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ORIGINAL RESEARCH

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Synthesis of new 1,3,4-oxadiazole and benzothiazolylthioether derivatives of 4-arylmethylidene-3-substituted-isoxazol-5(4H)-one as potential antimycobacterial agents

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Abstract

A new series of 4-[(substituted benzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (**6a–g**) and 4-(substituted benzylidene)-3-((benzo[*d*]thiazol-2-ylthio)methyl)isoxazol-5(4*H*)-one **(8a–g)** was synthesized. All the synthesized compounds were screened for antitubercular activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *Mycobacterium bovis* BCG (ATCC 35743) and antibacterial activity against *Escherichia coli* (NCIM 2576), *Pseudomonas flurescence* (NCIM 2059), *Staphylococcus aureus* (NCIM 2602), *Bacillus subtilis* (NCIM 2162). Amongst the synthesized 1,3,4-oxadiazole and benzothiazoyl thioether derivatives, compounds **6b** and **8b** showed excellent antimycobacterial activity and compounds **6b**, **8a**, **8b**, and **8d** showed excellent antibacterial activity against all tested antibacterial strains. The synthesized compounds were further evaluated for their cytotoxic activity against the HCT 116 and HeLa cancer cell lines. The 1,3,4-oxadiazole and benzothiazoyl thioether derivatives **6a–g** and **8a–g** did not show cytotoxicity.

Keywords Isoxazol-5(4H)-one · 1,3,4-Oxadiazole · Benzothiazol · Thioeteher · Antitubercular activity · Antibacterial activity

Introduction

Enormous numbers of antibiotics and chemotherapeutics drugs have contributed towards controlling human ailments. Since the last three decades, the complications of multidrug-resistant microorganisms have reached an alarming level in many countries around the world (Brown and Wright 2016; Aminov 2017; Powers 2004). The proliferation in antibiotic resistance due to numerous aspects has intensified the search for new, safe, and more efficient drugs which can prove to be dynamic against multidrug-resistant pathogens (Kunin and Ellis 2000; Cannon et al. 2009; Achkar and Fries 2010; Kathiravan et al. 2012).

The development of clubbed heterocyclic compounds is enormously valuable due to the pharmacological potential owned by the individual heterocycles themselves as well as a new one due to the reciprocal influence of the incorporated heterocycles. Compounds containing 1,3,4-oxadiazole nucleus showed broad spectrum of biological activities such as anti-inflammatory (Sahoo et al. 2014), antibacterial (Li et al. 2013), herbicidal (Tajik and Dadras 2011), insecticidal (Li and He 2012), and antifungal activities (Asan et al.

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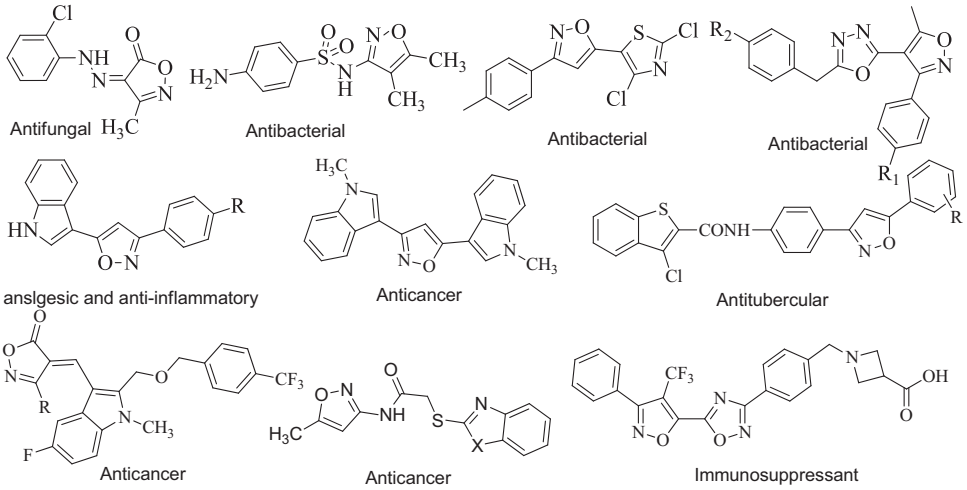

Fig. 1 Biologically active isoxazole derivatives

2012). Commercial fungicides or herbicides, such as triadimefon, triadimenol, flusilazole, and flupoxam (Guo et al. 2014) contains 1,3,4-oxadiazoles. On the other hand, the heterocyclic scaffold comprising isoxazole shows various biological activities, such as antibacterial (Badrey and Gomha 2014), anticonvulsant (Kaur et al. 2010), anticancer (Yong et al. 2014), anthelmintics (Mondal et al. 2012), anti-inflammatory (El-Hawash et al. 2014), adenosine antagonist (Choi et al. 2005), fungicidal (Reddy and Nagaraj 2008), herbicidal (Zhang et al. 2008), hypoglycaemic (Kumar et al. 2009), muscle relaxant (Tatee et al. 1986), nematocidal (Srinivas et al. 2010), insecticidal (Wang et al. 2011), antiviral (Muratov et al. 2011), antimicrobial (Gollapalli 2015), and antitubercular (Kozikowski et al. 2009) activities. (Fig. 1) Furthermore, benzothiazole nucleus containing molecules are also reported as anti-allergic (Musser et al. 1984), antitumor (Yoshida et al. 2005; Catriona et al. 2006), and anticancer (Bradshaw et al. 2002) and it was declared that these molecules can be used as an Aβ plaque imaging against for the study of patients with Alzheimer's disease (Kim et al. 2009).

Aryl and alkyl substituted thioethers are the backbone of many biologically active compounds reported for antibacterial (Macaev et al. 2005; McReynolds et al. 2004), antifungal (Chen et al. 2000), antioxidants in polymers (Coran 2003), anti-hyperplasia (Quaglia et al. 2002), and anticancer (Sugita et al. 2001) activities. 1,3,4-Oxadiazolin-2-thiones have earned a great deal of attention in heterocyclic chemistry as versatile intermediates due to the fact that the thiol group on the oxadiazole ring undergoes nucleophilic substitution reaction readily (Mekuskiene et al. 2003).

In view of the significance of isoxazole and 1,3,4-oxadiazole nucleus and in prolongation of our work (Abhale et al. 2015; Abhale et al. 2017) on hunt for bioactive heterocyclic compounds, in the present study, we have reported synthesis of 1,3,4-oxadiazole and benzothiazoyl thioether derivatives of 4-arylmethylidene-3-substituted-isoxazol-5(4H)-one as potential antimycobacterial agents.

