Synthesis and Antimicrobial Studies of Some 4-(Substituted)-Ethanoylamino-3-Mercapto-5-(4-Substituted) Phenyl-1,2,4-Triazoles

Neeraj Upmanyu[1], Sanjay Kumar[2], Kamal Shah[3] and Pradeep Mishra[3]

[1]Pharmaceutical Chemistry Division, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar-470003, India
[2]Defence Research Development and Establishment, Jhansi Road, Gwalior-474001, India
[3]GLA Institute of Pharmaceutical Research, Mathura-281406, India

ABSTRACT: Triazoles and triazoles with different substituent groups are found to possess diverse application in the field of medicine and industry. A series of 4-(substituted) ethanoylamino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazoles were synthesized as novel antimicrobial agents starting from different 4-substituted benzoic acids. The chemical structures of these newly synthesized compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, FAB^{+}-mass spectral data and elemental analyses. The antimicrobial activity of title compounds were examined against two gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and three fungi *(Candida albicans*, *Aspergillus niger* and *Fusarium oxysporum*) using disc diffusion method. Some of the compounds bearing methoxy group exhibited moderate to good antibacterial and antifungal activities.

Key words: 1, 2, 4-Triazoles, zone of inhibition, antibacterial, antifungal.

INTRODUCTION

Combat against bacterial and fungal infection has resulted in the development of a wide variety of antimicrobial drugs. After years of misuse and overuse of these drugs, microorganisms are becoming resistant resulting in a potent global health problem. It is evident that antimicrobial resistance is associated with an increase in mortality.[1] Hence, there is still a critical need for new antimicrobial agents to treat life-threatening diseases. In recent years active research has been initiated on heterocyclic chemistry and thus, scientists are engaged to synthesize new compounds related to various chemical groups like indole, 1,2,4-triazole, thiazole, imidazole, azetidinone, etc. 1,2,4-Triazoles have received considerable attention owing to their synthetic and effective biological importance. 1,2,4-Triazole derivatives are well known in medicinal chemistry due to their diverse biological properties like antibacterial, antifungal,[2-8] antitubercular,[9,10] anticancer,[11] anti-tumor,[12] anti-inflammatory[13-15], anticonvulsant,[16-17] urease inhibitor,[18] openers of Ca-activated potassium (Maxi-K) channel,[19] tubulin polymerization inhibitors,[20] ghrelin receptor antagonist[21] and antiviral activities.[22]

These findings prompted us to synthesize 4-(substituted) ethanoylamino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazoles **6-14a,b** having substitution on aromatic ring and different secondary amino groups were attached on ethanoyl moiety and these compounds were evaluated for antimicrobial activity.

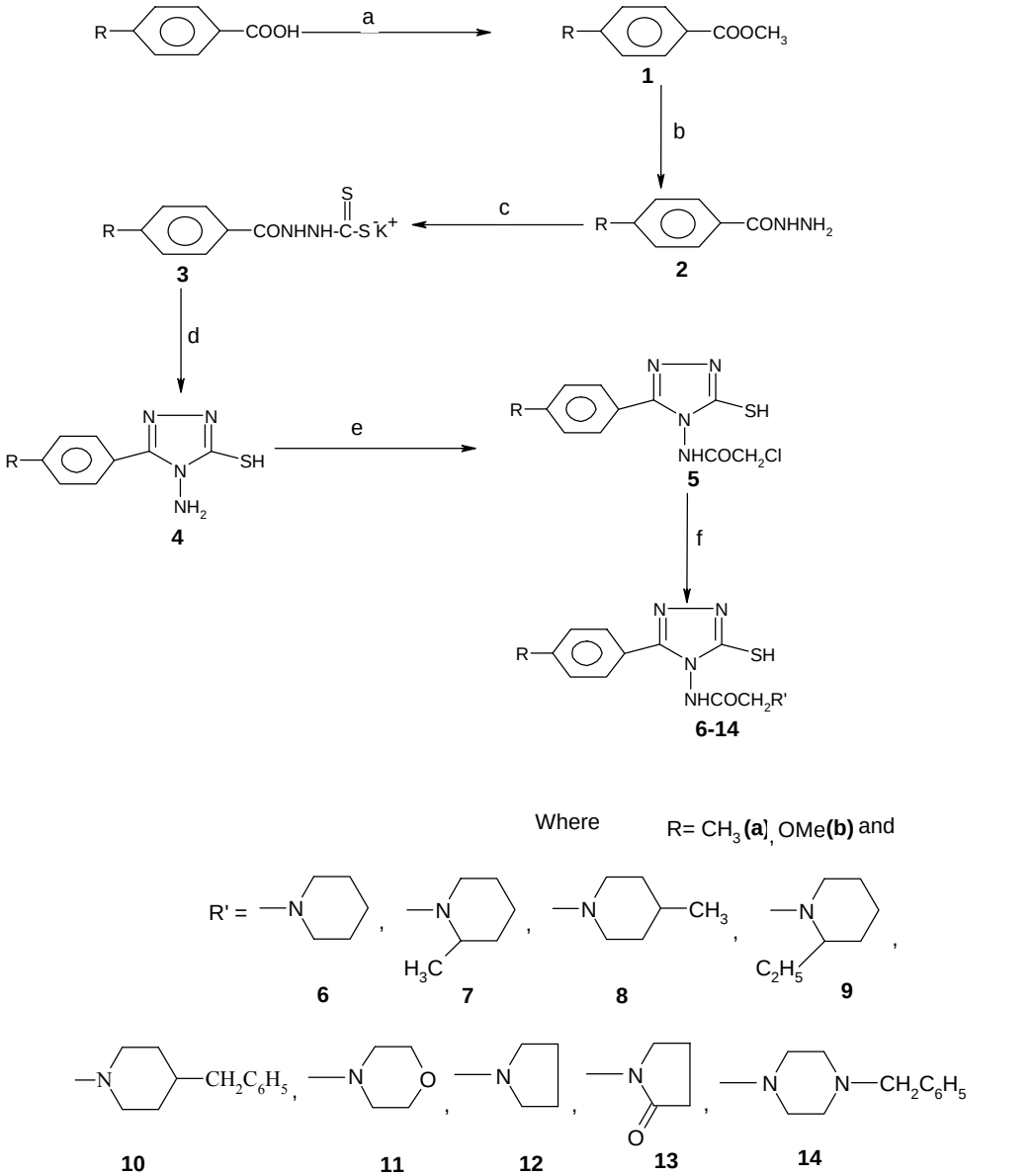
MATERIALS AND METHODS

Thin layer chromatography was used to observe completion of the reaction and purity of the synthesized compounds. Melting points were determined in open glass capillary tubes by using melting point apparatus and were uncorrected. IR spectra in KBr were recorded on a Nicolet-6700

Correspondence to: Neeraj Upmanyu
Mob. No.: 9425128642
Email: drneerajupmanyu@gmail.com

FTIR spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker avance III 400 MHZ spectrophotometer in DMSO-d_6/ $CDCl_3$ using TMS as an internal standard (chemical shifts are expressed in δ, ppm), Mass spectra were recorded on Jeol Sx 102/DA-600 mass spectrometer/data system using fast moving bombardment (FAB) technique and nitrogen analysis was recorded using elemental analyzer Elementar Vario EL III Carlo Erba 1108. The purity of the compounds was confirmed by TLC using Merck silica gel 60 F_{254} plates using a mixture of ethyl acetate and petroleum ether (1:1) and spots were visualized under UV radiation.

The synthesis of 4-(substituted ethanoyl) amino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazoles (**6a-o**) were prepared following stepwise procedures as mentioned in Figure 1.



