoles and further to our work on the development of bioactive heterocycles [23,24], herein the synthesis, molecular docking and assessment of antimicrobial properties of a library of 4,5-disubstituted-3-mercapto-1,2,4-triazoles are reported.

EXPERIMENTAL

Melting points were uncorrected and determined in open capillary tubes. The IR spectrum was performed by Shimadzu-8300 FTIR Spectrometer using KBr method. The ^{1}H NMR and ^{13}C NMR spectra were performed on Bruker 400 MHz, 100 MHz NMR spectrophotometer, respectively in DMSO-d_6. Thin layer chromatography was performed on silica gel (HF_{254} 200 mesh) using toluene: ethyl acetate as eluent and visualized in UV chamber.

General method for the synthesis of substituted esters (2a-c): Substituted aromatic acids (**1a-c**) (1 mmol) were dissolved in 5 mL of ethanol and refluxed for 3 h in the catalytic amount of concentrated sulfuric acid. Reaction completion was observed by TLC (toluene:ethyl acetate = 7:3). Ethanol was evaporated and the resulting mass was neutralized with 5% $NaHCO_3$. The product was extracted into ether, washed with water and the ether was evaporated to get the product (**2a-c**), which was taken directly for the second step.

General method for the synthesis of substituted hydrazide (3a-c): Aromatic esters (**2a-c**) (1 mmol) and hydrazine hydrate (1 mmol) were dissolved in ethanol and the mixture was refluxed for 3-4 h. After the completion of the reaction by TLC (toluene: ethyl acetate = 7: 3), the mass was cooled and solid separated was filtered to give hydrazides (**3a-c**).

General procedure for the synthesis of substituted 3-mercapto 1,2,4-triazoles (6a-u): A mixture of substituted hydrazides (**3a-c**) (1 mmol) and isothiocyanates (**4a-g**) (1 mmol) was refluxed for 2 h in ethanol. After the completion of the reaction (TLC, toluene:ethyl acetate = 7:3), the mass was cooled to room temperature and solid formed was filtered to give compounds **5a-u**. These compounds **5a-u** were refluxed with 2 mL NaOH solution (5%) for 4 h. After the completion of the reaction checked by TLC (toluene:ethyl acetate = 7:3), the mass was cooled and acidified (6N HCl). The product formed was filtered and recrystallized (ethanol) to get substituted 3-mercapto-1,2,4-triazoles (**6a-u**).

4-Benzyl-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol) and (isothiocyanatomethyl)benzene (**4a**) 0.15 g (1 mmol), as white solid to yield 0.25 g (86%), m.p.: 263-265 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 812 (CCl), 1328 (C=S), 1488 (C=N), 3442 (NH). ^{1}H NMR δ ppm: 5.3 (s, 2H, -CH_2), 7.28-7.33 (m, 5H, ArH), 7.38 (d, 2H, ArH), 7.45 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 50.5, 126.5, 126.8, 127.1, 128.1, 128.9, 129.85, 135.5, 136.5, 149.1, 179.2.

5-(4-Chlorophenyl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6b): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol) and isothiocyanatobenzene (**4b**) 0.14 g (1 mmol), as white solid to yield 0.25 g (88%), m.p.: 254-256 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 825 (CCl), 1326 (C=S), 1588 (C=N), 3011 (ArCH), 3440 (NH). ^{1}H NMR δ ppm: 7.31-7.37 (m, 3H, ArH), 7.37 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.50 (d, 2H, ArH) 14.19 (s, 1H, NH). ^{13}C NMR δ ppm: 125.5, 127.7, 129.1, 129.8, 129.9, 130.5, 134.8, 135.7, 148.9, 169.1.

4,5-*Bis*(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6c): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol) and 1-chloro-4-isothiocyanatobenzene (**4c**) 0.17 g (1 mmol), as white solid to yield 0.23 g (72%), m.p.: 240-242 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 825 (CCl), 1326 (C=S), 1590 (C=N), 3012 (ArCH), 3440 (NH). ^{1}H NMR δ ppm: 7.02 (d, 2H, ArH), 7.22 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.50 (d, 2H, ArH) 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 125.2, 128.2, 129.2, 129.6, 133.6, 133.9, 135.2, 135.7, 149.2, 169.2.

4-(3-Chlorophenyl)-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6d): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol) and 1-chloro-3-isothiocyanatobenzene (**4d**) 0.17 g (1 mmol), as white solid to yield 0.20 g (65%), m.p.: 260-262 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 816 (CCl), 1324 (C=S), 1562 (C=N), 3020 (Ar CH), 3449 (NH). ^{1}H NMR δ ppm: 6.80 (d, 1H, ArH), 7.05-7.22 (m, 3H, ArH), 7.41 (d, 2H, ArH), 7.49 (d, 2H, ArH) 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 127.1, 128.8, 129.2, 129.5, 130.6, 131.2, 134.6, 135.2, 135.6, 138.2, 149.3, 168.1.

4-Benzyl-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6e): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol) and (2-isothiocyanatoethyl)benzene (**4e**) 0.16 g (1 mmol), as white solid to yield 0.24 g (77%), m.p.: 230-232 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 810 (CCl), 1330 (C=S), 3015 (ArCH), 3482 (NH). ^{1}H NMR δ ppm: 2.83 (t, 2H, -CH_2), 3.50 (t, 2H, -CH_2), 7.25-7.4 (m, 7H, ArH), 7.61 (d, 2H, ArH), 14.0 (s, 1H, NH). ^{13}C NMR δ ppm: 41.8, 52.2, 125.8, 126.7, 127.6, 128.6, 128.9, 129.3, 135.6, 139.4, 148.8, 175.2.

