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ORIGINAL ARTICLE

Synthesis and *in vitro* antibacterial activity of new oxoethylthio-1,3,4-oxadiazole derivatives

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Oxoethylthio-1,3,4-oxadiazole;
Anti-tubercular;
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Abstract In the present investigation, a series of 2(4-pyridyl)-5[(aryl/heteroarylamino)-1-oxoethyl]thio-1,3,4-oxadiazole were synthesized using isonicotinohydrazide and substituted aryl/heteroaryl amines using pyridine as solvent. Newly synthesized compounds were tested for their *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis H37Rv* using the BACTEC 460 radiometric system. Among the synthesized compounds, compounds 2(4-pyridyl)-5((2-nitrophenylamino)-1-oxoethyl)thio-1,3,4-oxadiazole (**5e**), 2(4-pyridyl)-5((4-nitrophenylamino)-1-oxoethyl)thio-1,3,4-oxadiazole (**5g**) and 2(4-pyridyl)-5((2-pyrrolylamino)-1-oxoethyl)thio-1,3,4-oxadiazole (**5k**) produced highest efficacy and exhibited >90% inhibition at a concentration of 0.0077, 0.0052 and 0.0089 μM, respectively. All the new compounds were pharmacologically evaluated for their *in vitro* Antimicrobial activity.

1. Introduction

Tuberculosis (TB) is one of the most common infectious diseases known by the mankind. About 32% of the world's population is infected by *Mycobacterium tuberculosis* – the main causal agent of TB. Every year, approximately 8 million of the infected people develop active TB, and 2 million die (Gustavo, 2006). The incidence of TB infection has steadily risen in the last decade (WHO progress report, 2011); World Health Organization estimates that about 30 million people will be infected by *M. tuberculosis* within the next 20 years. The re-emergence of TB infection has been further complicated by an increase in the prevalence of drug-resistant TB cases. Current control efforts are severely hampered due to *M. tuberculosis*

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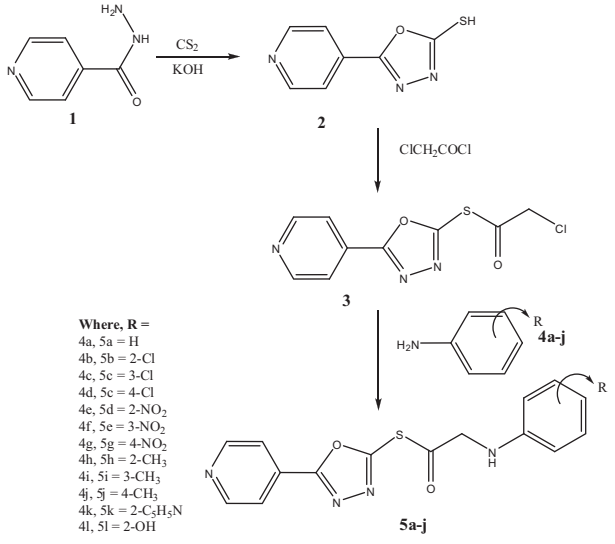
being a leading opportunistic infection in patients with acquired immune deficiency syndrome (AIDS) and the spreading of multidrug-resistant strains (MDR-MTB). Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to the first-line drugs: isoniazid (INH), rifampicin (RIF), ethambutol (ETH), streptomycin (STR), and pyrazinamide (PYR) (Kathryn et al., 2009).

The ever-increasing drug resistance, toxicity, and side effects of currently used anti-tuberculosis drugs, and the absence of their bactericidal activity highlight the need for new, safer, and more effective antimycobacterial compounds (Mudassar et al., 2010). Since no effective vaccine is available, the major strategy to combat the spreading of TB is not only chemotherapy (Sullivan et al., 2006), but also to develop new agents with potent sterilizing activity with short duration (Ballell et al., 2005).