Reagents and conditions : a) CH_3OH and conc. H_2SO_4, b) NH_2 $NH_2.H_2O$, c) CS_2 and alc. KOH, d) NH_2 $NH_2.H_2O$ and C_2H_5OH, e) $ClCH_2COCl$, f) different secondary amines

Scheme 1. Synthesis of the title compounds (**6-14 a,b**)

General procedure for methyl esters (1a,b). Substituted carboxylic acid (0.1 mol) was taken in methanol (100 ml) in a round bottom flask and conc. sulphuric acid (5.7 ml) was added to that. The mixture was refluxed for 4-6 hr. Excess of methanol was then distilled off. After cooling the contents were transferred to separating funnel containing 100 ml of distilled water. The synthesized ester was repeatedly extracted several times with carbon tetrachloride (30 ml). The combined organic layer was washed with solution of sodium-bi-carbonate (20%) to remove any unreacted acid. After washing with distilled water the organic layer was dried over anhydrous $MgSO_4$. Carbon tetrachloride was then distilled off under reduced pressure to get the ester, which was recrystallized from absolute alcohol.

Methyl 4-methylbenzoate (1a). Yield 94%, mp: 34-36ºC. IR (KBr), υ cm^{-1}: 2856, 2960 (CH_3), 1286, 1121 (C-O-C), 1722 (C=O).

Methyl 4-methoxybenzoate (1b). Yield 78%, mp: 46-48 ºC. IR (KBr), υ, cm^{-1}: 2852, 2958 (CH_3), 1280,1118 (C-O-C), 1720 (C=O).

General procedure for hydrazides (2a,b). Hydrazine hydrate (99%) (5.7 ml, 0.15 mol) was taken in a flat bottom flask and solution of methyl ester **1a,b** (0.1 mol) in ethanol was added dropwise with gentle stirring. After complete addition, the mixture was transferred into a round bottomed flask and refluxed for 4-6 hr. Ethanol was distilled off under reduced pressure. The precipitate formed was filtered and after drying recrystallized from ethanol.

4-Methyl benzohydrate (2a). Yield 93%, mp: 110-112ºC. IR (KBr), υ cm^{-1}: 1650 (C=O), 3050 (Ar-CH), 3304 (NH), 3435, 3345 (NH_2).

4-Methoxy benzohydrate (2b). Yield 92%, mp: 132-134ºC. IR (KBr), υ cm^{-1}: 1642 (C=O), 3070 (Ar-CH), 3310 (NH), 3428, 3350 (NH_2).

General procedure for potassium 3-aroyl dithiocarbazate (3a,b). A mixture of potassium hydroxide (0.15 mol), 100 ml of absolute ethanol and 0.1 mol of the aroylhydrazide **2a,b** were treated with 0.15 mol of carbon disulfide. This mixture was further diluted with 75 ml of absolute ethanol and stirred for 12-16 hr. The solvent was distilled off under reduced pressure. The salts, prepared as described above, were obtained in nearly quantitative yield and were employed without further purification.

General procedure for 4-amino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole (4a,b). A suspension of 0.1 mol of the potassium salt **3a,b** in absolute alcohol, 0.2 mol of hydrazine hydrate (99%) and water (6 ml) was refluxed for 2 to 3 hr. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide gas and a homogenous solution resulted. Cold distilled water (100 ml) was added and the solution was acidified with concentrated hydrochloric acid. The precipitated solid was filtered, washed with 2 × 30 ml portions of cold water and recrystallized from aqueous ethanol (50%).

4-Amino-3-mercapto-5-(4-methylphenyl)-1,2,4-triazole (4a). Yield 75%, mp: 210-212ºC. IR (KBr), υ cm^{-1}: 1350 (C-N), 1540 (C=N), 3000 (Ar-CH), 2550 (SH), 3312 (NH), 3432, 3346 (NH_2).

4-Amino-3-mercapto-5-(4-methoxyphenyl)-1,2,4-triazole (4b). Yield 78%, mp: 201-203ºC. IR (KBr), υ, cm^{-1}: 1345 (C-N), 1538 (C=N), 3050 (Ar-CH), 2540 (SH), 3310 (NH), 3438, 3352 (NH_2), 1262, 1092 (C-O-C).

General procedure for 4-(chloro-acyl amino)-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole. (5a,b). Compound 4-amino-3-mercapto-5-(p-substituted) phenyl-1,2,4-triazole **4a,b** (0.1 mol) was taken in dioxane (50 ml) in a two necked round bottom flask fitted with reflux condenser and a separating funnel. Chloroacetyl chloride 8.75 ml (0.11 mol) was taken in dioxane (25 ml approx), in the separating funnel. The chloroacetyl chloride was added in small portions to the vessel. After the complete addition, the contents of the flask was refluxed for an hour or so. After cooling the contents were poured on crushed ice. The precipitated product was filtered and washed several times with ice cold distilled water and recrystallized from absolute ethanol.

4-(chloroacyl amino)-3-mercapto-5-(4-methyl phenyl)-1,2,4-triazole. (5a). Yield 73%, mp: 176-

178ºC. IR (KBr), υ cm^{-1}: 1350 (C-N), 1540 (C=N), 745 (C-Cl), 3000 (Ar-CH), 2550 (SH), 3305 (NH).

4-(chloro-acyl amino)-3-mercapto-5-(4-methoxy-phenyl)-1,2,4-triazole. (5b). Yield 56%, mp: 164-166ºC. IR (KBr), υ cm^{-1}: 1350 (C-N), 1540 (C=N), 745 (C-Cl), 3000 (Ar-CH), 2550 (SH), 3305 (NH), 1272, 1084 (C-O-C).

General procedure for title compounds (6-14a,b). Compound 4-(chloroacyl-amino)-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole **5a,b** (0.025 mol) and respective amine (0.030 mol) along with the triethylamine (0.030 mol) were taken in a round bottomed flask in benzene (75 ml approx). The contents were refluxed for 3-4 hr. The precipitated triethylamine hydrochloride was separated out. The organic layer was washed several times with distilled water to remove last traces of hydrochloride. Benzene was distilled off under vacuum and the crude product was separated. Purification of the compounds was done by repeated crystallization from appropriate solvents and melting point was determined. Physico-chemical properties of these synthesized compounds are shown in Table 1.

6a: 4-(piperidin-1'yl) ethanoylamino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR(KBr, υ cm^{-1}): 3100 (Ar-H), 2949.9 (C-H), 1800 (C-C overtone), 2521 (S-H), 1660 (C=O), 1370 (C-N), 1530 (C=N str), 3300 (N-H), 1617.1 (Ar-C=C). ^{1}H NMR (DMSO-d_6, δ ppm): δ 1.19-1.76 (m, 10H, piper.), 2.31 (s, 3H, Ar-CH_3), 3.15 (s, 2H, $COCH_2$), 7.13-7.15(d, *J*=8.7, 2H, Ar-H), 7.99-8.00 (d, *J*=8.5, 2H, Ar-H), 9.87 (s, 1H, SH), 10.13 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): δ 163.5 (C_3 and C_5 triazole), 133, 126, 129.5, 141.5, 19.97 ($C_{1'}$,$C_{2'}$ and $C_{6'}$, $C_{3'}$ and $C_{5'}$, $C_{4'}$, $C_{4'a}$), 178.4 (C of NHCO), 58 (C of CH_2N), 49.3, 25.4 and 27.86 (C_a and C_e, C_b and C_d ,C_c of piperidine). FABMS *m/z:* 331 (M+1).