5-(4-Chlorophenyl)-4-(4-fluorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6f): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol), 1-fluoro-4-isothiocyanatobenzene(**1f**) 0.16 g (1 mmol), as white solid to yield 0.28 g (92%), m.p.: 243-245 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 856 (CCl), 1159 (CF), 1350.08 (C=S), 1596.9 (C=N), 3014 (ArCH), 3670 (NH). ^{1}H NMR δ ppm: 7.3-7.4 (m, 4H, ArH), 7.45 (d, 2H, ArH), 7.55 (d, 2H, ArH), 14.18 (s, 1H, NH). ^{13}C NMR δ ppm: 116.1, 125.1, 129.2, 129.6, 130.6, 134.6. 135.1, 148.8, 161.2, 166.9.

5-(4-Chlorophenyl)-4-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6g): Obtained from 4-chlorobenzohydrazide (**1a**) 0.16 g (1 mmol) and 1-isothiocyanato-4-nitrobenzene (**4g**) 0.18 g (1 mmol), as white solid to yield 0.23 g (70%), m.p.: 238-240 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 812 (CCl), 1355 (C=S), 1520 (NO_2), 3018 (Ar CH), 3492 (NH). ^{1}H NMR δ ppm: 6.62 (d, 2H, ArH), 7.41 (d, 2H, ArH), 7.6 (d, 2H, ArH), 8.0 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 124.6, 126.7, 129, 129.5, 134.5, 136.1, 141.9, 144.0, 149.1, 167.2.

4-Benzyl-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6h): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and (isothiocyanatomethyl)benzene (**4a**) 0.15 g (1 mmol), as white solid to yield 0.26 g (90%), m.p.: 249-251 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1342.30 (C=S), 1596.95 (C=N), 3020 (Ar CH), 3498(NH). ^{1}H NMR δ ppm: 3.77 (s, 3H, OCH_3), 5.33 (s, 2H, CH_2), 7.0 (d, 2H, ArH), 7.22 (t, 3H, ArH), 7.35 (d, 2H, ArH), 7.45 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 47.1, 55.6, 114.82, 120.8, 126.95, 127.94, 130.0, 131.9, 135.5, 148.6, 161.15, 180.1.

5-(4-Methoxyphenyl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6i): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and isothiocyanatobenzene (**4b**) 0.14 g (1 mmol), as a white solid to yield 0.25 g (88%), m.p.: 268-270 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1340 (C=S), 1555 (C=N), 3019 (Ar CH), 3489 (NH). ^{1}H NMR δ ppm: 3.73 (s, 3H, OCH_3), 6.10(d, 2H, ArH), 6.91(d, 1H, ArH), 7.05(d, 2H, ArH), 7.19 (d, 2H, ArH), 7.39 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 57.1, 114.8, 119.2, 128.1, 128.5, 130.1, 131.9, 135.2, 148.5, 161.8, 167.2.

4-(4-Chlorophenyl)-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6j): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and 1-chloro-4-isothio-

cyanatobenzene (**4c**) 0.17 g (1 mmol), as white solid to yield 0.24 g (76%), m.p.: 222-224 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 812 (CCl), 1330 (C=S), 1548 (C=N), 3018 (ArCH), 3496 (NH). ^{1}H NMR δ ppm: 3.79 (s, 3H, OCH_3), 6.20 (d, 2H, ArH), 7.0 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.5 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 57.1, 115.0, 120.1, 129.1, 129.6, 132.4, 133.8, 135.8, 148.9, 161.1, 169.2.

4-(3-Chlorophenyl)-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6k): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and 1-chloro-3-isothiocyanatobenzene (**4d**) 0.17 g (1 mmol), as white solid to yield 0.20 g (65%), m.p.: 260-262 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 816 (CCl), 1338 (C=S), 1556 (C=N), 3011 (ArCH), 3490 (NH). ^{1}H NMR δ ppm: 3.83 (s, 3H, OCH_3), 6.0 (d, 1H, ArH), 6.70 (s, 1H, ArH), 6.85 (d, 1H, ArH), 7.0 (d, 2H, ArH), 7.14 (t, 1H, ArH), 7.52 (d, 2H, ArH), 14.12 (s, 1H, NH). ^{13}C NMR δ ppm: 58.1, 114.2, 120.8, 128.6, 129.9, 131.0, 131.4, 135.1, 136.0, 138.3, 149.2, 162.0, 167.3.

5-(4-Methoxyphenyl)-4-(2-phenylethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6l): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and (2-isothiocyanatoethyl)benzene (**4e**) 0.17 g (1 mmol), as white solid to yield 0.21 g (69%), m.p.: 268-270 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1348 (C=S), 1568 (C=N), 3017 (ArCH), 3430 (NH). ^{1}H NMR δ ppm: 2.78 (t, 2H, CH_2), 3.66 (t, 2H, CH_2), 3.86 (s, 3H, OCH_3), 7.0 (d, 2H, ArH), 7.20-7.30 (m, 3H, ArH), 7.40 (d, 2H, ArH), 7.52 (d, 2H, ArH), 14.12 (s, 1H, NH). ^{13}C NMR δ ppm: 41.2, 51.3, 56.8, 114.4, 120.1, 124.9, 127.4, 128.6, 131.2, 139.5, 147.5, 161.7, 180.9.

4-(4-Fluorophenyl)-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6m): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and 1-fluoro-4-isothiocyanatobenzene (**4f**) 0.16 g (1 mmol), as white solid to yield 0.25 g (85%), m.p.: 245-247 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1120 (CF), 1350 (C=S), 1504 (C=N), 3448 (NH). ^{1}H NMR δ ppm: 3.74 (s, 3H, OCH_3), 6.92 (d, 2H, ArH), 7.36 (d, 2H, ArH), 7.42 (d, 2H, CH), 7.45 (d, 2H, CH), 14.06 (s, 1H, NH). ^{13}C NMR δ ppm: 56.3, 114.2, 116.1, 120.9, 131.1, 132.3, 135.3, 148.5, 161.9, 164.4, 168.3.

