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Received Date : 29-Jul-2016

Revised Date : 23-Nov-2016

Accepted Date : 10-Dec-2016

Article type : Research Article

Synthesis and biological evaluation of 1,2,4-triazole-3-thione and 1,3,4-oxadiazole-2-thione as anti-mycobacterial agents

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ABSTRACT

Resistance among dormant mycobacteria leading to multi-drug resistant (MDR) and extremely-drug resistant(XDR) tuberculosis is one of the major threats. Hence, a series of 1,2,4-triazole-3-thione and 1,3,4-oxadiazole-2-thione derivatives (**4a-5c**) have been synthesised and screened for their anti-tubercular activity against *Mycobacterium tuberculosis* H37Ra (H37Ra). Thetriazolethiones**4b** and **4v** showed high anti-tubercular activity (both MIC and IC_{50}) against the dormant H37Ra by *in vitro* and *ex vivo*. They were shown to have more specificity towards mycobacteria than other gram-negative and gram-positive pathogenic bacteria. The cytotoxicity was almost insignificant up to 100 µg/mL against THP-1, A549 and PANC-1 human cancer cell lines, and solubility was high in aqueous solution, indicating the potential of developing these compounds further as novel therapeutics against tuberculosis infection.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.12939

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Tuberculosis (TB) is a devastating disease of poverty, with 95% of the 1.4 million TB deaths occurring in the developing world and resulting in about 10 million orphaned children [1]. Furthermore, the emergence of a drug resistant microorganism responsible for TB, especially multidrug-resistant one along with the lethal combination of TB and HIV-1 infection, makes this disease one of the greatest global health challenges facing us today [2,3]. Therefore, there is an urgent need for new anti-tubercular drugs and drug targets to reduce the time for treatment and to identify agents that will be effective against intracellular persisting Mycobacterium tuberculosis. Although, several compounds are currently in advanced phases of clinical trials [4], despite a few safety concerns about its use, bedaquiline (TMC-207) achieved accelerated approval by the FDA for the treatment of MDR-TB in 2012, becoming the first new TB drug (from a novel class) since 1966 [5,6].

In this regard, it has been established that heterocyclic compounds play an important role in designing new class of structural entities for medicinal applications [7,8]. Among pharmacologically important heterocyclic compounds, 1,2,4-triazole and its derivatives are attracted considerable attention due to their interesting biological activities [9-21]. These azoles exert their anti-tubercular activity through inhibition of cytochrome P450 gene CYP121 (CYP121) by a mechanism in which the heterocyclic nitrogen (N-3 of imidazole or N-4 of 1,2,4-tiazole) binds to the sixth coordination site of the heme iron atom of the porphyrin in the substrate-binding site of the enzyme [22,23]. These results prompted us to take-up the 1,2,4-triazoles motif as an active pharmacophore for further diversification to exploit its anti TB potential. In this regard, we have reported novel triazole derivatives that have potent anti-mycobacterial activity owing to their known inhibition of sterol synthesis [24,25]. In continuation to our research project aimed at the development of anti-tubercular agents herein, we report the synthesis of 1,2,4-triazolethiones and 1,3,4-oxadiazoles thiones (compounds **4a-4z** and **5a-5c**, respectively) and their anti-tubercular activity against the

dormant and active stages of *M. tuberculosis* H37Ra (MTB). Furthermore, cytotoxicity studies in three different human cancer cell lines, THP-1, A549, and Panc-1, were carried out as well to evaluate their specificity for *M.tuberculosis*.

Experimental section

Chemistry

The routes employed forsynthesis of substituted 5-aryl-4*H*-1,2,4-triazole-3-thione derivatives **4** are outlined in **Schemes 1 and 2**. The acid chloride **2** was prepared by reacting aromatic carboxylic acid **1** with thionyl chloride. The thiosemicarbazide was acylated with acid chloride yielding intermediate **3**, which, without isolation, upon treatment with ethanolic or aqueous potassium hydroxide, cyclised to yield the compound 5-aryl-4*H*-1,2,4-triazole-3-thione **4** in moderate yields (Scheme1)[26-28]. Some triazolethiones could not be synthesised by the above procedure. Instead, they were prepared via ester derivatives as depicted in **Scheme 2**. The aroylhydrazides**3** were obtained by reaction with hydrazine hydrate in EtOH. Treatment of the aroylhydrazide**3** under basic conditions (KOH/EtOH) yielded the corresponding potassium aroyldithiocarbazate. The resulting intermediate **3** was refluxed in aqueousammonia to obtain compounds **4u-z.** Aroylhydrazides**3** were reacted with CS_2 in ethanolic KOH followed by acidification with HCl to yield 1,2,4-oxadiazole thiones**5a-c**. Homogeneity of the compounds was determined by thin-layer chromatography (TLC), and the structures of the newly synthesised compounds were characterised by infrared (IR) spectroscopy, ^{1}H-NMR, ^{13}C-NMR, and mass spectral analysis.

Methods

S1. General procedure for preparation of 4(a-z)

To a cold solution of substituted benzoic acid (1.0 mmol, 1 equiv.) in dry DCM (25 mL) with catalytic amount of DMF, thionyl chloride (1.5 mmol, 1.5 equiv.) was added drop-wise. The reaction mixture was refluxed for 4 h. The solvent was removed under reduced pressure. To the crude residue of the acid chloride in pyridine (25 mL), thiosemicarbazide (1.5 mmol, 1.5equiv.) was added portion-wise over a period of 10 min and then stirred at room temperature for 16 h. Pyridine was removed under reduced pressure. The crude residue was dissolved in 10% aqueous KOH (25 mL), and the resulting mixture was heated at 80°C-100°C for 3 h. After completion of the reaction, the mixture was cooled 0°C to 0°C and neutralised with 10% HCl. The precipitate was filtered, washed with water, and dried to yield compounds **4a-t**.