One of the most effective first-line anti-TB drugs is INH. Many analogs featuring the structure of INH have been synthesized and tested as antimycobacterials. In a critical review published recently, the existence of more than 3000 compounds based on the INH core was reported, about 66% of them being hydrazones (Ballell et al. 2005; Janin, 2007; Scior and Garces-Eisele, 2006). It has also been reported that conversion of INH to oxadiazoles produces the corresponding 5-substituted-3H-1,3,4-oxadiazol-2-thione and 3H-1,3,4,-oxadiazol-2-ones derivatives, which are characterized by high activity against *M. tuberculosis strain H37Rv* (Wilder-Smith, 1966; Maria et al., 2005). Also oxadiazoles conform to an important class of heterocyclic compounds with a wide range of biological activities such as antiviral (Maria et al., 2005), tyrosinase inhibitors (Tan et al., 2006), antimicrobial (Khan et al., 2005; Gaonkar et al., 2006; Mojahidul et al., 2008; Pachhamia and Parekh, 1988), cathepsin K inhibitors (Palmer et al., 2006), fungicidal (Mojahidul et al., 2008; Li et al., 2006), antineoplastic properties (Aboraia et al., 2006). Accordingly, their synthesis and transformations have been a focus of interest for a long time.

Herein, we report the synthesis of some 2(4-pyridyl)-5[(aryl/heteroarylamino)-1-oxoethyl] thio-1, 3, 4-oxadiazole derivatives (Scheme 1) and their *in vitro* antimicrobial activity and anti tubercular activity.



Scheme 1

2. Experimental

2.1. General

All the melting points were determined with a PMP–DM scientific melting point apparatus and are uncorrected. The purity of compounds was checked by TLC (0.5 mm thickness) using silica gel-G coated Aluminum plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. IR spectra (ν max in cm^{-1}) were recorded on a Perkin Elmer spectrum BX series FT-IR spectrometer using the KBr or Nujol technique. ^1H NMR spectra were recorded on a Bruker WM 400 FT MHz NMR instrument, using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The elemental analysis (C, H, N) of compounds was performed at SICART, Vallabh Vidyanagar, Anand, Gujarat (India) on Carlo Erba1108 elemental analyzer. Their results were found to be in good agreement with the calculated values.

2.2. Chemistry

The building blocks 2(4-pyridyl)-5-mercapto-1,3,4-oxadiazole(2) and 2(4-pyridyl)-5(2-chlorooacetyl)mercapto-1,3,4-oxadiazole(3) were prepared according to reported procedure (Khan et al., 2004) (Scheme 1).

2.2.1. General procedure for the synthesis of 2(4-Pyridyl)-5[(2-aryl amino)-1-oxoethyl] thio-1,3,4-Oxadiazole(5a-i)

In pyridine (30 ml.), a mixture of compound 3 (0.01 mol) and aryl/heteroaryl amine (4a-l) (0.01 mol) was refluxed for 4–5 h. Excess of pyridine was distilled off. The resulting residue was poured in ice-cold water to get the crude product. It was extracted with CH_2Cl_2 (30 × 3 ml.). After evaporation of CH_2Cl_2 at room temperature, the final product was separated. The progress of reaction was monitored by TLC using methanol:toluene (10:1) as eluent.

2.2.1.1. 2(4-pyridyl)-5[(2-phenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole(5a). Yield 67%, M.p. 168–169 °C. IR, ν (cm^{-1}): 3425 (NH str. of 2 °amine), 3076 (ArH), 1707, 1637 (C═O str.), 1534, 1485, 1450 (1,3,4-oxadiazole ring), 1171 (C—O—C str.), 1053 (C—O str. of 1,3,4-oxadiazole ring), 1257.20 (C—S—C str.), 1485 (C═N str.). ^1H NMR (400 MHz, DMSO-d_6), δ ppm: 6.40–7.06 (m, 5H, ArH, aryl ring), 4.01 (s, 1H, NH), 4.14 (s, 2H, CH_2), 7.62(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 8.64(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.2–151.7(C_1—C_5), 197.2(C_{14},C═O), 6.1(C_{15}, CH_2), 148.9(C_{12}), 149.6(C_{13}), 111.5–143.5(C_6—C_{11}). Anal. Calcd for $C_{15}H_{12}N_4O_2S$: C, 57.68; H, 3.87; N, 17.94; S, 10.27. Found: C, 57.54; H, 3.76; N, 17.76; S, 10.22.