5-(4-Methoxyphenyl)-4-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione(6n): Obtained from 4-methoxybenzohydrazide (**1b**) 0.16 g (1 mmol) and 1-isothiocyanato-4-nitrobenzene (**4g**) 0.18 g (1 mmol) as white solid to yield 0.21 g (65%), m.p.: 276-278 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1510 (NO_2), 1328 (C=S), 1528 (C=N), 3454 (NH). ^{1}H NMR δ ppm: 3.79 (s, 3H, OCH_3), 6.58 (d, 2H, ArH), 7.01(d, 2H, ArH), 7.45 (d, 2H, ArH), 8.31 (d, 2H, ArH). ^{13}C NMR δ ppm: 57.1, 114.1, 120.9, 123.8, 131.2, 135.3, 140.1, 145.5, 149.3, 161.7, 167.8.

4-Benzyl-5-[(4-methylphenoxy)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6o): Obtained from 2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and (isothiocyanatomethyl)benzene (**4a**) 0.15 g (1 mmol), as white solid to yield 0.27 g (87%), m.p.: 241-243 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1120 (C-O), 1325 (C=S), 1581 (C=N), 3435 (NH). ^{1}H NMR δ ppm: 2.40 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 5.51(s, 2H, CH_2), 6.79 (d, 2H, ArH), 7.01 (d, 2H, ArH), 7.20-7.25 (m, 3H, ArH), 7.40 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 22.3, 53.1, 76.5, 114.6, 126.5, 127.1, 128.9, 130.2, 131.5, 135.7, 155.3, 156.8, 183.5.

5-[(4-Methylphenoxy)methyl]-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6p): Obtained from 2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and isothiocyanatobenzene (**4b**) 0.14 g (1 mmol), as white solid to yield 0.25 g (83%), m.p.: 245-247 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1128 (CO), 1323 (C=S), 1548 (C=N), 3497 (NH). ^{1}H NMR δ ppm: 2.32 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.20-6.75 (m, 3H, ArH), 6.84 (d, 2H, ArH), 6.15 (d, 2H, ArH), 7.25(d, 2H, ArH), 14.2 (s, 1H, NH). ^{13}C NMR δ ppm: 21.3, 76.8, 115.3, 127.9, 128.5, 129.3, 130.2, 132.5, 134.6, 153.7, 157.0, 167.5.

4-(3-Chlorophenyl)-5-[(4-methylphenoxy)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6q): Obtained from 2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and 1-chloro-4-isothiocyanatobenzene (**4c**) 0.17 g (1 mmol), as white solid to yield 0.22 g (67%), m.p.: 231-233 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 816(CCl), 1326 (C=S), 1581 (C=N), 3488 (NH). ^{1}H NMR δ ppm: 2.30 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.22 (d, 2H, ArH), 6.84 (d, 2H, ArH), 7.0 (d, 2H, ArH), 7.22 (d, 2H, ArH). 14.3 (s, 1H, NH). ^{13}C NMR δ ppm: 24.3, 75.8, 114.6, 128.1, 128.9, 130.4, 132.1, 134.3, 136.5, 154.8, 156.7, 165.4.

4-(3-Chlorophenyl)-5-[(4-methylphenoxy)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6r): Obtained from 2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and 1-chloro-3-isothiocyanatobenzene (**4d**) 0.17 g (1 mmol) as white solid to yield 0.24 g (72%), m.p.: 254- 256 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 815 (CCl), 1328 (C=S), 1551 (C=N), 3450 (NH). ^{1}H NMR δ ppm: 2.32 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 6.0 (d, 2H, ArH), 6.79 (s, 1H, ArH), 6.85 (d, 2H, ArH), 7.10 (d, 2H, ArH), 7.18 (d, 1H, ArH), 14.2 (s, 1H, NH). ^{13}C NMR δ ppm: 21.2, 75.5, 114.5, 127.9, 130.0, 130.3, 130.8, 131.0, 131.5, 134.5, 137.8, 154.5, 156.9, 165.3.

5-[(4-Methylphenoxy)methyl]-4-(2-phenylethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6s): Obtained from 2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and (2-isothiocyanatoethyl)benzene (**4e**) 0.17 g (1 mmol), as white solid to yield 0.25 g (79%), m.p.: 259-261 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1200 (C-O), 1330 (C=S), 1554 (C=N), 3496 (NH). ^{1}H NMR δ ppm: 2.30 (s, 3H, CH_3), 4.79 (t, 2H, CH_2), 3.60 (t, 2H, CH_2), 4.12 (s, 2H, CH_2), 6.80 (d, 2H, ArH), 7.10 (d, 2H, ArH), 7.25-7.29 (m, 3H, ArH), 7.50 (d, 2H, ArH), 14.2 (s, 1H, NH). ^{13}C NMR δ ppm: 21.9, 39.9, 52.1, 77.1, 114.4, 125.4, 127.1, 129.0, 129.7, 130.7, 140.0, 153.8, 156.8, 178.6.

4-(4-Fluorophenyl)-5-[(4-methylphenoxy)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6t): Obtained from 2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and 1-fluoro-4-isothiocyanatobenzene (**4f**) 0.16 g (1 mmol), as white solid to yield 0.27 g (86%), m.p.: 224-226 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1112 (CF), 1215 (C-O), 1326 (C=S), 1558 (C=N), 3468 (NH). ^{1}H NMR δ ppm: 2.29 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 6.88 (d, 2H, ArH), 7.10 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.43 (d, 2H, ArH), 14.0 (s, 2H, NH). ^{13}C NMR δ ppm: 23.4, 75.6, 115.0, 116.2, 128.8, 129.3 130.5, 136.2, 153.8, 157.0, 162.1, 168.5.

5-[(4-Methylphenoxy)methyl]-4-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6u): Obtained from

2-(4-methylphenoxy)acetohydrzide (**1c**) 0.17 g (1 mmol) and 1-isothiocyanato-4-nitrobenzene (**4g**) 0.18 g (1 mmol), as white solid to yield 0.20 g (59%), m.p.: 231-233 ºC. FTIR (KBr, ν_{max}, cm^{-1}): 1218 (C-O), 1330 (C=S), 1560 (C=N), 3482 (NH). ^1H NMR δ ppm: 2.32 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.42 (d, 2H, ArH), 6.88 (d, 2H, ArH), 7.10 (d, 2H, ArH), 8.10 (d, 2H, ArH), 14.1 (s, 1H, NH). ^{13}C NMR δ ppm: 21.2, 76.5, 114.5, 121.1, 129.9, 130.5, 133.5, 140.0, 144.2, 154.8, 156.3, 167.6.