5-(4-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4a)

Yield: 79%; Mp: 290-293°C; IR (KBr): 539, 618, 672, 725, 839, 883, 985, 1105, 1294, 1459, 1557, 1611, 1655, 1908, 2855, 3196, 3306 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.57 (d, *J* = 8.6 Hz, 2H), 7.90 (d, *J* = 8.6 Hz, 2H); ^{13}C-NMR (DMSO-D6): 127.9, 128.6, 128.7, 131.2, 139.4, 168.0; HRMS: *m/z*= 212.0049,calcd. $C_8H_6ClN_3S$, found 212.0044 $[M+H]^+$.

5-(4-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4b)

Yield: 72%; Mp: 238-242°C; IR (KBr): 711, 792, 854, 950, 1020, 1073, 1113, 1171, 1245, 1305, 1341, 1375, 1457, 1515, 1582, 2705, 2747, 2858, 2952, 3350 cm^{-1}; ^{1}H-NMR (DMSO-D6): 8.16 (d, *J* = 8.8 Hz, 2H), 8.32 (d, *J* = 8.8 Hz, 2H); ^{13}C-NMR (DMSO-D6): δ 124.6, 127.0, 129.2, 131.3, 148.8, 167.9; HRMS: *m/z*= 223.0290, calcd.$C_8H_6N_4O_2S$, found 223.0284 $[M+H]^+$.

5-(4-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4c)

Yield: 81%; Mp: 265-268°C; IR (KBr): 547, 617, 735, 843, 1161, 1231, 1316, 1457, 1515, 1576, 1610, 2677, 2858, 2922, 3058, 3332 cm^{-1}; ^{1}H-NMR (DMSO-D6): δ 7.37 (t, *J* = 8.5 & 17.6 Hz, 2H), 7.93-7.97 (m, 2H), 13.70 (s,1H), 13.87 (s,1H); ^{13}C-NMR (DMSO-D6): δ 115.7, 115.2, 116.4, 116.6, 122.3, 128.3, 128.4, 130.5, 149.6, 162.3, 164.8, 167.2; HRMS: *m/z* = 196.0345 calcd.$C_8H_6FN_3S$, found 196.039 $[M+H]^+$.

5-(2-methyl-3-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4d)

Yield: 73%; Mp: 235°C; IR (KBr): 554, 611, 725, 803, 867, 975, 1032, 1252, 1307, 1370, 1459, 1527, 1578, 1612, 2858, 2922, 3061, 3384 cm^{-1} ^{1}H-NMR (DMSO-D6): δ 2.97 (s, 3H), 7.95 (d, J= 7.7 Hz, 1H), 8.02 (d, J= 7.6 Hz, 1H), 8.13 (m,1H); ^{13}C-NMR (DMSO-D6): δ 20.03, 124.7, 125.8, 129.9, 130.1, 134.2, 148.94, 149.92, 167.98.

5-(3-methyl-4-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4e)

Yield: 81%; Mp: 261-263°C; IR (KBr): 581, 615, 749, 791, 832, 881, 967, 993, 1032, 1067, 1098, 1234, 1334, 1375, 1456, 1572, 2855 cm^{-1}; ^{1}H-NMR (DMSO-D6): δ 2.55 (s, 1H), 7.93 (dd, *J*=8.02 Hz, 1H),8.01 (s, 1H), 8.11 (dd, *J* =8.02, 1H), 13.89 (s,1H), 14.03 (s,1H); ^{13}C-NMR (DMSO-D6): δ 20.2, 124.9, 125.98, 129.99, 130.3, 134.3, 149.07, 150.06, 168.1; HRMS: *m/z*= 237.0439,calcd. $C_9H_8N_4O_2S$, found 237.0441 $[M+H]^+$.

5-(4-(trifluoromethyl)phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4f)

Yield: 79%; Mp: 283°C; IR (KBr): 552, 599, 699, 763, 850, 940, 966, 1017, 1069, 1122, 1182, 1329, 1375, 1455, 1527, 1578, 1497, 2676, 2856 cm^{-1}; ^{1}H-NMR (DMSO-D6): δ 7.85 (dd, *J* = 8.1 Hz, 2H), 8.10 (dd, *J* = 8.1 Hz, 2H), 13.89 (s, 1H), 14.10 (s, 1H); ^{13}C-NMR (DMSO-D6): δ 122.7, 125.5, 125.8, 126.3, 126.3, 126.7, 127.6, 129.5, 130.4, 130.5, 130.8, 134.8, 142.1, 146.4, 149.3, 166.4, 167.7. HRMS: *m/z*= 246.0313, calcd.$C_9H_6F_3N_3S$, found 246.0307 $[M+H]^+$.

5-(3-(trifluoromethyl)phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4g)

Yield: 76%; Mp: 120-122°C;IR (KBr): 653, 699, 746, 811, 915, 979, 1082, 1127, 1173, 1229, 1323, 1369, 1457, 1502, 1574, 1614, 1695, 2586, 2921, 3054 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.71-7.91 (m, 2H), 8.22 (d, *J* = 7.3 Hz, 2H), 8.27 (s, *J* = 1.3 Hz, 1H), 13.83 (s, 1H), 14.04 (s, 1H);^{13}C-NMR: 122.5, 122.6, 125.4, 125.7, 126.7, 127.3, 129.7, 130.3, 130.6, 132.1, 133.4, 149.2, 166.2, 167.7. HRMS: *m/z*= 246.0313, calcd. $C_9H_6F_3N_3SF3S$, found 246.0307 $[M+H]^+$.

