Development of 1,2,4-Triazole-5-Thione Derivatives as Potential Inhibitors of Enoyl Acyl Carrier Protein Reductase (InhA) in Tuberculosis.

Dhagash Vora, Neha Upadhyay, Kalpana Tilekar, Viral Jain and C S Ramaa*

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth’s College of Pharmacy, Navi Mumbai-400614, Maharashtra, India.

Abstract

Tuberculosis (TB) ranks second, next to AIDS making it most formidable disease in the present age. One of the crucial enzymes involved in cell wall synthesis of Mycobacterium tuberculosis, InhA (enoyl acyl carrier protein reductase), one of the crucial enzymes involved in cell wall synthesis of Mycobacterium tuberculosis, has been authenticated as an effective target for anti-mycobacterial drug development. In the current work, novel derivatives of 1,2,4-triazole-5-thione rationally designed, synthesized and spectrally characterized as promising InhA inhibitors. Anti-mycobacterial potential was determined by resazurin microtiter assay using Mtb H₃₇Rv strain. The mechanism of action of these compounds was confirmed by InhA enzyme inhibition studies. 6b, the most active compound of the series displayed MIC of 0.19 µM in resazurin microtiter assay and InhA inhibition with IC₅₀ of 90 nM.

Keywords: Mycobacterium tuberculosis; 1,2,4-Triazole-5-thiones; InhA inhibition; ADME; REMA.

Introduction

Amongst the most formidable diseases of the present age, tuberculosis (TB) ranks second, next to AIDS, and it needs to be looked upon with utmost priority. The World Health Organization’s (WHO) 2018 global report identified Mycobacterium tuberculosis (Mtb) infection as one of the major causes of global mortality and morbidity and the resulting disease, TB, caused an estimated 1.6 million deaths out of 11.1 million people who fell ill with it in 2017 (1). In 2018, major cases of new tuberculosis arose in Asia, which contributed 60% of new cases globally. Looking at India, around 100 people die every day due to TB (2, 3). This situation has further worsen owing to an increase in multidrug resistant tuberculosis and extensively drug resistant tuberculosis (MDRTB and XDRTB respectively) cases in the past decade. Adding to the more fatal aspect of TB, recently, cases of totally drug-resistant tuberculosis (TDR-TB) have been identified wherein the patients do not respond to any of the available anti-TB drugs thereby making TB the nastiest and most noxious diseases than ever before (4).

Mtb comprises unique fatty acids, the mycolic acids, which are oddly lengthy chain β-hydroxy fatty acids with a long α-alkyl side chain (5). These are the chief building blocks of the protecting layer in the cell envelope of Mtb. Mycolic acids in Mtb are formed by a saturated short fatty acyl chain of 20-26 carbon atoms and a long meromycolic acid chain of 50-60...
carbon atoms. The biosynthesis of mycolic acids is achieved by the fatty acid synthase system (FAS) in M. tuberculosis. Mtb has unique FAS-I and FAS-II fatty acids biosynthetic pathways which are distinct from other bacteria. Amongst the various enzymes involved in FAS-II pathway, the NADH-dependent trans-2-enoyl acyl carrier protein reductase (InhA) is the main catalyst in biosynthesis of mycolic acid. Many literature reports have highlighted InhA as a prime molecular target of isoniazid (1), which is used as a first-line agent in the treatment of TB. INH is a prodrug, which is activated by KatG (a catalase-peroxidase) by oxidation to an acyl radical which binds covalently to co-substrate, NAD'. The INH-NAD' complex then acts as an effective InhA inhibitor (6-8).

The necessity for INH activation unlocked a way-in for the development of Mtb drug resistance. Thus, direct inhibition of InhA, will bypass the compulsory activation step of INH, and will serve as a promising target in the development of novel agents in anti-tubercular therapy. Other than triclosan (2) (9), which is a nonselective and relatively weak agent, three series of direct InhA inhibitors, namely, diphenyl ether derivatives (3) (10-12), pyrrolidine carboxamide derivatives (4), and piperazine derivatives (arylamides) (5) (Figure 1), have exhibited potent in-vitro activity. In this work, we report our findings of 1,2,4-triazole-5-thiones and 1,3,4-oxadiazole-2-thiones as a novel series of InhA inhibitors.

Experimental

Chemistry

General

Chemicals and solvents of LR grade were used for synthesis and purchased from Research Lab, S D Fine and Sigma suppliers in India. The reactions were monitored using pre-coated TLC plates (Merck pre-coated Silica Gel 60 F254) using various solvent systems. Veego melting point apparatus was used for recording of Melting points which were uncorrected. The synthesized compounds were structurally characterized using FTIR, NMR. Infrared spectroscopy was carried on the Shimadzu FT/IR-8400S. HNMR spectra were determined by Varian (300 MHz and 600MHz) and Bruker (400 MHz and 500MHz) NMR spectroscopies, whereas 13C-NMR were recorded on Varian (75 MHz) and Bruker (100 MHz and 125 MHz) NMR spectroscopies. Chemical shifts values are described in ppm (δ) against TMS as internal standard. The designations for signals are as follows: s-singlet; d-doublet; dd-doublet of doublet; t-triplet; and m-multiplet. General synthetic scheme (Scheme 1) was followed.

Figure 1. InhA inhibitors from literature; Isoniazid (1), Triclosan (2), Diphenyl ether derivative (3), Pyrrolidine carboxamide derivative (4) and Piperazine derivative (5).
for the synthesis of 1,2,4-triazole-5-thione and 1,3,4-oxadiazole-2-thione derivatives.

**Synthesis of 5-pyridin-4-yl-3H-(1,3,4)-oxadiazole-2-thione (4)**

In ethanolic solution of KOH (0.1 mol), isonicotinic acid hydrazide 3 (0.1 mol) was dissolved and to this carbon disulfide (0.1 mol) was added drop wise. The reaction was then refluxed for 10-12 h. After completion the mixture was poured over crushed ice and acidified with conc. HCl. The precipitate was filtered and recrystallized by using ethanol (13, 14).

Yellow crystalline solid; Yield: 71%; M.p.: 263-265 °C; FT-IR (KBr, cm⁻¹): 3325 (N-H stretch, oxadiazole), 2968, 2933 (aromatic C-H stretch), 1637 (C=N stretch, oxadiazole), 1612, 1560 (aromatic C=C stretch), 1165 (C=S stretch, oxadiazole), 1016 (C-O-C stretch, oxadiazole); 

1H NMR (400 MHz, DMSO-d₆): δ 8.82 (d, J = 4.0 Hz, 2H, pyridine), 7.82 (d, J = 4.0 Hz, 2H, pyridine)
13C NMR (100 MHz, DMSO-d₆): δ 177.05 (1C, C-2 [C=S], oxadiazole), 158.78 (1C, C-5, oxadiazole), 150.85, 129.70, 119.62 (5C, pyridine).

**Scheme 1.** Experimental scheme for synthesis of 1,2,4-triazole-5-thione derivatives and 1,3,4-oxadiazole-2-thione derivatives; i. CS₂, Δ, EtOH, KOH, ii. NH₂-NH₂.H₂O, Δ, Glacial acetic acid, iii. K₂CO₃, DCM, iv. K₂CO₃, DMF, RT, v. K₂CO₃, DMF, RT.
2-thione (4) (0.05 mol) was refluxed with hydrazine hydrate (99%, 75 mL) for 4 h. The cooled reaction mixture was quenched with ice-cold water followed by acidification with glacial acetic acid, to get crude which was purified by recrystallization from ethanol [15].

