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Research Article
Biological Sciences

SYNTHESIS, CHARACTERIZATION AND ANTIMYCOBACTERIAL ACTIVITY OF SOME NEWER 1, 2, 4-TRIAZOLE DERIVATIVES

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ABSTRACT

Due to their useful application in different areas of biological activity and as industrial intermediates eighteen 1,2,4-thiadiazole derivatives were synthesized using appropriate synthetic route with different substitutions. All of the synthesized compounds have been confirmed by elemental analyses, IR and ^{13}C NMR spectral data. Antimycobacterial activity of the synthesized compounds was carried out and percentage reduction in relative light units (RLU) was calculated using luciferase reporter phages (LRP) assay. Percentage reduction in relative light units (RLU) for rifampicin was also calculated. The test compounds showed significant antitubercular activity against Mycobacterium tuberculosis H37Rv and clinical isolates: S, H, R, and E resistant M. tuberculosis, when tested in vitro. Most of the compounds exhibited significant antimycobacterial activity. Against Mycobacterium tuberculosis H_{37}Rv compound B6 found to be most potent while, against Clinical isolate: S, H, R & E resistant M. tuberculosis (MDR) compound A5 caused maximum percentage reduction in light units at 50 µg/mL concentration.

KEY WORDS

1,2,4-Triazole, Rifampicin, LRP assay, Mycobacterium tuberculosis.

INTRODUCTION

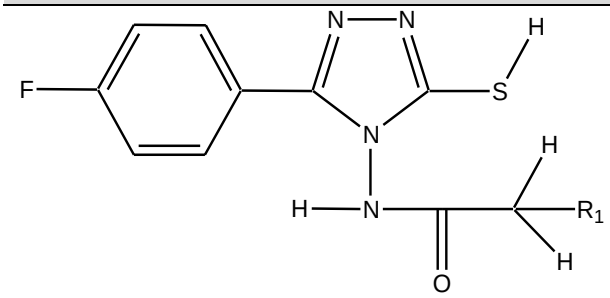
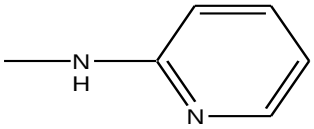
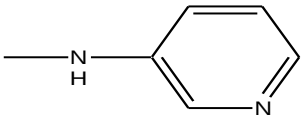
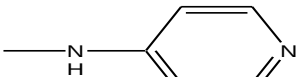
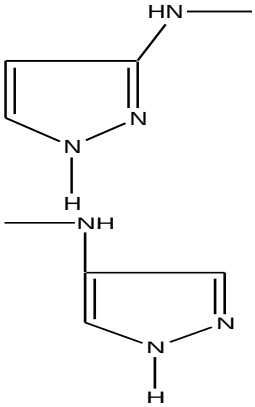
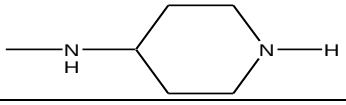
In recent years, the number of life-threatening infectious diseases caused by multi-drug resistant Gram-positive and Gram-negative pathogen bacteria has reached an alarming level in many countries around the world[1,2]. Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis* with high mortality rates[3-5]. Among the threats of infectious diseases, the tuberculosis alone is the greatest cause of mortality worldwide; roughly two million peoples were killed by tuberculosis every year [6]. In addition; HIV-1 and 2 infections has been a major factor in the actual resurgence of tuberculosis[7]. Multidrug-resistant (MDR) strains of *M. tuberculosis* are another problem of antitubercular chemotherapy, two most effective chemotherapeutic agents rifampin and isonicotinic acid hydrazide (INH) are now reported to be resistant against *M. tuberculosis*[8]. Currently, there is an overwhelming need to develop new structural

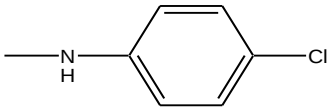
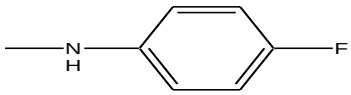
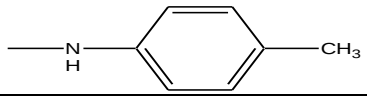
classes of antitubercular agents that allow shorter and more effective therapies[3, 9-11].

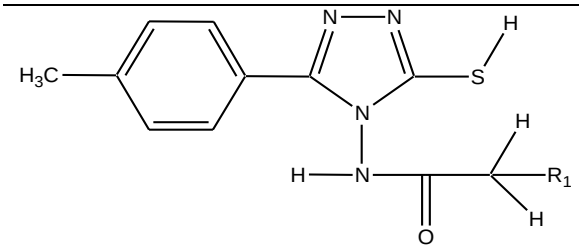
Various 1,2,4-triazoles found extensive investigations due to their useful application in different areas of biological activity and as industrial intermediates such as antiasthmatic[12], hypnotic[13], cytotoxic[14] and hypotensive[15-16], anti-inflammatory activity[17-18], anticonvulsant[19] and plant growth regulator anti coagulants[20].

A large number of 1,2,4-triazoles containing systems also showed remarkable anti-infectious profile viz. antibacterial[21], antimycobacterial[22], antifungal[23], antiviral (ribavirin)[24], antifungal (fluconazole)[25], antimicrobial[26], antibacterial[27], insecticidal[28] and HIV-1 protease inhibitors[29] activities. Present work is a further effort with 1,2,4-triazole derivatives.