5-(4-nitro-3-(trifluoromethyl)phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4h)

Yield: 79%; Mp: 138-141°C; ^{1}H-NMR (DMSO-D6): 8.1-8.2 (m, 21), 8.56-8.66 (m, 2H), ^{13}C-NMR: 122.8, 128.09, 128.3, 130.3, 131.8, 134.5, 147.04, 148.9, 149.1, 165.2, 167.8.

5-(4-chloro-2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4i)

Yield: 81%; Mp: 205°C;NMR (DMSO-D6): 2.44 (s, 3H), 7.42 (dd,*J* = 8.3 & 1.6 Hz, 1H), 7.47 (d, *J* = 1.6 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 13.66(s, 1H), 13.74 (s, 1H) ^{13}C-NMR: 21.06, 128.8, 130.4, 131.1, 133.7, 136.4, 149.8, 167.07 HRMS: *m/z*= 226.0206, calcd. $C_9H_8ClN_3S$, found 226.0200 $[M+H]^+$.

5 -(2-chloro-5-(trifluoromethyl)phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4j)

Yield: 81%; Mp: 185°C: P1PH NMR (DMSO-D6): 7.80 (d, *J* = 8.7 Hz, 1H), 7.92 (d, *J* = 8.7 Hz, 1H), 8.12 (s, 1H) ^{13}C-NMR: 126.4, 128.6, 128.91, 129.3, 131.9, 132.3, 136.96, 147.6, 164.5, 167.5

5-(3-chloro-2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4k)

Yield: 81%; Mp: 177-189°C;IR (KBr): 673, 721, 761, 796, 914, 975, 1019, 1075, 1115, 1158, 1253, 1303, 1380, 1454, 1503, 1574, 1647, 2651, 2857, 3116 cm^{-1}; ^{1}H-NMR (DMSO-D6): 2.52 (s, 3H), 7.27-7.40 (m, 1H), 7.51-7.71 (m, 2H), 13.69 (s, 1H), 13.79 (s, 1H); ^{13}C-NMR: 127.7, 127.92, 128.05, 128.96, 131.6, 150.4, 150.6, 167.07; HRMS: *m/z*= 226.0206,calcd. $C_9H_8ClN_3S$, found 226.0200 $[M+H]^+$.

5-(5-chloro-2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4l)

Yield: 79%; Mp: 230 °C;IR (KBr): 557, 608, 655, 731, 818, 981, 1036, 1073, 1159, 1376, 1457, 1598, 1703, 2858, 2923,3163, 3347 cm^{-1}; ^{1}H-NMR (DMSO-D6): 2.44 (s, 3H), 7.33-7.55 (m, 2H), 7.73-7.79 (m, 1H), 13.69 (s, 1H), 13.80 (s, 1H); ^{13}C-NMR: 21.06, 128.8, 130.4, 131.1, 133.7, 136.4, 149.8, 167.07; HRMS: *m/z* = 226.0206, calcd. $C_9H_8ClN_3S$, found 226.9551 $[M+H]^+$.

5-(2-(trifluoromethyl)phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4m)

Yield: 86%; Mp:245°C;^{1}H-NMR (DMSO-D6): 7.66-7.97 (m, 4H). ^{13}C-NMR: 126.98, 129.8, 131.07, 132.6, 133.02, 133.3, 148.7, 166.8.

5-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4n)

Yield: 83%; Mp: 255°C, IR (KBr): 631, 732, 796, 840, 963, 1015, 1078, 1122, 1176, 1253, 1300, 1389, 1456, 1519, 1607, 2858, 2923, 3243 cm^{-1}; ^{1}H-NMR (DMSO-D6): 3.80 (s, 3H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H); ^{13}C-NMR: 55.6, 113.95, 114.7, 127.6, 129.6, 161.15. HRMS: *m/z*= 208.0545, calcd. $C_9H_9N_3OS$, found 208.0539 $[M+H]^+$.

5-(2-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4o)

Yield: 79%; Mp: 205°C; IR (KBr): 559, 608, 663, 731, 765, 968, 1040, 1159, 1304, 1375, 1458, 1595, 1711, 2858, 2923, 3054, 3127, 3331 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.44-7.68 (m, 4H), 13.81 (s, 1H); ^{13}C-NMR:128.4, 128.7, 132.3, 133.03, 149.8, 151.02, 166.4, 182.6; HRMS: *m/z* = 212.0049, calcd. $C_8H_6ClN_3SN3S$, found 212.0044 $[M+H]^+$.

5-(2-chloro-5-(trifluoromethyl)phenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4p)

Yield: 71%; Mp: 288°C, IR (KBr): 728, 773, 901, 963, 1036, 1083, 1162, 1265, 1308, 1374, 1457, 1577, 1600, 1657, 2059, 2663, 2850, 2923, 3057, 3192 cmP^{-1}; ^{1}H-NMR (DMSO-D6): 2.42 (s, 3H), 7.27-7.45 (m, 4H); ^{13}C-NMR: 21.3, 125.4, 126.6, 129.4, 130.7, 131.8, 137.3, 151.03, 166.9.