2.2.1.2. 2(4-pyridyl)-5[(2-chlorophenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole(5b). Yield 66%, M.p. 171–172 °C. IR, ν (cm^{-1}): 3435 (NH str. of 2 °amine), 3077 (ArH), 1718, 1627 (C═O str.), 1529, 1490, 1451 (1,3,4-oxadiazole ring), 1165 (C—O—C str.), 1059 (C—O str. of 1,3,4-oxadiazole ring), 1251.20 (C—S—C str.), 1487 (C═N str.), 723 (C—Cl str.).

^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.36–7.08 (m, 4H, ArH, aryl ring), 3.9 (s, 1H, NH), 4.16 (2H, CH_2), 8.66(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.58(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.7–151.9(C_1—C_5), 187.7(C_{14},C═O), 59.4,C_{15}(CH_2), 117.3(C_{11}); 112.5–144.6(C_6—C_{10}); 148. 5149. 4(C_{12},C_{13}). Anal. Calcd for $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.16; O, 9.23; S, 9.25. Found: C, 51.83; H, 3.29; N, 16.18; S, 9.33.

2.2.1.3. 2(4-pyridyl)-5[(3-chlorophenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole (5c). Yield 67%, M.p. 174–175 °C. IR, v (cm^{-1}): 3441 (NH str. of 2 °amine), 3056 (Ar—H), 1731, 1612 (C═O str.), 1539, 1483, 1457 (1,3,4-oxadiazole ring), 1170 (C—O—C str.), 1067.09 (C—O str. of oxadiazole ring), 1238 (C—S—C str.), 1490 (C═N str.), 718 (C—Cl str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.33–6.99 (m, 4H, ArH, aryl ring), 4.19 (s, 2H,—CH_2), 4.3 (s, 1H, NH), 8.62(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.62(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.3–151.7(C_1—C_5), 194.4(C_{14} C═O), 65.6(C_{15},CH_2), 148.1149.2(C_{12},C_{13}), 112.5–144.6(C_6—C_9), 133.4(C_{10}). Anal. Calcd for $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.16; O, 9.23; S, 9.25. Found: C, 51.84; H, 3.21; N, 16.18; S, 9.37.

2.2.1.4. 2(4-pyridyl)-5[(4-chlorophenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole(5d). Yield 63%, M.p. 168–169 °C. IR, v (cm^{-1}): 3438 (NH str. of 2 °amine), 3019 (Ar—H), 1722, 1599 (C═O str.), 1518, 1470, 1445 (1,3,4-oxadiazole ring), 1153 (C—O—C str.), 1023 (C—O str. of oxadiazole ring), 1201 (C—S—C str.), 1510 (C═N str.), 734 (C—Cl str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.40–7.01 (m, 4H, ArH, aryl ring), 4.18 (s, 2H, CH_2), 3.8 (s, 1H, NH), 8.67(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.59(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.2–151.7(C_1—C_5); 187.7(C_{14},C═O), 59.9(C_{15},CH_2), 148.2149.7(C_{12},C_{13}), 121.9(C_9), 128.5–143.4(C_6—C_8), 112.9138.2(C_{10},C_{11}). Anal. Calcd for $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.16; S, 9.25. Found: C, 51.89; H, 3.24; N, 16.18; S, 9.34.

2.2.1.5. 2(4-pyridyl)-5[(2-nitrophenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole (5e). Yield 72%, M.p. 181–182 °C. IR, v (cm^{-1}): 3417 (NH str. of 2 °amine), 3099 (Ar—H), 1728, 1620 (C═O str.), 1546, 1463, 1448 (1,3,4-oxadiazole ring), 1183 (C—O—C str.), 1040 (C—O str. of oxadiazole ring), 1229 (C—S—C str.), 1490 (C═N str.), 1523 (NO_2 str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.70–7.96 (m, 4H, ArH, aryl ring), 4.14 (s, 2H, CH_2), 4.1 (s, 1H, NH), 8.63(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.62(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 114.4–149.8(C_1—C_{11}), 187.7(C_{14}), 59.2(C_{15}); 150.3149.4 (C_{12},C_{13}), 161.8161.7(C_{14},C═O), 55.9(C_{15},CH_2). Anal. Calcd for $C_{15}H_{11}N_5O_4S$: C, 50.42; H, 3.10; N, 19.60; S, 8.97. Found: C, 50.32; H, 3.14; N, 19.67; S, 8.84.