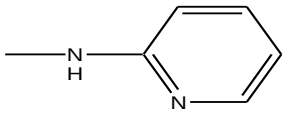
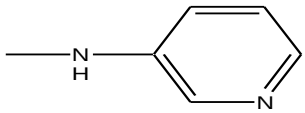
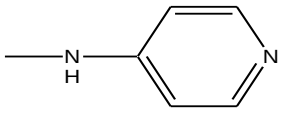
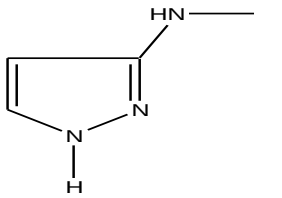
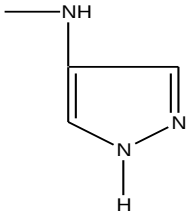
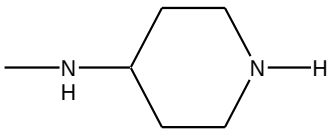
Table 1:Melting point, yield, molecular weight, molecular formula and types of substitutions on the synthesized compounds.

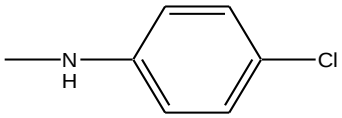
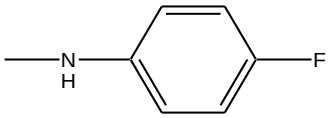
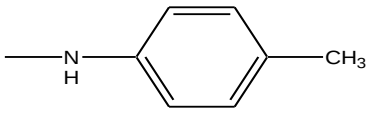
Comp.	Chemical name	Mol. Formula	Mol. Wt.	Yield (%)	Melting Point (°C)
					
General structure for compounds A1-A9					
A-1		$C_{15}H_{13}FN_6OS$	344.37	57	245-247
A-2		$C_{15}H_{13}FN_6OS$	344.37	61.7	243-245
A-3		$C_{15}H_{13}FN_6OS$	344.37	57.2	262-264
A-4		$C_{13}H_{12}FN_7OS$	333.35	52	251-253
A-5		$C_{13}H_{12}FN_7OS$	333.35	43	249-251
A-6		$C_{15}H_{19}FN_6OS$	350.42	53.5	263-265

A-7		$C_{16}H_{13}ClFN_5OS$	377.82	48	262-264
A-8		$C_{16}H_{13}F_2N_5OS$	361.37	48.3	265-267
A-9		$C_{17}H_{16}FN_5OS$	357.41	52.7	269-271



General structure for compounds B1-B9

B-1		$C_{16}H_{16}N_6OS$	340.40	35	175-177
B-2		$C_{16}H_{16}N_6OS$	340.40	39.2	179-181
B-3		$C_{16}H_{16}N_6OS$	340.40	41.2	195-197
B-4		$C_{14}H_{15}N_7OS$	329.38	33	164-166
B-5		$C_{14}H_{15}N_7OS$	329.38	31.2	155-157
B-6		$C_{16}H_{22}N_6OS$	346.45	32	153-155

B-7		$C_{17}H_{16}ClN_5OS$	373.86	40	>150
B-8		$C_{17}H_{16}FN_5OS$	357.41	37.1	131-133
B-9		$C_{18}H_{19}N_5OS$	353.44	39	147-149

MATERIALS AND METHODS

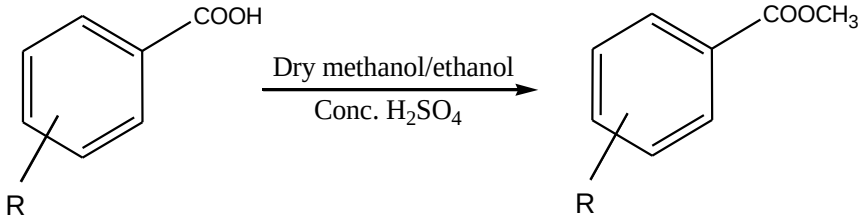
General

Starting material for the synthesis of 5-aryl-4-amino-3-mercapto-4-H-1,2,4-triazoles and Synthesis of 5-aryl-4-(chloroacetylamino)-3-mercapto-4H-1,2,4- triazoles was prepared starting from aromatic carboxylic acid. First of all the aromatic carboxylic acid was converted into corresponding esters using dry methanolor ethanol and concentrated sulphuric acid. subsequently the prepared esters were transformed into the hydrazide using hydrazine hydrate in ethanol. After this the potassium dithiocarbazinate were prepared using CS_2/KOH in ethanol. In the later part of the synthesis the side chain of dithiocarbazinate was cyclised using hydrazine hydrate under refluxing conditions. In the second last step of the synthesis the H of NH2 was replaced with COCH2Cl using chloro acetyl chloride. Finally synthesis of amino derivative of 5-aryl-4-(chloroacetylamino)-3-mercapto-4H-1,2,4-triazoles was carried out using respective amines under reflux conditions.

The synthesized compounds were subjected to qualitative tests for nitrogen, sulphur and halogen wherever desired. Quantitative analysis for nitrogen and carbon was done by Elemental Vario EL III Carlo Erba 1108. IR spectrum were recorded on Shimadzu IR Affinity-1 IR spectrophotometer in KBr pellets. NMR spectra were recorded on C^{13} Advance Brucher DRX 300 MHz spectrometer. Mass spectra were recorded on Jeol Sx 102/DA-6000 mass spectrometer using fast atomic bombardment (FAB) technique. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using silica gel G in various solvent system like hexane and ethyl acetate (6:4), visualization was done using iodine vapors in an iodine chamber or in 30% sulphuric acid. Melting point, yield, molecular weight, molecular formula and types of substitutions on the synthesized compounds are represented in **Table 1**.