5-(p-tolyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4q)

Yield: 84%; Mp: 255°C; IR (KBr): 723, 813, 1019, 1075, 1118, 1230, 1304, 1375, 1458, 1524, 1580,1610, 1711, 2859, 2923, 3084, 3167, 3265,3365 cm^{-1}; ^{1}H-NMR (DMSO-D6): 2.33 (s, 3H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H), 13.64 (s, 1H), 13.78 (s, 1H);^{13}C-NMR: 21.1, 122.9, 125.8, 129.8, 140.7, 150.5, 166.99;HRMS: *m/z* = 192.0595, calcd.$C_9H_9N_3S$, found 192.0590 $[M+H]^+$.

5-(3-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4r)

Yield: 77%; Mp: 154°C; IR (KBr):551, 664, 719, 751, 851, 907, 1141, 1262, 1304, 1382, 1423, 1468, 1570, 1696, 2548, 2599, 2658, 2707, 2952, 3082 cm^{-1}; ^{1}H-NMR (DMSO-D6): 6.19-6.39 (m, 2H), 6.57-6.63 (m, 2H); ^{13}C-NMR (DMSO-D6): 124.8, 125.9, 127.92, 130.89, 131.6, 134.4, 149.5, 167.8. HRMS: *m/z* = 212.0049, calcd.$C_8H_6ClN_3S$, found 212.0044 $[M+H]^+$.

5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione(4s)

Yield: 71%; Mp: 265°C; IR (KBr):557, 607, 695, 786, 906, 966, 1031, 1079, 1171, 1260, 1375, 1470, 1550, 1598, 1635, 1691, 2922, 3135, 3414 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.43-7.66 (m, 3H), 7.90 (dd, *J* = 1.8 & 8.4 Hz, 2H), 13.70 (s, 1H), 13.86 (s, 1H). ^{13}C-NMR (DMSO-D6): 125.97, 126.2, 129.6, 131.1, 150.7, 167.5. HRMS: *m/z* = 178.0439, calcd. C8H7N3S, found 178.0433 $[M+H]^+$.

5-(m-tolyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4t)

Yield: 79%; Mp: 280°C; IR (KBr):544, 611, 694, 768, 861, 982, 1165, 1226, 1294, 1375, 1457, 1568, 1620, 1668, 2670, 2728, 2858, 2922, 3107 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.28-7.38(m, 2H), 7.72 (t, *J* = 5.5 & 7.8 Hz, 2H), 13.70 (s, 1H), 13.86 (s, 1H); ^{13}C-NMR (DMSO-D6): 21.2, 123.1, 125.6, 126.4, 129.2, 131.5, 138.7, 150.5, 167.1. HRMS: *m/z* = 192.0595, calcd. $C_9H_9N_3S$, found 192.0590 $[M+H]^+$.

S2. General procedure for preparation of 4u-z

To a solution of ester (1 mmol, 1 equiv.), hydrazine hydrate (3 mmol, 3.0 equiv.) was added drop-wise. The mixture was refluxed for 5 h; after completion of the reaction, a solid product was formed, and the excess solvent was removed under reduced pressure. Potassium hydroxide (1.5 mmol, 1.5 equiv.) was dissolved in absolute ethanol (25mL); the crude residue obtained from the previous step, acylhydrazine (1 mmol, 1 equiv.), and carbon disulphide (1.5 mmol, 1.5 equiv.) were added drop-wise, and the reaction mixture was refluxed for 10h. After completion of the reaction as monitored by TLC, the solvent was evaporated under reduced pressure, and the residue was used for next step.To the crude residue, 25% ammonia solution (25 mL) was added and refluxed for 10 h. The reaction mixture was acidified with 10% HCl solution. The precipitate was filtered, washed with water, and dried to give compounds **4u-z**.

5-(4-hydroxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4u)

Yield: 76%; Mp: 214°C; IR (KBr): 534, 696, 737, 837, 932, 970, 1071, 1202, 1269, 1347, 1444, 1517, 1608, 1919, 2857, 2923, 3364 cm^{-1}; ^{1}H-NMR (DMSO-D6): 6.94 (dd, *J* = 8.8 Hz, 2H), 7.71 (dd, *J* = 8.8, 2H), 10.42 (s, 1H); ^{13}C-NMR (DMSO-D6): 113.3, 116.4, 128.3, 161.1, 161.3, 177.2.

5-(4-aminophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(4v)

Yield: 78%; Mp: 243°C; IR (KBr): 636, 692, 730, 832, 945, 968, 1066, 1118, 1164, 1295, 1366, 1457, 1508, 1614, 2852, 3318, 3346, 3393, 3442 cm^{-1}; ^{1}H-NMR (DMSO-D6): 6.73 (dd, *J*= 8.4 Hz, 2H), 7.56 (dd,*J* = 8.4 Hz, 2H);^{13}C-NMR (DMSO-D6): δ 109.9, 114.7, 127.9, 151.4, 161.6, 176.8; HRMS: *m/z* = 194.0626, calcd. $C_8H_8N_4S$, found 194.0621 $[M+H]^+$.

5-(furan-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4w)

Yield: 72%; Mp: 285°C; IR (KBr): 551, 595, 641, 740, 757, 826, 289, 972, 1019, 1077, 1167, 1375, 1456, 1636, 1778, 2724, 2850 cm^{-1}; ^{1}H-NMR (DMSO-D6): 6.67 (dd, *J* =1.7 & 3.4 Hz, 1H), 7.11 (d,*J* = 3.4 Hz, 1H), 7.89 (s, 1H), 13.67 (s, 1H), 13.88 (s, 1H); ^{13}C-NMR (DMSO-D6): δ 112.1, 112.2, 140.6, 143.3,145.6, 166.7; HRMS: *m/z* = 194.0626, calcd.for $C_8H_{10}N_4S$, found 194.0621 $[M+H]^+$.