Pale yellow crystalline solid; Yield: 68%; M.p.: 256-258 °C; FT-IR (KBr, cm⁻¹): 3271, 3163 (N-H stretch, Amine [NH₃]), 3234 (N-H stretch, triazole), 3090, 3057 (aromatic C-H stretch), 1606 (C=N stretch, triazole), 1572, 1556 (aromatic C=C stretch), 1217 (C=S stretch, triazole); ¹H NMR (500 MHz, DMSO-d₆): δ 8.77 (dd, J = 5.0, 1.5 Hz, 2H, pyridine), 8.03 (dd, J = 4.5, 1.5 Hz, 2H, pyridine), 5.86 (s, 2H, NH) ¹³C NMR (125 MHz, DMSO-d₆): δ 168.15 (1C, C=O, amide), 147.82 (1C, C-3, triazole), 150.62, 133.36, 122.01 (5C, pyridine).

General synthetic procedure for the preparation of 2-chloro-N-aryl or heteroaryl acetamides (2a-2v)

We have previously published synthetic procedure and characterization of 2a-2v in our different reports (16-19).

General synthetic procedure for the preparation of 6a-6v

A mixture of 4-amino-3-(pyridin-4-yl)-1H-(1,2,4)-triazole-5-thione (5) (0.05 mol) and 2-chloro-N-(aryl or heteroaryl) acetamides (2a-2v) (0.05 mol) along with anhydrous potassium carbonate (K₂CO₃) (0.075 mol) was stirred in dimethyl formamide (DMF) at room temperature. After completion of reaction (monitored by TLC), ice cold water was added to precipitate solid which was filtered and purified by recrystallization in appropriate solvent.

2-(4-Amino-3-(pyridin-4-yl)-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-yl)-N-(phenyl acetamide (6a)

White amorphous solid; Recrystallizing solvent: Ethanol; Yield: 84%; M.p.: 237-238 °C; FT-IR (KBr, cm⁻¹): 3234, 3176 (N-H stretch, Amine [NH₃]), 3296 (N-H stretch, amide), 3051, 3032 (aromatic C-H stretch), 2974, 2920, 2874 (aliphatic C-H stretch, CH₃ and CH₂), 1664 (C=O stretch, amide), 1203 (C=S stretch, triazole); ¹H NMR (500 MHz, DMSO-d₆): δ 10.27 (s, 1H, NH), 8.74 (dd, J = 4.5, 1.5 Hz, 2H, pyridine), 8.00 (dd, J = 4.5, 1.5 Hz, 2H, pyridine), 7.47 (d, J = 8.5 Hz, 2H, phenyl), 7.12 (d, J = 8.0Hz, 2H, phenyl), 6.33 (s,2H, NH₃), 4.18 (s, 2H, CH₂), 2.25 (s, 3H, CH₃); ¹³C NMR (125 MHz, DMSO-d₆): δ 166.03 (1C, C=O, amide), 155.21 (1C, C=S, triazole), 153.44, 136.74, 132.90, 129.61, 119.57 (6C, phenyl), 136.74, 132.90, 129.61, 119.57 (6C, phenyl), 36.61 (1C, CH₂), 20.87 (1C, CH₃). Anal.Calc. for C₁₆H₁₄N₄O₂S: C, 56.45; H, 4.74; N, 24.69.

2-(4-Amino-3-(pyridin-4-yl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-(4-bromo-2-fluorophenyl)acetamide (6c)

White amorphous solid; Recrystallizing solvent: Isopropanol; Yield: 74%; M.p.: 276-278 °C; FT-IR (KBr, cm⁻¹): 3246, 3182 (N-H stretch, Amine [NH₃]), 3340 (N-H stretch, amide), 3043, 3009 (aromatic C-H stretch), 2931 (aliphatic C-H stretch, CH₃), 1674 (C=O stretch, amide), 1336 (C-F stretch), 1176 (C=S stretch, triazole); ¹H NMR (600 MHz, DMSO-d₆): δ 10.25 (s, 1H, NH), 8.71 (d, J = 5.4 Hz, 2H, pyridine), 7.98 (d, J = 5.4 Hz, 2H, pyridine), 7.90 (t, J = 8.7 Hz, 1H, phenyl), 7.60 (d, J = 10.8 Hz, 1H, phenyl), 7.37 (d, J = 9.0Hz, 1H, phenyl), 6.30 (s, 2H,
2-(4-Amino-3-(pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(4-methoxyphenyl) acetamide (6d)

Dark brown amorphous solid; Recrystallizing solvent: Ethanol; Yield: 79%; M.p.: 256-258°C; FT-IR (KBr, cm⁻¹): 3234, 3174 (N-H stretch, Amine [NH]), 3290 (N-H stretch, amide), 3132, 3041 (aromatic C-H stretch, CH), 2962, 2920, 2881 (aliphatic C-H stretch, CH and CH₂), 1658 (C=O stretch, amide), 1247 (C-O-C stretch, 1188 (C=S stretch, triazole); ¹H NMR (500 MHz, DMSO-d₆): δ 10.22 (s, 1H, NH), 8.74 (d, J = 6.0 Hz, 2H, pyridine), 8.01 (d, J = 5.5 Hz, 2H, phenyl), 7.49 (d, J = 9.0 Hz, 2H, phenyl), 6.89 (d, J = 9.0 Hz, 2H, phenyl), 6.33 (s, 2H, NH), 4.16 (s, 2H, CH₂), 3.33 (s, 3H, CH₃); ¹³C NMR (125 MHz, DMSO-d₆): δ 165.76 (1C, C=O, amide), 154.43 (1C, C=O, amide), 149.89 (1C, C=O, amide), 149.39 (1C, C-3, triazole), 151.96, 133.85, 122.51 (5C, pyridine), 139.46, 129.70, 125.24, 121.21, 119.68, 115.27 (6C, phenyl), 129.84 (1C, CH₃), 36.15 (1C, CH₃). Anal. Calc. for C₁₅H₁₂BrFN₂O₄: C, 42.56; H, 2.86; N, 19.86.

Pale brown amorphous solid; Recrystallizing solvent: Ethanol; Yield: 66%; M.p.: 218-220°C; FT-IR (KBr, cm⁻¹): 3284, 3223 (N-H stretch, Amine [NH]), 3346 (N-H stretch, amide), 3115, 3037 (aromatic C-H stretch), 2918, 2872 (aliphatic C-H stretch, CH₂), 1662 (C=O stretch, amide), 1180 (C=O stretch, triazole), 742 (C=Cl stretch); ¹H NMR (500 MHz, DMSO-d₆): δ 10.17 (s, 1H, NH), 8.74 (d, J = 6.0 Hz, 2H, pyridine), 8.00 (d, J = 6.0 Hz, 2H, pyridine), 7.62 (d, J = 8.5 Hz, 2H, phenyl), 7.38 (d, J = 9.0 Hz, 2H, phenyl), 6.33 (s, 2H, NH), 4.20 (s, 2H, CH₂); ¹³C NMR (125 MHz, DMSO-d₆): δ 166.49 (1C, C-5 [C=S], triazole), 155.18 (1C, C=O, amide), 150.54 (1C, C-3, triazole), 152.46, 134.29, 121.72 (5C, pyridine), 138.20, 129.17, 127.49, 121.08 (6C, phenyl), 36.15 (1C, CH₃). Anal. Calc. for C₁₅H₁₆ClN₂O₄: C, 53.92; H, 4.52; N, 23.58.
2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(3-chloro-4-methylphenyl) acetamide (6h)

Buff solid; Recrystallizing solvent: Ethanol; Yield: 85%; M.p.: 214-216 °C; FT-IR (KBr, cm⁻¹): 3263, 3182 (N-H stretch, Amine [NH₂]), 3298 (N-H stretch, amide), 3099, 3037 (aromatic C-H stretch), 2958, 2922, 2864 (aliphatic C-H stretch, CH₃ and CH₂), 1664 (C=O stretch, amide), 155.21 (N-H stretch, Amine [NH]), 1495, 1481 (C=O stretch, triazole), 1346, 1287 (3C, pyridine), 116.58 (6C, phenyl), 103.43 (2C, CH₂), 92.43 (1C, CH), 81.52 (s, 1H, CH), 73.52 (1C, C=O, amide), 6.32 (s, 2H, NH). Anal. Calc. for C₁₇H₁₆N₅O₅S: C, 56.45; H, 4.74; N, 24.69.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(3-chlorophenyl) acetamide (6j)