7a: 4-(2'-methyl-piperidin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 3100 (Ar-H), 2949.9 (C-H), 1800 (C-C overtone), 2524 (S-H), 1660 (C=O), 1387 (C-N), 1588 (C=N), 1380 (C-H), 3350 (N-H). ^{1}H NMR (DMSO-d_6, δ ppm): δ 1.13 (s, 3H, CH_3- piper.), 1.31-2.56 (m, 14H, CH, CH_2, & CH_3), 7.01-7.03 (d, *J*=8.3, 2H, Ar-H), 8.03-8.05 (d, *J*=8.9, 2H, Ar-H), 9.67 (s, 1H, SH), 10.01 (s, 1H, CONH).^{13}C-NMR (DMSO-d_6, δ ppm): δ 153 (C_3 and C_5 triazole), 133, 126, 129, 141, 19 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 172 (C of NHCO), 57 (C of CH_2N), 44, 30, 29, 22, 50 and 18 (C_a, C_b, C_c ,C_d , C_e and C_f of 2-Methyl piperidine). FABMS *m/z*: 345 (M+1).

8a: 4-(4'-methyl-piperidin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 3100 (Ar-H), 2949.9 (C-H), 1850 (C-C overtone), 2550 (S-H), 1673 (C=O), 1412 (C-N), 1540 (C=N), 3300 (N-H), 1460 (C-H). ^{1}H NMR (DMSO-d_6, δ ppm): 1.11 (s, 3H, CH_3- piper.), 1.38-2.49 (m, 14H, CH, CH_2, & CH_3), 6.72-7.18 (m, 7H, Ar-H), 8.03-8.05 (d, *J*=8.8, 2H, Ar-H), 9.69 (s, 1H, SH), 10.08 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 148 (C_3 and C_5 triazole), 129, 121, 126, 141, 20 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$, and $C_{4'}$ and $C_{4'a}$), 178 (C of NHCO), 59 (C of CH_2N), 44, 31, 29, 21, 50 and 19 (C_a, C_b, C_c ,C_d, C_e and C_f of 2-Methyl piperidine).FABMS *m/z*: 345 (M+1).

9a: 4-(2'-Ethyl-piperidin-1'yl)ethanoylamino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 3100 (Ar-H str), 2949.2 (C-H str), 1900 (C-C overtone), 2520 (S-H str), 1660 (C=O str), 1410 (C-N), 1550 (C=N str), 3350 (N-H), 1479 (C-H). ^{1}H NMR (DMSO-d_6, δ ppm): 0.71-1.68 (m, 11H, CH_2), 2.78-2.89 (m, 6H, CH, CH_2 & Ar-CH_3), 7.11-7.13 (d, *J*=9.7, 2H, Ar-H), 8.40-8.42 (d, *J*=8.5, 2H, Ar-H), 9.91 (s, 1H, SH), 9.99 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 142 (C_3 and C_5 triazole), 133, 126, 129, 137, 21 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 169 (C of NHCO), 57 (C of CH_2N), 58, 34, 22, 26, and 44 (C_a and C_b, C_c and C_d and C_e of 2-Ethyl piperidine), 9 (C_g of methyl group of 2-Ethyl piperidine). FABMS *m/z:* 359 (M+1).

10a: 4-(4'-benzyl-piperidin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 3024 (Ar-H), 1800 (C-C overtone), 2530 (S-H), 1670 (C=O str), 1617 (Ar-C=C), 1360 (C-N), 1540 (C=N str), 1499 (C-H), 3340 (N-H). ^{1}H NMR (DMSO-d_6, δ ppm): 1.05-1.76 (m, 11H, CH_2), 2.39 (s, 3H, Ar-CH_3), 3.10 (s, 2H,

$COCH_2$), 6.72-7.18(m, 7H, Ar-H), 7.71-7.73 (d, *J*=7.9, 2H, Ar-H), 8.99 (s, 1H, SH), 9.87 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 148 (C_3 and C_5 triazole), 133, 126, 129, 137, 21 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 172 (C of NHCO), 58 (C of CH_2N), 44, 28, 36 (C_a ,C_e and C_b ,C_d and C_c of piperidine) 42.3 (C_f of benzyl piperidine), 139, 128.5, 125, ($C_{a'}$ and $C_{b'}$, $C_{C'}$, C_e, $C_{f'}$ and $C_{d'}$ of phenyl ring of benzyl piperidine). FABMS *m/z:* 421 (M+1).

11a: 4-(Morpholin-1'-yl) ethanoylamino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 1410 (C-N), 1540 (C=N),3100 (Ar-H), 1850 (C-C overtone), 2540 (S-H str), 1660 (C=O), 1068 (C-O), 3360 (N-H). ^{1}H NMR (DMSO-d_6, δ ppm): 2.36-2.59 (t, 2x2H, CH_2-morph.), 2.41-2.69 (m, 7H, CH_2-morph. & Ar-CH_3), 3.13 (s, 2H, $COCH_2$), 7.16-7.18 (d, *J*=10.0, 2H, Ar-H), 8.15-8.17 (d, *J*=9.8, 2H, Ar-H), 9.89 (s, 1H, SH), 10.07 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 138 (C_3 and C_5 triazole), 133, 126, 130, 142, 22 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 166 (C of NHCO), 67 (C of CH_2N), 65, 71 (C_a ,C_e and C_b ,C_d of morpholine). FABMS *m/z:* 333(M+1).

12a: 4-(pyrrolidin-1'yl) ethanoylamino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 1378 (C-N), 1550 (C=N), 3050 (Ar-H), 2920.1 (C-H), 1850 (C-C overtone), 2541 (S-H), 1660 (C=O), 1618 (Ar-C=C), 1507 (C-H). ^{1}H NMR (DMSO-d_6, δ ppm): 1.09-1.17 (t, 2x2H, CH_2-pyrr.), 1.19-1.24 (t, 2x2H, CH_2-pyrr.), 2.36 (s, 3H, Ar- CH_3), 3.10 (s, 2H, $COCH_2$), 7.09-7.11 (d, *J*=9.9, 2H, Ar-H), 8.10-8.12 (d, *J*=8.9, 2H, Ar-H), 9.78 (s, 1H, SH), 10.13 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6 δppm) : 144 (C_3 and C_5 triazole), 133, 126.1, 130, 137, 22 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 171 (C of NHCO), 66 (C of CH_2N), 52, 23 ($C_{a'}$,$C_{d'}$ and $C_{b'}$,$C_{c'}$ of pyrrolidine). FABMS *m/z:* 317 (M+1).

13a: 4-(pyrrolidin-2'one-1'yl) ethanoylamino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 1410 (C-N), 1530 (C=N), 3000 (Ar-H), 2920 (C-H), 1850 (C-C overtone), 2520 (S-H), 1660 (C=O), 1700 (C=O γ-butyrolactam). ^{1}H NMR (DMSO-d_6, δ ppm): 2.06-2.41 (m, 7H, CH_2-pyrr. & Ar-CH_3), 3.49-3.68 (m, 4H, CH_2-pyrr. & $COCH_2$), 7.19-7.21 (d, *J*=9.1, 2H, Ar-H), 8.23-8.25 (d, *J*=9.5, 2H, Ar-H), 9.68 (s, 1H, SH), 10.13 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 148 (C_3 and C_5 triazole), 133, 126, 129, 142, 21 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$ respectively), 178 (C of NHCO), 52 (C of CH_2N), 73, 30, 17, and 43 (C_a , and C_b and C_c and C_d of pyrrolidinone respectively). FABMS *m/z :* 331 (M+1).