Material and methods

Chemistry

Experimental

The reaction progress was monitored by thin layer chromatography (TLC). Physical constants of the pure compounds were determined in capillary tubes in silicon oil bath using a Veego melting point apparatus and are uncorrected. The synthesized compounds were analyzed using NMR and the spectra were recorded on Varian mercury XL-300 or Bruker spectrometer instruments; tetramethylsilane was used as an internal standard and chemical shifts are reported in δ units. Mass spectra were recorded on a Brucker LC-MS QP MS-3200Q trap spectrometer with an ionization potential of 70 eV.

General procedure for synthesis of 4-[(substituted benzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (6a–g)

The solution of 4-[(aryl)-methylidene-]-3-chloro-methyl-5 (4*H*)-isoxazolone (**4a–g**, 1 mmol), sodium hydrogen carbonate (1 mmol) and 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (**5**, 1 mmol) in dry ethanol was refluxed for 3 h. After the completion of the reaction (TLC) the mixture was cooled to room temperature, the solid product was separated then filtered and washed with cold ethanol. The product (**6a–g**) thus obtained was further purified by crystallization from aqueous ethanol. Similar procedure was used for the synthesis of **8a–g** using benzothiazole-2-thiol (**7**).

4-(4-hydroxybenzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (6a) ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.76 (s, 2H, S–CH_2-isoxazole), 7.03 (d, $J = 8.9$ Hz, 2H, Ar–H), 7.89 (d, $J = 6.0$ Hz, 2H, Py–H), 8.22 (s,1H, =CH), 8.46 (d, $J = 8.9$ Hz, 2H, Ar–H), 8.81(d, $J = 6.0$ Hz, 2H, Py–H), 11.46 (bs, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.97 (CH_2, –S–CH_2-isoxazole), 110.96 (C, Ar C-1), 116. 42 (CH, Ar C-3 and C-5), 119.99 (CH, Py C-3 and C-5), 124.43 (C, isoxazole C-4), 129.94 (CH, =CH), 137.94 (CH, Ar C-2 and C-6), 150.89 (CH, Py C-2 and C-6), 152.73 (C, Ar C-4), 161.10 (C, Py C-4), 163.76 (C, isoxazole C-3), 164.01 (C, oxadiazole C-5), 164.80 (C, oxadiazole C-2), 168.59 (C, isoxazole C=O), MS(*m*/*z*): 381.0573 $(M+H)^+$.

4-(4-methoxybenzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (6b): IR(KBr): 1760, 1610, 1580, 1568, 1179 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 3H, OCH_3), 4.73 (s, 2H, S–CH_2-isoxazole), 7.09 (d, $J = 8.9$ Hz, 2H, Ar–H), 7.88 (d, $J =$ 6.1 Hz, 2H, Py–H), 8.22 (s, 1H, =CH), 8.54 (d, $J = 8.9$ Hz, 2H, Ar–H), 8.80 (d, $J = 6.1$ Hz, 2H, Py–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.78 (CH_2, S–CH_2–isoxazole), 55.59 (CH_3, OCH_3), 112.52 (C, Ar C-1), 114.45 (CH, Ar C-3 and C-5), 119.73 (CH, Py C-3 and C-5), 125.71 (C, isoxazole C-4), 129.92 (CH, =CH), 137.25 (CH, Ar C-2 and C-6), 150.59 (CH, Py C-2 and C-6), 152.10 (C, Ar C-4), 160.47 (C, Py C-4), 163.72 (C, isoxazole C-3), 163.90 (C, oxadiazole C-5), 164.66 (C, oxadiazole C-2), 168.09 (C, isoxazole C=O); MS(*m*/*z*): 395.0815 $(M+H)^+$.

4-(4-hydroxy-3-methoxybenzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazole-ylthio)methyl) isoxazol-5(4H)-one, (6c) ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 3H, OCH_3), 4.73 (s, 2H, S–CH_2-isoxazole), 6.98 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.90 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.92 (d, $J = 6.1$ Hz, 2H, Py–H), 8.11 (s, 1H, =CH), 8.51 (s, 1H, Ar–H), 8.81 (d, $J =$ 6.1 Hz, 2H, Py–H), 11.06 (bs, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.97 (CH_2, –S–CH_2–isoxazole), 111.96 (C, Ar C-1), 114.42 (CH, Ar C-2), 115. 88 (CH, Ar C-5), 119.99 (CH, Py C-3 and C-5), 120.2 (CH, Ar C-6), 124.43 (C, isoxazole C-4), 129.90 (CH, =CH),148.91 (CH, Ar C-4), 150.89 (CH, Py C-2 and C-6), 151.35 (CH, Ar C-3), 161.10 (C, Py C-4), 163.76 (C, isoxazole C-3), 164.01 (C, oxadiazole C-5), 164.80 (C, oxadiazole C-2), 168.59 (C, isoxazole C=O); MS(*m*/*z*): 411.0685 $(M+H)^+$.

4-(4-methylbenzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazole-2-ylthio)methyl)isoxazol-5(4H)-one, (6d) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 4.70 (s, 2H, S–CH_2-isoxazole), 7.12 (d, $J = 8.7$ Hz, 2H, Ar–H), 7.90 (d, $J = 6.2$ Hz, 2H, Py–H), 8.24 (s, 2H, =CH), 8.50 (d, $J =$ 8.7 Hz, 2H, Ar–H), 8.84 (d, $J = 6.2$ Hz, 2H, Py–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.03 (CH_3,-CH_3), 26.75 (CH_2, S–CH_2–isoxazole), 112.50 (C, Ar C-1), 114.46 (CH, Ar C-3 and C-5), 119.70 (CH, Py C-3 and C-5), 125.75 (C, isoxazole C-4), 129.95 (CH, =CH), 137.30 (CH, Ar C-2 and C-6), 150.55 (CH, Py C-2 and C-6), 152.15 (C, Ar C-4), 160.44 (C, Py C-4),163.70 (C, isoxazole C-3), 163.95 (C, oxadiazole C-5), 164.70 (C, oxadiazole C-2), 168.01 (C, isoxazole C=O); MS(*m*/*z*): 379.0788 $(M+H)^+$.

4-(benzo[d][1,3]dioxol-5-ylmethylene)-3-(((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)methyl) isoxazol-5(4H)-one, (6e) ^{1}H NMR (400 MHz,$CDCl_3$) δ 4.71 (s, 2H, –S–CH2-isoaxzole), 6.08 (s, 2H, –OCH_2O–), 6.82 (d, $J = 6.1$ Hz, 2H, Py–H), 7.35–7.39 (m, 1H, Ar–H), 7.73–7.51 (m, 2H, Ar–H), 7.89 (s, 1H, =CH), 7.95 (d, 2H, $J = 6.1$ Hz, Py–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.31 (CH_2, S–CH_2-isoxazole), 101.28 (CH_2, –OCH_2O–), 112.10 (CH, Ar C-6), 116.32 (CH, Ar C-3), 119.69 (CH, Ar C-4), 121.45 (CH, Py C-3 and C-5), 124.98 (C, isoxazole C-4), 128.53 (C, Ar C-5), 148.13 (C, Ar C-2), 148.50 (C, oxadiazole C-2), 148.64 (C, Ar C-1), 151.16 (CH, Py C-2), 151.32 (CH, Py C-4), 161.28 (C, isoxazole C-3), 164.93 (C, oxadiazole C-5), 168.36 (C, isoxazole C=O); MS(*m*/*z*): 409.0530 $(M+H)^+$.

