Tropical Biomedicine 39(3): 467-475 (2022)
https://doi.org/10.47665/tb.39.3.019

RESEARCH ARTICLE

Synthesis, characterization, anti-mycobacterial activity and *in silico* study of new 2,5-disubstituted-1,3,4-oxadiazole derivatives

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ARTICLE HISTORY

Received: 24 June 2022
Revised: 12 September 2022
Accepted: 12 September 2022
Published: 30 September 2022

ABSTRACT

A series of new 2,5-disubstituted-1,3,4-oxadiazole derivatives (**5a-j** and **6a-j**) have been designed and synthesized in four-steps. Sixteen compounds among the twenty compounds are reported for the first time. The compounds were characterized and confirmed by the FTIR, 1D- and 2D-NMR and HRMS analyses, and were tested against *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* H37Ra. Compound **5d** was the most active against *M. smegmatis* with MIC value of 25 µM, and exhibited cidal activity with MBC of 68 µM, respectively. The time-kill assay showed the good killing rate at 77% with the combination of isoniazid (INH). In addition, checkboard assay confirmed the interaction of compound **5d** was categorised as additive. Docking simulation has been performed to position **5d** into the pantothenate synthetase active site with binding free energy value –8.6 kcal mol^{-1}. It also occupied the same active site as that of standard native ligand with similar interactions, which clearly indicate their potential as pantothenate synthetase inhibitor.

Keywords: 2,5-disubstituted-1,3,4-oxadiazoles; anti-mycobacterial; *Mycobacterium smegmatis*; *Mycobacterium tuberculosis* H37Ra; molecular docking.

INTRODUCTION

Tuberculosis (TB) is an infectious respiratory disease caused by *Mycobacterium* tuberculosis (CDC, 2016). This airborne disease is transmitted via droplets formed by coughing, talking, or singing of an infected person. TB is one of the major public health problems worldwide. According to the World Health Organization (WHO), approximately 1.5 million people worldwide died due to TB, and 10 million was infection in 2020 (WHO, 2021). In 2020, South-East Asia Region reported the highest new TB cases, with 43%, followed by African Region (25%) and Western Pacific (18%) (WHO, 2021). This situation are the huge obstacles to achieve the Sustainable Development Goals (SDGs) especially SDG 'Health Goal' (Goal No.3) that recently adopted at the United Nations to "End TB" by 2035 (Lönnroth & Raviglione, 2015).

So far, the only vaccine against TB, Bacillus Calmette-Guerin (BCG) vaccine is unable to extend to adults, thus making susceptible people at higher risk of being infected (CDC, 2016). The rapid increase in multidrug-resistant TB (MDR-TB) supersedes other risk factors. MDR-TB was known to be resistant to at least isoniazid and rifampicin, two of the first-line anti-TB drugs (WHO, 2021). This phenomenon worsens by the emergence of extensively drug-resistant TB (XDR TB) which is resistant to isoniazid and rifampicin, as well as any fluoroquinolone and at least one of three injectable second-line drugs, i.e., amikacin, kanamycin, or capreomycin. Hence, the rampant emergence of MDR-TB and XDR-TB makes the discovery of new drug a priority to prevent the generation of new resistant mycobacterial strains (Koul *et al*., 2011). The discovery and development of new drugs is a continuous process in the effort to combat TB. Many classes of old drugs that were discovered decades ago have now become new antibiotic candidates of chemically re-engineered molecules. The new drugs, which originally derived from the existing drug had undergone remodeling in their chemical structures to improve their anti-bacterial strength.

1,3,4-oxadiazole is better known and widely studied owing to its many significant chemical and biological properties. Substituted 1,3,4-oxadiazole derivatives display various biological activities including antibacterial (Koul *et al*., 2011; Song *et al*., 2018), antimicrobial (Khalilullah *et al*., 2016; Rubab *et al*., 2016; Chortani *et al*., 2020; Hannoun *et al*., 2020), anti-mycobacterial (Navarrete-Vazquez *et al*., 2007; Karabanovich *et al*., 2016; Ambhore *et al*., 2019), antifungal (Wani *et al*., 2015; Shi *et al*., 2020), anti-inflammatory (Abd-Ellah *et al*., 2017; Zheng *et al*., 2020), and anti-viral activities (Li *et al*., 2011b). Among the substituted 1,3,4-oxadiazole derivatives; 5-substituted-1,3,4-oxadiazole-2-thioether continuously draws interest for the development of new

drug moieties and have increasing importance as compounds with biological activities including anti-TB agents. Thus, this current work focused on the modification of 1,3,4-oxadiazole scaffold and the investigation of their anti-mycobacterial activity using *M. smegmatis* and *M. tuberculosis* H37Ra as surrogate TB organisms.

MATERIALS AND METHODS

Chemistry

Unless otherwise noted, materials were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), Acros Organics (Fair Lawn, NJ, USA), and Merck Chemical Co. (Darmstadt, Germany), used without purification. Column chromatography was performed using silica gel 60 (Merck, 40-63 µm). For thin-layer chromatography, TLC aluminium sheets (Merck, silica gel 60 F_{254}) were used. The spots were visualized under UV of 254–366 nm. All reactions were carried out in heat-dried glassware under a dry nitrogen atmosphere unless otherwise stated. All liquids transfer was conducted using standard syringe or cannula techniques. All spectral data were obtained on the following instruments: NMR spectra were obtained using Bruker Advance 500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer system (Bruker Bioscience, Billerica, MA, USA). Data were analyzed via Top Spin 3.6.1 software package. Chemical shifts were internally referred to as the solvent signals in $CDCl_3$ (^{1}H δ 7.26; ^{13}C δ 77.0), acetone-d_6 (^{1}H δ 2.05; ^{13}C δ 29.9 and 206.7), DMSO-d_6 (^{1}H δ 2.50; ^{13}C δ 39.5), and tetramethylsilane (TMS) signal at 0.00 ppm. Chemical shift values are mentioned in δ (ppm) and coupling constants (*J*) are given in Hz. The mass spectra were recorded with a Waters Xevo QTOF MS (Waters Corporation, Milford, MA, USA), and it is reported in *m/z*. The infrared (IR) spectra were obtained through Perkin Elmer FT-IR spectrometer RX1 (Perkin Elmer, Waltham, MA, USA). Melting points were determined on the Buchi B-542 apparatus by an open capillary method and are uncorrected.

General procedure for the synthesis of 2-alkylbenzysulfanyl-5-substituted-1,3,4-oxadiazoles derivatives (5a-j and 6a-j)

A solution of an alkylating agent (1 mmol) and tetrabutylammonium bromide, TBAB (0.05 mmol) in CH_2Cl_2 (5 mL) was added to a solution of the corresponding 5-substituted-1,3,4-oxadiazole-2-thiols (**4a-j**) (1.1 mmol) and sodium hydroxide (1.25 mmol) in H_2O (5 mL). The reaction mixture was stirred slowly at room temperature overnight. Upon completion, the organic layer was separated, washed with water (2 × 7 mL), and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The precipitate obtained was recrystallized from EtOH to afford compounds **5a-j** and **6a-j** (40-92% yields). For the known compounds, the spectroscopic data were compared with literatures.

*2-(Benzylsulfanyl)-5-phenyl-1,3,4-oxadiazole (**5a**)*

Cream powder, 92% yield; mp 116-118 °C; IR υ / cm^{-1} 3192, 3063, 2917, 1654, 1344, 1260, 1070, 691; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.62 (s, 2H, CH_2-H7"), 7.31 (t, *J* 7.5 Hz, 1H, Ar-H4"), 7.38 (t, *J* 7.5 Hz, 2H, Ar-H3", H5"), 7.55 (d, *J* 7.5 Hz, 2H, Ar-H2", H6"), 7.6 (t, *J* 8.0 Hz, 2H, Ar-H3', H5'), 7.62 (t, *J* 8.0 Hz, 1H, Ar-H4'), 8.20 (d, *J* 8.0 Hz, 2H, Ar-H2', H6'); ^{13}C NMR (125 MHz, Acetone-d_6) δ 36.2, 123.8, 126.4, 127.9, 128.7, 129.1, 129.3, 131.8, 136.6, 163.6, 165.6; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{13}N_2OS^+$ [M + H]$^+$: 269.0749, found: 269.0752; All data were compared with the reported literature (Lo Monte *et al*., 2013).