14a: 4-(1'-Benzyl-piperazin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methyl) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 1370 (C-N), 1540 (C=N), 3100 (Ar-H), 2925 (C-H), 1800 (C-C overtone), 2541 (S-H), 1660 (C=O), 3436 (N-H), 1074 (C-H piperazine), 1597 (Ar-C=C). ^{1}H NMR (DMSO-d_6, δ ppm): δ 2.36-2.89 (m, 11H, CH_2-piperazin & Ar-CH_3), 3.14 (s, 2H, $COCH_2$), 3.59 (s, 2H, Ar-CH_2), 7.01-7.58 (m, 7H, Ar-H), 7.99-8.01 (d, *J*=8.3, 2H, Ar-H), 8.61 (s, 1H, SH), 9.95 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 143.5 (C_3 and C_5 triazole), 133, 126.1, 129.9, 137, 21.8 ($C_{1'}$ and $C_{2'}$, $C_{6'}$, and $C_{3'}$, $C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 171 (C of NHCO), 59.3 (C of CH_2N), 60, 55 (C_a ,C_e and C_b ,C_d of piperazine) 59 (C_f of benzyl), 136, 129, 128, 127 ($C_{a'}$ and $C_{b'}$, $C_{f'}$ and $C_{c'}$, $C_{e'}$ and $C_{d'}$ of phenyl ring of benzyl piperazine). FABMS *m/z:* 422 (M+1).

6b: 4-(piperidin-1'yl) ethanoylamino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 691 (C-H), 1519 (C=N), 3314 (N-H), 3050 (Ar-H), 2840 (C-H Anisole), 1251 (C-O-C asymm.), 1072 (C-O-C symm.), 2520 (S-H str), 1714 (C=O), 1467 (CH-Piper.). ^{1}H NMR (DMSO-d_6, δ ppm): 1.19-1.76 (m, 10H, piper.), 3.15 (s, 2H, $COCH_2$), 3.75 (s, 3H, Ar-OCH_3), 7.11 (d, 2H, Ar-H), 7.90 (d, 2H, Ar-H), 9.81 (s, 1H, SH), 10.08 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 161 (C_3 and C_5 triazole), 128, 127, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 178 (C of NHCO), 59 (C of CH_2N), 53, 24 and 26 (C_a, C_e, and C_b,C_d and C_c of piperidine). FABMS *m/z:* 347 (M+1).

7b: 4-(2'-methyl-piperidin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 692 (C-H), 1350 (C-N), 1516 (C=N), 3050 (Ar-H str), 2947 (C-H Anisole), 1252

(C-O-C asymm.), 1073 (C-O-C symm.), 2520 (S-H), 1719 (C=O), 1611 (C=C Ar.), 1440 (CH-Piper.). ^{1}H NMR (DMSO-d_6, δ ppm): 1.13 (s, 3H, CH_3- piper.), 1.31-2.56 (m, 11H, CH, CH_2), 3.72 (s, 3H, Ar-OCH_3), 7.06 (d, 2H, Ar-H), 8.10 (d, 2H, Ar-H), 9.76 (s, 1H, SH), 10.05 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 161 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 169 (C of NHCO), 56 (C of CH_2N), 41, 19, 35, 23 and 26 (C_a and C_b, and C_c, and C_d and C_e of 2-Methyl piperidine), 39 (C_f of methyl group). FABMS *m/z:* 361 (M+1).

8b: 4-(4'-methyl-piperidin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 699 (C-H), 1351 (C-N), 1525 (C=N)3050 (Ar-H), 2835 (C-H anisole), 1254 (C-O-C asymm.), 1073 (C-O-C symm.), 2554 (S-H), 1660 (C=O), 1467 (CH-Piper.). ^{1}H NMR (DMSO-d_6, δ ppm): 1.14 (s, 3H, CH_3- piper.), 1.34-2.45 (m, 11H, CH & CH_2), 3.88 (s, 3H, Ar-OCH_3) 6.74-7.20 (m, 7H, Ar-H), 8.08 (d, 2H, Ar-H), 9.71 (s, 1H, SH), 10.03 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 170 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 178 (C of NHCO), 55 (C of CH_2N), 50, 32, 26 and 20 (C_a, C_e, and C_b,C_d and C_c and C_f of 4-Methyl piperidine). FABMS *m/z:* 361 (M+1).

9b: 4-(2'-Ethyl-piperidin-1'yl) ethanoylamino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 693 (C-H), 1351 (C-N), 1551 (C=N str), 3046 (Ar-H), 2840 (C-H Anisole), 1253 (C-O-C asymm.), 1068 (C-O-C symm.), 2554 (S-H), 1660 (C=O), 3315 (N-H), 1467 (CH-Piper.). ^{1}H NMR (DMSO-d_6, δ ppm): 0.74-1.71 (m, 11H, CH_2), 2.76-2.87 (m, 3H, CH & CH_2), 3.79 (s, 3H, Ar-OCH_3), 7.08 (d, 2H, Ar-H), 8.38 (d, 2H, Ar-H), 9.89 (s, 1H, SH), 9.98 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 161 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$), 178 (C of NHCO), 56 (C of CH_2N), 57, 29, 24, 25 and 50 (C_a and C_b, and C_c, and C_d and C_e of 2-Ethyl piperidine), 27 (C_f of methylene group), 9 (C_g of methyl group). FABMS *m/z:* 375 (M+1).

10b: 4-(4'-benzyl-piperidin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 692 (C-H), 1350 (C-N), 1519 (C=N), 3050 (Ar-H), 2981 (C-H Anisole), 1251 (C-O-C asymm.), 1072 (C-O-C symm.), 2520 (S-H), 1716 (C=O), 1466 (CH-Piper.). ^{1}H NMR (DMSO-d_6, δ ppm): 1.05-1.76 (m, 11H, CH_2), 3.59 (s, 3H, Ar-OCH_3), 3.14 (s, 2H, $COCH_2$), 6.75-7.21 (m, 7H, Ar-H), 7.66 (d, 2H, Ar-H), 8.97 (s, 1H, SH), 9.93 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 161 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$ respectively), 178 (C of NHCO), 60 (C of CH_2N), 50, 29 and 32 (C_a,C_e and C_b,C_d and C_c of piperidine respectively), 40 (C_f of benzyl-piperidine), 139, 124 and 126 ($C_{a'}$ and $C_{b'}$, $C_{C'}$, $C_{e'}$, $C_{f'}$ and $C_{d'}$ of benzyl-piperidine). FABMS *m/z:* 437 (M+1).

11b: 4-(Morpholin-1'-yl) ethanoylamino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 699 (C-H), 1352 (C-N), 1522 (C=N), 3100 (Ar-H), 2970 (C-H Anisole), 1253 (C-O-C asymm.), 1040 (C-O-C symm.), 2520 (S-H), 1660 (C=O), 1060 (C-O str. of cyclic ether), 1501 (CH-Morph.), 1615 (C=C Ar.). ^{1}H NMR (DMSO-d_6, δ ppm): 2.33-2.56 (t, 2x2H, CH_2-morph.), 2.38-2.66 (m, 4H, CH_2-morph.), 3.13 (s, 2H, $COCH_2$), 3.85 (s, 3H, Ar-OCH_3) 7.14 (d, 2H, Ar-H), 8.11 (d, 2H, Ar-H), 9.92 (s, 1H, SH), 10.11 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 161 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$ respectively), 178 (C of NHCO), 60 (C of CH_2N), 57 and 72 (C_a,C_e and C_b,C_d of morpholine respectively). FABMS *m/z:* 349 (M+1).