Molecular docking: Molecular docking has been carried out to study the antimicrobial potency of synthesized triazoles using Schrödinger's Maestro molecular modelling package. All the synthesized molecules and standard drugs were optimized using LigPrep. The protein structure was prepared using the protein preparation wizard. Prepared ligands were docked against CYP51. Fluconazole is considered as reference ligand to create a target grid. The low-energy ligands were docked against the generated target grid. ADME properties were studied using QikProp software. Best ligands are selected based on the glide scores, which help to predict the binding affinity of ligands.

Antimicrobial activity: Newly synthesized chemical entities were assessed for antimicrobial properties against bacterial strains (two Gram-negative bacteria *Escherichia coli* and *Serratia* and Gram-positive *Staphylococcus aureus*) and fungal cultures *Aspergillus niger* and *Aspergillus flavus* maintained at 4 ºC.

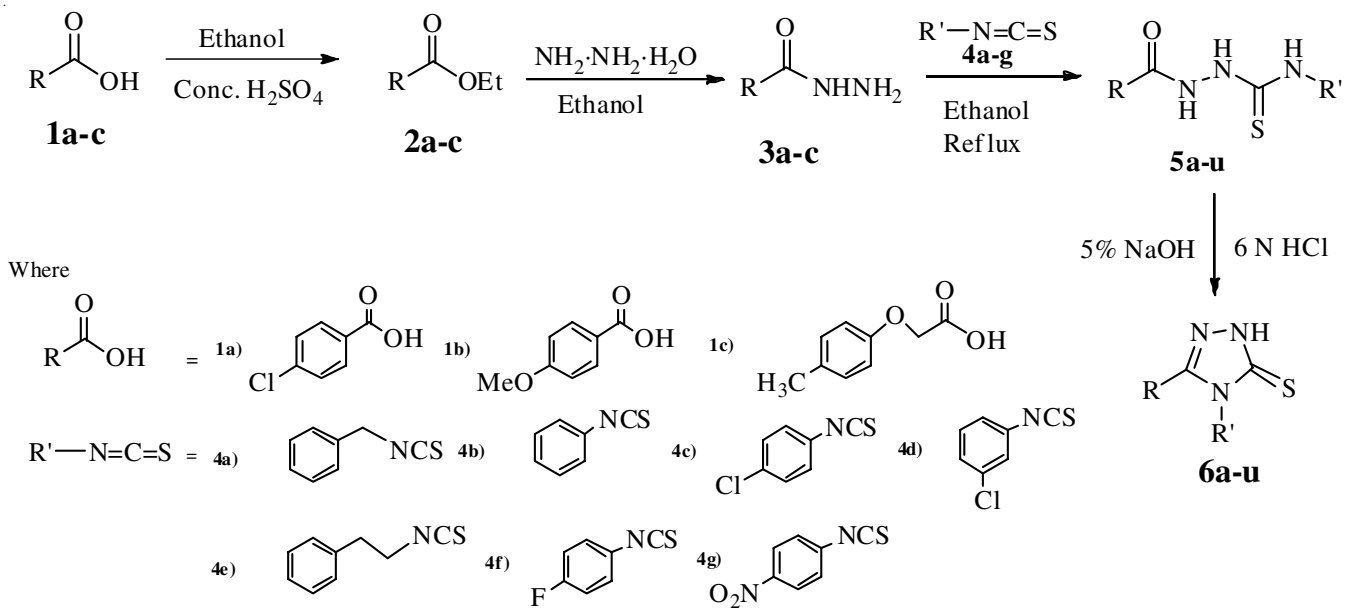
Preparation of the bacterial inoculum: Loop-full of each bacterial culture from stock-culture maintained at 4 ºC was added to 10 mL of nutrient broth separately in a sterile condition. Cultures were incubated at 37 ± 1 ºC overnight. After 24 h of incubation, these were taken as inoculum for the disc diffusion method.

Preparation of samples and media: Synthesized compounds were labelled appropriately and dissolved in DMF to make of 1 mg mL^{-1}. This was serially diluted to make 10 µg/mL. Preparation of Mueller-Hinton agar media and potato dextrose agar media were done as per the standard procedure. Mueller-Hinton agar medium composed of beef infusion 300 g/L, Casein acid hydrolysate 17.5 g, agar 17 g, starch 1.5 g, sodium chloride 5 g, sterile water 1000 mL growth medium for bacteria and potato 200 g, dextrose 20 g, agar 20 g to prepare potato dextrose agar medium to grow the fungal cultures.

Antimicrobial susceptibility test by disc diffusion method: Compounds were assessed for their activity by disc diffusion method using Whatman No.1 filter paper of 6 mm diameter discs. The assessment of an inhibition zone was proportional to the microbial susceptibility to the compound present in the disc. Sterile media was transferred to labelled Petri plates and allowed. A 200 µL of microbial cultures were placed on sterile plates and inoculum was spread using a sterile glass rod. Similarly for fungal culture, potato dextrose agar media was transferred in sterile condition to each labelled petri plate and allowed to solidify. Using sterile inoculation fungal cells were taken from stock and dispersed in labelled sterile tubes containing sterile water. From this 200 µL of each fungal culture, was placed on the surface of solid media and inoculum was spread using a sterile glass rod. Discs were placed accordingly using sterile forceps. Commercially available gentamycin and fluconazole discs were used as a positive control for bacterial and fungal culture, respectively. A 10 µL of DMF was used as a negative control for both cultures. After inoculation, plates were kept for 1 h at room temperature in the inoculation hood for pre-incubation distribution to reduce the effects of difference in time between the applications of the different samples. Plates were incubated at 37 ºC for 24-48 h for bacteria and 3 days incubation at 25 ºC for fungal cultures.