4-(4-(dimethylamino)benzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazole-2-ylthio)methyl) isoxazol-5(4H)-one, (6f) IR (KBr): 1718, 1571, 1541, 1512, 1274, 1197 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 (s, 6H, –$N(CH_3)_2$) 4.58 (s, 2H, S–CH_2-isoxazole), 6.72 (d, $J = 6$ Hz, 2H, Ar–H), 7.61 (s, 1H, =CH), 7.87 (d, $J = 5.5$ Hz, 2H, Py–H), 8.43(d, $J =$ 6 Hz, 2H, Ar–H), 8.81 (d, $J = 5.5$ Hz, 2H, Py–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.46 (CH_2, S–CH_2-isoxazole), 40.20 (CH_3,–$N(CH_3)_2$), 107.68 (C, Ar C-1), 111.76 (CH, Ar C-3 and C-5), 120.12 (CH, Py C-3 and C-5), 121.68 (C, isoxazole C-4), 130.42 (CH, =CH), 138.45 (CH, Ar C-2 and C-6), 150.10 (CH, Py C-2 and C-6), 150.95 (C, Ar C-4), 154.86 (C, Py C-4), 159.86 (C, isoxazole C-3), 164.91 (C, oxadiazole C-5), 169.84 (C, isoxazole C=O); MS(*m*/*z*): 409.1060 $(M+H)^+$.

4-((1H-indol-3-yl)methylene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazole-2-ylthio)methyl)isoxazol-5(4H)-one, (6g) IR(KBr): 3291, 1714, 1592, 1574, 1473, 1217, 1190 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.81 (s, 2H, –S–CH_2-isoaxzole), 7.31–7.33 (m, 2H, Ar–H), 7.55–7.58 (m, 1H, Ar), 7.88 (d, $J = 6.1$ Hz, 2H, Py–H), 8.13–8.15 (m, 1H, Ar–H), 8.52 (s,1H, Ar–H), 8.77 (d, $J = 6.1$ Hz, 2H, Py–H), 12.86 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.78 (CH_2, S–CH_2-isoxazole), 110.52 (C, Ar C-3), 113.45 (CH, Ar C-8), 119.02 (CH, Ar C-5), 119.73 (CH, Py C-3 and C-5), 120.22 (CH, Ar C-7), 122.25 (CH, Ar C-6), 125.71 (C, isoxazole C-4), 125.99 (C, Ar C-4), 129.92 (CH, =CH), 130.81 (CH, Ar C-2), 135.45 (C, Ar C-9), 150.59

(CH, Py C-2 and C-6), 160.47 (C, Py C-4), 163.72 (C, isoxazole C-3), 163.90 (C, oxadiazole C-5), 164.66 (C, oxadiazole C-2), 168.09 (C, isoxazole C=O); MS(*m*/*z*): 404.0745 $(M+H)^+$.

4-(4-hydroxybenzylidene)-3-((benzo[d]thiazol-2-ylthio) methyl)isoxazol-5(4H)-one, (8a) ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.78 (s, 2H, S–CH_2-isoaxzole), 7.00 (d, J = 8.8 Hz, 2H, Ar–H), 7.39 (td, J = 7.6 and 1.0 Hz, 1H, benzo[*d*]thiazol-H), 7.48 (td, J = 7.6 and 1.0 Hz, 1H, benzo[*d*] thiazol-H), 7.88 (d, J = 7.6 Hz, 1H, benzo[*d*]thiazol-H), 8.04 (d, J = 7.6 Hz, 1H, benzo[*d*]thiazol-H), 8.16 (s, 1H, =CH), 8.45 (d, J = 8.8 Hz, 2H, Ar–H), 11.30 (bs, 1H,-OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.39 (CH_2, S–CH_2-isoxazole), 111.33 (C, Ar C-1), 116.40 (CH, Ar C-3 and C-5), 121.34 (C, benzothiazole C-5), 121.96 (C, benzothiazole C-8), 124.42 (C, benzothiazole C-6), 124.79 (C, benzothiazole C-7), 126.49 (C, isoxazole C-4), 135.01 (C, benzothiazole C-9), 137.91 (CH, Ar C-2 and C-6), 152.38 (CH, =CH), 152.61 (C, Ar C-4), 161.39 (C, benzothiazole C-4), 164.60 (C, benzothiazole C-2), 164.76 (C, isoxazole C-3), 168.61 (C, isoxazole C=O); MS(*m*/*z*): 369.0289 $(M+H)^+$.

4-(4-methoxybenzylidene)-3-((benzo[d]thiazol-2-ylthio) methyl)isoxazol-5(4H)-one, (8b) IR (KBr): 1744, 1590, 1509, 1270, 1233, 1189 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 (s, 3H, OCH_3), 4.69 (s, 2H, S–CH_2-isoaxzole), 6.91 (d, J = 8.8 Hz, 2H, Ar–H), 7.35 (t, J = 7.6 and 1.1 Hz, 1H, benzo[*d*]thiazol-H), 7.47 (t, J = 7.6, 1.1 Hz, 1H, benzo[*d*] thiazol-H), 7.77 (d, J = 7.6 Hz, 1H, benzo[*d*]thiazol-H), 7.90 (s, 1H, =CH), 7.94 (d, J = 7.6 Hz, 1H, benzo[*d*]thiazol-H), 8.30 (d, J = 8.8 Hz, 2H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.35 (C, S–CH_2-isoxazole), 55.75 (CH_2, OCH_3), 113.38 (C, Ar C-1), 114.70 (CH, Ar C-3 and C-5), 121.43 (C, benzothiazole C-5), 121.54 (C, benzothiazole C-8), 124.94 (C, benzothiazole C-6), 125.96 (C, isoxazole C-4), 126.48 (C, benzothiazole C-7), 135.67 (C, benzothiazole C-9), 137.46 (CH, Ar C-2 and C-6), 151.26 (CH, =CH), 152.481 (C, Ar C-4), 161.28 (C, benzothiazole C-4), 164.89 (C, benzothiazole C-2), 165.02 (C, isoxazole C-3), 168.59 (C, isoxazole C=O); MS(*m*/*z*): 383.0524 $(M+H)^+$.