White crystalline solid; Recrystallizing solvent: Ethanol; Yield: 55%; M.p.: 184-186 °C; FT-IR (KBr, cm⁻¹): 3344, 3261 (N-H stretch, Amine [NH₂]), 3367 (N-H stretch, amide), 3047, 2989 (aromatic C-H stretch), 2929 (aliphatic C-H stretch, CH₃), 1668 (C=O stretch, triazole), 1334 (C-F stretch), 1180 (C=O stretch, amide), 1495, 1481 (C=O stretch, triazole), 1346, 1287 (3C, pyridine), 116.58 (6C, phenyl), 103.43 (2C, CH₂), 92.43 (1C, CH), 81.52 (s, 1H, CH), 73.52 (1C, C=O, amide), 6.32 (s, 2H, NH). Anal. Calc. for C₁₇H₁₆ClN₅OS: C, 49.93; H, 3.63; N, 22.39.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(3-chlorofluoromethylphenyl) acetamide (6k)

White amorphous solid; Recrystallizing solvent: Ethanol; Yield: 55%; M.p.: 184-186 °C; FT-IR (KBr, cm⁻¹): 3344, 3261 (N-H stretch, Amine [NH₂]), 3367 (N-H stretch, amide), 3047, 2989 (aromatic C-H stretch), 2929 (aliphatic C-H stretch, CH₃), 1668 (C=O stretch, triazole), 1334 (C-F stretch), 1180 (C=O stretch, amide), 1495, 1481 (C=O stretch, triazole), 1346, 1287 (3C, pyridine), 116.58 (6C, phenyl), 103.43 (2C, CH₂), 92.43 (1C, CH), 81.52 (s, 1H, CH), 73.52 (1C, C=O, amide), 6.32 (s, 2H, NH). Anal. Calc. for C₁₇H₁₆ClF₅N₅OS: C, 44.81; H, 2.82; N, 19.60.
2-((4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(3-methoxyphenyl)acetamide (6d)

Yellow amorphous solid; Recrystallizing solvent: Isopropanol; Yield: 70%; M.p.: 222-224 °C; FT-IR (KBr, cm⁻¹): 3232, 3176 (N-H stretch, Amine [NH]), 3290 (N-H stretch, amide), 3037 (aliphatic C-H stretch, 1576, 1467, 730 (C=S stretch, triazole); 1H NMR (600 MHz, DMSO-d6): δ 10.82 (s, 1H, NH), 8.91 (d, J = 4.8 Hz, 2H, pyridine), 7.97 (d, J = 5.4 Hz, 2H, pyridine), 7.26 (s, 1H, phenyl), 7.20 (t, J = 8.1 Hz, 1H, phenyl), 7.09 (d, J = 8.4 Hz, 1H, phenyl), 6.63 (d, J = 7.8 Hz, 1H, phenyl), 6.31 (s,2H, NH), 4.16 (s, 2H, CH), 3.70 (s, 3H, CH3); 13C NMR (100 MHz, DMSO-d6): δ 165.79 (1C, C-5 [C=S], triazole), 154.41 (1C, C=O, amide), 149.49 (1C, C-3, triazole), 151.80, 134.03, 121.24 (5C, pyridine), 159.40, 139.56, 129.13, 118.85, 108.78, 105.11 (6C, phenyl), 54.72 (1C, CH3), 36.78 (1C, CH3). Anal. Calc. for C22H16F3N2O2S: C, 53.92; H, 4.52; N, 23.58.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(4-fluorophenyl)acetamide (6m)

Orange amorphous solid; Recrystallizing solvent: Ethanol; Yield: 72%; M.p.: 227-229 °C; FT-IR (KBr, cm⁻¹): 3232, 3176 (N-H stretch, Amine [NH]), 3290 (N-H stretch, amide), 3037 (aliphatic C-H stretch, 1576, 1467, 726 (1C, C=O, amide), 149.49 (1C, C-3, triazole), 151.80, 134.03, 121.24 (5C, pyridine), 159.40, 139.56, 129.13, 118.85, 108.78, 105.11 (6C, phenyl), 54.72 (1C, CH3), 36.78 (1C, CH3). Anal. Calc. for C16H16F3N2O2S: C, 53.92; H, 4.52; N, 23.58.

Buff solid; Recrystallizing solvent: Ethanol; Yield: 90%; M.p.: 227-229 °C; FT-IR (KBr, cm⁻¹): 3265, 3201 (N-H stretch, Amine [NH]), 3362 (N-H stretch, amide), 3070, 3041 (aromatic C-H stretch), 2933 (aliphatic C-H stretch, CH3), 1680 (C=O stretch, amide), 1317 (C-F stretch), 1172 (C=S stretch, triazole); 1H NMR (500 MHz, DMSO-d6): δ 10.74 (s, 1H, NH), 8.74 (d, J = 6.60 Hz, 2H, pyridine), 8.00 (d, J = 6.0Hz, 2H, pyridine), 7.81 (d, J = 8.5 Hz, 2H, phenyl), 7.72 (d, J = 8.0 Hz, 2H, phenyl), 6.73 (s,2H, NH), 4.25 (s, 2H, CH3); 13C NMR (100 MHz, DMSO-d6): δ 166.33 (1C, C-5 [C=S], triazole), 154.57 (1C, C=O, amide), 149.28 (1C, C-3, triazole), 151.75, 133.88, 121.31 (5C, pyridine), 142.02, 134.33, 125.67, 118.90 (6C, phenyl), 124.33 (1C, CF3), 36.47 (1C, CH3). Anal. Calc. for C16H16F3N2O2S: C, 48.73; H, 3.32; N, 21.31.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(pyridin-2-yl)acetamide (6a)

Pale yellow amorphous solid; Recrystallizing solvent: Isopropanol; Yield: 69%; M.p.: 212-214 °C; FT-IR (KBr, cm⁻¹): 3261, 3171 (N-H stretch, Amine [NH]), 3335 (N-H stretch, amide), 3105, 3063 (aromatic C-H stretch), 2980, 2933 (aliphatic C-H stretch, CH3), 1676 (C=O stretch, amide), 1188 (C=S stretch, triazole); 1H NMR (600 MHz, DMSO-d6): δ 10.77 (s, 1H, NH), 8.71 (d, J = 4.8 Hz, 2H, pyridine), 8.31 (d, J = 4.8 Hz, 1H, pyridine[amide]), 7.97 (d, J = 5.4 Hz, 2H, pyridine), 7.77 (t, J = 7.5 Hz, 1H, pyridine[amide]), 7.10 (t, J = 6.3 Hz, 2H, pyridine[amide]), 6.31 (s,2H, NH), 4.22 (s, 2H, CH3); 13C NMR (100 MHz, DMSO-d6): δ 167.66 (1C, C-5 [C=S], triazole), 154.58 (1C, C=O, amide), 147.87 (1C, C-3, triazole), 149.95, 133.86, 121.24 (5C, pyridine), 151.94, 151.65, 138.08, 119.50, 113.42 (5C, pyridine [amide]), 36.83 (1C, CH3). Anal. Calc. for C16H18F3N2O2S: C, 51.36; H, 4.00; N, 29.95.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(6-methylpyridin-2-yl)acetamide (6p)

Dark brown amorphous solid; Recrystallizing solvent: Isopropanol; Yield: 56%; M.p.: 230-232 °C; FT-IR (KBr, cm⁻¹): 3271, 3228 (N-H stretch, Amine [NH]), 3346 (N-H stretch, amide), 3140,
2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(4-methylpyridin-2-yl) acetamide (6a)