2.2.1.6. 2(4-pyridyl)-5[(3-nitrophenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole (5f). Yield 70%, M.p. 183–184 °C. IR, v (cm^{-1}): 3433 (NH str. of 2 °amine), 3123 (Ar—H), 1715, 1624 (C═O str.), 1521, 1451, 1428 (1,3,4-oxadiazole ring), 1172 (C—O—C str.), 1058 (C—O str. of oxadiazole ring), 1260 (C—S—C str.), 1534 (C═N str.). 1512 (NO_2 str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.81–7.54 (m, 4H, ArH, aryl ring), 4.19 (2H,—CH_2), 4.3 (1H,—NH), 8.66(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.59(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.2–151.9(C_1—C_5); 187.4(C_{14},C═O), 59.2(C_{15},CH_2), 148. 9149.7(C_{12},C_{13}), 149.2(C_8); 107.4–144.9(C_6—C_{11}). Anal. Calcd for $C_{15}H_{11}N_5O_4S$: C, 50.42; H, 3.10; N, 19.60; S, 8.97. Found: C, 50.49; H, 3.01; N, 19.71; S, 8.89.

2.2.1.7. 2(4-pyridyl)-5[(4-nitrophenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole (5g). Yield 73%, M.p. 179–180 °C. IR, v (cm^{-1}): 3456 (NH str. of 2 °amine), 3130 (Ar—H), 1720, 1641 (C═O str.), 1524, 1466, 1405 (1,3,4-oxadiazole ring), 1159 (C—O—C str.), 1050 (C—O str. of oxadiazole ring), 1272 (C—S—C str.), 1534 (C═N str.), 1531 (NO_2 str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.67–7.98 (m, 4H, ArH, aryl ring), 4.16 (s, 2H, CH_2), 4.1 (s, 1H, NH), 8.64(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.63(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.7–151.9(C_1—C_5); 187.2(C_{14},C═O), 59.4(C_{15},CH_2), 148.9149.5(C_{12},C_{13}), 137.4(C_9), 112.9–150.2(C_6—C_{11}). Anal. Calcd for $C_{15}H_{11}N_5O_4S$: C, 50.42; H, 3.10; N, 19.60; S, 8.97. Found: C, 50.39; H, 3.18; N, 19.72; S, 8.72.

2.2.1.8. 2(4-pyridyl)-5[(2-tolyl)-1-oxoethyl]thio-1,3,4-oxadiazole (5h). Yield 68%, M.p. 191–192 °C. IR, v (cm^{-1}): 3452 (NH str. of 2 °amine), 3031 (Ar—H), 1712 (C═O str.), 1594, 1550, 1492, 1420 (1,3,4-oxadiazole ring), 1147 (C—O—C str.), 1051 (C—O str. of oxadiazole ring), 1251.03 (C—S—C str.), 1494 (C = N str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 2.37 (s, 3H, CH_3), 6.33–6.86 (m, 4H, ArH, aryl ring), 4.15 (s, 2H, CH_2), 3.8 (s, 1H, NH), 8.67(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.57(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.4–151.3(C_1—C_5), 187.3(C_{14},C═O), 59.3(C_{15},CH_2), 148.5149.0(C_{12},C_{13}), 124.7(C_{11}), 110.7–144.2(C_6—C_{10}); 11.9(C_{16}). Anal. Calcd for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.75; H, 4.46; N, 17.28; S, 9.64.

2.2.1.9. 2(4-pyridyl)-5[(3-tolyl)-1-oxoethyl]thio-1,3,4-oxadiazole (5i). Yield 65%, M.p. 186–187 °C. IR, v (cm^{-1}): 3450 (NH str. of 2 °amine), 3065 (Ar—H), 1708 (C = O str.), 1583, 1567, 1513, 1431 (1,3,4-oxadiazole ring), 1129 (C—O—C str.), 1068 (C—O str. of oxadiazole ring), 1257 (C—S—C str.), 1484 (C═N str.). ^{1}H NMR (400 MHz, DMSO—d_6), δ ppm: 2.34 (3H, CH_3), 6.20–6.94 (m, 4H, ArH, aryl ring), 4.13 (2H, CH_2),4.1 (1H, NH), 8.66(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.57(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.8–151.8(C_1—C_5); 187.7(C_{14},C═O), 59.1(C_{15},CH_2), 148.1149.2(C_{12},C_{13}), 137.2(C_{10}), 108.3–146.1(C_6—C_{11}), 21.3(C_{16}). Anal. Calcd for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.73; H, 4.37; N, 17.37; S, 9.68.