12b: 4-(pyrrolidin-1'yl) ethanoylamino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 699 (C-H), 1352 (C-N), 1530 (C=N), 3098 (Ar-H), 2981 (C-H Anisole), 1250 (C-O-C asymm.), 1080 (C-O-C symm.), 2578 (S-H), 1660 (C=O), 3350 (N-H), 1516 (CH-Pyrrol.), 1615 (C=C Ar.). ^{1}H NMR (DMSO-d_6, δ ppm): 1.08-1.16 (t, 2x2H, CH_2-pyrr.), 1.21-1.26 (t, 2x2H, CH_2-pyrr.), 3.10 (s, 2H, $COCH_2$), 3.86 (s, 3H, Ar-OCH_3), 7.09 (d, 2H, Ar-H), 8.13 (d, 2H, Ar-H), 9.81 (s, 1H, SH), 10.16 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm):

161 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$, respectively), 178 (C of NHCO), 59 (C of CH_2N), 51, 23 (C_a, C_d, and C_b,C_c, of pyrrolidine respectively). FABMS *m/z :* 333 (M+1).

13b: 4-(pyrrolidin-2'one-1'yl) ethanoylamino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 693 (C-H), 1351 (C-N), 1524 (C=N), 3050 (Ar-H), 2950 (C-H Anisole), 1251 (C-O-C asymm.), 1077 (C-O-C symm.), 2520 (S-H), 1660 (C=O), 1700 (C=O γ-butyrolactam), 1510 (C-H Pyrrol.). ^{1}H NMR (DMSO-d_6, δ ppm): 2.07-2.42 (m, 7H, CH_2-pyrr.), 3.45-3.64 (m, 4H, CH_2-pyrr. & $COCH_2$), 3.91 (s, 3H, Ar-OCH_3),7.18 (d, 2H, Ar-H), 8.22 (d, 2H, Ar-H), 9.73 (s, 1H, SH), 10.09 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 167 (C_3 and C_5 triazole), 129, 128, 115, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$, and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$ respectively), 178 (C of NHCO), 53 (C of CH_2N), 177, 36, 16, 40 (C_a and C_b and C_c, and C_d of pyrrolidine respectively). FABMS *m/z:* 347 (M+1).

14b: 4-(1'-Benzyl-piperazin-1'yl) ethanoyl-amino-3-mercapto-5-(4-methoxy) phenyl-1,2,4-triazole. FT-IR (KBr, υ cm^{-1}): 694 (C-H), 1352 (C-N), 1522 (C=N), 3100 (Ar-H), 2980 (C-H Anisole), 1253 (C-O-C asymm.), 1079 (C-O-C symm.), 1070 (C-H piperazine) 2520 (S-H), 1670 (C=O), 3367 (N-H), 1466 (CH-Piper.). 2.37-2.90 (m, 8H, CH_2-piperazin). ^{1}H NMR (DMSO-d_6, δ ppm): 3.16 (s, 2H, $COCH_2$), 3.62 (s, 2H, Ar-CH_2), 3.84 (s, 3H, Ar-OCH_3), 7.05-7.62 (m, 7H, Ar-H), 7.98 (d, 2H, Ar-H), 8.63 (s, 1H, SH), 9.98 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ ppm): 161 (C_3 and C_5 triazole), 129, 127, 116, 163, 56 ($C_{1'}$ and $C_{2'}$,$C_{6'}$ and $C_{3'}$,$C_{5'}$ and $C_{4'}$ and $C_{4'a}$ respectively), 178 (C of NHCO), 59 (C of CH_2N), 57, 55 and 60 (C_a, C_e and C_b, C_d and C_f of piperazine respectively), 62 (C_g 0f 1-benzyl piperazine), 136, 129, 128 and 127 ($C_{a'}$ and $C_{b'}$, $C_{f'}$ and $C_{c'}$, $C_{e'}$ and $C_{d'}$ of 1-benzyl-piperazine). FABMS m/z: 438 (M+1).

Antimicrobial activity

Antibacterial studies. The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-8793), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Bacillus subtilis* (ATCC-6633) [recultured] bacterial strains by disc diffusion method[23] at 70, 50 and 30 µg/ml concentrations, respectively. All the bacterial strains were procured from (Hi-media) ATCC, USA. A standard inoculum (1-2 $\times 10^7$ c.f.u/ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140ºC for an hour. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37ºC. Vancomycin and Amikacin were used as a standard drug. The inhibition zones were measured and compared with the controls.

Antifungal studies. Similarly, the newly prepared compounds were screened for their antifungal activity against *Candida albicans* (MTCC-227), *Aspergillus niger* (MTCC-3323) and *Fusarium oxysporum* (MTCC-2087) by paper disc method. All the fungal strains were procured from Institute of Microbial Technology, Chandigarh. For antifungal screening against *A. niger*, czapek yeast extract agar was used. Malt yeast agar (≈ pH 7.0) was employed as culture media against *C. albicans* and Potato sucrose agar was used as culture medium against *F.oxysporum*[24].

RESULTS AND DISCUSSION

Chemistry. The title compounds (**6-14a,b)** were synthesized according to Scheme 1. The structure of all the synthesized compounds were elucidated by spectral data. Benzoic acid derivatives were converted into their esters (**1a,b)** using methanol and catalytic amount of sulphuric acid. Esters (**1a,b)** on treatment with hydrazine hydrate yielded corresponding aroyl hydrazides (**2-a,b**). The IR spectra of (**2a,b)** showed the absence of ester stretching frequency, instead gave a band at around 1650 cm^{-1} for carbonyl group and showed sharp

Table 1. Physico-chemical properties of the synthesized compounds.

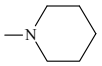
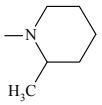
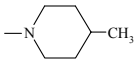
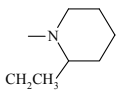
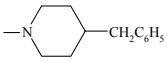
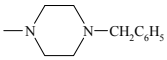
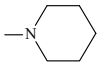
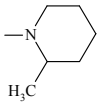
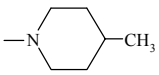
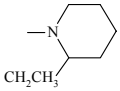
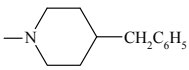
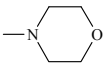
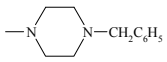
Code No.	R	R'	Yield (%)	M.P. (°C)	Molecular Formula	Nitrogen estimation found (calculated) (%)	Log P
6a	-CH_3		65	134	$C_{16}H_{21}N_5SO$	21.09 (21.13)	3.89
7a	-CH_3		72	126	$C_{17}H_{23}N_5SO$	20.23 (20.27)	4.38
8a	-CH_3		81	105	$C_{17}H_{23}N_5SO$	20.24 (20.27)	4.38
9a	-CH_3		63	122	$C_{18}H_{25}N_5SO$	19.52 (19.48)	4.91
10a	-CH_3		58	150	$C_{23}H_{27}N_5SO$	16.58 (16.61)	5.88
11a	-CH_3		63	148	$C_{15}H_{19}N_5SO_2$	21.04 (21.01)	2.57
12a	-CH_3		72	176	$C_{15}H_{19}N_5SO$	22.10 (22.06)	3.32
13a	-CH_3		70	116	$C_{15}H_{17}N_5SO_2$	21.16 (21.13)	3.40
14a	-CH_3		62	140	$C_{22}H_{26}N_6SO$	19.83 (19.89)	4.18
6b	-OCH_3		64	201	$C_{16}H_{21}N_5SO_2$	20.13 (20.16)	3.59
7b	-OCH_3		41	166	$C_{17}H_{23}N_5SO_2$	19.42 (19.38)	4.08
8b	-OCH_3		67	178	$C_{17}H_{23}N_5SO_2$	19.35 (19.38)	4.08
9b	-OCH_3		55	185	$C_{18}H_{25}N_5SO_2$	18.68 (18.65)	4.62
10b	-OCH_3		59	163	$C_{23}H_{27}N_5SO_2$	13.20 (13.23)	5.58
11b	-OCH_3		74	186	$C_{15}H_{19}N_5SO_3$	20.08 (20.04)	2.28
12b	-OCH_3		61	196	$C_{15}H_{19}N_5SO_2$	21.04 (21.01)	3.03
13b	-OCH_3		65	138	$C_{15}H_{17}N_5SO_3$	20.13 (20.16)	3.11
14b	-OCH_3		55	181	$C_{22}H_{26}N_6SO_2$	19.12 (19.16)	3.88

Table 2. Antibacterial activity of the synthesized compounds (6-14 a,b).