5-(pyridin-3-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4y)

Yield: 82%; Mp: 229°C; IR (KBr): 544, 630, 705, 810, 926, 1026, 1072, 1154, 1191, 1365, 1460, 1529, 1603, 2362, 2715, 2857, 2924 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.62 (dd, *J* = 4.9 & 7.9 Hz, 1H), 7.24 (dt, *J* = 1.9 & 8.9 Hz, 1H), 7.77 (d, *J*= 3.8 Hz, 1H), 9.01 (s, 1H); ^{13}C-NMR (DMSO-D6): 118.7, 123.9, 133.4, 146.3, 152.1, 158.4, 177.1.

S3. General procedure for preparation of 5(a-b)

To a solution of ester (1 mmol, 1 equiv.), hydrazine hydrate (3 mmol, 3.0 equiv.) was added drop-wise. The mixture was refluxed for 5 h; after completion of the reaction, a solid product was formed, and the excess solvent was removed under reduced pressure. Potassium hydroxide (1.5 mmol, 1.5 equiv.) was dissolved in absolute ethanol (25 mL). The crude residue obtained from previous step, acylhydrazine(1 mmol, 1 equiv.),and carbon disulfide (1.5 mmol, 1.5 equiv.) were added drop-wise,and the reaction mixture was refluxed for 10 h.After completion of the reaction as monitored by TLC, the residue was poured in ice-cold water and acidified with 10% HCl. The precipitate was filtered, washed with water, and dried to give compound **5**.

5-(4-chlorophenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione(5a)

Yield: 76%; Mp: 215°C; IR (KBr): 615, 671, 770, 835, 882, 983, 1102, 1168, 1359, 1467, 1608, 2717, 2845, 2924, 3192, 3303, 3544 cm^{-1}; ^{1}H-NMR (DMSO-D6): 7.6 (d, *J* = 8.2 Hz, 2H), 7.89 (d, *J* = 8.1 Hz, 2H); ^{13}C-NMR (DMSO-D6): 121.95, 128.4, 130.1, 137.5, 160.2, 177.98; MS (EI): *m/z* = 235[M^{+}+ Na].

5-(4-fluorophenyl)-1,3,4-oxadiazole-2-thione (5b)

Yield: 72%; Mp: 282°C; ^{1}H-NMR: 7.43 (t, J = 8.8 & 17.9 Hz, 2H), 7.93 (m, J= 8.8 & 17.9 Hz, 2H). ^{13}C-NMR (DMSO-D6): 117.1, 117.4, 119.7, 129.3, 129.4, 160.3, 163.4, 165.98, 177.96.

Biology

5-aryl-4*H*-1,2,4-triazole-3-thiones **4a-z** and **5a-c** were tested for their *in vitro* effect against *M. tuberculosis* H37Ra (strainATCC 25177), which is susceptible to control drugs (rifampicin, isoniazid, ethambutol, and pyrazinamide). In vitro Activity against MTB was determined using the XTT Reduction Menadione Assay (XRMA) and ex vivo in a THP1 infection modelby NR assay as per the protocol described previously [29-31].The minimum inhibitory concentration (MIC; in µg/mL) was recorded as the lowest concentration highest dilution of the compounds control drug that completely inhibited the growth of *Mycobacterium* cultures.

In vitro cytotoxicity of thiones(**4a-z** and **5a-c)** against THP-1, A549, and Panc-1 human cell lines was determined using the MTT assay as described previously [32,33].Briefly, the cells were seeded into a 96-well plate at a density of 1×10^4 per well for 24 h, followed by drug treatment for 48 h. Next, 5 mg/mL MTT were added to the medium, and the cells were incubated for 4 h at 37°C and 5% CO_2. After removing the culture medium, acidified isopropanol (0.04N HCl) was added. The absorbance was measured using a Molecular Probes plate reader at 570 nm. The percentage of cytotoxicity was calculated using the formula:

% cytotoxicity = [(control –test) / (control – blank)] × 100,

Where,

Control= cell growth in medium without thiones, but with DMSO,

Test= cell growth in presence of thiones,

Blank= culture medium without cells.

Each experiment was performed in triplicate, and the quantitative value was expressed as the average ± standard deviation.

Determining the solubility of acompound in water has become anessential early measurement in the drug discovery process. In this regard, the aqueous solubility of 5-aryl-4*H*-1,2,4-triazole-3-thiones **4b, 4c, 4f, 4j, 4t, 4v, and 4z** were measured according to instructions provided by Millipore using the 96-well plate format protocol (Figure 4).

Data were collected using a Molecular Devices SPECTRAmax® Plus microplate spectrometer. The ratio of filtrate vs. standard absorbance was calculated to quantify the aqueous solubility using the formula below:

$$\boldsymbol{IF}: \frac{(\sum \boldsymbol{AUat260,280,300,320,340,360nm}) - (\boldsymbol{AUat800nm})\boldsymbol{Filtrate}}{(\sum \boldsymbol{AUat260,280,300,320,340,360}) - (\boldsymbol{AUat800nm})\boldsymbol{Standard}} \approx \mathbf{1.0}$$

Then, Aqueous Solubility ≥ 500 µM

$$\boldsymbol{IF}: \frac{(\sum \boldsymbol{AUat260,280,300,320,340,360nm}) - (\boldsymbol{AUat800nm})\boldsymbol{Filtrate}}{(\sum \boldsymbol{AUat260,280,300,320,340,360}) - (\boldsymbol{AUat800nm})\boldsymbol{Standard}} \leq \mathbf{0.5}$$

Then, Aqueous Solubility ≤ 100 µM

$$\boldsymbol{IF}: \frac{(\sum \boldsymbol{AUat260,280,300,320,340,360nm}) - (\boldsymbol{AUat800nm})\boldsymbol{Filtrate}}{(\sum \boldsymbol{AUat260,280,300,320,340,360}) - (\boldsymbol{AUat800nm})\boldsymbol{Standard}} < \mathbf{1.0 \text{ and } > 5.0}$$

Then, 100 µM<Aqueous Solubility <500 µM.