Buff solid; Recrystallizing solvent: Ethanol; Yield: 63%; M.p.: 217-219 °C; FT-IR (KBr, cm⁻¹): 3271, 3236 (N-H stretch, Amine [NH]), 3342 (N-H stretch, amide), 1547 (1C, C=O, amide), 1492 (1C, C-3, triazole), 1518, 1336, 121.9 (5C, pyridine), 156.32, 151.84, 138.13, 118.63, 110.34 (5C, pyridine [amide]), 36.34 (1C, CH), 23.53 (1C, CH3). Anal. Calc. for C13H13N2OS: C, 52.77; H, 4.43; N, 28.72.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(5-methylisoxazol-3-yl) acetamide (6b)

Pale yellow amorphous solid; Recrystallizing solvent: Isopropanol; Yield: 56%; M.p.: 242-244 °C; FT-IR (KBr, cm⁻¹): 3236, 3136 (N-H stretch, Amine [NH]), 3331, 3136 (N-H stretch, amide), 1678 (C=O stretch, amide), 1570 (aromatic C=N stretch) 1199 (C=S stretch, triazole); ¹H NMR (600 MHz, DMSO-d₆): δ 6.30 (s, 2H, NH), 6.33 (s, 1H, NH), 8.74 (d, J = 6.0Hz, 2H, pyridine), 7.99 (d, J = 5.4 Hz, 2H, pyridine), 6.61 (s, 1H, isoxazole), 6.33 (s, 2H, NH), 4.20 (s, 2H, CH3), 2.38 (s, 3H, CH3); 13C NMR (100 MHz, DMSO-d₆): δ 166.19 (1C, C-5 [C=S], triazole), 154.47 (1C, C=O, amide), 149.87 (1C, C-3, triazole), 151.88, 133.88, 121.20 (5C, pyridine), 151.69, 144.72, 147.39, 120.44, 118.33 (5C, pyridine [amide]), 36.19 (1C, CH3), 20.90 (1C, CH3). Anal. Calc. for C14H13N2O2S: C, 47.12; H, 3.95; N, 29.59.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(5-methylbenzothiazol-3-yl) acetamide (6c)

Brown amorphous solid; Recrystallizing solvent: Ethanol; Yield: 45%; M.p.: 205-207 °C; FT-IR (KBr, cm⁻¹): 3286, 3203 (N-H stretch, Amine [NH]), 3356 (N-H stretch, amide), 3091, 3053 (aromatic C-H stretch), 2974, 2924 (aliphatic C-H stretch, CH3), 1670 (C=O stretch, amide), 1190 (C=S stretch, triazole); ¹H NMR (500 MHz, DMSO-d₆): δ 10.71 (s, 1H, NH), 8.74 (d, J = 5.5 Hz, 2H, pyridine), 8.68 (d, J = 4.5 Hz, 2H, pyridine), 8.00 (d, J = 5.5 Hz, 2H, pyridine), 7.21 (t, J = 4.5 Hz, 1H, pyrimidine), 6.33 (s, 2H, NH), 4.42 (s, 2H, CH3); 13C NMR (100 MHz, DMSO-d₆): δ 166.19 (1C, C-5 [C=S], triazole), 154.47 (1C, C=O, amide), 149.93 (1C, C=O, amide), 149.87 (1C, C-3, triazole), 149.66, 133.86, 121.21 (5C, pyridine), 157.54, 96.13 (3C, isoxazole), 35.44 (1C, CH3), 12.18 (1C, CH3). Anal. Calc. for C15H13N2O2S: C, 47.12; H, 3.95; N, 29.59.
Hz, 1H, benzothiazole), 7.25 (d, J = 7.2 Hz, 1H, benzothiazole), 7.19 (t, J = 7.5 Hz, 1H, benzothiazole), 6.33 (s, 2H, NH), 4.31 (s, 2H, CH), 3.29 (s, 3H, CH), 1.33 (m, 2H, pyridine); 13C NMR (100 MHz, DMSO-d6): δ 167.12 (1C, C-5 [C=S], triazole), 154.37 (1C, C-O, amide), 147.58 (1C, C-3, triazole), 149.95, 133.83, 121.22 (5C, pyridine), 159.79, 152.96, 131.10, 129.88, 126.50, 123.45, 118.87 (7C, benzothiazole), 34.73 (1C, CH), 25.38 (1C, CH). Anal. Calc. for C15H13N5O4S2: C, 51.37; H, 3.80; N, 24.67.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-thiazol-2-yl acetamide (6u)

Orange amorphous solid; Recrystallizing solvent: Ethanol; Yield: 42%; M.p.: 253-255°C; FT-IR (KBr, cm⁻¹): 3327, 3284 (N-H stretch amine [NH]), 3342 (N-H stretch, amide), 3151, 3099 (aromatic and heteroaromatic C-H stretch), 3051 (aliphatic C-H stretch, CH), 1683 (C=O stretch, amide), 1174 (C=S stretch, triazole); 1H NMR (600 MHz, DMSO-d6): δ 12.42 (s, 1H, NH), 8.71 (d, J = 5.4 Hz, 2H, pyridine), 7.96 (d, J = 5.4 Hz, 2H, pyridine), 7.47 (d, J = 3.0Hz, 1H, thiazole), 7.22 (d, J = 3.6 Hz, 1H, thiazole), 6.32 (s, 2H, NH), 4.26 (s, 2H, CH); 13C NMR (100 MHz, DMSO-d6): δ 166.10 (1C, C-5 [C=S], triazole), 154.42 (1C, C=O, amide), 149.93 (1C, C-3, triazole), 152.03, 133.83, 121.22 (5C, pyridine), 157.79, 137.57, 113.45 (3C, thiazole), 34.60 (1C, CH). Anal. Calc. for C21H15N5O4S2: C, 53.23; H, 3.33; N, 29.41.

2-(4-Amino-(3-pyridin-4-yl)-5-thioxo-4,5-dihydro-1H[1,2,4]triazol-1-yl)-N-(5-methylthiazol-2-yl) acetamide (6v)

Yellow amorphous solid; Recrystallizing solvent: Ethanol; Yield: 63%; M.p.: 250-252°C; FT-IR (KBr, cm⁻¹): 3281, 3192 (N-H stretch amine [NH]), 3360 (N-H stretch, amide), 3037 (aromatic C-H stretch), 2956, 2920 (aliphatic C-H stretch, CH, and CH2), 1687 (C=O stretch, amide), 1178 (C=S stretch, triazole); 1H NMR (600 MHz, DMSO-d6): δ 12.21 (s, 1H, NH), 8.71 (d, J = 5.4 Hz, 2H, pyridine), 7.96 (d, J = 5.4 Hz, 2H, pyridine), 7.13 (s, 1H, thiazole), 6.31 (s, 2H, NH), 4.23 (s, 2H, CH2), 2.32 (s, 3H, CH3); 13C NMR (100 MHz, DMSO-d6): δ 165.80 (1C, C-5 [C=S], triazole), 154.44 (1C, C=O, amide), 149.97 (1C, C-3, triazole), 152.02, 133.83, 121.22 (5C, pyridine), 155.96, 134.73, 126.33 (3C, thiazole), 35.54 (1C, CH3), 11.09 (1C, CH). Anal. Calc. for C15H13N5O4S2: C, 44.94; H, 3.77; N, 28.22.

General synthetic procedure for the preparation of 7a-7g.

A mixture of 5-pyridin-4-yl-3H-(1,3,4)oxadiazole-2-thione (4)(0.05 mol) and 2-chloro-N-(aryl or heteroaryl) acetamides (2o, 2s, 2u-2y) (0.05 mol) along with anhydrous potassium carbonate (K2CO3) (0.075 mol) was stirred in dimethyl formamide (DMF) at room temperature. After completion of reaction (monitored by TLC), ice cold water was added to precipitate out the product which was collected and purified by recrystallization from ethanol.