4-(4-hydroxy-3-methoxybenzylidene)-3-((benzo[d]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (8c) ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 4.77 (s, 2H, S–CH_2-isoxazole), 6.95 (d, J = 8.5 Hz, 1H, Ar–H), 7.38 (td, J = 7.6 and 1.1 Hz, 1H, benzo[*d*]thiazol-H), 7.48 (td, J = 7.6 and 1.1 Hz, 1H, benzo[*d*]thiazol-H), 7.86 (d, J = 8.5 Hz, 1H, Ar–H), 7.89 (d, J = 7.6 Hz, 1H, benzo[*d*]thiazol-H), 8.00 (d, J = 7.6 Hz, 1H, benzo[*d*]thiazol-H), 8.09 (s, 1H, =CH), 8.51 (s, 1H, Ar–H), 8.47 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.42 (CH_2, S–CH_2-isoxazole) 56.85 (CH_3, OCH_3), 112.30 (C, Ar C-1), 115.72 (CH, Ar C-5), 123.41 (C, benzothiazole C-5), 120.50 (C, benzothiazole C-8), 125.90 (C, benzothiazole C-6), 126.15 (C, benzothiazole C-7), 126.45 (C, isoxazole C-4), 135.71 (C, benzothiazole C-9), 137.48 (CH, Ar C-2 and C-6), 146.48 (CH, Ar C-3), 149.62 (C, Ar C-4), 151.30 (CH, =CH), 161.30 (C, benzothiazole C-4), 164.92 (C, benzothiazole C-2), 166.02 (C, isoxazole C-3), 169.59 (C, isoxazole C=O); MS(*m*/*z*): 399.0398 $(M+H)^+$.

4-(4-methylbenzylidene)-3-((benzo[d]thiazol-2-ylthio) methyl)isoxazol-5(4H)-one, (8d) ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.65 (s, 2H, S–CH_2-isoaxzole), 6.90 (d, J = 8.6 Hz, 2H, Ar–H), 7.38 (td, J = 7.5 and 1.2 Hz, 1H, benzo[*d*]thiazol-H), 7.45 (td, J = 7.5, 1.2 Hz, 1H, benzo[*d*]thiazol-H), 7.75 (d, J = 7.5 Hz, 1H, benzo[*d*]thiazol-H), 7.88 (s, 1H, =CH), 7.92 (d, J = 7.5 Hz, 1H, benzo[*d*]thiazol-H), 8.32 (d, J = 8.6 Hz, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.3 (C, CH_3), 27.33 (CH_2, S–CH_2-isoxazole), 113.32 (C, Ar C-1), 114.72 (CH, Ar C-3, C-5), 121.41 (C, benzothiazole C-5), 121.56 (C, benzothiazole C-8), 124.98 (C, benzothiazole C-6), 125.93 (C, benzothiazole C-7), 126.46 (C, isoxazole C-4), 135.68 (C, benzothiazole C-9), 137.45 (CH, Ar C-2 and C-6), 138.35 (C, Ar C-4), 151.25 (CH, =CH), 161.24 (C, benzothiazole C-4), 164.90 (C, benzothiazole C-2), 165.08 (C, isoxazole C-3), 168.51 (C, isoxazole C=O); MS(*m*/*z*): 367.0467 $(M+H)^+$.

4-((benzo[d][1,3]dioxol-5-yl)methylene)-3-((benzo[d]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (8e) ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.77 (s, 2H, S–CH_2-isoxazole), 6.20 (s, 2H, OCH_2O), 6.90 (d, J = 8.4 Hz, 1H, Ar–H), 7.38 (td, J = 7.7 and 1.2 Hz, 1H, benzo[*d*]thiazol-H), 7.48 (td, J = 7.7 and 1.2 Hz, 1H, benzo[*d*]thiazol-H), 7.86 (d, J = 8.4 Hz, 1H, Ar–H), 7.89 (d, J = 7.7 Hz, 1H, benzo[*d*]thiazol-H), 8.00 (d, J = 7.7 Hz, 1H, benzo[*d*]thiazol-H), 8.09 (s, 1H, =CH) 8.51 (s, 1H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.30 (CH_2, -CH_2-isoxazole), 110.50 (CH_2, –OCH_2O–), 112.30 (CH, Ar C-6), 116.72 (CH, Ar C-3), 119.69 (CH, Ar C-4), 123.41 (C, benzothiazole C-5), 120.50 (C, benzothiazole C-8), 125.90 (C, benzothiazole C-6), 126.15 (C, benzothiazole C-7), 126.45 (C, isoxazole C-4), 128.53 (C, Ar C-5), 135.71 (C, benzothiazole C-9), 146.48 (CH, Ar C-3), 148.13 (C, Ar C-2), 148.64 (C, Ar C-1), 151.30 (C, Ar C-4), 152.28 (CH, =CH), 161.24 (C, benzothiazole C-4), 164.92 (C, benzothiazole C-2), 166.02 (C, isoxazole C-3), 169.59 (C, isoxazole C=O); MS(*m*/*z*):397.0234 $(M+H)^+$.

4-(4-(dimethylamino)benzylidene)-3-((benzo[d]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (8f) IR (KBr): 1706, 1588, 1549, 1525, 1376, 1199 cm^{-1}; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 3.29 (s, 6H, $N(CH_3)_2$), 4.72 (s, 2H, S–CH_2-isoaxzole), 6.78 (d, $J = 9.3$ Hz, 2H, Ar–H), 7.37 (td, $J = 7.6$ and 1.1 Hz, 1H, benzo[*d*]thiazol-H), 7.47 (td, $J = 7.6$ and 1.1 Hz, 1H, benzo[*d*]thiazol-H), 7.87 (s, 1H, =CH), 7.92 (m, 2H, benzo[*d*]thiazol-H), 8.40 (d, $J = 8.6$ Hz, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.30 (CH_3, –N$(CH_3)_2$), 45.49 (CH_2, S–CH_2-isoxazole), 106.55 (C, Ar C-1), 111.55 (CH, Ar C-3 and C-5), 121.06 (C, benzothiazole C-5), 121.15 (C, benzothiazole C-8), 121.42 (C, benzothiazole C-6), 124.49 (C, benzothiazole C-7), 126.14 (C, isoxazole C-4), 134.97 (C, benzothiazole C-9), 137.86 (CH, Ar C-2,6), 150.72 (CH, =CH), 152.28 (C, Ar C-4), 154.50 (C, benzothiazole C-4), 160.94 (C, benzothiazole C-2), 164.70 (C, isoxazole C-3), 169.52 (C, isoxazole C=O); MS (*m*/*z*): 396.0762 $(M+H)^+$.