RESULTS AND DISCUSSION

The synthetic pathway adapted for the synthesis of targets compounds is represented in **Scheme-I**. Twenty-one 3-mercapto-1,2,4-triazole derivatives were synthesized and their antimicrobial activity was explored.



Scheme-I: Synthesis of 3-mercapto-1,2,4-triazole derivatives

Different aromatic acids (**1a-c**) were esterified by ethanol and concentrated sulphuric acid to yield esters **2a-c**. The esters were subsequently converted into hydrazides **3a-c** using hydrazine hydrate in ethanol. Treatment of compounds **3a-c** with various substituted isothiocyanates followed by intramolecular cyclization gave expected 3-mercapto-1,2,4-triazole derivatives in yields of 59-92%. All the synthesized compounds were characterized using spectral techniques. In IR spectra, the absence of the C=O absorption band in the region 1680-1640 cm^{-1} confirmed the formation of cyclic triazole moiety. The presence of bands in the region of 1350-1320 cm^{-1} and 3698-3430 cm^{-1} indicated the presence of C=S and NH functional groups, respectively. In ^{1}H NMR, the most downfield singlet signal observed in the region δ 14-14.2 ppm indicates the presence of NH proton and in all the compounds, the aromatic protons were observed in the region from δ 6.0 to 8.0 ppm. In ^{13}C NMR, the most deshielded C-atom attached to sulphur appeared in the region δ 165-169 ppm.

Molecular docking: Molecular docking study was done to investigate the binding interactions of synthesized triazoles with cytochrome P450 14a-sterol demethylases (CYP51) [25, 26]. This enzyme plays an important role in sterol biosynthesis. All the 21 molecules were docked against the fluconazole-bound CYP51 from *Mycobacterium tuberculosis* (PDB NO: 1EA1) resolved at 2.2 Å (Fig. 1). The active site of CYP51, contain triazole ring, which is placed perpendicular to the porphyrin plane. *N*-Atom of this triazole ring coordinate with the heme iron. The active site of CYP_{450} consists of amino acid residues as Tyr76, Leu321, ILE323, Met433, Leu324, Phe78, Met79, Ala256, Phe255, Arg96, Val435 and Hem 460. Among the docked ligands, ten ligands showed better glide score (-5.904 to -6.938) compared to standard drugs. The glide scores of all synthesized compounds are shown in Table-1. Compound **6k** showed appreciable glide score of -6.938 and showed three π-π stacking interactions with residue PHE-78, PHE-83 and HEM-460 (Fig. 2). Compound **6b** showed π-π stacking interactions with residues (TYR-76 and PHE-78) and π-cationic as well as salt bridge interactions with ARG-96. Also, π-π stacking and salt bridge interactions were observed between compound **6u** and HEM-460. Compound **6f** formed π-π stacking interactions with PHE-78 and TYR-76 and π-cationic and salt bridge inter-actions are formed between compound **6f** and ARG-96. Similarly, compound **6t** displayed π-π stacking and salt bridge interactions with HEM-460. Compound **6p** showed π-π stacking interactions with residues (TYR-76, PHE-78 and HEM-460) and salt bridge interactions with HEM-460. Compound **6c** displayed π-cationic stacking and salt bridge interactions with ARG-96 and π-π stacking with PHE-78 and HEM-460. In case of compound **6h**, π-π stacking interactions were observed with PHE-78, TYR-76, HEM-460, HIS-259 and salt bridge with ARG-96. Compound **6m** showed π-π stacking with PHE-78, HEM-460 and π-cationic stacking with ARG-96. Compound **6j** formed π-π stacking

TABLE-1
DOCKING SCORES OF THE SYNTHESIZED LIGANDS AND REFERENCE LIGAND

Compound	Glide score	PSA
6k	-6.938	39.924
6b	-6.860	31.661
6u	-6.855	84.449
6f	-6.634	31.667
6t	-6.614	39.455
6p	-6.548	39.452
6c	-6.388	31.666
6h	-6.071	40.226
6m	-5.904	39.886
6j	-5.856	39.881
6g	-5.348	76.684
Gentamycin	-12.86	186.304
Fluconazole	-5.324	74.748