*2-(Benzylsulfanyl)-5-(3'-nitrophenyl)-1,3,4-oxadiazole (**5b**)*

Light orange powder, 55% yield; mp 90-92 °C; IR υ / cm^{-1} 3045, 2978, 1655, 1501, 1385, 1255; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.65 (s, 2H, CH_2-H7"), 7.32 (t, *J* 7.5 Hz, 2H, Ar-H3", H5"), 7.37 (d, *J* 7.5 Hz, 2H, Ar-H2", H6"), 7.38 (t, *J* 7.5 Hz, 1H, Ar-H4"), 7.57 (d, *J* 7.7 Hz, 1H, Ar-H6'), 7.94 (t, *J* 7.7 Hz, 1H, Ar-H5'), 8.43 (dd, *J* 7.7, 1.8 Hz, 1H, Ar-H4'), 8.75 (d, *J* 1.8 Hz, 1H, Ar-H2'); ^{13}C NMR (125 MHz, Acetone-d_6) δ 36.9, 121.6, 125.2, 126.1, 128.3, 128.7, 128.9, 129.2, 130.4, 135.3. 148.6, 163.9, 165.2; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{12}N_3O_3S^+$ [M + H]$^+$: 314.0599, found: 314.0602.

*2-(Benzylsulfanyl)-5-(2'-Amino-3'-chlorophenyl)-1,3,4-oxadiazole (**5c**)*

Brown liquid, 70% yield; IR υ / cm^{-1} 2927, 2859, 1660, 1385, 1255, 1089, 865; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.91 (s, 2H, CH_2-H7"), 6.55 (d, *J* 8.5 Hz, 1H, Ar-H4'), 6.85 (t, *J* 8.5 Hz, 1H, Ar-H5'), 7.2 (m, 1H, Ar-H4"), 7.35 (d, *J* 8.0 Hz, 2H, Ar-H2", H6"), 7.36 (t, *J* 8.0 Hz, 2H, Ar-H3", H5"), 7.70 (d, *J* 8.5 Hz, 1H, Ar-H6'); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.2, 108.4, 115.2, 115.7, 124.3, 124.5, 128.3, 130.5, 133.0, 139.0, 145.5, 152.7, 167.2; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{13}ClN_3OS^+$ [M + H]$^+$: 318.0468, found: 318.0472.

*2-[(Benzylsulfanyl) 1,3,4-oxadiazol-2-yl] 5'-bromophenol (**5d**)*

Light brown powder, 67% yield; mp 97-99 °C; IR υ / cm^{-1} 3397, 3060, 2959, 1622, 1453, 1341, 1107, 748; ^{1}H NMR (500 MHz, $CDCl_3$-d_1) δ 4.58 (s, 2H, CH_2-H7"), 7.06 (d, *J* 8.8 Hz, 1H, Ar-H3'), 7.30 (t, *J* 8.3 Hz, 1H, Ar-H4"), 7.36 (t, *J* 8.3 Hz, 2H, Ar-H3", H5"), 7.49 (d, *J* 8.3 Hz, 2H, Ar-H2", H6"), 7.59 (dd, *J* 8.8, 2.5, Hz, 1H, Ar-H4'), 7.83 (d, *J* 2.5 Hz, 1H, Ar-H6'), 10.66 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$-d_1) δ 36.4, 110.7, 112.2, 119.9, 128.6, 129.1, 129.5, 131.4, 136.2, 137.1, 155.9, 163.7, 163.8; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{12}BrN_2O_2S^+$ [M + H]$^+$: 362.9803, found: 362.9801.

*2-(Benzylsulfanyl)-5-(4'-chlorophenyl)-1,3,4-oxadiazole (**5e**)*

White solid, 81% yield; mp 151-153 °C; IR υ / cm^{-1} 3120, 2878, 1619, 1255, 1089, 865; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.63 (s, 2H, CH_2-H7"), 7.32 (t, *J* 7.5 Hz, 1H, Ar-H4"), 7.37 (t, *J* 7.5 Hz, 2H, Ar-H3", H5"), 7.55 (d, *J* 7.5 Hz, 2H, Ar-H2", H6"), 7.64 (d, *J* 8.7 Hz, 2H, Ar-H3', H5'), 8.03 (d, *J* 8.7 Hz, 2H, Ar-H2' H6'); ^{13}C NMR (125 MHz, Acetone-d_6) δ 36.2, 122.6, 127.9, 128.1, 128.4, 128.7, 129.5, 136.6, 137.3, 163.9, 164.9; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{12}ClN_2OS^+$ [M + H]$^+$: 303.0358, found: 303.0355. All data were compared with the reported literature (Liu *et al*., 2012).

*2-(Benzylsulfanyl)-5-(4'-nitrophenethyl)-1,3,4-oxadiazole (**5f**)*

Brown powder, 81% yield; mp 128-130 °C; IR υ / cm^{-1} 3338, 2928, 1660, 1502, 1385, 1255, 1089, 657; ^{1}H NMR (500 MHz, Acetone-d) δ 3.14 (t, *J* 7.0 Hz, 2H, CH_2-H8'), 3.22 (t, *J* 7.0 Hz, 2H, CH_2-H7'), 4.59 (s, 2H, CH_2-H7"), 7.33 (m, 1H, Ar-H4"), 7.35 (m, 2H, Ar-H2", H6"), 7.37 (d, *J* 8.5 Hz, 2H, Ar-H3", H5"), 7.41 (d, *J* 8.7 Hz, 2H, Ar-H5', H3'), 8.18 (d, *J* 8.7 Hz, 2H, Ar-H2', H6'); ^{13}C NMR (125 MHz, Acetone-d) δ 26.0, 32.0, 36.7, 123.5, 128.1, 128.8, 129.1, 129.7, 135.5, 138.2, 146.8, 164.2, 166.3; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{17}H_{16}N_3O_3S^+$ [M + H]$^+$: 342.0912, found: 342.0909.

*(E)-2-(benzylsulfanyl)-5-(1-phenylprop-1-en-2'-yl)-1,3,4-oxadiazole (**5g**)*

Light yellow powder, 53% yield; mp 118-120 °C; IR υ / cm^{-1} 2935, 1656, 1181, 1063, 852; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.34 (d, *J* 1.4 Hz, 3H, CH_3-H3'), 4.73 (s, 2H, CH_2-H7"), 7.32 (m, 1H, Ar-H4"), 7.38 (t, *J* 7.5 Hz, 1H, Ar-H7'), 7.44 (s, 1H, Ar-H1'), 7.47 (t, *J* 7.5 Hz, 2H, Ar-H6', H8'), 7.53 (d, *J* 7.5 Hz, 2H, Ar-H5', H9'), 7.86 (d, *J* 8.7 Hz, 2H, Ar-H3", H5"), 8.24 (d, *J* 8.7 Hz, 2H, Ar-H2", H6"); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.4, 35.5, 120.9, 123.9, 128.5, 128.6, 129.4, 129.6, 130.1, 134.6, 135.4, 138.1, 164.7, 167.4; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{18}H_{17}N_2OS^+$ [M + H]$^+$: 309.1061, found: 309.1058.