2-(5-pyridin-4-yl-2-thioxo-[1,3,4]oxadiazol-3-yl)-N-pyridin-2-yl acetamide (7a)

Brown crystalline solid; Yield: 45%; M.p.: 216-218°C; FT-IR (KBr, cm⁻¹): 3204 (N-H stretch, amide), 3161, 3113 (aromatic C-H stretch), 2980, 2935 (aliphatic C-H stretch, CH3), 1682 (C=O stretch, amide), 1180 (C=S stretch, oxadiazole); 1H NMR (300 MHz, DMSO-d6): δ 10.94 (s, 1H, NH), 8.81 (dd, J = 4.5, 3.0Hz, 2H, pyridine), 8.34 (d, J = 4.8 Hz, 1H, pyridine [amide]), 8.02 (d, J = 8.4 Hz, 1H, pyridine [amide]), 7.88 (dd, J = 4.2, 2.7 Hz, 2H, pyridine), 7.76-7.82 (m, 1H, pyridine [amide]), 7.11-7.15 (m,1H, pyridine [amide]), 4.43 (s, 2H, CH3); 13C NMR (75 MHz, DMSO-d6): δ165.79 (1C, C=O, amide), 164.78 (1C, C-2 [C=S], oxadiazole), 163.65 (1C, C-5, oxadiazole), 148.11, 129.99, 119.94 (5C, pyridine), 151.54, 150.91, 138.38, 119.83, 113.47 (5C, pyridine [amide]), 36.61 (1C, CH3). Anal. Calc. for C14H11N2O2S: C, 53.66; H, 3.54; N, 22.35.

N-(5-methylisoxazol-3-yl)-2-(5-(pyridin-4-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) acetamide (7b)

White crystalline solid; Yield: 68%; M.p.: 232-234 °C; FT-IR (KBr, cm⁻¹): 3263 (N-H stretch, amide), 3018, 2972, 2912 (aliphatic C-H stretch, CH, and CH2), 1691 (C=O stretch, amide), 1572 (aromatic C=N stretch) 1172 (C=S stretch, oxadiazole); 1H NMR (400 MHz,
1,2,4-Triazole-5-Thione Derivatives As InhA inhibitors

2-(5-pyridin-4-yl-2-thioxo-1,3,4-oxadiazol-3-yl)-N-thiazol-2-yl acetamide (7c)

White crystalline solid; Yield: 61%; M.p.: 244-246 °C; FT-IR (KBr, cm⁻¹): 3194 (N-H stretch, amide), 3053 (aromatic C-H stretch), 2949, 2920 (aliphatic C-H stretch, CH₂), 1611 (C=O stretch, amide), 1576, 1520, 1467, 1425, 1388 (C=C stretch), 1276, 1220, 1174 (C=S stretch, thiocarbonyl), 1154, 1132, 1095, 1045, 1023, 960, 924, 853, 816 (C-O stretch, oxadiazole); ¹H NMR (300 MHz, DMSO-d₆): δ 8.90 (d, J = 4.5, 2.7 Hz, 2H, pyridine), 7.87 (dd, J = 3.0, 1.9 Hz, 1H, thiazole), 7.25 (d, J = 3.3 Hz, 1H, thiazole), 4.44 (s, 2H, CH₂), 1.67 (s, 3H, CH₃). Anal. Calc. for C₆H₅N₂O₂S: C, 52.02; H, 2.84; N, 21.93.

N-(5-nitrothiazol-2-yl)-2-(5-(pyridin-4-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamide (7f)

Brown crystalline solid; Yield: 56%; M.p.: 254-256 °C; FT-IR (KBr, cm⁻¹): 3250 (N-H stretch, amide), 3076 (aromatic C-H stretch), 2933 (aliphatic C-H stretch, CH₃), 1635 (C=O stretch, amide), 1161 (C=S stretch, oxadiazole); ¹H NMR (400 MHz, DMSO-d₆): δ 8.22 (d, J = 4 Hz, 2H, pyridine), 7.86 (s, 1H, thiazole), 7.89 (d, J = 3 Hz, 2H, pyridine), 4.54 (s, 2H, CH₂); ¹³C NMR (75 MHz, DMSO-d₆): 163.57 (1C, C=O, amide), 164.17 (1C, C-2 [C=S], oxadiazole), 150.95, 129.98, 119.99 (5C, pyridine), 141.76, 132.98, 120.88, 118.28, 117.58 (7C, benzothiazole), 38.66 (1C, CH₃). Anal. Calc. for C₁₆H₁₄N₅O₄S₂: C, 39.56; H, 2.21; N, 23.07.

2-(5-pyridin-4-yl-2-thioxo-[1,3,4]oxadiazol-3-yl)-N-benzothiazol-2-yl acetamide (7g)

Yellow crystalline solid; Yield: 53%; M.p.: 206-208 °C; FT-IR (KBr, cm⁻¹): 3184 (N-H stretch, amide), 3083 (aromatic C-H stretch), 2921 (aliphatic C-H stretch, CH₃), 1689 (C=O stretch, amide), 1167 (C=S stretch, oxadiazole); ¹H NMR (300 MHz, DMSO-d₆): δ 8.18 (d, J = 2.4 Hz, 2H, benzothiazole), 8.18 (dd, J = 4.65, 3.0 Hz, 2H, pyridine), 7.90 (dd, J = 9.0, 6.6 Hz, 1H, benzothiazole), 7.90 (d, J = 4.2, 2.7 Hz, 2H, pyridine), 7.71 (d, J = 9.0 Hz, 1H, benzothiazole), 4.41 (s, 2H, CH₂); ¹³C NMR (75 MHz, DMSO-d₆): δ 171.72 (1C, C=O, amide), 164.35 (1C, C-2 [C=S], oxadiazole), 163.30 (1C, C-5, oxadiazole), 150.90, 130.15, 119.95 (5C, pyridine), 140.76, 132.98, 120.88, 118.28, 117.58 (7C, benzothiazole), 38.66 (1C, CH₃). Anal. Calc. for C₁₆H₁₄N₅O₄S₂: C, 37.52; H, 2.10; N, 18.96.

N-(5-nitrothiazol-2-yl)-2-(5-(pyridin-4-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamide (7e)

Brown crystalline solid; Yield: 56%; M.p.: 254-256 °C; FT-IR (KBr, cm⁻¹): 3250 (N-H stretch, amide), 3076 (aromatic C-H stretch), 2933 (aliphatic C-H stretch, CH₃), 1635 (C=O stretch, amide), 1161 (C=S stretch, oxadiazole); ¹H NMR (400 MHz, DMSO-d₆): δ 8.22 (d, J = 4 Hz, 2H, pyridine), 7.86 (s, 1H, thiazole), 7.89 (d, J = 3 Hz, 2H, pyridine), 4.54 (s, 2H, CH₂); ¹³C NMR (75 MHz, DMSO-d₆): δ 163.57 (1C, C=O, amide), 164.17 (1C, C-2 [C=S], oxadiazole), 150.95, 129.98, 119.99 (5C, pyridine), 141.76, 132.98, 120.88, 118.28, 117.58 (7C, benzothiazole), 38.66 (1C, CH₃). Anal. Calc. for C₁₆H₁₄N₅O₄S₂: C, 39.56; H, 2.21; N, 23.07.