Comp.	*S. aures*			*B. subtilis*			*P. aeruginosa*			*E.coli*		
	70 µg/ml	50 µg/ml	30 µg/ml	70 µg/ml	50 µg/ml	30 µg/ml	70 µg/ml	50 µg/ml	30 µg/ml	70 µg/ml	50 µg/ml	30 µg/ml
6a	8.26 ±0.38	7.23 ±0.55	6.40 ±0.46	8.13 ±0.56	7.16 ±0.47	6.16 ±0.70	9.20 ±0.70	8.00 ±0.80	7.33 ±1.14	7.33 ±0.56	6.50 ±0.60	6.20 ±0.66
7a	9.13 ±0.56	8.13 ±0.56	7.16 ±0.70	9.20 ±0.70	8.06 ±0.65	7.17 ±0.56	10.12 ±0.56	9.23 ±0.65	8.26 ±0.68	8.23 ±0.60	7.13 ±0.56	6.30 ±0.46
8a	7.03 ±0.56	6.16 ±0.70	6.13 ±0.56	8.10 ±0.46	7.76 ±0.61	6.10 ±0.10	7.16 ±0.47	6.40 ±0.46	6.16 ±0.47	6.46 ±0.71	6.33 ±0.21	6.16 ±0.70
9a	8.03 ±0.56	7.10 ±0.70	6.16 ±0.80	7.03 ±0.56	6.16 ±0.70	6.13 ±0.56	8.96 ±0.55	7.10 ±0.56	6.63 ±0.56	8.40 ±0.56	7.10 ±0.70	6.46 ±0.71
10a	10.23 ±0.70	9.23 ±0.55	8.40 ±0.46	8.10 ±0.75	7.06 ±0.65	6.13 ±0.56	7.10 ±0.46	6.76 ±0.61	6.17 ±0.56	8.20 ±0.66	7.23 ±0.70	6.83 ±0.56
11a	12.26 ±0.38	10.23 ±0.55	9.40 ±0.46	11.53 ±0.55	10.27 ±0.56	9.10 ±0.76	13.13 ±0.56	12.20 ±0.70	11.16 ±0.47	12.20 ±0.56	11.50 ±0.56	10.06 ±0.56
12a	6.20 ±0.46	6.17 ±0.56	6.10 ±0.10	6.33 ±0.21	6.17 ±0.56	6.10 ±0.10	6.76 ±0.61	6.33 ±0.21	6.16 ±0.70	6.40 ±0.46	6.33 ±0.21	6.17 ±0.56
13a	7.13 ±0.65	6.20 ±0.53	6.10 ±0.10	7.40 ±0.30	6.40 ±0.46	6.13 ±0.56	8.06 ±0.65	7.80 ±0.56	6.10 ±0.56	7.03 ±0.65	6.13 ±0.56	6.10 ±0.56
14a	8.16 ±0.56	7.10 ±0.75	6.46 ±0.71	7.10 ±0.56	6.20 ±0.70	6.10 ±0.10	8.20 ±0.56	7.13 ±0.70	6.20 ±0.70	9.70 ±0.45	8.23 ±0.61	7.23 ±1.04
6b	10.30 ±0.56	9.23 ±0.70	8.20 ±0.56	9.20 ±0.56	8.30 ±0.65	7.26 ±0.90	11.23 ±0.55	9.20 ±0.56	8.13 ±0.65	10.26 ±0.56	9.16 ±0.56	8.26 ±0.65
7b	9.16 ±0.60	8.23 ±0.65	7.65 ±0.55	10.16 ±0.65	9.26 ±1.06	8.14 ±0.56	8.13 ±0.56	7.12 ±0.56	6.26 ±0.92	8.20 ±0.56	7.26 ±0.60	6.23 ±0.60
8b	8.03 ±0.56	7.06 ±0.65	6.20 ±0.70	7.03 ±0.75	6.40 ±0.30	6.10 ±0.10	8.03 ±0.75	7.10 ±0.76	6.20 ±1.10	9.03 ±0.56	8.16 ±0.56	7.30 ±0.56
9b	11.13 ±0.56	10.16 ±0.75	9.03 ±0.56	7.06 ±0.56	6.26 ±0.75	6.10 ±0.75	10.56 ±0.56	9.16 ±0.75	8.63 ±0.56	9.16 ±0.56	8.20 ±0.82	7.30 ±0.46
10b	8.23 ±0.56	7.16 ±0.56	6.33 ±0.55	8.23 ±0.60	7.43 ±0.70	6.16 ±0.47	9.23 ±0.56	8.13 ±0.56	7.33 ±0.56	10.16 ±0.56	9.13 ±0.56	8.80 ±0.56
11b	15.06 ±0.56	12.23 ±0.56	11.10 ±0.56	13.96 ±0.56	12.03 ±0.80	11.16 ±0.80	16.13 ±0.70	14.03 ±0.56	13.16 ±0.75	15.23 ±0.65	13.17 ±0.75	12.16 ±0.80
12b	9.10 ±0.70	8.23 ±0.56	7.10 ±0.56	7.43 ±0.70	6.33 ±0.21	6.13 ±0.65	8.10 ±0.56	7.26 ±0.56	6.56 ±0.56	7.03 ±0.56	6.26 ±0.65	6.06 ±0.56
13b	6.33 ±0.21	6.23 ±0.21	6.10 ±0.10	7.16 ±0.75	6.36 ±0.64	6.13 ±0.65	10.16 ±0.65	9.10 ±0.80	8.20 ±0.65	8.13 ±0.86	7.03 ±0.56	6.20 ±0.75
14b	10.06 ±0.70	9.10 ±0.80	8.20 ±0.75	8.06 ±0.56	7.17 ±0.56	6.20 ±0.56	10.23 ±0.70	9.10 ±0.56	8.23 ±0.56	14.80 ±0.56	12.20 ±0.65	11.20 ±0.70
Vancomycin			18.20 ±0.30			17.43 ±0.35						
Amikacin									22.10 ±0.36			21.27 ±0.56

bands in the region of 3300-3430 cm^{-1} due to -$NHNH_2$ group. Aroyl hydrazides (**2a,b)** on reacting with alcoholic potassium hydroxide and carbon di-sulfide yielded corresponding potassium 3-aroyl dithiocarbazate (**3a, b**). The IR spectra of **3a, b** showed the presence of C=S stretching at around 1129 cm^{-1} and C=O stretching of amide at around 1667 cm^{-1}. Potassium 3-aroyl dithiocarbazate (**3a,b)** on cyclization reaction with ethanol and hydrazine hydrate yielded 4-amino-3-mercapto-5-substituted-phenyl 1,2,4-triazole (**4a,b**). 1,2,4-Triazole showed C-N stretching at around 1380 cm^{-1} and C=N stretching at around 1530 cm^{-1}. Free thiol group which was attached to 1,2,4-triazole showed stretching band at around 2520-2580 cm^{-1} and 3200-3300 cm^{-1} due to amino group. Signal at around δ**e** 143 ppm confirmed the C-3 and C-5 carbon of 1,2,4-triazole ring. 4-amino-3-mercapto-5-substituted-phenyl 1,2,4-triazole (**4a,b**) on treatment with chloroacetyl chloride in dioxane yielded 4-(chloro-acetyl amino)-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole (**5a,b**). The IR spectra of **5a,b** showed

the disappearance of 3200-3300 stretching of amino group. Signals at around δe 165 ppm and 53 ppm confirmed the –NHCO and CH_2 group. 4-(Chloro-acetyl amino)-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole (**5a,b**) on treatment with different secondary amines in triethyl amine yielded title compounds (**6-14a,b**) (Scheme 1). IR spectra showed C-N and C=N stretching frequencies of triazole at around 1350 cm^{-1} and 1540 cm^{-1}, respectively. Aromatic C-H stretching of phenyl ring appeared around 3000-3100 cm^{-1}. Further ^{13}C-NMR spectra, which showed C_3 and C_5 signal of 1,2,4-triazole at around δe 145 ppm. Carbonyl carbon and methylene carbon of –NHCOCH_2N< were seen at δe 173 ppm and 56 ppm, respectively and ^{1}H NMR spectra, showed singlet of thiol group between 8.67-9.92 ppm, singlet of –CONH between 9.87-10.16 ppm.