*2-(Benzylsulfanyl)-5-(1'H-indol-2'-yl)-1,3,4-oxadiazole (**5h**)*

Light yellow powder, 60% yield; mp 178-180 °C; IR υ / cm^{-1} 3306, 3120, 2983, 1688, 1309, 1248, 1019, 739; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.48 (s, 1H, CH_2-H7"), 7.01 (t, *J* 8.3 Hz, 1H, Ar-H6'), 7.05 (d, *J* 1.2, 1H, Ar-H9'), 7.15 (m, 1H, Ar-H5'), 7.17 (t, *J* 8.4 Hz, 1H, Ar-H4"), 7.22 (t, *J* 8.4 Hz, 2H, Ar-H3", H5"), 7.39 (d, *J* 8.4 Hz, 2H, Ar-H2", H6"), 7.43 (t, *J* 8.3 Hz, 1H, Ar-H7'), 7.55 (d, *J* 8.1 Hz, 1H, Ar-

H4'), 10.9 (s, 1H, -NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.5, 105.2, 112.1, 120.6, 121.2, 121.5, 124.5, 127.8, 127.9, 128.6, 129.0, 136.5, 138.1, 160.8, 162.9; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{17}H_{14}N_3OS^+$ [M + H]$^+$: 308.0857, found: 308.0855. All data were compared with the reported literature (El Ashry *et al*., 2009).

*2-(Benzylsulfanyl)-5-(2'-naphthalen-1'-ol)-1,3,4-oxadiazole (**5i**)*
Cream powder, 89% yield; mp 108-110 °C; IR υ / cm^{-1} 2962, 2874, 1601, 1514, 1343, 1176, 1049, 702; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.72 (s, 2H, CH_2-H7"), 7.33 (m, 1H, Ar-H4"), 7.35 (d, *J* 8.2 Hz, 1H, Ar-H6'), 7.40 (d, *J* 8.8 Hz, 2H, Ar-H3", H5"), 7.46 (d, *J* 8.2 Hz, 1H, Ar-H8'), 7.52 (d, *J* 8.8 Hz, 2H, Ar-H2", H6"), 7.53 (d, *J* 8.2 Hz, 1H, Ar-H5'), 7.73 (t, *J* 8.2 Hz, 1H, Ar-H7'), 7.83 (d, *J* 8.2 Hz, 1H, Ar-H9'), 8.27 (d, *J* 8.2 Hz, 1H, Ar-H10'); ^{13}C NMR (125 MHz, Acetone-d_6) δ 35.4, 112.0, 124.6, 126.6, 127.5, 127.8, 128.3, 127.5, 128.7, 128.9, 129.0, 129.2, 135.2, 136.5, 152.8, 163.9, 165.1; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{19}H_{15}N_2O_2S^+$ [M + H]$^+$: 335.0854, found: 335.0850.

*2-(Benzylsulfanyl)-5-((1'H-indol-3'-yl)methyl)-1,3,4-oxadiazole (**5j**)*
Dark brown liquid, 70% yield; IR υ / cm^{-1} 3373, 2962, 1699, 1637, 1151, 1038, 743; ^{1}H NMR (500 MHz, Acetone-d_6) δ 2.89 (s, 2H, CH_2-H10'), 4.76 (s, 3H, CH_2-H7"), 7.00 (t, *J* 8.1 Hz, 1H, Ar-H6'), 7.1 (t, *J* 8.1 Hz, 1H, Ar-H7'), 7.22 (s, 1H, Ar-H2'), 7.33 (dd, *J* 8.1 Hz, 1H, Ar-H5'), 7.35 (d, *J* 8.1 Hz, 1H, Ar-H8'), 7.38 (t, *J* 7.5 Hz, 2H, Ar-H3", H5"), 7.39 (m, *J* 7.5 Hz, 1H, Ar-H4"), 7.43 (d, *J* 7.5 Hz, 2H, Ar-H2", H6"); ^{13}C NMR (125 MHz, Acetone-d_6) δ 31.4, 36.3, 106.6, 111.2, 118.9, 119.6, 122.2, 123.0, 128.6, 128.8, 129.0, 129.1, 129.6, 136.1, 167.6, 173.8; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{18}H_{16}N_3OS^+$ [M + H]$^+$: 322.1014, found: 322.1009. All data were compared with the reported literature (Song *et al*., 2021).

*2-(4"-Nitrobenzyl)sulfanyl)-5-phenyl-1,3,4-oxadiazole (**6a**)*
Cream powder, 90% yield; mp 118-120 °C; IR υ / cm^{-1} 3062, 2917, 1654, 1520, 1341, 1260, 1070, 691; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.72 (s, 2H, CH_2-H7"), 7.65 (t, *J* 7.5 Hz, 2H, Ar-H4'), 7.70 (t, *J* 8.2 Hz, 1H, Ar-H3', H5'), 7.78 (d, *J* 8.2 Hz, 2H, Ar-H2', H6'), 7.95 (d, *J* 8.8 Hz, 2H, Ar-H3", H5"), 8.22 (d, *J* 8.8 Hz, 2H, Ar-H2", H6"); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.4, 123.4, 124.1, 126.9, 129.9, 130.8, 132.6, 145.4, 147.4, 163.4, 165.9; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{12}N_3O_3S^+$ [M + H]$^+$: 314.0599, found: 314.0602.

*2-[(4"-Nitrobenzyl)sulfanyl)-5-(3'-nitrophenyl)]-1,3,4-oxadiazole (**6b**)*
Light orange powder, 55% yield; mp 90-92 °C; IR υ / cm^{-1} 3003, 2932, 1655, 1501, 1385, 1255; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.65 (s, 2H, CH_2-H7"), 7.32 (t, *J* 8.1 Hz, 3H, Ar-H3", H5"), 7.37 (d, *J* 8.1 Hz, 2H, Ar-H2", H6"), 7.57 (d, *J* 8.1 Hz, 1H, Ar-H6'), 7.94 (t, *J* 8.1 Hz, 1H, Ar-H5'), 8.43 (dd, *J* 8.1, 1.8 Hz, 1H, Ar-H4'), 8.75 (d, *J* 1.8 Hz, 1H, Ar-H2'); ^{13}C NMR (125 MHz, Acetone-d_6) δ 36.9, 121.6, 125.2, 126.1, 128.3, 128.7, 128.9, 129.2, 130.4, 135.3, 148.6, 163.9, 165.2; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{11}N_4O_5S^+$ [M + H]$^+$: 359.0451, found: 359.0455.

*2-(4"-Nitrobenzyl)sulfanyl)]-5-[(3'-Chloro-2'-aniline)-1,3,4-oxadiazole (**6c**)*
Brown solid, 70% yield; mp 45-48 °C; IR υ / cm^{-1} 2997, 2859, 1660, 1533, 1385, 1255, 1089, 865; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.91 (s, 2H, CH_2-H7"), 6.55 (d, *J* 8.2 Hz, 1H, Ar-H4'), 6.85 (t, *J* 8.2 Hz, 1H, Ar-H5'), 7.66 (d, *J* 8.2 Hz, 1H, Ar-H6'), 7.73 (d, *J* 8.5 Hz, 2H, Ar-H2", H6"), 8.22 (d, *J* 8.5 Hz, 2H, Ar-H3", H5"); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.2, 108.4, 115.2, 115.7, 124.3, 124.5, 128.3, 130.5, 133.0, 139.0, 145.5, 152.7, 167.2; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{12}ClN_4O_3S^+$ [M + H]$^+$: 363.0319, found: 363.0315.