N-(5-nitrothiazol-2-yl)-2-(5-(pyridin-4-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)acetamide (7f)

Brown crystalline solid; Yield: 56%; M.p.: 254-256 °C; FT-IR (KBr, cm⁻¹): 3250 (N-H stretch, amide), 3076 (aromatic C-H stretch), 2933 (aliphatic C-H stretch, CH₃), 1635 (C=O stretch, amide), 1161 (C=S stretch, oxadiazole); ¹H NMR (400 MHz, DMSO-d₆): δ 8.22 (d, J = 4 Hz, 2H, pyridine), 7.86 (s, 1H, thiazole), 7.89 (d, J = 3 Hz, 2H, pyridine), 4.54 (s, 2H, CH₂); ¹³C NMR (75 MHz, DMSO-d₆): δ 163.57 (1C, C=O, amide), 164.17 (1C, C-2 [C=S], oxadiazole), 150.95, 129.98, 119.99 (5C, pyridine), 141.76, 132.98, 120.88, 118.28, 117.58 (7C, benzothiazole), 38.66 (1C, CH₃). Anal. Calc. for C₁₆H₁₄N₅O₄S₂: C, 39.56; H, 2.21; N, 23.07.
pyridine), 7.66 (d, J = 9.0 Hz, 1H, benzothiazole), 7.54 (d, J = 2.7 Hz 1H, benzothiazole), 7.03 (dd, J = 9.0, 6.3 Hz 1H, benzothiazole), 4.48 (s, 2H, CH₃), 4.05 (q, 2H, OCH₂), 1.33 (s, 3H, CH₃); ¹³C NMR (75 MHz, DMSO-d₆): δ 166.20 (1C, C=O, amide), 164.30 (1C, C-2 [C=S], oxadiazole), 163.76 (1C, C-5, oxadiazole), 150.92, 129.99, 119.97 (5C, pyridine), 155.40, 132.79, 120.21, 121.20, 115.33, 105.35 (7C, benzothiazole), 63.57 (1C, O-CH₂), 35.86 (1C, CH₂), 14.68 (1C, CH₃). Anal. Calc. for C₁₈H₁₅N₅O₃S₂: C, 52.29; H, 3.66; N, 16.94.

Biological evaluation

*Mtb H₃Rv assay [Resazurin microtiter-based assay (REMA)]*

To determine the potency of a compound against *M. tuberculosis*, the compounds were dissolved and serially diluted in DMSO in a 384 well plate. 1 μL of compound was dispensed per well for a 10-point concentration response format using a BiomekFx liquid dispenser. To this, 40 μL of *M. tuberculosis* (3-5 x 10⁵ CFU/mL in 7H9 medium, 0.05% Tween 80, ADC, Casamino acids) were added with a multidrop dispenser. The plates were then incubated at 37 °C for 6 days. 10 μL of resazurin solution (20 mg/100 mL diluted 1:1 with 10% Tween 80) were added and the plates were incubated for an additional 24 h at 37 °C for color development. Absorbance in Spectramax at two wavelengths (575 & 610 nm) was measured and the MIC determined (Ratio of the absorbance values used for calculating 90% inhibition with respect to growth control).

*Mtb InhA enzyme inhibition assay (Fluorescence based assay)*

The compounds were dissolved in 100% dimethyl sulfoxide (DMSO). InhA (1.25 nM) was preincubated for ~15 min at room temperature with 0.050 mM NADH and inhibitor at a final concentration of 2% (v/v) dimethyl sulfoxide in 50μL reaction volume. The 50μL enzyme reaction contained PIPES- 30 mM, pH 7.5, NaCl - 50 mM, 0.005% Brij-35, DTT - 2mM, and EDTA- 0.1mM. The enzyme reaction was started by the addition of 150μl of dodecyl CoA synthesized in-house and the depletion of NADH was followed by measuring the fluorescence of NADH kinetically in the reaction for 30min at excitation at 340nm and emission at 420nm using Tecan Saffire II reader. The concentration at which 50% inhibition in enzyme activity was observed was reported as the IC₅₀.

Results and Discussion

**Designing**

Pan *et al.* elucidated a general binding model based on the binding modes of known InhA inhibitors and identified three key areas of interaction at the InhA active site (10). The first area includes the groups involved in the hydrogen bond interactions amongst the groups present on the inhibitors, *i.e.*, Tyr-158 and the 2'-hydroxyl functionality of nicotinamide ribose. The second region is the hydrophobic pocket wherein the hydrophobic contacts include Gly-96, Phe-97, Met-103, Gly-104, Phe-149, Ala-157, Met-161, Pro-193, Ala-198, Met-199, Ile-202, Val-203, Ile215, and Leu218 residues. The third region is present near the hydrogen bonding group of the inhibitor. This region is comparatively size constrained and exposed to solvent. It is also closer to non-polar as well as polar groups (cofactor phosphodiester bridge), in the substrate binding loop (eg. Ala-198). The selectivity of enzyme inhibition is extremely sensitive to the size and chemical nature of the substituent.

One important class among the previously identified anti-mycobacterial hits are the pyrrolidine carboxamide class of direct InhA inhibitors. Their overall chemical structure topology incorporates ring C as the hydrophobic binding region, a linker extending to six atom length, a hydrogen bonding carbonyl incorporated into ring B and finally, ring A serving as the size constrained region (Figure 2). In the present methodology, we hybridized in one molecular platform, the 1,2,4-triazole-5-thione core/linker to hydrogen bonding carbonyl group of amides to give the designed compound 6. The designed ligand thus has three parts:1,2,4-triazole-5-thione moiety (ring B) linked with acetamido group sandwiched between a left-hand pyridine (ring C) and a right-hand substituted aryl/heteroaryl moiety (ring A) as shown in Figure 2. The presence
of a pyridine nucleus in INH as hydrophobic
binding region in INH-NAD$^+$ adduct made it
an automatic choice as the left-hand ring A (6).
The selection of 1,2,4-triazole-5-thione nucleus
as ring B was based on the established literature
wherein 1,2,4-triazole nucleus has been proved
to elicit anti-mycobacterial activity (20, 21).
The selection of the right-hand substituent was
purely based on the inputs from the review by
Pan et al., wherein it was found that the TB
activity could be optimized at the right-hand
side.

**SwissADME predictions**

Drug development consists of calculation of
ADME profile, but determination of ADME by
actual experimentations for all the compounds
is very time consuming and tedious task. In
this situation, computer-models offer valid
replacements to (22) Thus, SwissADME web
tool is freely available and gives access to
the fast and robust models for prediction of
pharmacokinetics/physicochemical properties
and drug-likeness. Easy efficient input and
interpretation are key advantages of the tool. We
have used this tool to predict properties of our
compounds. All the compounds were observed
to follow the Lipinski’s rule of five (Table 1).
Judging the data, the molecules seem to be drug
like and may have good passive oral absorption.

An intuitive method offered by SwissADME
model is known as BOILED-Egg, which
simultaneously predicts two crucial ADME
parameters, \( \text{viz.} \) brain access (BBB) and passive
gastrointestinal absorption (HIA). Although
conceptually it looks very simple, as it relies
only on two descriptors, WLOGP (lipophilicity)
and TPSA (apparent polarity), the BOILED-Egg
has been verified to be a candid explanation
and competent translation to molecular design
in numerous drug discoveries (23). Out of
29 compounds, none was observed in egg
yolk (Yellow) region, predicting that all do
not penetrate the brain. 19 compounds were
observed to be absorbed well but not crossing
BBB (in the white), in all these molecules Ar/
Het substitution was phenyl/substituted phenyl
moiety.

Remaining 10 molecules (8r- 8v, 13c-13g)
were observed to be not absorbed and not
Vora D et al. / IJPR (2019), 18 (4):1742-1758

crossing BBB (outside the Egg) and may have poor bioavailability (Figure 3). When we closely observed the Ar/Het substitution, we found that all these 10 molecules have substituted/unsubstituted five/six membered heterocycle or substituted/unsubstituted heteroaryl fused ring system such as benzothiazole. Moreover, in biological screening these 10 molecules were found inactive or nearly inactive in both Mtbo and InhA enzyme inhibition assay.