Table 3. Antifungal activity of the synthesized compounds (6-14 a,b).

Comp.	*C. albicans*			*A. niger*			*F. oxysporum*		
	70 µg/ml	50 µg/ml	30 µg/ml	70 µg/ml	50 µg/ml	30 µg/ml	70 µg/ml	50 µg/ml	30 µg/ml
6a	7.13 ±0.75	6.40 ±0.46	6.26 ±0.60	10.13 ±0.65	9.16 ±0.75	7.26 ±0.65	9.03 ±1.05	8.30 ±0.70	7.23 ±0.65
7a	9.16 ±0.55	8.20 ±0.76	7.26 ±0.65	11.16 ±0.70	10.10 ±0.56	9.13 ±0.70	10.20 ±0.65	9.16 ±0.71	8.16 ±0.55
8a	7.13 ±0.71	6.17 ±0.65	6.06 ±0.65	7.03 ±0.55	6.33 ±0.21	6.16 ±0.70	9.06 ±0.60	8.16 ±0.75	7.26 ±0.65
9a	8.21 ±0.66	7.16 ±0.75	6.23 ±0.70	8.23 ±0.55	7.26 ±0.81	6.17 ±0.65	7.06 ±0.65	6.26 ±1.20	6.06 ±1.30
10a	7.13 ±0.65	6.33 ±0.55	6.26 ±0.65	8.16 ±0.60	7.16 ±0.75	6.26 ±0.60	7.13 ±0.65	6.33 ±0.55	6.16 ±0.75
11a	11.17 ±0.55	10.00 ±0.98	8.27 ±0.65	15.03 ±0.86	14.20 ±0.76	12.16 ±0.47	8.21 ±0.65	7.17 ±0.65	6.23 ±0.65
12a	7.10 ±0.56	6.20 ±0.72	6.13 ±0.65	7.20 ±0.65	6.16 ±0.75	6.10 ±0.10	7.76 ±0.61	6.40 ±0.46	6.26 ±0.60
13a	6.33 ±0.21	6.23 ±0.21	6.03 ±0.60	7.23 ±0.70	7.17 ±0.60	6.67 ±0.45	6.33 ±0.21	6.17 ±0.65	6.03 ±0.65
14a	8.26 ±0.65	7.46 ±0.71	6.23 ±0.65	9.13 ±0.55	8.23 ±0.60	7.13 ±0.65	8.97 ±0.65	7.26 ±0.75	6.03 ±1.36
6b	11.03 ±0.55	10.13 ±0.70	9.20 ±0.75	12.20 ±0.56	11.20 ±0.70	10.26 ±0.65	10.37 ±0.55	9.10 ± 0.75	8.06 ± 0.55
7b	12.03 ±0.55	11.26 ±0.65	10.13 ±0.70	14.10 ±0.65	12.93 ±1.22	12.13 ±0.65	11.10 ±0.70	10.23 ±0.65	9.23 ±0.65
8b	11.10 ±0.65	10.13 ±0.75	9.16 ±0.89	11.21 ±0.60	10.20 ±0.75	9.13 ±0.70	9.06 ±0.65	8.16 ±0.81	7.03 ±0.65
9b	13.21 ±0.56	12.13 ±0.65	11.06 ±0.65	9.13 ±0.75	8.00 ±0.70	7.03 ±0.70	7.06 ±0.60	6.26 ±0.65	6.16 ±0.70
10b	8.23 ±0.65	7.13 ±0.65	6.43 ±0.35	10.13 ±0.65	9.03 ± 0.55	8.13 ±0.70	8.16 ±0.65	7.20 ±0.66	6.20 ±0.66
11b	17.13 ±0.55	16.17 ±0.65	14.27 ±0.65	18.03 ±0.70	16.20 ±0.92	14.26 ±0.45	10.13 ±0.65	9.30 ±0.66	8.16 ±0.65
12b	7.80 ±0.66	6.36 ±0.64	6.23 ±0.70	9.16 ±0.65	8.13 ±0.61	7.23 ±0.70	8.03 ±0.80	7.13 ±0.75	6.23 ±0.65
13b	6.76 ±0.61	6.33 ±0.21	6.16 ±0.70	7.10 ±0.66	6.20 ±0.70	6.06 ±0.70	7.10 ±0.66	6.76 ±0.61	6.20 ±0.70
14b	10.13 ±0.60	9.06 ±0.65	8.23 ±0.70	11.86 ±0.55	10.13 ±0.80	8.96 ±0.55	6.36 ±0.64	6.23 ±0.70	6.10 ±0.10
Clotrim azole			21.87 ± 1.07			22.56 ±0.51			19.26 ±0.56

Antibacterial activity. The two series of 1,2,4-triazole derivatives (**6-14a,b**) were screened for *in vitro* antibacterial activity against two gram positive (*S. aureus* ATCC 25923, *B. subtilis* ATCC 6633)

and two gram negative (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 8793) recultured bacterial strains by disc diffusion method.[23] Vancomycin was used as standard drug for gram positive strains and amikacin was used as standard drug for gram negative strains. Results showed that compounds **11a, 6b**, **9b**, **11b** and **14b** exhibited good antibacterial activity against all tested bacterial strains. Compounds **8a**, **12a** and **13b** axhibited lesser degree of activity. These all active synthesized compounds were more effective against *S. aureus* and *P. aeruginosa* bacterial strains than the two others. The zone of inhibition of the tested compounds at different concentrations are shown in Table 2.

Antifungal activity. For *in vitro* antifungal activity, three fungal species *C. albicans,* MTCC 227, *A. niger* MTCC-3323 and *F. oxysporum,* MTCC-2087 were used and compared with standard drug clotrimazole. Most of the compounds showed good antifungal activity against *A. niger* compare to other two fungal strains. Compounds **7a**, **11a**, **6b**, **7b** and **11b** exhibited good antifungal activity against all tested fungal strains. Compounds **8a**, **12a**, **13a** and **13b** showed lesser degree of activity. The remaining compounds displayed poor activities against the fungal strains as compared to standard drug clotrimazole. The zone of inhibition of the tested compounds at different concentrations are shown in Table 3.

Log*p* values demonstrated the crucial role of lipophilicity in determining the antimicrobial activity. Increasing ring size of the compound leads to increase in the log*p* value, which further influences antimicrobial activity.