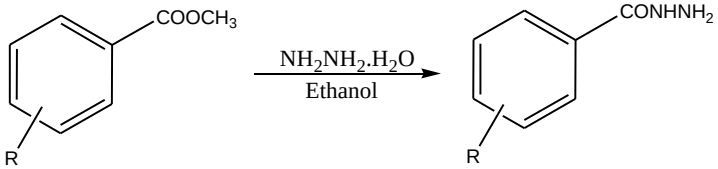
Syntheses of the target compounds **(A1-A9) & (B1-B9)** were performed by following steps.

Synthesis of ester from acid



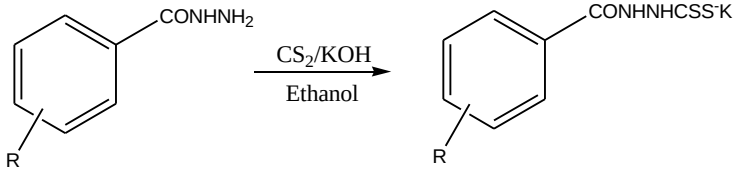
A mixture of substituted benzoic acid/hetero aromatic acid (0.3 mol), 130 mL of absolute alcohol and 3.3 mL of conc. H_2SO_4 was refluxed for 2 h on water bath. After completion of reaction, excess of ethanol was distilled off and content was transferred into separating funnel containing 310 mL distilled water. Carbon-tetrachloride (20 mL) was added, aqueous layer and ester layers were separated. Ester layer (lower layer) was taken in another separating funnel and shaken with a strong solution of sodium bicarbonate until all free acid was removed and no further evolution of carbon dioxide occur. Washed with water and dried by pouring into a small conical flask containing 7.5g magnesium sulphate. Corked the flask, shaken for 2 minutes then carbon tetrachloride was distilled off under reduced pressure. The resulting colorless liquid was collected and the completion of reaction was checked by TLC using hexane and ethyl acetate (6:4) and iodine vapours as a detecting reagent.

Synthesis of hydrazide of synthesized ester



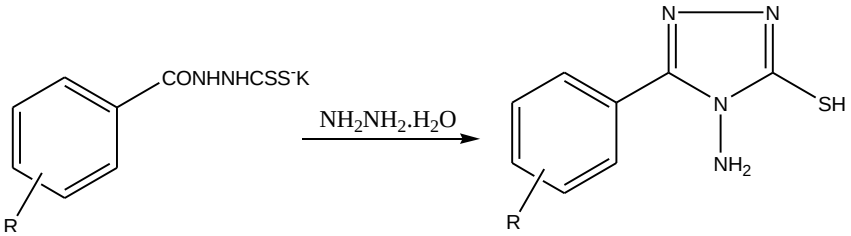
Synthesized aromatic/ heteroaromatic esters (0.1 mol) and 80% hydrazine hydrate (0.1 mol) was refluxed on a water bath for 15 min. Sufficient absolute ethanol was added to obtain a clear solution. Again the contents were refluxed for 2 h. Then excess alcohol was evaporated and solution was cooled**.** The solid obtained was separated and re-crystallized from ethanol to obtain the needle shaped crystals.

Synthesis of potassium dithiocarbazinate



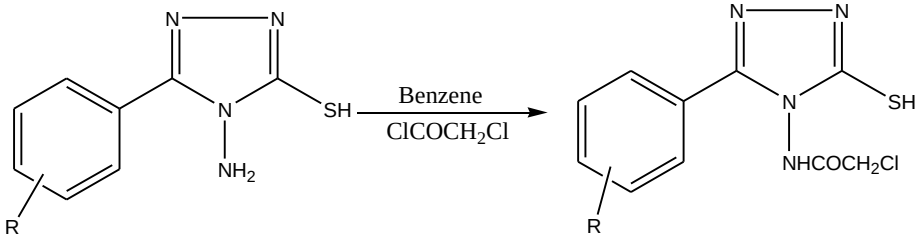
Substituted aromatic/heteroaromatic hydrazides (0.02 mol), KOH (0.012 mol) and CS_2 (0.015 mol) in absolute ethanol (350 mL) were stirred for 10 h. After the completion of reaction, ether (200 mL) was added. The obtained precipitate was filtered, washed and dried. The synthesized dithiocarbazinate was used for the next step without further purification.

Synthesis of 5-aryl-4-amino-3-mercapto-4-H-1,2,4-triazole



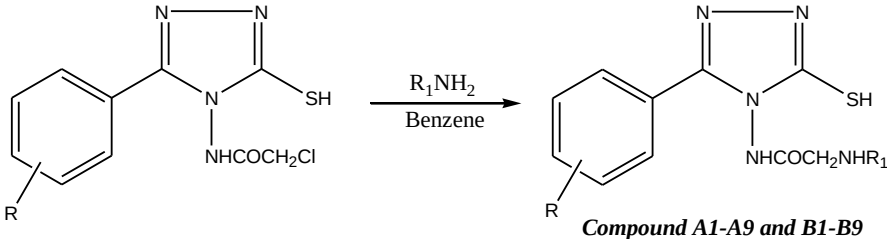
Substituted synthesized dithocarbazinate (0.1 mol), hydrazine hydrate (0.3 mol) and water (30 mL) was refluxed for 3h, H_2S was evolved during the reaction and clear solution resulted, sufficient cold water was added and then the mixture was cooled to 5°C. Acidified the cold solution with dil. HCl. Obtained precipitate was filtered, washed and re-crystallized from aqueous ethanol (50%).