*2-((4"-Nitrobenzyl)sulfanyl)-5-(5'-Bromo-2'-hydroxy)-1,3,4-oxadiazole) (**6d**)*
Light brown crystal powder, 78% yield; mp 129-131 °C; IR υ / cm^{-1} 3395, 2928, 1619, 1520, 1453, 1342, 1107, 748, 696; ^{1}H NMR (500 MHz, $CDCl_3$-d_1) δ 4.52 (s, 2H, CH_2-H7"), 6.94 (d, *J* 8.5 Hz, 1H, Ar-H3'), 7.44 (d, *J* 8.5 Hz, 1H, Ar-H4'), 7.60 (d, *J* 8.5 Hz, 2H, Ar-H3", H5"), 7.70 (d, *J* 8.5 Hz, 1H, Ar-H2", H6"), 8.5 (s, 1H, Ar-H6'); ^{13}C NMR (125 MHz, $CDCl_3$-d_1) δ 35.7, 110.8, 112.2, 119.8, 124.1, 128.6, 130.1, 136.5, 145.6, 147.4, 156.3, 162.9, 164.5; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{11}BrN_3O_4S^+$ [M + H]$^+$: 407.9653, found: 407.9649.

*2-((4"-Nitrobenzyl)sulfanyl)-5-(4'-Chlorophenyl)-1,3,4-oxadiazole (**6e**)*
Yellow powder, 80% yield; mp 110-112 °C; IR υ / cm^{-1} 3120, 2878, 1619, 1525, 1255, 1089, 865; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.47 (s, 2H, CH_2-H7"), 7.41 (d, *J* 8.6 Hz, 2H, Ar-H3", H5"), 7.53 (d, *J* 8.8 Hz, 2H, Ar-H2', H6'), 7.70 (d, *J* 8.8 Hz, 2H, Ar-H3', H5'), 7.97 (d, *J* 8.6 Hz, 2H, Ar-H2", H6"); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 40.1, 127.0, 128.9, 133.5, 134.8, 135.6, 142.0, 150.2, 152.1, 168.4, 169.9; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{15}H_{11}ClN_3O_3S^+$ [M + H]$^+$: 348.0209, found: 348.0205.

*2-((4"-Nitrobenzyl)sulfanyl)-5-(4'-nitrophenethyl)-1,3,4-oxadiazole (**6f**)*
Brown powder. 69% yield; mp 20-122 °C; IR υ / cm^{-1} 3538, 2928, 1660, 1502, 1385, 1255, 1069, 657; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.14 (t, *J* 7.1 Hz, 2H, CH_2-H7'), 3.22 (t, *J* 7.1 Hz, 2H, CH_2-H8'), 4.59 (s, 2H, CH_2-H7"), 7.52 (d, *J* 8.6 Hz, 2H, Ar-H2', H6'), 7.69 (d, *J* 8.6 Hz, 2H, Ar-H3", H5"), 8.13 (d, *J* 8.6 Hz, 2H, Ar-H3', H5'), 8.18 (d, *J* 8.6 Hz, 2H, Ar-H2", H6"); ^{13}C NMR (125 MHz, DMSO-d_6) δ 26.0, 31.4, 35.2, 123.9, 124.1, 130.2, 130.7, 145.4, 146.6, 147.3, 148.4, 162.8, 167.7; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{17}H_{15}N_4O_5S^+$ [M + H]$^+$: 387.0763, found: 387.0759.

*(E)-2-((4"-Nitrobenzyl)sulfanyl)- 5-(1-phenylprop-1'-en-22 -yl)-1,3,4-oxadiazole (**6g**)*
Yellow powder, 68% yield; mp 120-122 °C; IR υ / cm^{-1} 3103, 2935, 1656, 1508, 1181, 1063, 852; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.34 (d, *J* 1.4 Hz, 3H, CH_3-H2'), 4.73 (s, 2H, CH_2-H7"), 7.38 (t, *J* 7.5 Hz, 1H, Ar-H7'), 7.44 (s, 1H, Ar-H3'), 7.47 (t, *J* 7.5 Hz, 2H, Ar-H6', H8'), 7.53 (d, *J* 7.5 Hz, 2H, Ar-H5', H9'), 7.86 (d, *J* 8.7 Hz, 2H, Ar-H3", H5"), 8.24 (d, *J* 8.7 Hz, 2H, Ar-H2", H6"). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.4, 35.5, 120.9, 123.9, 128.5, 128.6, 129.4, 129.6, 130.1, 134.6, 135.4, 138.1, 164.7, 167.4; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{18}H_{16}N_3O_3S^+$ [M + H]$^+$: 354.0912, found: 354.0915.

*2-((4"-Nitrobenzyl)sulfanyl)-5-(1'H-Indol-2'-yl)-1,3,4-oxadiazole (**6h**)*
Light brown powder, 60% yield; mp 180-182 °C; IR υ / cm^{-1} 3307, 3078, 2963, 1684, 1517, 1340, 1245, 1019, 742; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.74 (s, 2H, CH_2-H7"), 7.10 (d, *J* 8.0 Hz, 1H, Ar-H5'), 7.17 (s, 1H, Ar-H9'), 7.26 (t, *J* 8.0 Hz, 1H, Ar-H6'), 7.49 (t, *J* 8.0 Hz, 1H, Ar-H7'), 7.67 (d, *J* 8 Hz, 1H, Ar-H4'), 7.79 (d, *J* 8.5 Hz, 2H, Ar-H3", H5"), 8.21 (d, *J* 8.5 Hz, 2H, Ar-H2", H6"), 12.22 (s, 1H, -NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.6, 105.6, 112.8, 120.9, 122.0, 124.1, 124.8, 127.7, 130.8, 131.2, 138.2, 145.4, 147.4, 161.2, 162.6; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{17}H_{13}N_4O_3S^+$ [M + H]$^+$: 353.0708, found: 353.0710.

*2-((4"-Nitrobenzyl)sulfanyl)-5-(2'-naphthalen-1'-ol)-1,3,4-oxadiazole (**6i**)*
Cream powder, 70% yield; mp 120-121 °C; IR υ / cm^{-1} 2962, 2874, 1601, 1514, 1343, 1176, 1049, 702; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.72 (s, 2H, CH_2-H7"), 7.37 (t, *J* 8.2 Hz, 1H, Ar-H6'), 7.38 (d, *J* 7.8 Hz, 1H, Ar-H9'), 7.53 (t, *J* 8.2 Hz, 1H, Ar-H5'), 7.77 (t, *J* 8.2 Hz, 1H, Ar-H7'), 7.81 (d, *J* 8.8 Hz, 2H, Ar-H3", H5"), 7.96 (d, *J* 8.1 Hz, 1H, Ar-H8'), 8.22 (d, *J* 8.8 Hz, 2H, Ar-H2", H6"), 8.38 (d, *J* 7.8 Hz 1H, Ar-H10'), 10.43 (s, 1H, OH); ^{13}C NMR (125 MHz, Acetone-d_6) δ 35.4, 111.1, 112.8, 124.3, 124.3, 124.5, 126.4, 127.3, 129.0, 129.1, 130.8, 136.2, 145.5, 147.4, 152.9, 163.4, 165.1; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{19}H_{14}N_3O_4S^+$ [M + H]$^+$: 380.0705, found: 380.0703.

2-[(4"-Nitrobenzylsulfanyl)-5-(1'H-indol-3'-yl)methyl)]-1,3,4-oxadiazole ***(6j)***

Dark brown liquid, 40% yield; IR υ / cm^{-1}: 3373, 3122, 2962, 1699, 1637, 1509, 1309, 1151, 1038, 743; ^{1}H NMR (500 MHz, Acetone-d_6) δ 2.89 (s, 2H, CH_2-H10'), 4.76 (s, 3H, CH_2-H7"), 7.00 (t, *J* 8.1 Hz, 1H, Ar-H6'), 7.10 (t, *J* 8.1 Hz, 1H, Ar-H7'), 7.22 (s, 1H, Ar-H2'), 7.33 (d, *J* 8.1 Hz, 1H, Ar-H5'), 7.35 (d, *J* 8.1 Hz, 1H, Ar-H8'), 7.38 (t, *J* 7.6 Hz, 2H, Ar-H3", H5"), 7.43 (d, *J* 7.6 Hz, 2H, Ar-H2", H6"); ^{13}C NMR (125 MHz, Acetone-d_6) δ 31.4, 36.3, 106.6, 111.2, 118.9, 119.6, 122.2, 123.0, 128.6, 128.8, 128.9, 129.1, 129.6, 136.1, 167.6, 173.8; HRMS (TOF-ES$^+$) *m/z*, calcd. for $C_{18}H_{15}N_4O_3S^+$ $[M + H]^+$: 367.0865, found: 367.0867.