In the present study, we evaluated the antimicrobial and anti-proliferative activity for a set of thiones. In a preliminary screening, the anti-tubercular and antimicrobial activities of the thiones were assessed at concentrations of 30, 10, and 3 µg/mL. Then, dose-dependent

inhibition assays were performed at concentrations of 100, 30, 10, 3, 1, 0.3, 0.1, and 0.03 μg/mL to determine the MIC values (Table S1).

All of the triazolethione compounds **(4a-z and 5a-c)** tested in present study exhibited anti-tubercular activities against the dormant and active phases of MTB. The MIC values against the dormant stage ranged from 0.46to 30 μg/mL, while the values against the active stage ranged from 9.05 to 30 μg/mL. The IC_{50}values against the dormant stage ranged from <0.03 to 26.54 μg/mL, while the values against the active stage ranged from <0.03 to 30 μg/mL(refer table S1). The triazole compounds **4b, 4c, 4f, 4j, 4t, 4v, and 4z** showed the highest anti-tubercular activities (both MIC and IC50; Table S1) against the dormant phase of MTB. The other substituted triazole compounds also had significant anti-tubercular activity (both MIC and IC_{50}) against the dormant phase. Although the triazole thione compounds **4b**, **4c**, **4f**, **4j**, **4t**, **4v**, and **4z** showed MIC values of <5 μg/mL against the dormant phase of MTB, they exhibited lower potencies than the standards (rifampicinand isoniazid) used as control drugs. Compound **4v** was found to be more active than the other compounds with an MIC value of 0.46μg/mL against the dormant phage of MTB. Since these triazolethione compounds are efficacious against intracellular *M. tuberculosis*, they have the potential to be developed as anti-tubercular drug candidates.

The synthesised compounds **4a-z** and **5a-c** were assayed further for their cytotoxic activity in three different human cancer cell lines, THP-1, A549, and Panc-1, after 48-h exposure to the tested compounds using the MTT assay [34,35].As shown in Figure 2, the data reveal that all 5-aryl-4*H*-1,2,4-triazole-3-thiones tested show low cytotoxicity (0 to 100 μg/mL) in human cancer cell lines. The anti-tubercular activities against the dormant phase of MTB and the non-toxic properties in THP-1, A549, and Panc-1 cell lines of **4a**, **4b, 4c, 4f, 4j, 4t, 4v, and 4z** demonstrate that these compounds can be used as potential dormant stage inhibitors.

The aqueous solubility of triazolethione derivatives **(4a-z and 5a-c)** was measured using the Millipore 96-well plate format protocol (PC2445EN00). The solubility of the thione compounds was calculated according to the US pharmacopeia definition of solubility. The solubility of thiones **4a-f** was >500 to 1280 μM and that of **4g-5c** was >1280 to 3200 μM, while the solubility of testosterone and furosemide was 365 μM and 500 μM, respectively. The solubility results demonstrate that these synthetic thione compounds are highly soluble in aqueous solution for oral drugs. The solubility values were somewhat reproducible. In case of the reference compounds, the solubility values also were reproducible and not significantly different from those reported in the literature.(Figure 5)

Our SAR investigation began with preparation of a series of analogues in which the 3 position of a 1, 2, 4 triazole thione was fixed as aromatic substituent. The structure activity relationship (SAR) of the molecular diversity is summarized in Table 1. The MIC values against *M. tuberculosis* H37Ra were obtained in the range of 0.46 - > 100 μg/ml. The presence of versatile substituent's on 3 position of a 1, 2, 4 triazole thione skeleton was significantly affected on ant-tubercular activity. Thione para substituted -NH_2 (electron donating) and -NO_2 (electron withdrawing) analogs were found to be considerably potent at MIC 0.46 and 0.64 μg/mL. Next; the influence of hydrophilic or lipophilic variants on the parent structure was probed. The thione analogs in which aromatic ring constituted with different electron withdrawing groups, compounds 4c, 4e, 4f, 4g, 4h, 4i, 4j, 4q, 4t, 4z and 5c displayed superior activity with MIC values < 16 and > 1 μg/mL. The aromatic ring containing para Chloro (4 –Cl) substituent at , for instance 4a, 4k, 4l, 4r and 5a were found to be weakly actives with MIC > 30 μg/mL. In the same context, 2-substituted aromatic ring of different analogs, 4d, 4m, 4n, 4o, 4p were also found to be weakly actives with MIC > 30 μg/mL. Also, few analogs such as 4w, 4x, 4y and 4z thione substitution with hetroatomic aromatic ring were less active (MIC > 30 μg/mL). The compound 5a and 5b with oxadiazole thione at 4-substitution exhibited almost very less anti-TB activity. This trend has clearly indicated that not only the presence of -NO_2 and -NH_2 but its site-selectivity in the particular ring is also essential to generate a 'lead' molecule.