Synthesis of 5-aryl-4-(chloroacetylamino)-3-mercapto-4H-1,2,4- triazole



In a two necked flask fitted with reflux condenser containing 100 mL benzene and obtained compound (0.1M) and separating funnel containing chloro acetyl chloride in 30 mL benzene. The mixture was refluxed and chloro acetyl chloride was added in small portions. After addition of chloro acetyl chloride, solution was again refluxed for 5-6 h, cooled and thereafter contents were poured on crushed ice. The obtained precipitate was filtered, washed and recrystallized from absolute ethanol.

Synthesis of amino derivative of 5-aryl-4-(chloroacetylamino)-3-mercapto-4H-1,2,4-triazole



Compound A1-A9 and B1-B9

Synthesized substituted 5-aryl-4-(chloroacetylamino)-3-mercapto-1,2,4- triazole (0.03 mol), respective amines (0.03 mol) and 75 mL benzene was taken in round bottom flask. The contents were refluxed for 5-6 h and cooled. Filtered the precipitate and washed with distilled water several times to remove traces of hydrochloride. Product obtained was recrystallized from appropriate solvent.

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-(pyridin-2-yl amino)acetamide* (A1)**

ν_{max} (KBr) (cm^{-1}) 3048 (C-H str, Aromatic ring), 761 (C-H def (oop), Monosubstituted phenyl ring), 1591,1404 (C=C str, Aromatic ring), 1530 (C=N str, 1,2,4-triazole ring and pyridine ring), 1317 (C-N str, 1,2,4-triazole ring), 688,658 (C-S

str, 1,2,4-triazole ring), 1750 (C=O str, Amide-I), 1610 (N-H bending, Amide-II), 982 (C-F str, C-F str of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and C-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3, (C_y of methylene carbon), 161.1 (C_a of pyridine ring), 148.9 (C_c of pyridine ring), 113 (C_d of pyridine ring), 138 (C_e of pyridine ring), 108.9 (C_f of pyridine ring); Anal. Calcd for $C_{15}H_{13}FN_6OS$: C, 52.32; N, 24.40. Found: C, 52.19; N, 24.12. Mass (ES+) spectra of compound exhibited molecular ion peak at m/z 345 (M^+).

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-(pyridin-3-yl amino)acetamide* (A2)**

ν_{max} (KBr) (cm^{-1}) 3054 (C-H str, Aromatic ring), 790 (C-H def (oop), Monosubstituted phenyl ring), 1581,1420 (C=C str, Aromatic ring), 1520 (C=N str, 1,2,4-triazole ring and pyridine ring), 1313 (C-N str, 1,2,4-triazole ring), 675,708 (C-S str, 1,2,4-triazole ring), 1759 (C=O str, Amide-I), 1625 (N-H bending, Amide-II), 995 (C-F str, C-F str of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1'of phenyl ring), 128.6 (C-2' and c-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 145.1 (C_a of pyridine ring), 137.9 (C_b of pyridine ring), 139 (C_d of pyridine ring), 124.5 (C_e of pyridine ring), 121.5 (C_f of pyridine ring); Anal. Calcd for $C_{15}H_{13}FN_6OS$: C, 52.32; N, 24.40. Found: C, 52.22; N, 24.19. Mass (ES+) spectra of compound exhibited molecular ion peak at m/z 345 (M^+).

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-(pyridin-4-yl amino)acetamide* (A3)**

ν_{max} (KBr) 3085 (C-H str, Aromatic ring), 822 (C-H def (oop), Monosubstituted phenyl ring), 1581,1440 (C=C str, Aromatic ring), 1530 (C=N str, 1,2,4-triazole ring and pyridine ring), 1275 (C-N str, 1,2,4-triazole ring), 773,705 (C-S str, 1,2,4-triazole ring), 1740 (C=O str, Amide-I), 1645 (N-H bending, Amide-II), 990 (C-F str, C-F str of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and c-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 155.3 (C_a of pyridine ring), 109.8 (C_b of pyridine ring), 150.7 (C_c of pyridine ring), 150.7 (C_e of pyridine ring), 109.8 (C_f of pyridine ring);

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-(1H-pyrazol-3-yl amino)acetamide* (A4)**

ν_{max} (KBr) (cm^{-1}) 3098 (C-H str, Aromatic ring), 752 (C-H def (oop), Monosubstituted phenyl ring), 1620,1430 (C=C str, Aromatic ring), 1591 (C=N str, 1,2,4-triazole ring and pyrazole ring), 1326 (C-N str, 1,2,4-triazole ring), 752,709 (C-S str, 1,2,4-triazole ring), 1750 (C=O str, Amide-I), 1605 (N-H bending, Amide-II), 980 (C-F str, C-F str of phenyl ring), 3280 (N-H str, Secondary amine of pyrazole nucleus); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and c-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57 (C_y of methylene carbon), 154 (C_a of pyrazole ring), 132 (C_d of pyrazole ring), 91.5 (C_e of pyrazole ring)

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-(1H-pyrazol-4-yl amino)acetamide* (A5)**

ν_{max} (KBr) (cm^{-1}) 3020 (C-H str, Aromatic ring), 760 (C-H def (oop), Monosubstituted phenyl ring), 1516,1395 (C=C str, Aromatic ring), 1555 (C=N str, 1,2,4-triazole ring and pyrazole ring), 1326 (C-N str, 1,2,4-triazole ring), 760,680 (C-S str, 1,2,4-triazole ring), 1695 (C=O str, Amide-I), 1610 (N-H bending, Amide-II), 955 (C-F str, C-F str of phenyl ring), 3195 (N-H str, Secondary amine of pyrazole nucleus); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and c-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57 (C_y of methylene carbon), 130 (C_a of pyrazole ring), 122.4 (C_b of pyrazole ring), 122.4 (C_e of pyrazole ring)

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-(piperidin-4-yl amino)acetamide* (A6)**