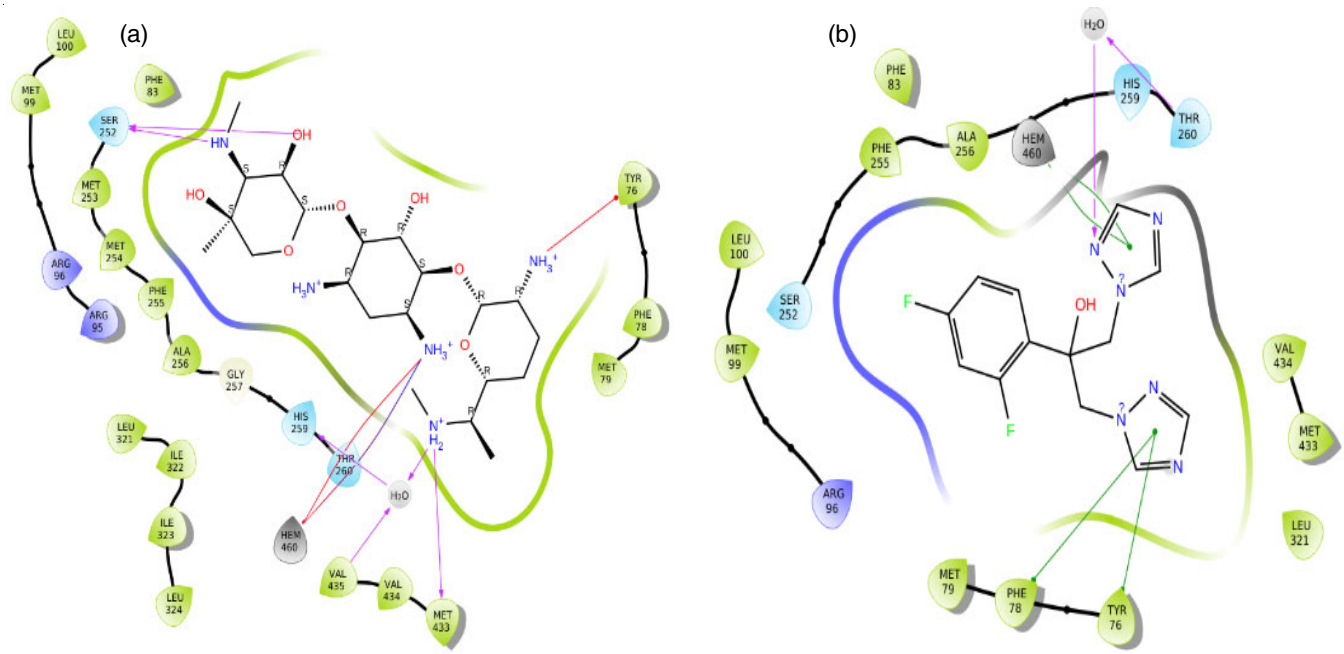


Fig. 1. Binding interactions of (a) gentamycin and (b) fluconazole with 1EA1

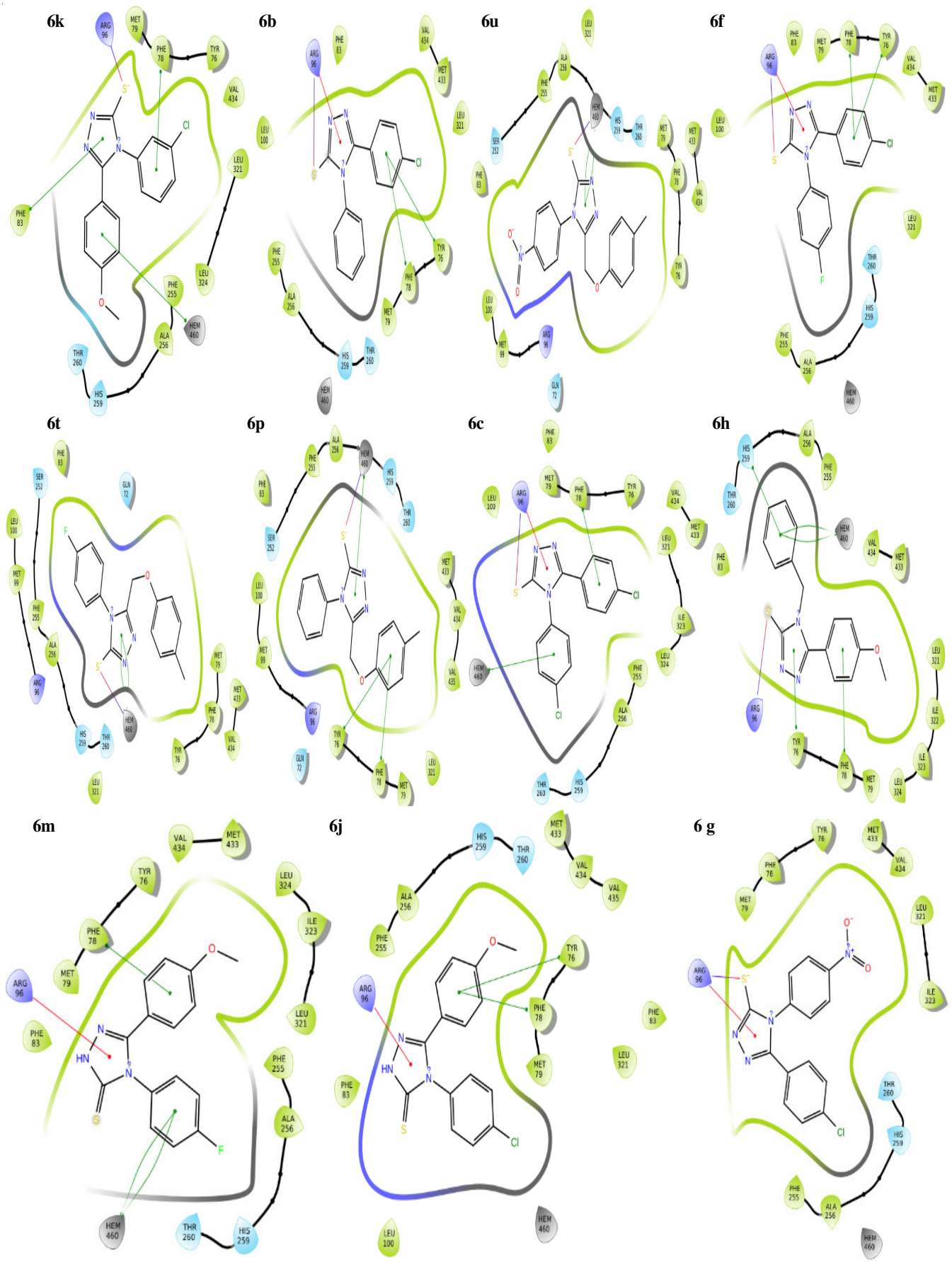


Fig. 2. Binding interactions of synthesized ligands **6k, 6b, 6u, 6f, 6t, 6p, 6c, 6h, 6m, 6j** and **6 g** with 1EA1

TABLE-2
PHARMACOKINETIC PROPERTIES OF ALL SYNTHESIZED COMPOUNDS

Compound	HBD (less than 5)	HBA (less than 10)	MW (less than 500 Dalton)	log P (less than 5)	Lipinski's rule follow
6k	0.8	2.75	317.792	4.436	Yes
6b	0.8	2.00	287.766	4.340	Yes
6u	0.8	3.75	342.372	3.833	Yes
6f	0.8	2.00	305.757	4.575	Yes
6t	0.8	2.75	315.364	4.772	Yes
6p	0.8	2.75	297.374	4.537	Yes
6c	0.8	2.00	322.211	4.837	Yes
6h	0.8	2.75	297.374	4.315	Yes
6m	0.8	2.75	301.338	4.188	Yes
6j	0.8	2.75	317.792	4.447	Yes
6g	0.8	3.00	332.764	3.643	Yes

with PHE-78, TYR 76 and π-cationic stacking with ARG-96. Compound **6 g** displayed π-cationic stacking and salt bridge with ARG-96. The polar surface area of nitrogen and oxygen atoms are also within the acceptable range (Table-1).