4-((1H-indol-3-yl)methylene)-3-((benzo[d]thiazol-2-ylthio) methyl)isoxazol-5(4H)-one, (8g) IR (KBr): 3297, 1716, 1592, 1574, 1473, 1370, 1343, 1217, 1195 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.81 (s, 2H, –S–CH_2-isoaxzole), 7.31–7.33 (m, 2H, Ar–H), 7.38 (td, $J = 7.7$ and 1.2 Hz, 1H, benzo[*d*]thiazol-H), 7.48 (td, $J = 7.7$ and 1.2 Hz, 1H, benzo [*d*]thiazol-H), 7.55–7.58 (m, 1H, Ar), 7.77 (d, $J = 6.1$ Hz, 2H, Py–H), 7.89 (d, $J = 7.7$ Hz, 1H, benzo[*d*]thiazol-H), 8.00 (d, $J = 7.7$ Hz, 1H, benzo[*d*]thiazol-H), 8.09 (s, 1H, =CH), 8.13–8.15 (m, 1H, Ar–H), 8.52 (s, 1H, Ar–H), 8.77 (d, $J = 6.1$ Hz, 2H, Py–H), 12.86 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.85 (CH_2, S–CH_2-isoxazole), 111.52 (C, Ar C-3), 114.45 (CH, Ar C-8), 118.88 (CH, Ar C-5), 120.18 (CH, Ar C-7), 120.50 (C, benzothiazole C-8), 122.25 (CH, Ar C-6), 123.41 (C, benzothiazole C-5), 124.70 (C, benzothiazole C-6), 125.60 (C, Ar C-4), 126.15 (C, benzothiazole C-7), 131.81 (CH, Ar C-2), 132.28 (C, isoxazole C-4), 134.45 (C, Ar C-9), 135.71 (C, benzothiazole C-9), 151.30 (CH, =CH), 154.50 (C, benzothiazole C-4), 164.92 (C, benzothiazole C-2), 166.02 (C, isoxazole C-3), 169.59 (C, isoxazole C=O); MS(*m*/*z*): 392.0448 $(M+H)^+$.

Biological assay

Antitubercular activity

In vitro antitubercular activity of synthesized compounds against *M. tuberculosis* H37Ra (ATCC 25177) was determined through the XTT reduction menadione assay (XRMA), reading absorbance at 470 nm as per the protocol described by (Singh et al. 2011; Khan and Sarkar 2008). In vitro antitubercular activity against *M. bovis* BCG (ATCC 35743) was performed using the nitrate reductase (NR) assay (Khan and Sarkar 2008) to estimate inhibition of *M. bovis* BCG by compounds absorbance for the NR assay was measured at 540 nm. The % inhibition was determined using the following formula:

$$\%\,\text{inhibition} = \left[\left(\begin{array}{l}\text{activity of mycobacteria without compounds}\\ -\text{activity of mycobacteria in the presence of compounds}\end{array}\right)\Big/ (\text{activity of mycobacteria without compounds} - \text{blank})\right] \times 100$$

Antibacterial activity

All bacterial cultures were first grown in Luria Burtony media at 37 °C at 180 rpm. Once the culture reaches 1 Optical Density (O.D.), it is used for an antibacterial assay. The Gram-negative and Gram-positive bacterial strains were obtained from NCIM, NCL, Pune. All the bacterial strains were grown in Luria Burtony medium from Hi Media, India. The antibacterial screening was performed in 96-well plates after 8 and 12 h for Gram-negative and Gram-positive bacteria, respectively (Singh et al. 2011; Khan and Sarkar 2008). Overall 0.1 % of 1 O.D. culture at 620 nm was used for screening inoculated culture was added into each well of 96-well plate containing the compounds to be tested and the optical density for each plate was measured at 620 nm.

To determine the minimum inhibitory concentration (MIC), the compounds were screened at 3, 10, and 30 µg/mL concentration. Each concentration was tested in duplicates in a single experiment and MIC_{90} values were calculated using Origin Pro Software.

Cytotoxic activity

Human Cancer cell lines Cervix adenocarcinoma Hela and colorectal carcinoma HCT 116 were obtained from NCCS, Pune and cell cultures were maintained under standard conditions. The cytotoxic activity in growth inhibition was determined at 30, 10, 3 µg mL^{-1} concentrations. The appropriate culture medium of 0.05% trypsin and 0.02% ethylene diamine tetra-acetic acid in PBS was diluted to obtain a cell density of 105 cells/mL. An aliquot of 100 µL of each suspension was seeded in 96-well cell culture plates and was incubated at 37 °C for 24 h in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator. The synthesized compounds at 3, 10, and 30 µg/mL concentrations were added to the wells containing cells (1 µL/well). Rifampicin and dimethyl sulphoxide were used as positive and negative control, respectively. The plates were further incubated for 48 h, then the solution containing the unattached cells was discarded and the wells were washed three times with 1 mL of PBS followed by addition of 10 µL of MTT (5 mg/mL in PBS) to adherent cells in growth medium. After 4 h at 37 °C for MTT cleavage, the formazan product was solubilised by addition of 100 µL of 0.04 N HCl in isopropanol. Absorbance was measured on a SpectraMax®

PLUS 384 plate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 570 nm. Each concentration was tested in triplicate in a single experiment. Percentage cytotoxicity was calculated using the following formula:

$$\%\,\text{cytotoxicity} = (\text{average of control} - \text{average of compound})/(\text{average of control} - \text{average of blank}) \times 100)$$

Result and discussion

A general route for the synthesis of 4-[(substituted benzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (**6a–g**) and 4-(substituted benzylidene)-3-((benzo[*d*]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (**8a–g**) is described in Scheme 1. Ethyl-4-chloroacetoacetate (**1**), hydroxylamine hydrochloride (**2**), and substituted aldehydes (**3a–g**) were agitated at 0 °C to room temperature in an aqueous condition which resulted the formation of 4-[(aryl)-methylidene]-3-chloro-methyl-5(4H)-isoxazolone **(4a–g)** derivatives (Chavan et al. 2015). 5-(Pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (**5**) was achieved by a ring closure reaction of analogous acid hydrazide with carbon disulfide in the presence of potassium hydroxide. 5-(Pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (**5**) and benzothiazole **(7)** retorton compounds **4a–g** in the presence of sodium hydrogen carbonate in dry ethanol furnished 4-[(substituted benzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (**6a–g**) and 4-(substituted benzylidene)-3-((benzo[*d*]thiazol-2-ylthio)methyl) isoxazol-5(4H)-one, **(8a–g)** respectively, in good yield. (Table 1).