ν_{max} (KBr) (cm^{-1}) 3120 (C-H str, Aromatic ring), 775 (C-H def (oop), Monosubstituted phenyl ring), 1610,1389 (C=C str, Aromatic ring), 1560 (C=N str, 1,2,4-triazole ring), 1269 (C-N str, 1,2,4-triazole ring), 733,698 (C-S str, 1,2,4-triazole ring), 1730 (C=O str, Amide-I), 1615 (N-H bending, Amide-II), 970 (C-F str, C-F str of phenyl ring), 3235 (N-H str, Secondary amine of piperidine nucleus); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and c-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 53 (C_y of methylene carbon), 50.2 (C_a of piperidine ring), 34.1 (C_b and C_f of piperidine ring), 43.1 (C_c and C_e of piperidine ring)

***2-[(4-chlorophenyl)amino]-N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]acetamide* (A7)**

ν_{max} (KBr) (cm^{-1}) 3105 (C-H str, Aromatic ring), 812 (C-H def (oop), Monosubstituted phenyl ring), 1593,1380 (C=C str, Aromatic ring), 1520 (C=N str, 1,2,4-triazole ring), 1274 (C-N str, 1,2,4-triazole ring), 740,653 C-S str, 1,2,4-triazole ring), 1752 (C=O str, Amide-I), 1610 (N-H bending, Amide-II), 945 (C-F str, C-F str of phenyl ring), 675 (C-Cl str, Phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and C-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 141.6 (C_a of phenyl ring), 113.7 (C_b and C_f of phenyl ring), 129.7 (C_c and C_e of phenyl ring), 122.2 (C_d of phenyl ring)

***2-[(4-fluorophenyl)amino]-N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]acetamide* (A8)**

ν_{max} (KBr) (cm^{-1}) 3055 (C-H str, Aromatic ring), 810 (C-H def (oop), Monosubstituted phenyl ring), 1596,1440 (C=C str, Aromatic ring), 1542 (C=N str, 1,2,4-triazole ring), 1334 (C-N str, 1,2,4-triazole ring), 759,690 (C-S str, 1,2,4-triazole ring), 1725 (C=O str, Amide-I), 1630 (N-H bending, Amide-II), 990 (C-F str, C-F str of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and C-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 139.1 (C_a of phenyl ring), 113.9 (C_b and C_f of phenyl ring), 116.3 (C_c and C_e of phenyl ring), 150.5 (C_d of phenyl ring)

***N-[5-(4-fluorophenyl)-3-mercapto-4H-1,2,4-triazol-4-yl]-2-[(4-methylphenyl)amino]acetamide* (A9)**

ν_{max} (KBr) (cm^{-1}) 3050 (C-H str, Aromatic ring), 760 (C-H def (oop), Monosubstituted phenyl ring), 1595,1405 (C=C str, Aromatic ring), 1530 (C=N str, 1,2,4-triazole ring), 1316 (C-N str, 1,2,4-triazole ring), 774,719 (C-S str, 1,2,4-triazole ring), 1755 (C=O str, Amide-I), 1625 (N-H bending, Amide-II), 980 (C-F str, C-F str of phenyl ring), 2920 (C-H str, Methyl group); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 132.1 (C-1' of phenyl ring), 128.6 (C-2' and C-6' of phenyl ring), 116 (C-3' and C-5' of phenyl ring), 162.1 (C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 140.5 (C_a of phenyl ring), 112.2 (C_b and C_f of phenyl ring), 130 (C_c and C_e of phenyl ring), 126.1 (C_d of phenyl ring), 20.9 (Methyl carbon of C_d of phenyl ring)

***N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-(pyridin-2-yl amino)acetamide* (B1)**

ν_{max} (KBr) (cm^{-1}) 3050 (C-H str, Aromatic ring), 760 (C-H def (oop), Monosubstituted phenyl ring), 1591,1404 (C=C str, Aromatic ring), 1530 (C=N str, 1,2,4-triazole ring and pyridine ring), 1315 (C-N str, 1,2,4-triazole ring), 690,650 (C-S str, 1,2,4- triazole ring), 1755 (C=O str, Amide-I), 1610 (N-H bending, Amide-II), 2935 (C-H str, Methyl group of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and C-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 161.1 (C_a of pyridine ring), 148.9 (C_c of pyridine ring), 113 (C_d of pyridine ring), 138 (C_e of pyridine ring), 108.9 (C_f of pyridine ring)

***N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-(pyridin-3-yl amino)acetamide* (B2)**

ν_{max} (KBr) (cm^{-1}) 3074 (C-H str, Aromatic ring), 820 (C-H def (oop), Monosubstituted phenyl ring), 1590,1410 (C=C str, Aromatic ring), 1562 (C=N str, 1,2,4-triazole ring and pyridine ring), 1310 (C-N str, 1,2,4-triazole ring), 650,720 (C-S str, 1,2,4-triazole ring), 1755 (C=O str, Amide-I), 1605 (N-H bending, Amide-II), 2950 (C-H str, Methyl group of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 145.1 (C_a of pyridine ring), 137.9 (C_b of pyridine ring), 139 (C_d of pyridine ring), 124.5 (C_e of pyridine ring), 121.5 (C_f of pyridine ring)

***N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-(pyridin-4-yl amino)acetamide* (B3)**

ν_{max} (KBr) (cm^{-1}) 3125 (C-H str, Aromatic ring), 808 (C-H def (oop), Monosubstituted phenyl ring), 1590 (C=C str, Aromatic ring), 1560 (C=N str, 1,2,4-triazole ring and pyridine ring), 1350 (C-N str, 1,2,4-triazole ring), 610,630 (C-S str, 1,2,4-triazole ring), 1759 (C=O str, Amide-I), 1605 (N-H bending, Amide-II), 2945 (C-H str, Methyl group of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 155.3 (C_a of pyridine ring), 109.8 (C_b and C_f of pyridine ring), 150.7 (C_c and C_e of pyridine ring)