2.2.1.10. 2(4-pyridyl)-5[(4-tolyl)-1-oxoethyl]thio-1,3,4-oxadiazole (5j). Yield 61%, M.p. 188–189 °C. IR, v (cm^{-1}): 3456 (NH str. of 2 °amine), 3065 (Ar—H), 1710 (C═O str.), 1573, 1556, 1522, 1443 (1,3,4-oxadiazole ring), 1123 (C—O—C str.), 1075 (C—O str. of oxadiazole ring), 1271 (C—S—C str.), 1478 (C═N str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 2.36 (s, 3H, —CH_3), 6.35–6.85 (m, 4H, ArH, aryl ring), 4.19 (s, 2H, CH_2), 4.3 (s, 1H, NH), 8.62(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.63(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C

NMR (100 MHz, DMSO-d_6), δ ppm: 120.3–151.7(C_1—C_5), 187.3(C_{14},C═O), 59.4(C_{15},CH_2), 148.7149.7(C_{12},C_{13}), 125.1(C_9), 21.4(C_{16}), 114.6–138.5(C_6—C_{11}). Anal. Calcd for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.72; H, 4.53; N, 17.29; S, 9.72.

2.2.1.11. 2(4-pyridyl)-5[(2-pyrrolylamino)-1-oxoethyl]thio-1,3,4-oxadiazole (5k). Yield 64%, M.p.201–202 °C. IR, ν (cm^{-1}): 3451 (NH str. of 2 °amine), 3051 (Ar—H), 1716 (C═O str.), 1576, 1580, 1526, 1423 (1,3,4-oxadiazole ring), 1143 (C—O—C str.), 1048 (C—O str. of oxadiazole ring), 1269 (C—S—C str.), 1484 (C═N str.) 1326 (NH str. of pyridine). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 6.58–8.12 (m, 4H, ArH, aryl ring), 4.15 (s, 2H, CH_2), 3.7 (s, 1H, NH), 8.67(d, *J* = *9* Hz, 2H, CH, 4-pyridyl ring), 7.57(d, *J* = *9* Hz, 2H, CH, 4-pyridyl ring), 4.54–4.97 (m, 4H, CH, pyrrolyl ring), 6.7 (s, 1H, NH, pyrrolyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 121.6–151.2(C_1—C_5); 199.7(C_{12},C═O), 59.8(C_{13},CH_2), 148.2149.7(C_{10},C_{11}); 18.8–75.7(C_6—C_9). Anal. Calcd for $C_{14}H_{11}N_5O_2S$: C, 53.66; H, 3.54; N, 22.35; S, 10.23. Found: C, 53.78; H, 3.68; N, 22.54; S, 10.14.

2.2.1.12. 2(4-pyridyl)-5[(2-hydroxylphenylamino)-1-oxoethyl]thio-1,3,4-oxadiazole (5l). Yield 72%, M.p.200–202 °C. IR, ν (cm^{-1}): 3454 (NH str. of 2 °amine), 3061 (Ar—H), 1721 (C═O str.), 1570, 1586, 1526, 1412 (1,3,4-oxadiazole ring), 1134 (C—O—C str.), 1054 (C—O str. of oxadiazole ring), 1280 (C—S—C str.), 1478 (C═N str.). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 5.1 (s, 1H, OH), 6.24–6.57 (m, 4H, CH, aryl ring), 4.19 (s, 2H,CH_2), 4.3 (s, 1H, NH), 8.67(d, J = 9 Hz, 2H, CH, 4-pyridyl ring), 7.57(d, J = 9 Hz, 2H, CH, 4-pyridyl ring). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 120.7–151.9(C_1—C_5), 187.2(C_{14},C═O), 59.1(C_{15},CH_2), 148.8, 149.9(C_{12},C_{13}), 109.3–142.7(C_6—C_{11}). Anal. Calcd for $C_{14}H_{11}N_5O_2S$: C, 54.87; H, 3.68; N, 17.06; S, 9.77. Found: C, 54.70; H, 3.61; N, 17.24; S, 9. 72.