Anti-mycobacterial Activity (MIC and MBC evaluations)

Bacterial culture

M. smegmatis (ATCC 14468) and *M. tuberculosis* H37Ra (ATCC 25177), were purchased from American Type Culture Collection (ATCC, USA). Their stock cultures were maintained on Middlebrook 7H10 (M7H10) agar slants supplemented with oleic acid, albumin, dextrose and catalase (OADC) enrichment and in Middlebrook 7H9 (M7H9) broth supplemented with albumin, dextrose and catalase (ADC) enrichment at 4 °C and -25 °C, respectively. The test cultures were activated from the stock cultures by culturing on the M7H10 agar and incubated for two days for *M. smegmatis* and 10 days for *M. tuberculosis* H37Ra at 37 °C in 4% CO_2.

Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the bacterium. 5-substituted-1,3,4-oxadiazole-2-thiol derivatives (**5a-j** and **6a-j**) were screened for anti-mycobacterial MIC values against *M. smegmatis* and *M. tuberculosis* H37Ra. The test was performed by using the TEMA method as previously described in Mohamad *et al*. (2011, 2018) and Wong *et al*. (2021). The test concentrations of the compounds were in the range of 68 µM and 2300 µM. The control drug used for *M. smegmatis* was streptomycin with an initial concentration of 6.25 µg mL^{-1}. Isoniazid was used as the control drug for *M. tuberculosis* H37Ra with an initial concentration of 1.25 µm mL^{-1}. The MIC was determined after the addition of MTT into the microplate. A colour change from yellow to purple was observed and the MIC value was recorded as the lowest concentration of compounds and drugs that remained yellow colour.

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of 5-substituted-1,3,4-oxadiazole-2-thiol derivatives (**5a-j** and **6a-j**) were determined after the MIC evaluation. The MBC is defined as the lowest concentration of an individual compound or drug that is lethal towards the mycobacteria. A loopful of the culture broth from all wells ≥ MIC value in microplates were streaked onto M7H10 agar plates in replicates after initial incubation prior the addition of MTT reagent. The streaking was done by using sterilized inoculating loops. The agar plates were sealed and incubated for 3 days for *M. smegmatis* and 28 days for *M. tuberculosis* H37Ra at 37 °C in 4% CO_2. The growth of mycobacteria was observed. The MBC was interpreted as the lowest concentration, which showed no growth of the mycobacteria.

Drug interaction checkerboard assay of compounds 5c and 5d

Compounds **5c** and **5d** were selected for interaction study with four first-line anti-TB drugs using the checkerboard TEMA assay. The assay was performed in three microtitre plates labelled Microplate 1 (drug dilution), Microplate 2 (compounds dilution) and Microplate 3 (interaction study). The outer of the well of the microtitre plates were filled with sterilised distilled water. A volume of 150 µL of M7H9 broth was added into wells in Microplate 1, except for the first row (Row G). The drug solution at a concentration that was 16 times higher than the MIC concentration was added into Row G and Row F in a volume of 150 µL. A two-fold serial dilution of the drug was performed across the y-axis starting from Row F to Row B. The excess 150 µL solution was discarded from Row B. In Microplate 2, the dilution was carried out similar to that in Microplate 1 with a slight difference. The wells were added with 50 µL of M7H9 broth, except for the first column (Column 2). The compound solution of 50 µL with 16 times higher in MIC concentration was added in Column 2 and Column 3. Two-fold serial dilution of the compound was done across the x-axis starting from Column 3 to Column 7. The excess 50 µL solution from column 7 was discarded.

For the combination study, a volume of 50 µL from Microplates 1 and 2 were transferred into Microplate 3 following their position of the wells. Column 8 until 10 were reserved for drug control. Column 11 was used as a positive control where 100 µL of M7H9 broth was added into all wells. A volume of 100 µL of log-phase bacterial inoculum was then added. The microplates were covered with lids and sealed with parafilm and incubated at 37 °C in 4% CO_2 for 24 hours. A volume of 50 µL of freshly prepared MTT reagent was added into all test wells after 24 hours of incubation. The microplates were sealed and incubated for another 24 hours. A resulting yellow colour in the well indicated that the growth of the mycobacteria was inhibited, while purple colour indicated growth of mycobacteria. The assay was performed in triplicate.

The total fractional inhibitory concentration index (ΣFICI) can be calculated by the following formula:

$$FICI = \frac{MIC_A\ Combination}{MIC_A\ Alone} + \frac{MIC_B\ Combination}{MIC_B\ Alone}$$

Where A = compound, and B = first-line anti-TB drug

The FIC index was used to classify drug interactions between compounds towards the drug (Li *et al*., 2011a; Singh *et al*., 2000). The drug interactions were defined as synergy if the ΣFIC index was ≤ 0.5, additive if the ΣFICI was from 0.5 to 4, and antagonistic if the FICI was > 4.

Time-kill assay of compound 5c and 5d

The cidal effect of the most active compounds **5c** and **5d** in combination with INH on the growth kinetics of *M. smegmatis* was evaluated by performing the time-kill assay (Balouiri *et al*., 2016). Four sets of bottles containing M7H9 broth in duplicates were prepared and labelled. A volume of 0.5 mL of the mycobacterial inoculum at McFarland Standard 2 was added into all bottles. Bottle 1 contained broth and bacterial inoculum mixed with compound **5c** or **5d** at its MIC value; Bottle 2 contained broth and bacterial inoculum with INH at its MIC value; Bottle 3 contained broth and bacterial inoculum in combination with INH and compound **5c** or **5d** at their respective MICs; Bottle 4 (positive control) contained only broth and inoculum. The final total volume of each bottle was 5 mL. The cultured bottles were incubated at 37 °C in 4% CO_2 for 72 hours. Culture samples were taken every 8-hour interval to do colony counts using the drop plate method. A volume of 20 µL of culture sample was drawn out of the bottles for a serial dilution up to 10^{-5}. Then, 10 µL from each dilution (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) was dropped in triplicate onto two sets of M7H10 agar plates. The droplets were left to dry at room temperature then the plates were sealed and incubated at 37 °C in 4% CO_2 for 72 hours. The colonies formed on the agar were counted and colony-forming unit (CFU) per mL was calculated. The results plotted as a percentage of colonies compared to the time 0 versus time.

Molecular docking studies

In an effort to further elucidate the possible mechanism by which the newly synthesized oxadiazole derivatives could exhibit their antimycobacterial activity, molecular docking studies on *M. tuberculosis* target enzyme pantothenate synthetase was carried out. The docking protocol has been carried out using AutoDock 4.2 to identify appropriate binding modes and conformation of the ligand molecules. The X-ray crystal structures of the target enzyme pantothenate synthetase (PDB code: 3IVX, resolution-1.73) was retrieved from the PDB and used for molecular modelling studies (Hung *et al*., 2009). A dataset of the target compounds was sketched using ChemDraw Ultra 13.0 and converted into 3D structures using Hyperchem Pro 8.0 software (www.hyper.com). AutoDock tools (ADT) version 1.5.6 (www.autodock.scrips.edu) was used to prepare molecular docking. The grid box size was set to a dimension of 15.13'17.85'–3.57 in x, y, z coordinates to cover the active site of the enzyme while virtual screening was performed by AutoDock 4.2.5.1. The best binding conformation was selected from the docking log (.dlg) file for each ligand and further interaction analysis was done using PyMol and Discovery Studio Visualizer 4.0 (Osman *et al*., 2017).