***N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-(1H-pyrazol-3-yl amino)acetamide* (B4)**

ν_{max} (KBr) (cm^{-1}) 3060 (C-H str, Aromatic ring), 760 (C-H def (oop), Monosubstituted phenyl ring), 1591,1410 (C=C str, Aromatic ring), 1505 (C=N str, 1,2,4-triazole ring), 1305 (C-N str, 1,2,4-triazole ring), 620,700 (C-S str, 1,2,4-triazole ring), 1755 (C=O str, Amide-I), 1620 (N-H bending, Amide-II), 2975 (C-H str, Methyl group of phenyl ring), 3283 (N-H str, Secondary amine of pyrazole ring); **13C NMR (300 MHz, d):** ν_{max} (KBr) 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 26.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57 (C_y of methylene carbon), 154 (C_a of pyrazole ring), 132 (C_d of pyrazole ring), 91.5 (C_e of pyrazole ring)

N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-(1H-pyrazol-4-yl amino)acetamide **(B5)**

ν_{max} (KBr) (cm^{-1}) 3050 (C-H str, Aromatic ring), 770 (C-H def (oop), Monosubstituted phenyl ring), 1590,1405 (C=C str, Aromatic ring), 1530 (C=N str, 1,2,4-triazole ring), 1316 (C-N str, 1,2,4-triazole ring), 88,635 (C-S str, 1,2,4-triazole ring), 1725 (C=O str, Amide-I), 1645 (N-H bending, Amide-II), 2965 (C-H str, Methyl group of phenyl ring), 3285 (N-H str, Secondary amine of pyrazole ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57 (C_y of methylene carbon), 130 (C_a of pyrazole ring), 122.4 (C_b and C_e of pyrazole ring)

N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-(piperidin-4-yl amino)acetamide **(B6)**

ν_{max} (KBr) (cm^{-1}) 3080 (C-H str, Aromatic ring), 820 (C-H def (oop), Monosubstituted phenyl ring), 1510,1320 (C=C str, Aromatic ring), 1519 (C=N str, 1,2,4-triazole ring), 1251 (C-N str, 1,2,4-triazole ring), 615,674 (C-S str, 1,2,4-triazole ring), 1742 (C=O str, Amide-I), 1603 (N-H bending, Amide-II), 2905 (C-H str, Methyl group of phenyl ring), 3155 (N-H str, Secondary amine of piperidine ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 53 (C_y of methylene carbon), 50.2 (C_a of piperidine ring), 34.1 (C_b and C_f of piperidine ring), 43.1 (C_c and C_e of piperidine ring)

2-[(4-chlorophenyl)amino]-N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]acetamide **(B7)**

ν_{max} (KBr) (cm^{-1}) 3190 (C-H str, Aromatic ring), 812 (C-H def (oop), Monosubstituted phenyl ring), 1593,1380 (C=C str, Aromatic ring), 1520 (C=N str, 1,2,4-triazole ring), 1274 (C-N str, 1,2,4-triazole ring), 740,653 (C-S str, 1,2,4-triazole ring), 1752 (C=O str, Amide-I), 1610 (N-H bending, Amide-II), 2865 (C-H str, Methyl group of phenyl ring), 675 (C-Cl str, Phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 141.6 (C_a of phenyl ring), 113.7 (C_b and C_f of phenyl ring), 129.7 (C_c and C_e of phenyl ring)

2-[(4-fluorophenyl)amino]-N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]acetamide **(B8)**

ν_{max} (KBr) (cm^{-1}) 3150 (C-H str, Aromatic ring), 821 (C-H def (oop), Monosubstituted phenyl ring), 1586,1365 (C=C str, Aromatic ring), 1500 (C=N str, 1,2,4-triazole ring), 1313 (C-N str, 1,2,4-triazole ring), 614,674 (C-S str, 1,2,4-triazole ring), 1705 (C=O str, Amide-I), 1610 (N-H bending, Amide-II), 2825 (C-H str, Methyl group of phenyl ring), 960 (C-F str, Phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4' of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 139.1 (C_a of phenyl ring), 113.9 (C_b and C_f of phenyl ring), 116.3 (C_c and C_e of phenyl ring)

N-[3-mercapto-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl]-2-[(4-methylphenyl)amino]acetamide **(B9)**

ν_{max} (KBr) (cm^{-1}) 3010 (C-H str, Aromatic ring), 760 (C-H def (oop), Monosubstituted phenyl ring), 1580,1400 (C=C str, Aromatic ring), 1530 (C=N str, 1,2,4-triazole ring), 1315 (C-N str, 1,2,4-triazole ring), 680,655 (C-S str, 1,2,4-triazole ring), 1732 (C=O str, Amide-I), 1618 (N-H bending, Amide-II), 2950 (C-H str, Methyl group of phenyl ring); **13C NMR (300 MHz, d):** 148 (C-3 and C-5 of 1,2,4-triazole), 133.5 (C-1' of phenyl ring), 126.9 (C-2' and c-6' of phenyl ring), 129.7 (C-3' and C-5' of phenyl ring), 137.7 (C-4' of phenyl ring), 20.9 (Methyl carbon of C-4'and C_d of phenyl ring), 173 (C_x of carbonyl carbon), 57.3 (C_y of methylene carbon), 140.5 (C_a of phenyl ring), 112.2 (C_b and C_f of phenyl ring), 130 (C_c and C_e of phenyl ring)