2.3. In vitro antibacterial activity

Antibacterial activity (Srinivas et al., 2005), was investigated *in vitro* on gram +ve and gram −ve bacteria. The standard strains used in these tests were: *Staphylococcus aureus* (*MTCC 96*), *Bacillus subtlis (MTCC 121), Escherichia coli* (*MTCC 443*), and *Salomonella paratyphi A* (*MTCC 735*). A logarithmic phase culture of each bacterial strain was diluted with nutrient broth in order to obtain a density of 10^6 CFU mL^{-1}. Nutrient broth (pH 6.9) and Hinton agar (pH 7.4) were purchased from Hi-media Laboratory Pvt. Ltd. The test was performed in a 96-well microtiter plate in a final volume of 100 µl. Test compounds were dissolved in dimethyl sulfoxide (DMSO) at an initial concentration of 1000 µg mL^{-1} and serially diluted in the plate using the nutrient broth. Each well was then inoculated with the standardized bacterial suspension (10^6 CFU ml^{-1}) and incubated at 37.0 °C for 18–24 h. One well containing bacteria without sample (growth control), and one well only containing broth (sterility control) were also used. After the incubation, the growth (or its lack) of the bacteria was determined visually both in wells containing test compound and in the control wells. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC. In addition, 5 µL of the suspension from each well was inoculated in a Muller Hinton agar plate to control bacterial viability. Standard antibacterial agents such as Penicillin and Streptomycin were also screened under identical conditions for comparison. The minimum inhibitory concentration (MIC) values are presented in Table 1.

2.4. Antitubercular activity

The primary screening was conducted at (6.25 µg/ml) concentration against *M. tuberculosis H37Rv* (*ATCC27294*) in BACTEC 12B medium using the BACTEC 460 radiometric system (Interleid, 1991; Colins and Franzblau, 1997). Compounds demonstrating at least 90% inhibition in the primary screen were re-examined at lower concentration (MIC) in broth micro-dilution assay with Alamar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC50) in VERO at concentration equal to and greater than the MIC for *M. tuberculosis H37Rv* after 72 h of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation assay. The data of compounds are recorded in Table 2. The anti-tubercular activity was found in the range of 22–96% growth of inhibition.

3. Results and discussion

3.1. Chemistry

Using commercially available isonicotinohydrazide (1) new oxoethylthio-1,3,4-oxadiazole derivatives (5a-l) were synthesized. Building blocks 2(4-pyridyl)-5-mercapto-1,3,4-oxadiazole(2) and 2(4-pyridyl)-5(2-chlorooacetyl)mercapto-1,3,4-oxadiazole(3), were prepared according to reported procedure (Khan et al., 2004). Compound (3) undergoes nucleophilic substitution with various aryl/heteroaryl amines (4a-l)

Table 1 *In vitro* antibacterial activity of **5a-l**.

Compd.	R	MIC (µg/mL)			
		Gram +ve organism		Gram −ve organism	
		B.s	*S.a*	*E.c*	*S.t*
5a	H	>400	–	–	>100
5b	2-Cl	>100	>100	25	12.5
5c	3-Cl	50	25	>100	>100
5d	4-Cl	50	25	12.5	12.5
5e	2-NO_2	>100	>100	>200	>200
5f	3-NO_2	>400	>100	>400	>100
5g	4-NO_2	–	>200	>200	>400
5h	2-CH_3	>100	>200	50	25
5i	3-CH_3	>100	>100	>100	>100
5j	4-CH_3	>200	12.5	>200	25
5k	2-C_5H_5N	25	12.5	12.5	25
5l	2-OH	>200	>100	>100	>200
Penicillin		3.12	1.56	6.25	12.5
Streptomycin		6.25	6.25	6.25	3.12

Gram +ve Organisms, B.s.: *Bacillus subtilis* (*MTCC 121*), S.a.: *Staphylococcus aureus* (*MTCC 96*), Gram −ve Organisms, E.c.: *Escherichia coli* (*MTCC 443*), S.p.: *Salomonella paratyphi A* (*MTCC 735*).