The ADME analysis revealed that all the synthesized compounds follow Lipinski's rule. All the ligands have molecular weight less than 500 daltons. Compounds showed less than five (0.8) hydrogen bond donors and less than ten (2-3) hydrogen bond acceptors. Also, partition coefficient log P (o/w) values are within the acceptable range (3.643-4.837) (Table-2). These results indicate that the synthesized compounds may be potential antimicrobial candidates.

Antimicrobial activity: A series of synthesized triazoles was tested for antibacterial activity with gentamycin as a '+' control, DMF as a '–' control against bacterial species *E. coli*, *Serratia* (Gram-negative) and *S. aureus* (Gram-positive). All the plates were observed for the inhibition zone and zone diameter was measured and compared with the zone obtained for known standards after the incubation period. According to Table-3, among the series **6a-u** of tested compounds, **6b**, **6d**, **6h**, **6i**, **6k**, **6l**, **6m** did not show any inhibition zone, confirming no activity against any of tested microorganisms. Compound **6r** showed remarkable activity against all tested bacterial strains compared to the gentamycin. Also, compounds **6s** and **6u** showed moderate antibacterial activities against Gram-negative bacteria *E. coli* as well as *Serratia*, whereas **6j** and **6t** showed moderate activity against *S. aureus*.

Synthesized triazoles were also evaluated for antifungal activity by taking fluconazole as a positive control, DMF as a negative control and were tested against fungi species *A. niger*, *A. flavus*. In comparison with fluconazole, compounds **6s** and **6u** showed good to moderate inhibition zone for both *A. niger* and *A. flavus*. From the above observations, it is concluded that compound **6r** is highly active against both bacterial as well as fungal strains. Overall, results showed that some of the triazole derivatives possess significant antibacterial as well as antifungal activity.

From the results of Table-3, we could collect valuable data about SAR. Some 3-mercapto 1,2,4-triazole derivatives exhibited good antimicrobial activities. Among the synthesized molecules, compound **6r** took its position on the top of the series. The presence of the *meta*-chlorophenyl group at triazole moiety enhanced the antibacterial activity. Similarly, compounds **6j**, **6s**, **6t** and **6u** showed moderate activity against bacterial strains. Compounds having electron-withdrawing groups (-Cl, -F and -NO_2) attached to triazole moiety increased the antibacterial activity whereas compounds with electron-releasing groups decreased the activity. Compounds **6s** and **6u** showed good to moderate inhibition zone for both *A. niger* and *A. flavus* but compounds **6b, 6d, 6h, 6i, 6k, 6l** and **6m** didn't show any zone of inhibition for the tested bacterial and fungal strains.

TABLE-3
in vitro aNTIMICROBIAL ACTIVITY OF
3-MERCAPTO-1,2,4-TRIAZOLES (**6a-u**)

Compd.	Zone of inhibition (mm)				
	Bacteria			Fungi	
	E. coli	*Serratia*	*S. aureus*	*A. nigar*	*A. flavas*
6a	+	+	–	–	–
6b	–	–	–	–	–
6c	+	–	–	+	+
6d	–	–	–	–	–
6e	–	–	+	+	+
6f	+	+	+	+	+
6g	+	+	–	–	–
6h	–	–	–	–	–
6i	–	–	–	–	–
6j	+	+	++	+	+
6k	–	–	–	–	–
6l	–	–	–	–	–
6m	–	–	–	–	–
6n	–	–	+	–	–
6o	+	+	–	–	–
6p	–	–	–	+	+
6q	+	+	+	+	+
6r	+++	+++	+++	–	–
6s	++	+	–	+++	+
6t	–	–	++	–	–
6u	++	+++	–	+	+++
DMF	–	–	–	–	–
Gentamycin*	+++	+++	+++	–	–
Fluconazole*	–	–	–	+++	+++

DMF is used as a negative control. *Gentamycin and fluconazole are used as bacterial and fungal standards respectively.