The synthesis and biological evaluation of a series of 5-aryl-4H-1,2,4-triazole-3-thiones **4a-z** and **5a-c** as anti-tubercular agents have been discussed, and all were found to be active against the dormant stage of *Mycobacterium tuberculosis* H37Ra. The compounds **4b, 4c, 4f, 4j, 4t, 4v, and 4z** were highly active. Compounds **4b** and **4v** displayed the most potent overall anti-tubercular activity against dormant *Mycobacterium tuberculosis* H37Ra *in vitro* (MIC <0.7 μg/mL and IC_{50}<0.03 μg/mL). From the present study, compounds 4**b** and **4v** were found to have low cytotoxicity (up to100 μg/mL) in THP-1, A549, and PANC-1 human cancer cell lines and are highly soluble in aqueous solution. SAR of this work demonstrates that compounds **4b** and **4v** may be the best candidates for further investigation as potential drugs in the search for new, safe and effective anti-tubercular drugs.

Acknowledgment

This work was financially supported by CSIR, New Delhi, India, (Project Code: BSC0103, CSC0406). Authors also are thankful to Dr. P. R. Rajamohanan, NCL, Pune for his help on spectral analysis. Authors are also thankful to Professor Mamoru Koketsu, Gifu university, Japan for his help on spectra analysis.

Supplementary data

Supplementary data (experimental details, Proton and Carbon NMRs of new compounds and Tables of biological activity) associated with this article can be found, in the online version, at http://

References

[1] Global Tuberculosis Control: WHO Report. (2013)World Health Organization: Geneva, Switzerland.(2011)

[2] CorbettE.L., Watt C.J., Walker N., Maher D., Williams B.G., RaviglioneM.C., Dye C.(2003)The Growing Burden of Tuberculosis Global Trends and Interactions With the HIV Epidemic. Arch. Intern. Med;163:1009.

[3] Long R. (2000)Drug-resistant tuberculosis. Can. Med. Assoc. J.;163: 425.

[4] ZumlaA., NahidP., Cole S.T. (2013)Advances in the development of new tuberculosis drugs and treatment regimens. Nature Rev. Drug Discovery.;12: 388−404.

[5] Wong E.B., Cohen K.A., BishaiW.R. (2013)Rising to the challenge: new therapies for tuberculosis. Trends Microbiol.;21: 493−501.

[6] FieldS. K., Clin. (2013)Safety and efficacy of delamanid in the treatment of multidrug-resistant tuberculosis (MDR-TB)Med. Insights: Ther.;5: 137−149.

[7] Sachs G., Shin J.M., HowdenC.W. (2006)Review article: the clinical pharmacology of proton pump inhibitors. Pharmacol.Ther.;23: 2-8.

[8] GomtsyanA., Chem. (2012)Heterocycles in drugs and drugs discovery. Heterocycl. Compd.;48: 8-10.

[9] EswaranS., AdhikariA.V., ShettyN.S., (2009)Synthesis and antimicrobial activities of novel quinoline derivatives carrying 1,2,4-triazole moiety. Eur. J. Med. Chem.;44: 4637.

[10] DemirbasA., SahinD., DemirbasN., KaraogluS.A. (2009) Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities. Eur. J. Med. Chem.;44:2896-2903.

[11] Pal D.K., Singh V., PandeyD.D., MauryaR.K.(2014)Synthesis, characterization and Antimicrobial evaluation of some 1,2,4-triazole derivatives. Int. J. Pharm. Pharm. Sci.;6: 213-216.

[12] KucukguzelI., KucukguzelS.G., RollasS., KirazM. (2001) Some 3-Thioxo/Alkylthio-1,2,4-triazoles with a Substituted Thiourea Moiety as Possible Antimycobacterials. Bioorg. Med. Chem. Lett.;11:1703.

[13]KandemirliF., ShvetsN., UnsalanS., KucukguzelI., RollasS., KovalishynV., DimogloA. (2006)The Structure - Antituberculosis Activity Relationships Study in a Series of 5-(4-Aminophenyl)-4-Substituted-2,4-Dihydro-3h-1,2,4-Triazole- 3-Thione Derivatives. A Combined Electronic-Topological and Neural Networks Approach. Med Chem.;2:415-422.

[14] Garcia M.A., SantamariaS.M., CachoM., de la LlaveF.M., Julian M., Martinez A. , Pascual-Teresa B.D., Ramos A.(2005)Synthesis, Biological Evaluation, and Three-Dimensional Quantitative Structure−Activity Relationship Study of Small-Molecule Positive Modulators of Adrenomedullin**.** J. Med. Chem.;48:4068–4075.

[15] NavidpourL., ShafaroodiH., AbdiK., AminiM., GhahremaniM.H., DehpourA.R., ShafieeA.(2006)Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4H-1,2,4-triazoles as selective COX-2 inhibitors. Bioorg.Med. Chem.;14: 2507.

[16]Amir M., ShikhaK.(2004)Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl]acetic acid derivatives. Eur. J. Med. Chem.;39: 535.

[17] KumidhaD., Leonard J.T., MuthumaniM., ChidhambaranathanN., KalavathiT. (2013)Synthesis and evaluation of some 1,2,4-Triazole derivatives as anticonvulsant, anti-inflammatory and antimicrobial agents. Asian J. Pharm. Clin.;6:5-8.

[18] HouY.P., Sun J., Pang Z.H., LvP.C., Li D.D., Yan L., Zhang H.J., ZhengE.X., Zhao J., Zhu H.L.(2011)Synthesis and antitumor activity of 1,2,4-triazoles having 1,4-benzodioxan fragment as a novel class of potent methionine aminopeptidase type II inhibitors. Bioorg. Med. Chem.;19: 5948.