RESULTS AND DISCUSSION

Chemistry

The new series of 2,5-disubstituted-1,3,4-oxadiazole derivatives (**5a-j** and **6a-j**) was achieved as outlined in Scheme 1 in four-step reaction. The final products were synthesized in moderate to good yields between 40 92%. The chemical structures of the target compounds were fully characterized by the FTIR, HRMS analyses and with 1D- and 2D-NMR spectroscopic data.

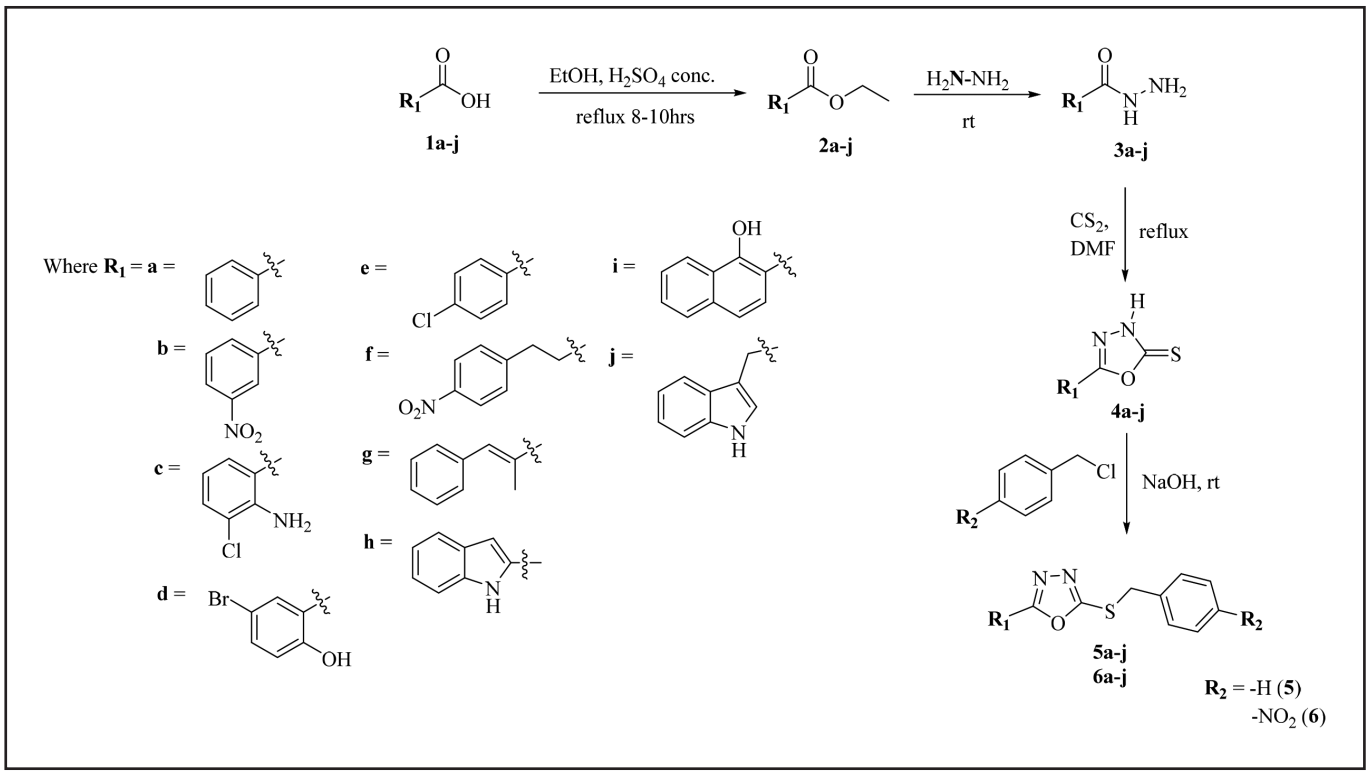
Anti-mycobacterial activity

Twenty 2,5-disubstituted-1,3,4-oxadiazole derivatives (**5a-j** and **6a-j**) were screened for anti-mycobacterial activity against two surrogates for *M. tuberculosis* species, namely *M. smegmatis* and *M. tuberculosis* H37Ra using the TEMA method. Their MIC and MBC values are shown in Table 1 with streptomycin (STR) and isoniazid (INH) as the control drugs.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of **5a-j** and **6a-j** against *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* H37Ra

Compounds	MIC and MBC (µM)			
	Mycobacterium smegmatis		*Mycobacterium tuberculosis* H37Ra	
	MIC	MBC	MIC	MBC
5a	186	NC	NI	–
5b	510	NC	NI	–
5c	50	78	NI	–
5d	25	68	275	NC
5e	165	NC	NI	–
5f	NI	–	NI	–
5g	NI	–	NI	–
5h	162	NC	NI	–
5i	299	NC	299	NC
5j	NI	–	NI	–
6a	1276	NC	319	NC
6b	279	NC	NI	–
6c	137	NC	NI	–
6d	244	NC	NI	–
6e	2300	NC	1152	NC
6f	NI	–	NI	–
6g	NI	NC	NI	–
6h	1135	NC	568	NC
6i	2110	NC	263	NC
6j	NI	–	NI	–
Control drugs	**Streptomycin (STR) 2.69 nM (1.56 µg mL^{-1})**		**Isoniazid (INH) 0.57 nM (0.08 µg mL^{-1})**	

"NI": No inhibition even at the highest test concentration of 2300 µM.
"NC": No cidal effect even at the highest test concentration of 2300 µM.
"–": Not tested.
MICs of control drugs were consistent againts test *Mycobacterium* species throughout the study, and tested in duplicate and independently at least twice.



Scheme 1. Synthetic pathway for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles.

Out of the aforementioned twenty synthesized compounds, fourteen compounds exhibited activity against *M. smegmatis* with MIC values in the range of 68 µM and 2300 µM. Compounds **5c** and **5d** were the most active compounds with the lowest MIC values of 50 µM and 25 µM, respectively. Three other compounds: **5e**, **5h**, and **6c** also exhibited moderate activity with MICs of 165 µM, 162 µM and 137 µM, respectively. Whilst none of the test compounds showed promising activity against *M. tuberculosis* H37Ra.

Based on the MIC results, the fourteen compounds (**5a-e**, **5h**, **5i**, **6a-e**, **6h** and **6i**) were further evaluated for their cidal activity (MBC) against *M. smegmatis*, while compounds **5d**, **5i**, **6a**, **6e**, **6h** and **6i** were evaluated against *M. tuberculosis* H37Ra. The MBC values show that only two compounds, **5c** and **5d** have cidal activity against *M. smegmatis* at the tested concentration with MBCs of 78 µM and 68 µM, respectively. The remaining compounds did not show any cidal effects even at the highest test concentration of 2300 µM. Hence, compounds **5c** and **5d** were selected for further investigation.

The results of the *in vitro* anti-mycobacterial evaluation revealed that almost all compounds bearing the benzylsulfanyl-1,3,4-oxadiazole moiety (**5a-j**), exhibited good activity against *M. smegmatis*. The results suggest that the incorporation of the benzylsulfanyl moiety is necessary for good anti-mycobacterial activity against *M. smegmatis* as reported by Karabanovich *et al*. (2016). However, the 4-nitrobenzyl derivatives (**6a-j**) exhibited poor anti-mycobacterial effects. The anti-mycobacterial potencies of the 2-benzylsulfanyl-5-substituted-1,3,4-oxadiazole derivatives in series **5** were found to be considerably higher than those of 2(4-nitrobenzylsulfany)-5-substituted-1,3,4-oxadiazole derivatives in series **6**. Furthermore, the halogen groups such as bromine in the benzene ring of the 1,3,4-oxadiazole core appeared to have a negligible influence on the antimycobacterial activities. Whereas compounds with aliphatic substituents at R_1 position i.e **5f-g**, **5j**, **6f-g** and **6j** were not active against *M. smegmatis* even at the highest test concentration of 2300 µM.