Conclusion

A series of 3-mercapto-1,2,4-triazole derivatives (**6a-u**) were synthesized, characterized and investigated for their antibacterial activities against *E. coli, Serratia, S. aureus* and antifungal activities against *A. nigar*, *A. flavas.* Some of the molecules exhibited good antimicrobial activity. Among them, compound **6r, 6s** and **6u** proved to be the better compounds. The molecular docking study showed that the synthesized compounds showed appreciable interactions with 1EA1. The ADME properties of compounds were also within an acceptable range. These results suggested that the synthesized triazole derivatives may emerge as a valuable lead series which might be useful as antibacterial and antifungal agents by structural modifications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D. Dheer, V. Singh and R. Shankar, *Bioorg. Chem.*, **71**, 30 (2017); https://doi.org/10.1016/j.bioorg.2017.01.010
2. X.-D. Deng, M.-X. Song, Y. Zheng and Z.-S. Quan, *Eur. J. Med. Chem.*, **73**, 217 (2014); https://doi.org/10.1016/j.ejmech.2013.12.014
3. A. Ayati, S. Emami and A. Foroumadi, *Eur. J. Med. Chem.*, **109**, 380 (2016); https://doi.org/10.1016/j.ejmech.2016.01.009
4. T. Plech, B. Kapron, J.J. Luszczki, A. Paneth, A. Siwek, M. Kolaczkowski, M. Zolnierek and G. Nowak, *Eur. J. Med. Chem.*, **86**, 690 (2014); https://doi.org/10.1016/j.ejmech.2014.09.034
5. A. Aziz Ali, D. Gogoi, A.K. Chaliha, A.K. Buragohain, P. Trivedi, P.J. Saikia, P.S. Gehlot, A. Kumar, V. Chaturvedi and D. Sarma, *Bioorg. Med. Chem. Lett.*, **27**, 3698 (2017); https://doi.org/10.1016/j.bmcl.2017.07.008
6. K. Shiva Raju, S. Anki Reddy, G. Sabitha, V. Siva Krishna, D. Sriram, K. Bharathi Reddy and S. Rao Sagurthi, *Bioorg. Med. Chem. Lett.*, **29**, 284 (2019); https://doi.org/10.1016/j.bmcl.2018.11.036
7. A. Mermer, N. Demirbas, Y. Sirin, H. Uslu, Z. Özdemir and A. Demirbas, *Bioorg. Chem.*, **78**, 236 (2018); https://doi.org/10.1016/j.bioorg.2018.03.017
8. I. Saadaoui, F. Krichen, B. Ben Salah, R. Ben Mansour, N. Miled, A. Bougatef and M. Kossentini, *J. Mol. Struct.*, **1180**, 344 (2019); https://doi.org/10.1016/j.molstruc.2018.12.008
9. H. Bayrak, A. Demirbas, N. Demirbas and S.A. Karaoglu, *Eur. J. Med. Chem.*, **44**, 4362 (2009); https://doi.org/10.1016/j.ejmech.2009.05.022
10. S. Nanjunda Swamy, Basappa, G. Sarala, B.S. Priya, S.L. Gaonkar, J. Shashidhara Prasad and K.S. Rangappa, *Bioorg. Med. Chem. Lett.*, **16**, 999 (2006); https://doi.org/10.1016/j.bmcl.2005.10.084
11. M.M. Sekhar, U. Nagarjuna, V. Padmavathi, A. Padmaja, N.V. Reddy and T. Vijaya, *Eur. J. Med. Chem.*, **145**, 1 (2018); https://doi.org/10.1016/j.ejmech.2017.12.067
12. S.G. Küçükgüzel and P. Çikla-Süzgün, *Eur. J. Med. Chem.*, **97**, 830 (2015); https://doi.org/10.1016/j.ejmech.2014.11.033
13. H.N. Nagesh, K.M. Naidu, D.H. Rao, J.P. Sridevi, P. Yogeeswari, D. Sriram and K.V.G. Chandra Sekhar, *Bioorg. Med. Chem. Lett.*, **23**, 6805 (2013); https://doi.org/10.1016/j.bmcl.2013.10.016
14. K.D. Thomas, A.V. Adhikari, I.H. Chowdhury, E. Sumesh and N.K. Pal, *Eur. J. Med. Chem.*, **46**, 2503 (2011); https://doi.org/10.1016/j.ejmech.2011.03.039
15. W.S. Horne, M.K. Yadav, C.D. Stout and M.R. Ghadiri, *J. Am. Chem. Soc.*, **126**, 15366 (2004); https://doi.org/10.1021/ja0450408
16. M.A. Pfaller, *Am. J. Med.*, **125**, S3 (2012); https://doi.org/10.1016/j.amjmed.2011.11.001
17. G.S. Hassan, S.M. El-Messery, F.A. Al-Omary, S.T. Al-Rashood, M.I. Shabayek, Y.S. Abulfadl, E.-S.E. Habib, S.M. El-Hallouty, W. Fayad, K.M. Mohamed, B.S. El-Menshawi and H.I. El-Subbagh, *Eur. J. Med. Chem.*, **66**, 135 (2013); https://doi.org/10.1016/j.ejmech.2013.05.039
18. B. Yadagiri, S. Gurrala, R. Bantu, L. Nagarapu, S. Polepalli, G. Srujana and N. Jain, *Bioorg. Med. Chem. Lett.*, **25**, 2220 (2015); https://doi.org/10.1016/j.bmcl.2015.03.032
19. S.M. El-Khawass and N.S. Habib, *J. Heterocycl. Chem.*, **26**, 177 (1989); https://doi.org/10.1002/jhet.5570260131
20. A.S. Shawali, I.F. Zeid, M.H. Abdelkader, A.A. Elsherbini and F.M.A. Altalbawy, *J. Chin. Chem. Soc.*, **48**, 65 (2001); https://doi.org/10.1002/jccs.200100012
21. B. Namratha and S.L. Gaonkar, *Int. J. Pharm. Pharm. Sci.*, **6**, 73 (2014).
22. M.M. Slaihim, F.S.R. Al-Suede, M. Khairuddean, M.B. Khadeer Ahamed and A.M. Shah, *J. Mol. Struct.*, **1196**, 78 (2019); https://doi.org/10.1016/j.molstruc.2019.06.066
23. S.L. Gaonkar and K.M.L. Rai, *Tetrahedron Lett.*, **46**, 5969 (2005); https://doi.org/10.1016/j.tetlet.2005.06.007
24. K. Sunitha, M. Hemshekhar, S.L. Gaonkar, M. Sebastin Santhosh, M. Suresh Kumar, Basappa, B.S. Priya, K. Kemparaju, K.S. Rangappa, S. Nanjunda Swamy and K.S. Girish, *Basic Clin. Pharmacol. Toxicol.*, **109**, 292 (2011); https://doi.org/10.1111/j.1742-7843.2011.00725.x
25. S. Bano, M.S. Alam, K. Javed, M. Dudeja, A.K. Das and A. Dhulap, *Eur. J. Med. Chem.*, **95**, 96 (2015); https://doi.org/10.1016/j.ejmech.2015.03.031
26. G. Singh, A. Arora, A. Singh, P. Kalra, S. Rani, K. Singh, I.K. Maurya and R.S. Mandal, *ChemistrySelect*, **3**, 1942 (2018); https://doi.org/10.1002/slct.201703051