[19]Lin R., Connolly P.J., Huang S., Wetter S.K., Lu Y., Murray W.V., Emanuel S.L. GruningerR.H., Fuentes-PesqueraA.R., RuggC.A., Middleton S.A., JolliffeL.K. (2005)1-Acyl-1H-[1,2,4]triazole-3,5-diamine analogues as novel and potent anticancer cyclin-dependent kinase inhibitors: synthesis and evaluation of biological activities. J. Med. Chem.; 48: 4208-4211.

[20] SaadH.A., Osman N.A., MoustafaA.H. (2011)Synthesis and Analgesic Activity of Some New Pyrazoles and Triazoles Bearing a 6,8-Dibromo-2-methylquinazoline Moiety. Molecules; 16: 10187-10201.

[21] ShashikalaD.K., RamaiahM., VanitaG.K., VeenaK., VaidyaV.P. (2011)Synthesis and analgesic activity of triazolothiadiazoles and triazolothiadiazines encompassing 3–nitronaphtho[2,1-b]furan. Chem. Pharm. Res.;3: 445-451.

[22] BellamineA., LepeshevaG.I., WatermanM.R.(2004)Fluconazole binding and sterol demethylation in three CYP51 isoforms indicate differences in active site topology.J. Lipid Res.;45: 2000–2007.

[23] DunfordA.J., McLean K.J., SabriM., Harriet E.S., HeyesD.J., ScruttonN.S., Munro A.W., (2007)Rapid P450 Heme Iron Reduction by Laser Photoexcitation of Mycobacterium tuberculosis CYP121 and CYP51B1: Analysis of CO complexation reactions and reversibility of the P450/P420 equilibrium. J. Biol. Chem.;282: 24816-24824.

[24] SarkarD., DeshpandeS.R., MaybhateS.P., LikhiteA.P., SarkarS., Khan A., ChaudhryP.M., ChavanS.R.A method of screening anti-tubercular compounds. Patent no: WO-2012123971 A2.

[25] SarkarD., LikhiteA.P., Joshi R.A., Joshi R.R., KhedkarV. (2014)Novel Anti-tubercular agents. NF-0233.

[26] GolovlyovaS.M., MoskvichevY.A., AlovE.M., KobylinskyD.B., ErmolaevaV.V.(2001) Synthesis of novel five-membered nitrogen containing heterocyclic compounds from derivatives of arylsulfonyl- and aryl thioacetic acid and –propionoc acid. Chem. Heterocycl.Compd.;37:1102.

[27] LabanauskasL., UdrenaiteE., GaidelisP., BrukstusA.(2004)Synthesis of 5-(2-,3- and 4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol derivatives exhibiting anti-inflammatory activityFarmaco.; 59:255.

[28] OzturkS., AkkurtM., CansizA.,KoparirM.,SekerciM.,Heinemann F.W. (2004)4-(4-Chlorophenyl)-3-(furan-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione. Acta.Cryst.; E60: O425.

[29] SarkarD., Singh U.(2011)A novel screening method based on menadione mediated rapid reduction of tetrazolium salt for testing of anti-mycobacterial agents. J. Microbiol. Methods;84:202.

[30] Khan A., SarkarS., SarkarD.(2008)Bactericidal activity of 2-nitroimidazole against the active replicating stage of Mycobacterium bovis BCG and Mycobacterium tuberculosis with intracellular efficacy in THP-1 macrophages. Int. J. Antimicrob. Agents; 32: 40.

[31] SarkarS., SarkarD. (2012)Potential use of nitrate reductase as a biomarker for the identification of active and dormant inhibitors of Mycobacterium tuberculosis in a THP1 infection model. J. Bio Mol. Screen.; 17:966.

[32] CiapettiG., CenniE., PratelliL., PizzoferratoA. (1993)II vifro evaluation of cell/biomaterial teraction by MTI' assay. Biomaterials;14: 359-364.

[33] MosmannT. (1983)Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth.;65:55-63.

[34] DzoyemJ.P., Guru S.K., PiemeC.A., KueteV., Sharma A., Khan I.A., SaxenaA.K., VishwakarmaR.A. (2013)Cytotoxic and antimicrobial activity of selected Cameroonian edible plants. BMC Complement.Altern. Med.;13: 78.

[35] Alley M.C., ScudieroD.A., Monks A., HurseyM.L., Czerwinski M.J., Fine D.L., Abbott B.J., Mayo J.G., Shoemaker R.H., Boyd M.R. (1988)Evaluation of a Soluble Tetrazolium /Formazan Assay for Cell Growth and Drug Sensitivity in Culture Using Human and Other Tumor Cell Lines. Cancer Res.;48: 589.

Figures

Figure 1 *In vitro*: Dose-dependent inhibition of 4b (a) on dormant *Mycobacterium tuberculosis* H37Ra compared with Rifampicin (b) as standard drug.

Figure 2 *In vitro*: Dose-dependent inhibition of 4v (a) on active *Mycobacterium tuberculosis* H37Ra compared with Rifampicin (b) as standard drug.

Figure 3 *Ex vivo:* Dose-dependent inhibition of 4b and 4v (a) on dormant *Mycobacterium tuberculosis* H37Ra compared with Rifampicin (b) as standard drug.

Figure 4 Schematic representation of the aqueous solubility screening assay.

Figure 5 Percentage cytotoxicity of thiones at 100µg/mL against human cell lines