Drug interaction of compounds 5c and 5d

The most active compounds **5c** and **5d** were analyzed further for their interactions with four first-line anti-TB drugs: rifampicin (RIF), isoniazid (INH), streptomycin (STR), and ethambutol (EMB) by using the checkerboard method. The ΣFICI showed that all combinations of **5c** and **5d** produced an additive interaction with the first-line drugs against *M. smegmatis* as shown in Table 2 (Rand *et al*., 1993; Bollenbach *et al*., 2015). The additive interaction of compounds **5c** and **5d** could be due to the presence of amino (NH_2) and hydroxy (OH) group, respectively. These substituents create a secure molecular structure, which give a rise to a very complex hyperfine structure, thus increasing the reactivity of the aromatic ring based on the electron donating effect. Therefore, it could be inferred that halogen, amino, and hydroxyl groups in compounds **5c** and **5d** could enhance the anti-mycobacterial activity of the oxadiazole core.

Time-kill evaluation of compounds 5c and 5d

Time-kill kinetic study was carried out to assess the killing rates of compounds **5c** and **5d** alone, and in combination with INH on the growth of *M. smegmatis.* INH and compound **5c** alone and compound **5c** in combination with INH showed a similar trend with no significant difference in the rate of killing (Figure 1). The treatments immediately caused a killing rate of 30% at 16 hours, then the colony counts drastically decreased to 12% until the end of the study period at 72 hours. A killing rate of 88% was achieved at the end of the study in all treatments groups.

The results of *in vitro* time-kill assay for compound **5d** in combination with INH against *M. smegmatis* are showed in Figure 2. Similar to **5c**, the combination of compound **5d** with INH at their respective MICs caused a gradual decrease by 58.81% (41.18% killing rate) in the colony counts at 48th hour, then slightly decrease until the end of the study period 72nd hour (Figure 2). In summary, **5c** and **5d** were shown to be effective in inhibiting the growth of *M. smegmatis* when in combination with INH as similar to the individual compounds.

Table 2. Interaction of compounds **5c** and **5d** with first-line anti-tuberculosis drugs against *M. smegmatis*

Combination	ΣFICI	Interaction
5c + Rifampicin	2.5	Antagonistic
5c + Isoniazid	0.625	Additive
5c + Streptomycin	1.125	Transition
5c + Ethambutol	1.125	Transition
5d + Rifampicin	1.125	Transition
5d + Isoniazid	0.625	Additive
5d + Streptomycin	1.5	Transition
5d + Ethambutol	1.5	Transition

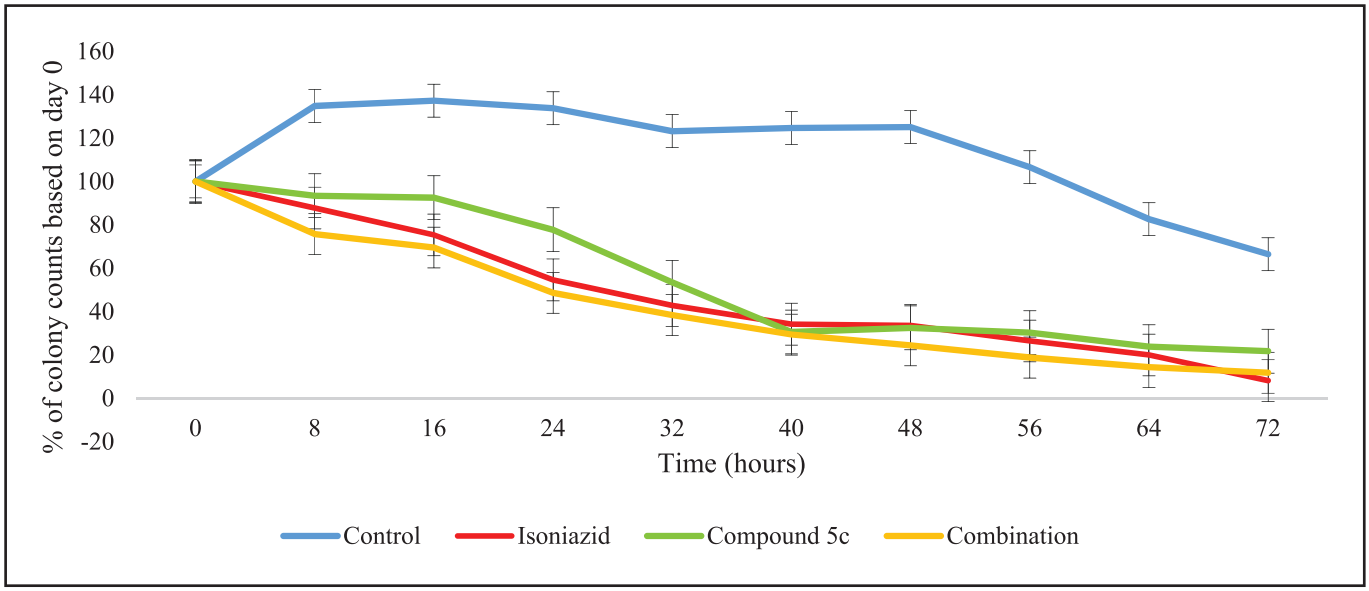


Figure 1. Time-kill curves of Mycobacterium smegmatis treated with compound **5c** (MIC: 25 µg/mL), isoniazid (MIC: 25 µg mL^{-1}, and compound **5c** (MIC: 25 µg mL^{-1}) in combination with isoniazid (MIC: 25 µg mL^{-1}) over a period of 72 hours.

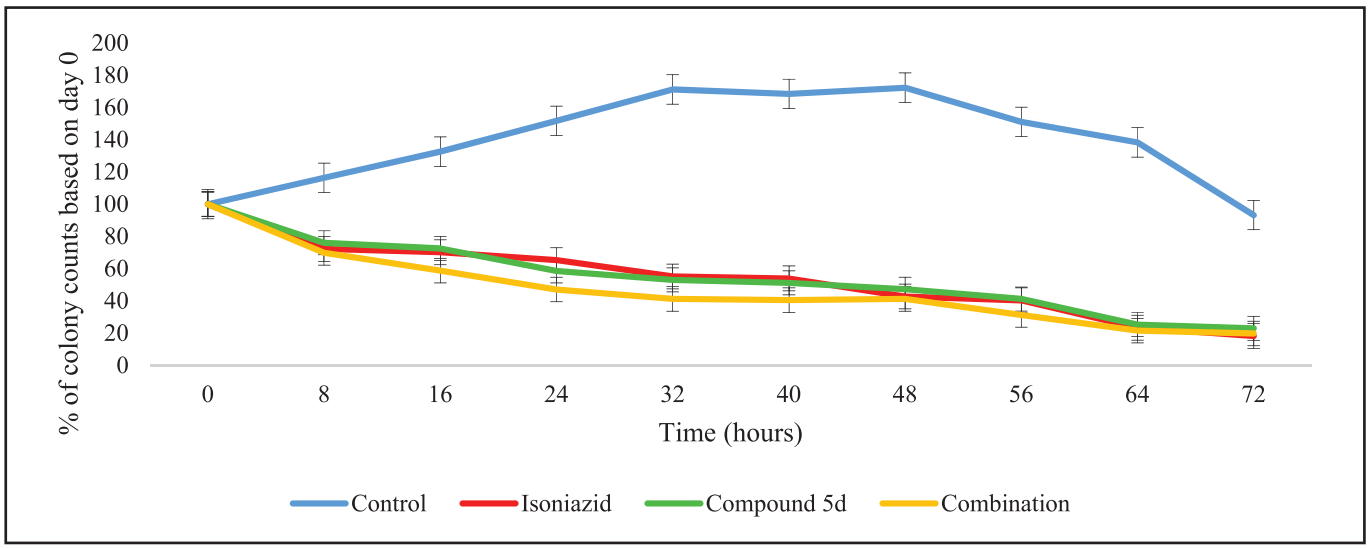


Figure 2. Time-kill curves of *Mycobacterium smegmatis* treated with compound **5d** (MIC: 25 µg mL^{-1}), isoniazid (MIC: 25 µg mL^{-1}) and compound **5d** (MIC: 25 µg mL^{-1}) in combination with isoniazid (MIC: 25 µg mL^{-1}) over a period of 72 hours.