The reaction of 4-[(4-methoxyphenyl)-methylidene-]-3-chloro-methyl-5(4*H*)-isoxazolone **(4b)** with 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (**5**) in the presence of sodium hydrogen carbonate in dry ethanol gave 4-[(4-methoxybenzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (**6b**) in 92% yield. The

Scheme 1 Synthesis of compounds **6a–g** and **8a–g**

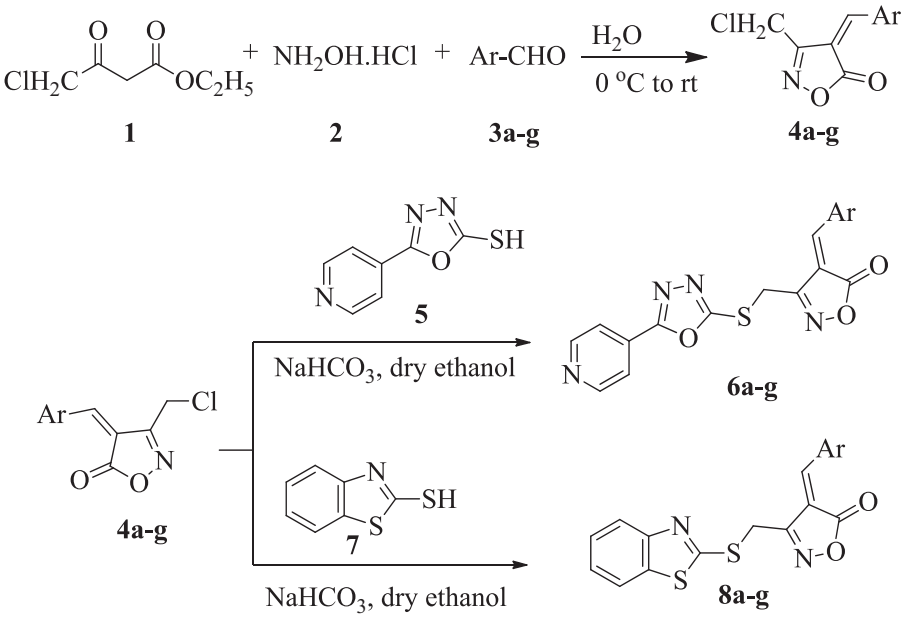

Table 1 Yields and physical constant of compound **6a–g** and **8a–g**

Comp.	Ar	Yield[a] (%)	M.P. (°C)	Comp.	Ar	Yield[a] (%)	M.P. (°C)
6a	4-OH C_6H_4	88	202	**8a**	4-OH C_6H_4	96	200
6b	4-OCH_3 C_6H_4	92	186	**8b**	4-OCH_3 C_6H_4	88	175
6c	3-OCH_3,4-OH C_6H_3	98	168	**8c**	3-OCH_3,4-OH C_6H_3	89	199
6d	4-CH_3 C_6H_4	90	179	**8d**	4-CH_3 C_6H_4	86	178
6e	3,4-OCH_2O–C_6H_3	90	198	**8e**	3,4-OCH_2O– C_6H_3	89	199
6f	4-$N(CH_3)_2$ C_6H_4	96	202	**8f**	4-$N(CH_3)_2$ C_6H_4	88	225
6g	3-Indole	78	192	**8g**	3-Indole	85	248

[a]Isolated Yield

infrared spectrum of compound **6b** displayed an absorption band at 1749 cm^{-1}, indicating the presence of lactone C=O. The absorbance band at 1182 cm^{-1} represents the thioether (C–S–C) linkage, this observation clearly indicating the formation of desired product **6b**. The ^{1}H NMR spectrum of **6b** exhibited a sharp signal at δ 7.88, which was assigned to one olefin proton. The two singlets at δ 3.94 and 4.73 are attributed to $–OCH_3$ and $–CH_2S$, respectively. Two doublets at δ 7.09 and 8.55 with $J = 8.9$ Hz corresponds to ortho and meta protons of 4-methoxyphenyl ring and two doublets at δ 7.88 and δ 8.80 with $J = 6.08$ Hz corresponds to the protons of 4-substituted pyridine. The ^{13}C NMR of compound **6b** showed two peaks in the aliphatic region at δ 26.78 and 55.59 due to $–SCH_2–$ and $–OCH_3$, respectively. The isoxazolone carbons resonated at δ 129.92 and 168.09 due to olefinic and isoxazole carbonyl group carbons, respectively. The mass spectrum of compound **6b** displayed a signals at *m/z* 395.0815 corresponding to the $[M+H]^+$ ions which are in accordance with the predicted values.

The reaction of 4-[(4-methoxyphenyl)-methylidene-]-3-chloro-methyl-5(4H)-isoxazolone (**4b**)with benzothiazole (**7**) in the presence of sodium hydrogen carbonate in dry ethanol gives 4-(4-methoxybenzylidene)-3-((benzo[*d*]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one (**8b**) in 88% yield, (Table 2). The structure of compounds was confirmed by spectroscopic methods.

The ^{1}H NMR spectrum of **8b** exhibited a signal at δ 7.90 which was assigned to one olefinic proton. Two singlets at δ 3.72 and 4.69 were attributed to $–OCH_3$ and $–CH_2S–$, respectively. Two doublets at δ 6.91 and 8.30 with $J =$ 8.8 Hz was assigned to 4-methoxyphenyl protons. The aromatic protons of benzothiazole resonated at δ 7.35–7.94. The ^{13}C NMR spectrum of compound **8b** showed peaks at δ 27.35 and δ 55.75 due to $–SCH_2–$ and $–OCH_3$, respectively. The isoxazolone carbons showed two peaks at δ 126.48 and 168.59 due to olefinic carbon and isoxazole carbonyl group. The structure was further confirmed by mass spectrum that displayed a signal at *m/z* 383.0524 corresponding to $[M+H]^+$.

All the synthesized compounds were characterized by spectroscopic analysis and screened for their antimycobacterial and antimicrobial activity.

Biological screening

Antimycobacterial activity

The antimycobacterial activity for the synthesized compounds were evaluated by measuring % inhibition of growth against *M. tuberculosis* H37Ra and *M. bovis* (BCG) in liquid medium. In vitro activity studies against *M. tuberculosis* and *M. bovis* BCG were performed using the XRMA (Singh et al. 2011; Khan and Sarkar 2008) and NR assays (Sarkar and Sarkar 2012; Liu and Hou 2012), respectively.