CONCLUSION

Compounds having piperidine, 2-ethyl piperidine, morpholine and benzyl piperazine group at position 4 of C_2 acetamido group of the triazole ring system, displayed good antibacterial activity. SAR studies were also suggesting that the majority of the compounds having 4-methoxy phenyl group at position 5 of the triazole ring showed good antibacterial activity. Antifungal screening confirmed that compounds with piperidine, 2-methyl piperidine and morpholine have significant antifungal activity. Structure activity relationship also reveals that majority of the compounds having para-methoxy phenyl group at position 5 of the triazole ring showed good antifungal activity than the methyl group. So, it seems from antimicrobial results that substitution at phenyl position and at 4^{th} position of triazole ring played vital role in increasing antimicrobial activity.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Defence Research Development and Establishment, Gwalior for providing necessary facility and also to Dr. Urmila Tuteja, Head, Microbiology Division, DRDE, Gwalior and Head, Dept. of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar (M.P.).

REFERENCES

1. Hof, H. 2008. Is there a serious risk of resistance development to azoles among fungi due to the widespread use and long-termapplication of azole antifungals in medicine? *Drug Res. Updat.* **11**, 25-31.
2. Mathew, V., Keshavayya, J., Acharya, V.P.V. and Reddy, B.M. 2006. Heterocyclic system containing bridgehead nitrogen atom: synthesis and pharmacological activities of some substituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles. *Eur. J. Med. Chem.* **41**, 1048-1058.
3. Turan-Zitouni, G., Kaplancıklı, Z.A., Yıldız, M.T. and Demet Kaya, P.C. 2005. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[*N*-(2-thiazolyl) acetamido]thio-4H-1,2,4-triazole derivatives. *Eur. J. Med. Chem.* **40**, 607-613.
4. Upmanyu, N., Kumar, S., Kharya, M.D., Shah, K. and Mishra P. 2011. Synthesis and antimicrobial evaluation of some novel 1,2,4-triazole derivatives. *Acta Pol. Pharm. Drug Res.* **68**, 213-221.
5. El Rayes, S.M. 2010. Convenient synthesis and antimicrobial activity of some novel amino acid coupled triazoles. *Molecules.* **15**, 6759-6772.
6. Ezabadi, I.R., Canoutsis, C., Zoumpoulakis, P., Geronikaki, A., Sokovic, M., Glamocilija, J. and Ćirić, A. 2008. Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: synthesis, biological evaluation, lipophilicity, and conformational studies. *Bioorg. Med. Chem.* **16**, 1150-1161.
7. Kaplancıklı, Z.A., Turan-Zitouni, G., Özdemir, A. and Revial, G. 2008. New triazole and triazolothiadiazine derivatives as possible antimicrobial agents. *Eur. J. Med. Chem.* **43**, 155-159.

8. Serdar, M., Güumrükçüğlu, N., Karaoğlu, Ş.A. and Demĭrbaş, N. 2007. Synthesis of some novel 3,5-diaryl-1,2,4-triazole derivatives and investigation of their antimicrobial activities. *Turk. J. Chem.* **31**, 315-326.
9. Shiradkar, M.R., Murahari, K.K., Gangadasu, H.R., Suresh, T., Kalyan, C.A., Panchal, D., Kaur, R., Burange, P., Ghogare, J., Mokale, V. and Raut, M. 2007. Synthesis of new S-derivatives of clubbed triazolyl thiazole as anti-Mycobacterium tuberculosis agents. *Bioorg. Med. Chem.* **15**, 3997-4008.
10. Shiradkar, M., Kumar, G.V.S., Dasari, V., Tatikonda, S., Akula, K.C. and Shah, R. 2007. Clubbed triazoles: A novel approach to antitubercular drugs. *Eur. J. Med. Chem.* **42**, 807-816.
11. Sztanke, K., Tuzimski, T., Rzymowska, J., Pasternak, K. and Kandefer-Szerszen, M. 2008. Synthesis, determination of the lipophilicity, anticancer and antimicrobial properties of some fused 1,2,4-triazole derivatives. *Eur. J. Med. Chem.* **43**, 404-419.
12. Romagnoli, R., Baraldi, P.G., Cruz-Lopez, O., Cara, C.L., Carrion, M.D., Brancale, A., Hamel, E., Chen, L., Bortolozzi, R., Basso, G. and Viola, G. 2010. Synthesis and antitumor activity of 1,5-disubstituted 1,2,4-triazoles as Cis-restricted combretastatin analogues. *J. Med. Chem.* **53**, 4248-4258.
13. Kumar, H., Javed, S.A., Khan, S.A. and Amir, M. 2008. 1,3,4-Oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxy acetic acid: Synthesis and preliminary evaluation of biological properties. *Eur. J. Med. Chem.* **43**, 2688-2698.
14. Abdel-Megeed, A.M., Abdel-Rahman, H.M., Alkaramany, G.S. and El-Gendy, M.A. 2009. Design, synthesis and molecular modeling study of acylated 1,2,4-triazole-3-acetates with potential anti-inflammatory activity. *Eur. J. Med. Chem.* **44**, 117-123.
15. Navidpour, L., Shafaroodi, H., Abdi, K., Amini, M., Ghahremani, M.H., Dehpourd, A.R. and Shafieea, A. 2006. Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4H-1,2,4-triazoles as selective COX-2 inhibitors. *Bioorg. Med. Chem.* **14**, 2507–2517.
16. Küçükgüzel, I., Küçükgüzel, Ş.G., Rollas, S., Ötük-Sanıs, G., Özdemir, O., Bayrak, I., Altug, T. and Stables, J.P. 2004. Synthesis of some 3-(Arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4H-1,2,4-triazole derivatives and their anticonvulsant activity. *IL FARMACO*. **59**, 893-901.
17. Cui, X.S., Chen, J., Chai, K.Y., Lee, J.S. and Quan, Z.S. 2009. Synthesis and anticonvulsant evaluation of 3-substituted-4-(4-hexyloxyphenyl)-4*H*-1,2,4-triazoles. *Med. Chem. Res.* **18**, 49-58.
18. Akhtar, T., Hameed, S., Khan, K.M., Khan, A. and Choudhary, M.I. 2010. Design, synthesis, and urease inhibition studies of some 1,3,4-oxadiazoles and 1,2,4-triazoles derived from mandelic acid. *J. Enz. Inhibit. Med. Chem.* **25**, 572-576.
19. Romine, J.L., Martin, S.W., Gribkoff, V.K., Boissard, C.G., Dworetzky, S.I., Natale, J., Li, Y., Gao, Q., Meanwell, N.A. and Starrett, J.E. 2002. 4,5-Diphenyltriazol-3-ones: Openers of large-conductance Ca^{2+}-activated potassium (Maxi-K) channels. *J. Med. Chem.* **45**, 2942-2952.
20. Zhang, Q., Peng, Y., Wang, X.I., Keenan, S.M., Arora, S. and Welsh, W.J. 2007. Highly potent triazole-based tubulin polymerization inhibitors. *J. Med. Chem.* **50**, 749-754.
21. Moulin, A., Demange, L., Ryan, J., Mousseaux, D., Sanchez, P., Bergé, G., Gagne, D., Perrissoud, D., Locatelli, V., Torsello, A., Galleyrand, J.C., Fehrentz, J.A. and Martinez J. 2008. New trisubstituted 1,2,4-triazole derivatives as potent ghrelin receptor antagonists. 3. synthesis and pharmacological *in vitro* and *in vivo* evaluations. *J. Med. Chem.* **51**, 689-693.
22. Witkowski, J.T., Robins, R.K., Sidwell, R.W. and Simon, L.N. 1972. Design, synthesis, and broad spectrum antiviral activity of 1-.beta.-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and related nucleosides. *J. Med. Chem.* **15**, 1150-1154.
23. Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. 1975. *Medicinal Microbiology*, 12th Eds., **2**, Churchill Livingstone, London, pp. 196-202.
24. Microbial Type Culture Collection and Gene Bank (MTCC), 2000. Institute of Microbial Technology (IMTECH), Chandigarh, 5th Ed., Medium No-137, pp. 130-138.