Table 2 Anti-tubercular activity of the synthesized compounds against *M. tuberculosis H37Rv*.

Compd.	Primary screen (6.25 μg/mL)	% inhibition	Concentration (μM)	Actual MIC (μg/mL)	C log P*
5a	>6.25	64	0.0354	–	0.4996
5b	>6.25	12	0.1640	–	1.5150
5c	>6.25	32	0.1706	–	1.5150
5d	>6.25	28	0.1735	–	1.5150
5e	>6.25	92	0.0077	6.05	0.8964
5f	>6.25	86	0.0132	5.92	0.8964
5g	>6.25	96	0.0052	6.00	0.8964
5h	>6.25	63	0.1130	–	0.9986
5i	6.25	62	0.1138	–	0.9986
5j	>6.25	64	0.1133	–	0.9986
5k	>6.25	96	0.0089	5.77	−0.8943
5l	6.25	69	0.1184	–	−9.1673
Isoniazid	>6.25	98	0.025 – 0.05		−0.6680

* C log P = calculated using Chemdraw ultra software.

in presence of pyridine as solvent to give (5a-l). The structures of various synthesized compounds were assigned on the basis of elemental analysis, IR, ^{1}H & ^{13}C NMR spectral analysis (Fig. 1). These compounds were also screened *in vitro* for their antibacterial and anti-tubercular activity.

3.2. In vitro antibacterial activity

Compounds (**5b**), (**5h**) with 2-Cl & 2-CH_3 substitution respectively to the phenyl nucleus exhibited moderate to good activity against gram-negative organisms and compounds (**5c**), (**5d**) with 3-Cl & 4-Cl substitution respectively to the phenyl nucleus exhibited moderate to good activity against gram-positive organisms only. Compound (**5j**) with 4-CH_3 exhibits moderate to good activity against *Staphylococcus aureus* (*MTCC 96*) and *Salomonella paratyphi A* (*MTCC 735*). While the compound (**5k**) with 4-C_5H_5N exhibited remarkable antibacterial activity against all tested organisms. The remaining compounds showed poor or no activity, even at concentrations of 100 μg/ml or 400 μg/ml.

3.3. In vitro anti-tubercular activity

All newly synthesized oxoethylthio-1,3,4-oxadiazole derivatives (**5a-l**) were tested for their anti-mycobacterial activity *in vitro* against *M. tuberculosis H37Rv* using the BACTEC 460 radiometric system. The results are summarized in Tables 2 with INH, a standard used for comparison. Among the newly synthesized compounds, compounds (**5e**), (**5g**) and (**5k**) produced highest efficacy and exhibited >90% inhibition at a concentration of 0.0077, 0.0052 and 0.0089 μM. Thus, the para substituted groups substitution derivatives displayed relatively higher inhibitory activity in general. On the other hand with chloro, methyl group (5b), (5c), (5d) and unsubstituted phenyl group (5a) showed relatively low inhibitory activity against *M. tuberculosis H37Rv*. Instead (Cl) group and (CH_3) group substitution at phenyl ring analog worsens the anti-mycobacterial activity. All the newly synthesized compounds (5a-r) were tested for cytotoxicity (IC_{50}) in VERO cells at (62.5 μg/ml) concentrations. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation method. All the active compounds were found to be non-toxic.

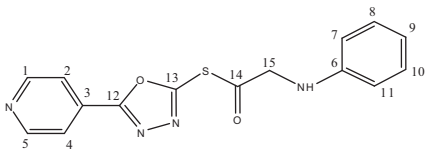


Figure 1 Numbering Pattern for 13C NMR Spectra.

4. Conclusion

Among the newer derivatives, it is conceivable that some of the derivatives that displayed promising anti-mycobacterial activity can be further modeled to exhibit better potency than the standard drugs. Thus, the new synthesized oxoethylthio-1,3,4-oxadiazole derivatives may provide valuable leads for developing new antibacterial agents.

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