The antibacterial activity (Singh et al. 2011; Khan and Sarkar 2008) of synthesized compounds was evaluated against the standard Gram-negative bacteria, *E. coli*, *P. flurescence* and Gram-positive bacteria, *S. aureus B. subtilis*. In a preliminary antimycobacterial evaluation, all the compounds were screened at 30 µg/mL concentration. The

Table 2 Antimycobacterial activity in % inhibition at 30 µg/mL of compounds **6a–g** and **8a–g**

Compound	*M. tuberculosis H37Ra*	*M. bovis BCG*	*E. coli*	*P. flurescence*	*S. aureus*	*B. subtilis*
6a	4.2	3.71	65.68	64.55	76.56	66.38
6b	**96.9**	**93.39**	**97.7**	**97.73**	**97.15**	**99.27**
6c	–	4.29	55.13	53.39	60.62	53.6
6d	40.2	–	62.02	65.32	61.24	67.17
6e	**89.9**	**82.33**	35.32	31.09	31.54	35.92
6f	13.4	–	**80.26**	**89.42**	**82.15**	**87.21**
6g	43.4	3.59	61.08	64.38	66.4	62.66
8a	**99.4**	**94.49**	**92.23**	**93.98**	**92.87**	**91.94**
8b	**96.5**	**96.36**	**91.13**	**91.24**	**90.87**	**92.51**
8c	**80**	**87.15**	40.78	40.73	39.73	38.63
8d	**92.3**	**95.27**	**90.4**	**92.77**	**95.27**	**94.68**
8e	26.1	30.02	30.01	32.49	36.29	36.47
8f	49.1	48.99	84.73	81.65	87.4	84.04
8g	16.1	50.16	22.75	23.47	25.35	23.07
Rifampicin	**99.5**	**99.00**				
Ampicillin			**97.00**	**96.6**	**95.6**	**98.5**

– = Inactive

Bold values indicates good activity

Table 3 Antimycobacterial activities in MIC_{90} (μg/mL) of compounds **6a–g** and **8a–g**

Compound	*M. tuberculosis* H37Ra	*M. bovis* BCG	*E. coli*	*P. flurescence*	*S. aureus*	*B. subtilis*
6a	>30	>30	>30	>30	>30	>30
6b	**7.46**	**15.01**	**2.93**	**7.17**	**6.63**	**3.2**
6c	**nd**	>30	>30	>30	>30	>30
6d	>30	>30	>30	>30	>30	>30
6e	>30	>30	>30	>30	>30	>30
6f	>30	>30	>30	>30	>30	>30
6g	>30	>30	>30	>30	>30	>30
8a	**25.42**	**12.33**	**24.1**	**20.13**	**11.25**	**11.58**
8b	**7.9**	**12.22**	**11.25**	**19.5**	**11.23**	**12.19**
8c	>30	>30	>30	>30	>30	>30
8d	**29.15**	**9.34**	**23.5**	**20.97**	**16.84**	**21.88**
8e	>30	>30	>30	>30	>30	>30
8f	>30	>30	>30	>30	>30	>30
8g	>30	>30	>30	>30	>30	>30
Ampicillin			**1.45**	**4.36**	**1.0**	**10.32**
Kanamycin			**1.62**	**0.49**	**>30**	**1.35**
Rifampicin	**0.75**	**0.81**				

nd = not determined

Bold values indicates good activity

in vitro screening (% inhibition) results against microorganisms tested are summarized in Table 2.

Active compounds were further screened for MIC. The best potential hits in the series were further evaluated for cytotoxicity. Rifampicin was used as positive control for antimycobacterial. Ampicillin and Kanamycin were used as positive control for antibacterial activity. The MIC in μg/mL is summarized in Table 3.

The antimycobacterial activity result analysis provided some lead molecules with good to excellent antimycobacterial activity. The results revealed that, among the compounds 4-[(substituted benzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (**6a–g**), compound **6a** (Ar = 4-OH-C_6H_4) was found inactive against both mycobacterium strains, while the 4-OH group when substituted by 4-OCH_3 in compound **6b** (Ar = 4-OCH_3–C_6H_4) registered excellent activity against *M. tuberculosis* H37Ra with MIC 7.46 μg/mL concentration. Compound **6b** also reported good activity against *M. bovis* with MIC 15.01 μg/mL concentration. It was noticed that the 4-OCH_3 group in compound **6b** (Ar = 4-OCH_3–C_6H_4) when substituted by the 3,4-O–CH_2–O– group in compound **6e** (Ar = 3,4-OCH_2O–C_6H_3) activity decreases against both mycobacterium strains. Whereas the compounds **6c** (Ar = 3-OCH_3-4-OH–C_6H_3), **6f** (Ar = 4-$N(CH_3)_2$–C_6H_4) and **6g** (Ar = 3-indol) were found less active against both strains. Among the compounds 4-(substituted benzylidene)-3-((benzo[*d*]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one (**8a–g**), compound **8a** (Ar = 4-OH-C_6H_4) registered good activity against *M. tuberculosis* H37Ra with MIC 25.42 μg/mL and excellent activity against *M. bovis* with the MIC 12.33 μg/mL concentration. It was noted that when 4-OH group was substituted by 4-OCH_3 in compound **8b** (Ar = 4-OCH_3–C_6H_4) there was threefold increase in the activity against *M. tuberculosis* H37Ra with MIC 7.9 μg/mL and the activity was retained against *M. bovis* with MIC 12.22 μg/mL. The 4-OH group of compound **8a** when substituted by 3-OCH_3, 4-OH in compound **8c** (Ar = 3-OCH_3-4-OH–C_6H_3) showed moderate activity against both mycobacterium strains, whereas, the 4-OH group of compound **8a** was substituted by 4-CH_3 in compound **8d** (Ar = 4-CH_3–C_6H_4) showed decrease in activity against *M. tuberculosis* H37Ra with MIC 29.15 μg/mL and increase in activity against *M. bovis* with MIC 9.34 μg/mL. Compounds **8e** (Ar = 4-OCH_2O–C_6H_3), **8f** (Ar = 4-N$(CH_3)_2$–C_6H_4), and **8g** (Ar = 3-indol) were found less active against both mycobacterium strains.

It is necessary to mention that the 4-methoxybenzylidene group on isoxazol-5(4H)-one in compounds **6b** and **8b** were found effective against the *M. tuberculosis* H37Ra and *M. bovis*.

From the antibacterial activity results, it was also noticed that most of the compounds showed excellent to moderate activity. Among the compounds 4-(substituted benzylidene)-3-[(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-methyl]isoxazol-5(4H)-one (**6a–g**), compounds **6a** (Ar = 4-OH-C_6H_4) showed moderate activity against all the screened antibacterial strains. The 4-OH group of

compound **6a**, when substituted by 4-OCH_3 in compound **6b** (Ar = 4-OCH_3-C_6H_4), reported excellent activity against all bacterial strains. 4-(4-Methoxybenzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (**6b**) registered excellent activity against Gram-negative bacterial strains *E. coli* (MIC 2.93 µg/mL) and *P. flurescence* (MIC 7.17 µg/mL) which are only twofold less than the standard drug Ampicillin. Compound **6b** (Ar = 4-OCH_3–C_6H_4) also showed excellent activity against Gram-positive bacterial strains *S. aureus* (MIC 6.63 µg/mL) and *B. subtilis* (MIC 3.2 µg/mL) which is threefold more potent than the reference drug Ampicillin. Compounds **6c** (Ar = 3-OCH_3-4-OH–C_6H_3), **6d** (Ar = 4-CH_3–C_6H_3), **6e** (Ar = 3,4-OCH_2O–C_6H_3) **6f** (Ar = 4-$N(CH_3)_2$–C_6H_4) and **6g** (Ar = 3-indol) reported moderate activity against bacterial strains.