Table 1. SwissADME prediction data.

| Code | TPSA | Log P | Log S | ESOL2 | GI absorption | Lipinski #violations | Lead likeness #violations |
|------|------|-------|-------|-------|----------------|----------------------|---------------------------|
| 6a   | 122.85 | 1    | -2.65 | Soluble | High | 0 | 0 |
| 6b   | 122.85 | 1.66 | -2.94 | Soluble | High | 0 | 0 |
| 6c   | 122.85 | 1.76 | -3.71 | Soluble | High | 0 | 1 |
| 6d   | 132.08 | 1.14 | -2.71 | Soluble | High | 0 | 1 |
| 6e   | 122.85 | 1.88 | -3.48 | Soluble | High | 0 | 1 |
| 6f   | 122.85 | 1.52 | -3.24 | Soluble | High | 0 | 1 |
| 6g   | 122.85 | 1.9 | -3.24 | Soluble | High | 0 | 1 |
| 6h   | 122.85 | 2.17 | -3.53 | Soluble | High | 0 | 1 |
| 6i   | 122.85 | 1.66 | -2.94 | Soluble | High | 0 | 0 |
| 6j   | 122.85 | 1.52 | -3.24 | Soluble | High | 0 | 1 |
| 6k   | 122.85 | 2.12 | -4.08 | Moderately soluble | Low | 0 | 1 |
| 6l   | 132.08 | 1.14 | -2.71 | Soluble | High | 0 | 1 |
| 6m   | 122.85 | 1.39 | -2.81 | Soluble | High | 0 | 0 |
| 6n   | 122.85 | 1.88 | -3.48 | Soluble | High | 0 | 1 |
| 6o   | 135.74 | 0.36 | -2.19 | Soluble | High | 0 | 0 |
| 6p   | 135.74 | 1.02 | -2.51 | Soluble | High | 0 | 0 |
| 6q   | 135.74 | 1.02 | -2.48 | Soluble | High | 0 | 0 |
| 6r   | 148.63 | -0.27 | -1.79 | Very soluble | Low | 0 | 0 |
| 6s   | 148.88 | 0.17 | -2.14 | Soluble | Low | 0 | 0 |
| 6t   | 163.98 | 1.64 | -3.82 | Soluble | Low | 0 | 1 |
| 6u   | 163.98 | -0.14 | -2.29 | Soluble | Low | 0 | 0 |
| 6v   | 163.98 | 0.54 | -2.6 | Soluble | Low | 0 | 0 |
| 7a   | 117.93 | 0.48 | -2.85 | Soluble | High | 0 | 0 |
| 7b   | 131.07 | -0.13 | -2.79 | Soluble | High | 0 | 0 |
| 7c   | 146.17 | -0.02 | -2.94 | Soluble | Low | 0 | 0 |
| 7d   | 146.17 | 0.25 | -3.26 | Soluble | Low | 0 | 0 |
| 7e   | 191.99 | -0.06 | -3.19 | Soluble | Low | 0 | 1 |
| 7f   | 146.17 | 1.38 | -4.18 | Moderately soluble | Low | 0 | 1 |
| 7g   | 155.4 | 1.07 | -4.47 | Moderately soluble | Low | 0 | 1 |

a- Topological polar surface area b- Log of partition coefficient (P), c- Log solubility, d-estimated aqueous solubility, e- Gastrointestinal.
Chemistry

The synthetic pathway used to achieve the target compounds has been delineated in Scheme 1. The construction of the target compounds, 6a-6v, involved the synthesis of 5-(pyridin-4-yl)-3H-(1,3,4)-oxadiazole-2-thione (4) by refluxing isoniazid with carbon disulfide in the presence of ethanolic KOH. Compound 4 on further treatment with 99% hydrazine hydrate yielded 4-amino-3-(pyridin-4-yl)-1H-(1,2,4)-triazole-5-thione (5). Both 4 and 5 were obtained in good yield. It has been observed that compound 4 can undergo thiol-thione tautomerism and thereby could also exist in thiol form (24). However, compounds 4 and 5 exist in thione form which was confirmed by FT-IR and 13C-NMR data. Compounds 2a-2y were obtained by N-chloroacetylation of various aromatic and heteroaromatic amines (1a-1y) using weak base such as K2CO3 in dichloromethane (DCM) as solvent. The final step involved the condensation between 2a-2v and 5 mediated by anhydrous K2CO3 and DMF to afford the target compounds 6a-6v. Compounds 7a-7g from condensation between 4 and 2o, 2s, 2u-2y were also attempted in synthesis, as steps towards the derivation of structure-activity relationships (SAR) and lead identification. The reactions were monitored for completion by thin layer chromatography. The structures of newly synthesized compounds were confirmed by spectral data -1H NMR, 13C NMR, and FTIR.

FT-IR spectra of these derivatives displayed characteristic absorption in the range of 3360-3176 cm⁻¹ corresponding to NH(s) vibration of free amino group as well as NH(s) vibration of the amide group. In addition, they also exhibited characteristic absorption peak in the range of 1687-1660 cm⁻¹ corresponding to C=O(s) of amide group and in the range of 1213-1172 cm⁻¹ corresponding to C=S(s). Proton NMR spectra of compounds 6a-6v showed two characteristic doublets in a region of δ=8.71-8.74 and 7.96-8.01 ppm corresponding to four hydrogen atoms of K2CO3, and DMF to afford the target compounds 6a-6v. Compounds 7a-7g from condensation between 4 and 2o, 2s, 2u-2y were also attempted in synthesis, as steps towards the derivation of structure-activity relationships (SAR) and lead identification. The reactions were monitored for completion by thin layer chromatography. The structures of newly synthesized compounds were confirmed by spectral data -1H NMR, 13C NMR, and FTIR.

FT-IR spectra of these derivatives displayed characteristic absorption in the range of 3360-3176 cm⁻¹ corresponding to NH(s) vibration of free amino group as well as NH(s) vibration of the amide group. In addition, they also exhibited characteristic absorption peak in the range of 1687-1660 cm⁻¹ corresponding to C=O(s) of amide group and in the range of 1213-1172 cm⁻¹ corresponding to C=S(s). Proton NMR spectra of compounds 6a-6v showed two characteristic doublets in a region of δ=8.71-8.74 and 7.96-8.01 ppm corresponding to four hydrogen atoms of
Biological evaluation
The MIC and IC_{50} values have been shown in Table 2. From the data it was observed that there is direct correlation between the antitubercular activity and InhA inhibition. The molecules which exhibited higher InhA inhibition have also led to higher antitubercular activity in Mtb assay. Similarly compounds with less inhibition of InhA have shown poor antitubercular activity. This indicates that the antitubercular activity is due to the inhibition of InhA enzyme and thus we can say that our newly designed compounds specifically target InhA enzyme (Figure 4).

In the aromatic series of 1,2,4-triazole-5-thiones, compounds 6b, and 6g-6i showed the best activity both in terms of MIC and InhA IC_{50} while the activity decreased in compounds 6c-6f and 6j-6n. The plausible reason could be justified by considering compound 6a as the reference. The unsubstituted phenyl ring of 6a does not show any remarkable inductive effect whereas the electron donating methyl group present at 4-position and 3,4-positions of the compounds 6b and 6g respectively elicits positive inductive (+I) effect which seems to be essential for the activation of the carbonyl group due to the inhibition of InhA enzyme and thus the carbonyl group would not be that much activated and the same is reflected in a mild decrease in the activity.

However, moving on from six-membered aromatic to six-membered and five-membered heterocyclic ring systems (6o-6v), the activity drastically decreases with compound 6t being inactive. The inactivity of 6t can be attributed to two reasons; the first being the strong electron withdrawing thiazole ring of benzothiazole and...
the second reason could be the bulk of the overall benzothiazole ring.

Considering the seven oxadiazole molecules 7a-7g, compounds 7a-7e are very slightly active while 7f-7g are inactive thereby indicating that there is no significant change in the activity when compared to their triazole counterparts. This suggests that the size of ring A has more dominating effect on the final activity compared to the type of linker viz, triazole or oxadiazole.