LUCIFERASE REPORTER PHAGES (LRP) ASSAY

Fifty-microliter bacterial suspension equivalent to MacFarlands No.2 standard was added to 400 ml of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this setup was incubated for 72 h at 37 °C. After incubation 50 ml of the high titer luciferase reporter phage (phAE129) and 400 ml of 0.1 M $CaCl_2$ were added to all the vials and this setup was incubated at 37 °C for 4 h. After incubation, 100 ml of the mixture was taken from each tube into a luminometer cuvette and equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10 s of integration in the Luminometer (Monolight 2010). Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with the control. The experiment was repeated when the mean RLU of the control was less than 1000.All the newly synthesized compounds were assayed *in-vitro* for antitubercular activity against *M. tuberculosis* H37Rv and clinical isolates: S, H, R, and E resistant *M. tuberculosis*. In case of antimycobacterial activity % reduction in relative light units (RLU) was calculated using luciferase reporter phages (LRP) assay at two different concentrations (50 and 100 µg/ml) using Rifampicin as a reference standard.

RESULTS AND DISCUSSION

The observed percentage reduction in RLU is tabulated in **Table 2**. Compound is considered to be an antimycobacterial if fifty percent reduction in the relative light units (RLU) is observed when compared to the control using a luminometer.

Table 2:% reduction in relative light units (RLU) of the synthesized compounds

Compound	% reduction in RLU			
	Mycobacterium tuberculosis $H_{37}Rv$		Clinical isolate: S, H, R & E resistant *M. tuberculosis*(MDR)	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
A1	0	25.73	6.25	22.74
A2	10.70	46.08	26.06	39.06
A3	34.07	47.06	38.07	47.82
A4	28.16	39.33	43.55	46.85
A5	28.51	40.01	47.36	48.79
A6	20.93	35.90	11.02	36.64
A7	6.76	12.32	32.12	10.32
A8	32.65	42.23	33.52	29.65
A9	7.67	12.08	23.24	12.32
B1	30.12	34.23	12.67	23.65
B2	6.34	7.52	12.23	14.65
B3	34.45	39.67	36.76	43.56
B4	9.67	21.84	23.37	16.43
B5	18.52	47.89	21.09	31.67
B6	32.12	48.86	42.23	32.66
B7	12.65	19.06	27.65	19.89
B8	5.76	12.65	32.90	23.67
B9	3.60	22.89	24.67	42.60
Rifampicin (2 µg/mL)	81.91		34.44	

***Where S- Streptomycin, H- Isoniazid, R- Rifampicin, E- Ethambutol and MDR- Multi Drug Resistant.**

The % reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at 50 µg/ml ranged from 0 to 34.07 for series A and 3.60 to 34.45 for series B. At 100 µg/ml dose the % reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at 100 µg/ml ranges from 12.08 to 47.06for series A and 7.52 to 48.86 for series B. None of the compound gave more than 50 % reduction in relative light units (RLU) against *M. tuberculosis* H37Rv at 50 µg/ml. These results indicate the low potential nature of the compounds towards TB. Percentage reduction in relative light units (RLU) for clinical isolates: S, H, R, and E resistant *M. tuberculosis* is 6.25 to 47.36 for series A and 12.23 to 42.23 for series B. At 100 µg/ml dose the % reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at100 µg/ml ranges from 10.32 to 48.79 for series A and 14.65 to 43.56 for series B.

Against *Mycobacterium tuberculosis* $H_{37}Rv$ compound B6 found to be most potent at 100 µg/mL concentration while compound B3 shown the maximum potency at 50 µg/mL concentration.

Against Clinical isolate: S, H, R & E resistant *M. tuberculosis* (MDR) compound A5 caused maximum percentage reduction in light units at

50 µg/mL concentration while it also shown to be most potent compound at 100 µg/mL concentration.

All the compounds except compound A1, were found to be active against both the *Mycobacterium tuberculosis* strain with variable magnitude of activities. Overall the study shows that further derivatisation on the same line must be carried out to get the idea for proper designing of the 1,24-triazoles against the *Mycobacterium tuberculosis.*

CONCLUSION

In the present investigation 1,2,4-triazoles were synthesized and evaluated for their antimycobacterial activity. In total eighteen 1,2,4-thiadiazole derivatives were synthesized with different substitutions. All of the synthesized compounds have been confirmed by elemental analyses, IR and ^{13}C NMR spectral data. Almost all the compounds exhibited significant antimycobacterial activity. Against *Mycobacterium tuberculosis* $H_{37}Rv$ compound B6 found to be most potent at 100 µg/mL concentration while compound B3 shown the maximum potency at 50 µg/mL concentration. While, against Clinical isolate: S, H, R & E resistant *M. tuberculosis* (MDR) compound A5 caused maximum percentage reduction in light units at 50 µg/mL concentration while it also shown to be most potent compound at 100 µg/mL concentration.