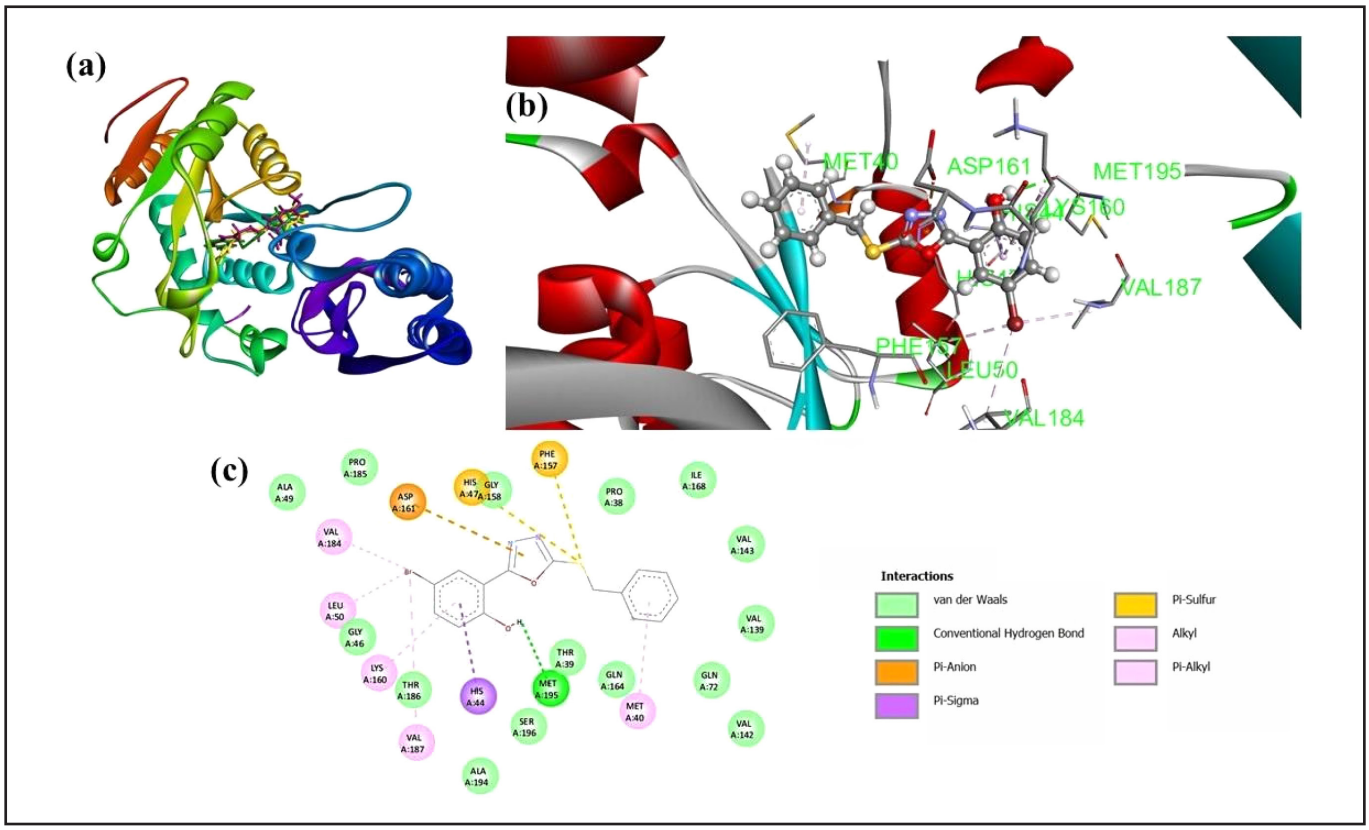


Figure 3. Figure showing the results of docking studies of oxadiazole derivatives at the active site of pantothenate synthetase (PDB ID: 3IVX). (a) Compound **5c** (green), **5d** (yellow) and native ligand (magenta) at the active site of pantothenate synthetase. (b) compound **5d** at the active site of pantothenate synthetase showing one hydrogen bond (green line). (c) 2D binding interactions of compound **5d** showing different types of interactions.

***In-silico* molecular docking studies**

To further investigate the mechanism of promising anti-mycobacterial activities of compounds **5c** and **5d**, molecular docking studies were performed on the enzyme pantothenate synthetase using AutoDock program. The enzyme pantothenate synthetase is a validated drug target in *M. tuberculosis* and is an indispensable for the bacterial cell growth. The crystal structure of pantothenate synthetase in complex with native ligand, 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1*H*-indol-1-yl)acetic acid was retrieved from the RCSB repository (PDB ID: 3IVX) [20]. Molecular docking studies were performed using Lamarckian genetic algorithm-implemented program and the procedure was validated by redocking the native ligand at the active site (RMSD 2.0). Results of molecular docking studies showed that the binding free energies of compounds **5c**, **5d** and native ligand were -8.4 kcal mol^{-1}, -8.6 kcal mol^{-1} and -10.3 kcal mol^{-1}, respectively. Both the compounds **5c** and **5d** occupied the same active site as that of standard native ligand with similar interactions. This clearly indicates that the oxadiazole derivatives are promising inhibitors of pantothenate synthetase. The docked conformations of the two ligands **5c** and **5d** bound to the active site of pantothenate synthetase are shown in Figure 3. Detailed interactions studies of compound **5d** showed that it formed one

hydrogen bond with amino acid Met195. The oxadiazole ring formed a π-anion interaction with the Asp161, phenyl ring formed a π-sigma bonding, whereas the terminal benzyl ring formed a π-alkyl bonding. The thioether linkage formed two π-sulphur bonds with the His47 and Phe157 amino acids. The other active site residues Leu50, Val184 and Val187 also formed alkyl interactions with the bromo group. Thus, the antimycobacterial effect of oxadiazole derivatives could be due to the inhibition of pantothenate synthetase.

CONCLUSION

A new 2,5-disubstituted-1,3,4-oxadiazole derivatives (**5a-j** and **6a-j**) were synthesized with moderate to good yields (40-92%). Anti-mycobacterial activity of synthesized compounds was evaluated against surrogate tubercle organism (*M. smegmatis* and *M. tuberculosis* H37Ra). Among them, only **5c** and **5d** exhibited potent inhibitory effect against *M. smegmatis* with MBC values of 78 µM and 68 µM, respectively. The time-kill assay further showed that the killing rates of combination (**5c** or **5d** with INH) and drug alone (**5c** or **5d** or INH) towards the growth of *M. smegmatis* were essentially the same (77–82%). Hence, the time-kill assay supported the finding of the checkerboard assay, in which the interaction was categorized as additive. Therefore, compounds **5c** and **5d** showed promising potential as anti-tuberculosis drug lead worthy of further investigation.

ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support from the Universiti Sains Malaysia USM RUI grant (1001/PKIMIA/8011072) and Ministry of Higher Education of Malaysia (MyBrain15 Scheme–MyMaster).

Conflict of Interest

The authors declare that they have no conflict of interest.

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