As discussed in the designing section, the ring A is the size constrained region and therefore any increase or decrease in the size of the ring which form the optimum, would adversely affect the final activity of the compound. Thus, based on the results obtained, the optimum size of the ring A seems to be six-membered aromatic ring.

### Conclusions

Various 1,2,4-triazole derivatives targeting InhA were designed, synthesized and spectrally characterized using IR, $^1$H NMR and $^{13}$C NMR spectroscopy. The resazurin microtiter assay (REMA) of the characterized compounds on the *Mycobacterium tuberculosis* H$_3$7Rv strain identified promising candidates in the series and their mechanism of action validated by the InhA enzyme inhibitory studies. The compounds 6b, 6g-6i were found to be promising and compound 6b was identified as hit with Mtb H$_3$7Rv MIC of 0.19 µM and InhA IC$_{50}$ value of 0.09 µM (90 nM). SwissADME predictions were found to be correlating structures with the biological activity and most of the compounds exhibited drug-likeliness.

There is a scope for further optimization of the identified hits to obtain more potent direct InhA inhibitors, which could serve as the plausible leads for further drug development.

### Acknowledgement

We would like to thank National Institute for Research in Tuberculosis, Chennai, India for helping us to conduct biological evaluation.

---

| Compound Code | Mtb MIC (µM) | InhA IC$_{50}$ (µM) | Compound Code | Mtb MIC (µM) | InhA IC$_{50}$ (µM) |
|---------------|--------------|----------------------|---------------|--------------|----------------------|
| 6a            | 12.5         | 0.68                 | 6p            | 100          | 86                   |
| 6b            | 0.19         | 0.09                 | 6q            | 50           | 42                   |
| 6c            | 3.12         | 6.15                 | 6r            | >100         | >100                 |
| 6d            | 25           | 10.78                | 6s            | 50           | 36                   |
| 6e            | 12.5         | 5.95                 | 6t            | >100         | >100                 |
| 6f            | 12.5         | 1.2                  | 6u            | >100         | >100                 |
| 6g            | 0.39         | 0.19                 | 6v            | 50           | 47                   |
| 6h            | 6.25         | 0.34                 | 7a            | 50           | 54                   |
| 6i            | 0.39         | 0.12                 | 7b            | 50           | 24                   |
| 6j            | 6.25         | 3.1                  | 7c            | 50           | 37                   |
| 6k            | 12.5         | 6.36                 | 7d            | 100          | 67                   |
| 6l            | 50           | 23                   | 7e            | 100          | 82                   |
| 6m            | 12.5         | 6.3                  | 7f            | >100         | 98                   |
| 6n            | 6.25         | 3.14                 | 7g            | >100         | >100                 |
| 6o            | 100          | 76                   | INH           | 0.05         | NA                   |
References

(1) Global tuberculosis report 2018. Available from: URL: https://www.who.int/tb/publications /global_report/en.

(2) Tuberculosis: Key Facts. Available from: URL: http://www.who.int/mediacentre/factsheets/fs104/en/index.html.

(3) Tuberculosis-WHO India. Available from: URL: http://www.whoindia.org/EN /Section3/Section123.html.

(4) Udwadia ZF, Amale RA, Ajbani KK and Rodrigues C. Totally drug-resistant tuberculosis in India. Clin. Infect. Dis. (2012) 54: 579-81.

(5) Asselineau J and Lederer E. Structure of the Mycolic Acids of Mycobacteria. Nature (1950) 166: 782-3.

(6) Rozwarski DA Grant GA, Barton DH, Jacobs WR JR and Sacchettini JC. Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. Science (1998) 279: 98-102.

(7) Lei B, Wei CJ and Tu SC. Action Mechanism of Antitubercular Isoniazid Activation by mycobacterium tuberculosis katG, isolation, and characterization of inhA inhibitor. J. Biol. Chem. (2000) 275: 2520-6.

(8) Rawat R, Whitty A and Peter JT. The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the Mycobacterium tuberculosis enoyl reductase: Adduct affinity and drug resistance. Proc. Natl. Acad. Sci. U. S. A. (2003) 100: 13881-6.

(9) Parikh SL, Xiao G and Tonge PJ. Inhibition of InhA, the Enoyl Reductase from Mycobacterium tuberculosis, by Triclosan and Isoniazid. Biochemistry (2000) 39: 7645-50.

(10) Pan P and Tonge PJ. Targeting InhA, the FASII enoyl-ACP reductase: SAR studies on novel inhibitor scaffolds. Curr. Topics Med. Chem. (2012) 12: 672-693.

(11) Luckner SR, Liu N, Am-Ende CW, Tonge PJ and Kisker C. A slow, tight-binding inhibitor of InhA, the enoyl-ACP reductase from Mycobacterium tuberculosis. J. Biol. Chem. (2010) 285: 14330-7.

(12) Am Ende CW, Knudson SE, Liu N, Childs J, Sullivan TJ, Boyne M, Xu H, Gergina Y, Knudson DL, Johnson F, Peloquin CA, Slayden RA and Tonge PJ. Synthesis and in-vitro antitubercular activity of B-ring modified diaryl ether InhA inhibitors. Bioorg. Med. Chem. Lett. (2008) 18: 3029-33.

(13) Amir M, Kumar H and Javed SA. Condensed bridgehead nitrogen heterocyclic system: Synthesis and pharmacological activities of 1,2,4-triazole-[3,4-b]-1,3,4-thiadiazole derivatives of ibuprofen and biphenyl-4-yl-oxy acetic acid. J. Med. Chem. (2008) 43: 2056-66.

(14) Amir M and Shikha K. Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-(2,6-dichloroanilino) phenylacetic acid derivatives. Eur. J. Med. Chem. (2004) 39: 535-45.

(15) Reid JR and Heindel ND. Improved syntheses of 5-substituted-4-amino-3-mercapto-(4H)1,2,4-triazoles. J. Heterocycl. Chem. (1976) 13: 925-6.

(16) Patil V, Tilekar K, Sonali MM, Mohan R and Ramaa CS. Synthesis and primary cytotoxicity evaluation of new 5-benzylidene-2,4-thiazolidinedione derivatives. Eur. J. Med. Chem. (2010) 45: 4539-44.

(17) Bhunusahni U, Saranya R, Keerthana S, Pushkar K, Kiran C, Chatterjee S and Ramaa CS. 5-benzylidene-2,4-thiazolidinedione derivatives: Design, synthesis and evaluation as inhibitors of angiogenesis targeting VEGFR-2. Bioorg. Chem. (2016) 67: 139-47.

(18) Joshi H, Marulkar K, Gotia V and Ramaa CS. Hydroxy cinnamic acid derivatives as partial PPARγ agonists: In silico studies, synthesis and biological characterization against chronic myeloid leukemia cell line (K-562). Anti-Cancer Agents Med. Chem. (2017) 17: 524-41.

(19) Kabir A, Tilekar K, Upadhya N and Ramaa CS. Novel antraquinone derivatives as dual inhibitors of topoisomerase 2 and casein kinase 2: In silico studies, synthesis and biological evaluation on human leukemic cell lines. Anti-Cancer Agents Med. Chem. (2018) 18: 1551-62.

(20) Joshi SD, Vagdevi HM, Vaidya VP and Gadaginamath GS. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antituberular agents. Eur. J. Med. Chem. (2008) 43: 1989-96.

(21) Zhou CH and Wang Y. Recent researches in triazole compounds as medicinal drugs. Curr. Med. Chem. (2012) 19: 239-80.

(22) Daina, A. SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. Sci. Reports (2016) 7: 42717.

(23) Daina, A and Zoete, V. A BOILED-Egg to Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. ChemMedChem (2016) 11: 1117–21.

(24) Aydogan F, Turgut Z and Ocal N. Synthesis and electronic structure of new ary1-and -alkyl-substituted 1,3,4-oxadiazole-2-thione derivatives. Turk. J. Chem. (2002) 26: 159-69.

This article is available online at http://www.ijpr.ir