Among the compounds 4-(substituted benzylidene)-3-((benzo[*d*]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one (**8a–g**), compound **8a** (Ar = 4-OH-C_6H_4), reported good activity against Gram-negative bacterial strains *E. coli* (MIC 24.1 µg/mL) and *P. flurescence* (MIC 20.13 µg/mL) and excellent activity against Gram-positive bacterial strains *S. aureus* (MIC 11.25 µg/mL) and *B. subtilis* (MIC 11.58 µg/mL). It was observed that, when 4-OH group was substituted by 4-OCH_3 in compound **8b** (Ar = 4-OCH_3-C_6H_4), the activity against *E. coli* increased by twofolds with MIC 11.25 µg/mL while the activity was retained against *P. flurescence* (MIC 19.5 µg/mL), *S. aureus* (MIC 11.23 µg/mL) and *B. subtilis* (MIC 12.19 µg/mL). Whereas the 4-OH group of compound **8a** when substituted by 4-CH_3 in compound **8d**, the activity was retained against *E. coli* (MIC 23.5 µg/mL) and *P. flurescence* (MIC 20.97 µg/mL) while activity decreased against *S. aureus* (MIC 16.84 µg/mL) and *B. subtilis* (MIC 21.88 µg/mL). Compounds **8c** (Ar = 3-OCH_3-4-OH–C_6H_3), **8e** (Ar = 4-OCH_2–O–C_6H_3), **8f** (Ar = 4-$N(CH_3)_2$–C_6H_4) and **8g** (Ar = 3-indol) were found active against all tested bacterial strains.

It is worth mentioning that compounds 4-(4-methoxybenzylidene)-3-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (**6b**) and 4-(4-methoxybenzylidene)-3-((benzo[d]thiazol-2-ylthio)methyl)isoxazol-5(4H)-one, (**8b**) reported excellent antimycobacterial activity and compound **6b** also showed excellent antibacterial activity against all tested strains. The structure activity relationship revealed that isoxazolone pharmacophore substituted by 4-methoxybenzylidene at 4 position and 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)methyl at 3 position, respectively showed excellent antimycobacterial and antibacterial activity.

Cytotoxic activity

The synthesized compounds were further evaluated against two human cancer cell lines (cervix adenocarcinoma HeLa and colorectal carcinoma HCT 116) to check the toxicity of these compounds using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide MTT assay with 48 h exposure time of the tested compounds. Rifampicin was used as a positive control. From the cytotoxicity activity result analysis (Table 4), it was worth to mention that all screened thioether derivatives are found less cytotoxic against HeLa and HCT 116 cell lines and the active compounds are leads as antimicrobials.

Table 4 Cytotoxic activity of compounds **6a–g** and **8a–g**

Compound	HeLa			HCT 116		
	30 µg/mL	10 µg/mL	3 µg/mL	30 µg/mL	10 µg/mL	3 µg/mL
6a	49.77	–	–	37.58	17.20	2.31
6b	–	–	–	12.95	4.16	–
6c	–	–	–	–	–	–
6d	–	–	–	–	–	–
6e	–	–	–	34.89	17.34	16.90
6f	–	–	–	–	–	–
6g	–	–	–	40.73	27.19	16.45
8a	70.97	–	–	51.97	25.88	8.46
8b	41.27	–	–	33.56	–	–
8c	–	–	–	–	–	–
8d	80.80	0.33	–	65.55	20.92	10.91
8e	75.80	–	–	22.81	–	–
8f	–	–	–	–	–	–
8g	–	–	–	39.98	17.60	–
Rifampicin	**25.50**	**20.10**	**15.80**	**22.15**	**16.50**	**10.12**

– = Inactive

Table 5 ADME prediction of compounds **6a–g** and **8a–g**

Comp.	MW	TPSA	iLOGP	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor	Lipinski #violations
6a	380.4	136	2.44	High	No	Yes	Yes	Yes	No	0
6b	394.4	125	2.83	High	No	Yes	Yes	Yes	Yes	0
6c	410.4	134.23	2.99	High	No	Yes	Yes	Yes	Yes	0
6d	378.4	115.77	3.03	High	No	Yes	Yes	Yes	No	0
6e	408.4	134.23	2.99	High	No	Yes	Yes	Yes	Yes	0
6f	407.4	119.01	3.03	High	No	Yes	Yes	Yes	Yes	0
6g	403.4	136	2.44	High	No	Yes	Yes	Yes	No	0
8a	368.4	125.32	2.59	High	No	Yes	Yes	Yes	Yes	0
8b	382.5	114.32	3.44	High	No	Yes	Yes	Yes	Yes	0
8c	398.5	119.45	2.98	High	No	Yes	Yes	Yes	Yes	0
8d	366.5	105.09	3.19	High	No	Yes	Yes	Yes	Yes	0
6e	396.4	123.55	3.27	High	No	Yes	Yes	Yes	Yes	0
8f	395.5	108.33	3.24	High	No	Yes	Yes	Yes	Yes	0
8g	391.5	120.88	2.86	High	No	Yes	Yes	Yes	Yes	0

ADME Prediction

ADME of the newly synthesized compounds were studied via using online free portal www. swissadme.ch to check the possible violation of any drug-like properties (SwissADME 2017). All compounds following the Lipinski's rule of 5 so have potential to be developed as drug. The synthesized compounds are showed high absorption at GI tract, not crossing the blood brain barrier. Solubility of compounds in water and lipid plays a very important role in the distribution of drug. All compounds are lipid soluble and poorly soluble in water except **6a**, **8a**, and **8f** which are moderately soluble in water. But theses have high absorption by GI. The dissolution in vivo will be rate limiting step in drug absorption. Drug metabolism is dependent on cytochrome p450 enzymes. Those drugs that are metabolized by CYP2D6 to inactive metabolites, CYP2D6 inhibitors may result in toxicity. These compounds are not showing inhibitory action to CYP2D6 but these are the inhibitors of rest of the isozymes of cytochrome p450 family (Table 5).

Conclusion

In this work, synthesis and biological screening of new 1,3,4-oxadiazole and benzothiazolyl thioether derivatives of 4-arylmethylidene-3-substituted-isoxazol-5(4H)-one are reported. From the antimycobacterial results, it can be concluded that, compounds with 4-methoxybenzylidene at 4 position and 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)methyl at 3 position of isoxazolone, showed potential antimycobacterial and antibacterial activities. Also, benzothiazolyl thioether at 3 position of isoxazolone and –OH or $–CH_3$ group on 4 position of benzyledene showed good to excellent activity against all the tested strains. It is noteworthy that, compounds **6b** and **8b** could serve as lead molecules, as they reveal good antimycobacterial as well as antibacterial activity. Thus, these results will be enlightening to researchers for further study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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