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REFERENCES

1. Berber I, Cokmus C, Atalan E, Comparison of Staphylococcus species, Cellular and extracellular proteins, Mikrobiologiia, 72:54–9, (2003).
2. Mitscher LA, Pillai SP, Gentry EJ, Shankel DM, Multiple drug resistance, Med Res Rev, 19:477–496 (1999).
3. Vohra R, Gupta M, Chaturvedi R, Singh Y, Attack on the scourge of tuberculosis: patented drug targets, Recent Pat Antiinfect Drug Discovery, 1:95-106 (2006).
4. Janin YL, Antituberculosis drugs: ten years of research, Bioorg Med Chem, 15:2479-2513 (2007).
5. Kaufmann SH, Schaible UE, 100th anniversary of Robert Koch's Nobel Prize for the discovery of the tubercle bacillus, Trends Microbiol, 13:469-475 (2005).
6. Williams BG and Dye C, Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS, Science, 301:1535-1537 (2003).
7. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C, The growing burden of tuberculosis: global trends and interactions with the HIV epidemic, Arch Intern Med, 163:1009–1021 (2003).
8. Zignol M, Hosseini MS, Wright A, Lambregts-van WC, Nunn P, Watt CJ, and Williams BG, Global incidence of multidrug-resistant tuberculosis, J Infect Dis, 194:479-485 (2006).
9. Jain A, Mondal R, Extensively drug-resistant tuberculosis: Current challenges and threats, FEMS Immunol, Medi. Microbiol, 53: 145-150 (2008).
10. Duncan K, Barry III CE, Prospects for new antitubercular drugs, Curr Opin Microbiol, 7:460-465 (2004).
11. Lamichhane G, Novel targets in M. tuberculosis: search for new drugs, Trends Mol. Med, 17: 25-33, (2011).
12. Naito Y, Akahoshi F, Takeda S, Okada T, Kajii M, Nishimura H, Sugiura M, Fukaya C, Kagitani Y, Synthesis and pharmacological activity of triazole derivatives inhibiting eosinophilia. J Med Chem, 39:3019-3029 (1996).
13. Hester JB, Rudzik AD, Kamdar BV, 6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepines which have central nervous system depressant activity, J Med Chem, 14:1078-1081 (1971).
14. Milton NGN, Inhibition of catalase activity with 3-amino-triazole enhances the cytotoxicity of the Alzheimer's amyloid-β peptide, Neurotoxicol, 22:767-774 (2001).
15. Burell G, Evans JM, Hadley MS, Hicks F, Stemp G, Benzopyran potassium channel activators related to cromakalim - heterocyclic amide replacements at position 4, Bioorg Med Chem Lett, 4:1285-1290, (1994).
16. Ghorab MM, Abdel Hamide SG, Ali GM, El-Sayed H, Synthesis and Insecticidal Activity of Some New 3[4(3H)-

Quinazolinone-2-yl)thiomethyl]-1,2,4-triazole-5-thiols, Pestic Sci, 48:31-35 (1996).
17. Mullican MD, Wilson MW, Conner DT, Kostlan CR, Schrier DJ, Dyer RD, Design of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazoles as orally active nonulcerogenic anti-inflammatory agents, J Med Chem, 36:1090–1099 (1993).
18. Tozkoparan B, Kupeli E, Yesilada E, Ertan M, Preparation of 5-aryl-3-alkylthio-l,2,4-triazoles and corresponding sulfones with antiinflammatory–analgesic activity, Bioorg Med Chem, 15:1808–1814 (2007).
19. Kelley JL, Koble CS, Davis RG, Mc Lean EW, Soroko FE, Cooper BR, 1-(Fluorobenzyl)-4-amino-1H-1,2,3-triazolo[4,5-c]pyridines: synthesis and Anticonvulsant activity, J Med Chem, 38:4131–4134, (1995).
20. Singh RJ, Singh DK, Syntheses and biological activity of some 3, 5-diaryl-4H-1, 2, 4-triazole derivatives, IJPBS, 2:1-6, (2010)
21. Foroumadi A, Mansouri S, Kiani Z, Rahamani A, Synthesis and in vitro antibacterial evaluation of N-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] piperazinyl quinolones, Eur J Med Chem, 38:851–854 (2003).
22. Kucukguzel I, Kucukguzel SG, Rollas S, Kiraz M, Some 3-thioxo/alkylthio-1,2,4-triazoles with a substituted thiourea moiety as possible antimycobacterials, Bioorg Med Chem Lett, 11:1703–1707 (2001).
23. Heubach G, Sachse B, Buerstell H, 1,2,4-Triazole derivatives. Ger. Offen. 2, 826, 760; Chem Abstr 92:181200h. (1975).
24. De Clercq E, Antiviral drugs in current clinical use, J Clin Virol, 30:115-133 (2004).
25. Collin X, Sauleau A, Coulon J, 1,2,4-Triazolo mercapto and aminonitriles as potent antifungal agents, Bioorg Med Chem Lett, 13:2601-2605 (2003).
26. Kidwai M, Sapra P, Misra P, Saxena RK, Singh M, Microwave assisted solid support synthesis of novel 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazepines as potent antimicrobial agents, Bioorg Med Chem, 9:217-220 (2001).
27. Papakonstantinou-Garoufalias S, Pouli N, Marakos P, Chytyroglou-Ladas A, Synthesis antimicrobial and antifungal activity of some new 3-substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl)-1*H*-1,2,4-triazole, Farmaco, 57:973-977 (2002).
28. Ghorab MM, Abdel- Hamide SG, El-Gaby MSA, El-Sayed SM, Synthesis and Effect of Some New [1,2,4]Triazolo[4,3-a]quinazolin-5(4H)-ones and Related Compounds on Ehrlich Ascites Carcinoma Cells, Acta Pharm, 49:1-10 (1999).
29. Thompson SK, Eppley AM, Frazee JS, Darcy MG, Lum RT, Tomaszeck TA, Ivanoff LA, Morris JF, Sternberg EJ, Lambert DM, Fernandez AV, Patteway SR, Meek TD, Metcalf BW, Gleason JG, Synthesis and antiviral activity of a novel class of HIV-1 protease inhibitors containing a heterocyclic P_1'-P_2' amide bond isostere, Bioorg Med Chem Lett, 4:2441-2446 (1994).

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