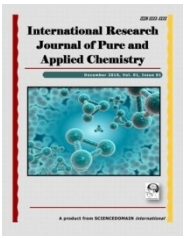

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Biological Evaluation and Spectral Studies of Asymmetrical 3,5-Disubstituted-1,2,4-Oxadiazoles

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Authors' contributions

This work was carried out in collaboration with all authors. All the authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

In present communication, we report the synthesis of 3,5-disubstituted-1,2,4-oxadiazoles derivatives from amino acid derivative with excellent 80-95% yields via a fast and easy procedure under mild conditions. Structures of all the newly synthesized compounds were elucidated based on their elemental analyses and various spectroscopic methods. The in vitro activities of these compounds against bacteria and fungi were evaluated by the well diffusion and the minimum inhibitory concentration (MIC) methods. Some of the synthesized derivatives were found active as compare to standard drugs.

Keywords: 4,5,6,7-Tetrahydrothieno [3,2-c] pyridine; amino acids; amidoximes; chiral-1,2,4-oxadiazoles; antimicrobial activities.

1. INTRODUCTION

1,2,4-oxadiazole is widely used as biologically active heterocyclic compounds, and is often used in drug discovery, bioisosteric replacements of esters and amides, [1] and as dipeptide mimetics [2] in a number of pharmacologically important molecules. They can also be found

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in a number of biologically important drugs, such as muscarinic agonists, [3] serotoninergic (5-HT3) antagonists, [4] benzodiazepine receptor agonists and dopamine ligands. [5] Numerous 1,2,4-oxadiazoles have been suggested as potential agonists for cortical muscarinic, [6] benzodiazepine, [7] and 5-HT1D (5-hydroxytryptamine) receptors [8] and histamine H3 receptors. [9] They show their activity as antirhinoviral agents, growth hormone secretagogues, [10] anti-inflammatory agents [11] and antitumor agents. [12] They also inhibit the SH2 domain of tyrosine kinase, [13] monoamine oxidase, [14] human nuetrophil elastase [15] and human DNA topoisomerases. [16] Tropane derivatives of 1,2,4-oxadiazoles display high affinity for the cocaine binding site of the dopamine transporter. [5]

4,5,6,7-Tetrahydrothieno [3,2-c]pyridine [THTP] and its derivatives, e.g. clopidogrel [17] and ticlopidine, [18] are well acknowledged for their non-cytotoxic and complement inhibition properties. A series of THTPs have been evaluated for their human phenylethanolamine N-methyltransferase inhibitory potency and affinity for the α2-adrenoceptor. [19].

Looking to the unique medicinal applications of both 1,2,4-oxadiazole and THTP compounds, our main concern was to synthesize such compounds that show higher biological activities. Hence, the present communication comprises the mild one-pot condensation of amidoximes with various aromatic aldehydes to synthesize series of 1,2,4-oxadiazoles (compounds 5a-h). Amidoxime was synthesized from THTP and amino acids.

2. MATERIALS AND METHODS

The elemental analyses were performed by Vario EL CHN elemental analyzer. The FT-IR spectra were recorded on Perkin Elmer Spectrum GX spectrophotometer using KBr pellets. The ^{1}H, ^{13}C NMR, and DEPT-135 spectra were recorded on Bruker 400 MHz instrument using DMSO-d6 as solvent. The MS-CI spectrum were recorded on Shimadzu LC–MS 2010 eV spectrometer in acetonitrile. The melting points were checked by standard open capillary method and are found uncorrected.

2.1 Synthesis of 2-[2-(6,7-Dihydrothieno[3,2-c]Pyridin-5(4H)-yl)-2-Oxoethyl Amino] Propanenitrile (3)

An equimolecular mixture of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride **1** (0.01 mol/1.755 g) and 2-(2-cyanopyrrolidin-1-yl)acetyl chloride **2** (0.01 mol/1.726 g) was dissolved in anhydrous acetonitrile (20 mL) using few drops of triethylamine (TEA) as base under constant stirring, and the reaction mixture was refluxed for 2.5 h. After completion of the reaction, reaction mixture was poured in ice-cold water to obtain a light yellow product, which was filtered, air dried and purified by column chromatography (petroleum ether: ethanol/ 30:70) and recrystallized from ethanol to afford a white crystalline compound designated as **3**. Yield: 83 %; m.p. 160-163ºC; IR (KBr, cm^{-1}): 3187 (-NH), 3082 (C-H str., aromatic), 2862 (C-H str. aliphatic), 2205 (-CN), 1665 (>C=O of amide), 1543 (C=C, asymmetric, str.), 1478-1474 (C=C str. ring), 1226 (C-N str.), 753 (C-H def, aromatic), 724 (C-S-C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.18 (3H, d, J = 6.2Hz, H12), 2.65 (2H, t, J = 6.0Hz, H7), 3.59 (2H, t, J = 6.0Hz, H6), 3.73 (2H, s, H4), 3.90 (2H, J = 7.2, d, H9), 4.42 (1H, m, H11), 6.70 (1H, br, NH), 6.84 (1H, d, J = 7.2Hz, H3), 7.12 (1H, d, J = 7.2Hz, H2); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 20.98, 24.40, 37.92, 42.51, 43.32, 48.48, 113.20, 118.60, 119.12, 130.43, 132.23, 168.00. Anal. Calcd. for $C_{12}H_{15}N_3OS$: C, 57.18; H, 6.06; N, 16.85; S, 12.86. Found: C, 57.13; H, 5.98; N, 16.76; S, 12.75; LC-MS (m/z): 249.33.

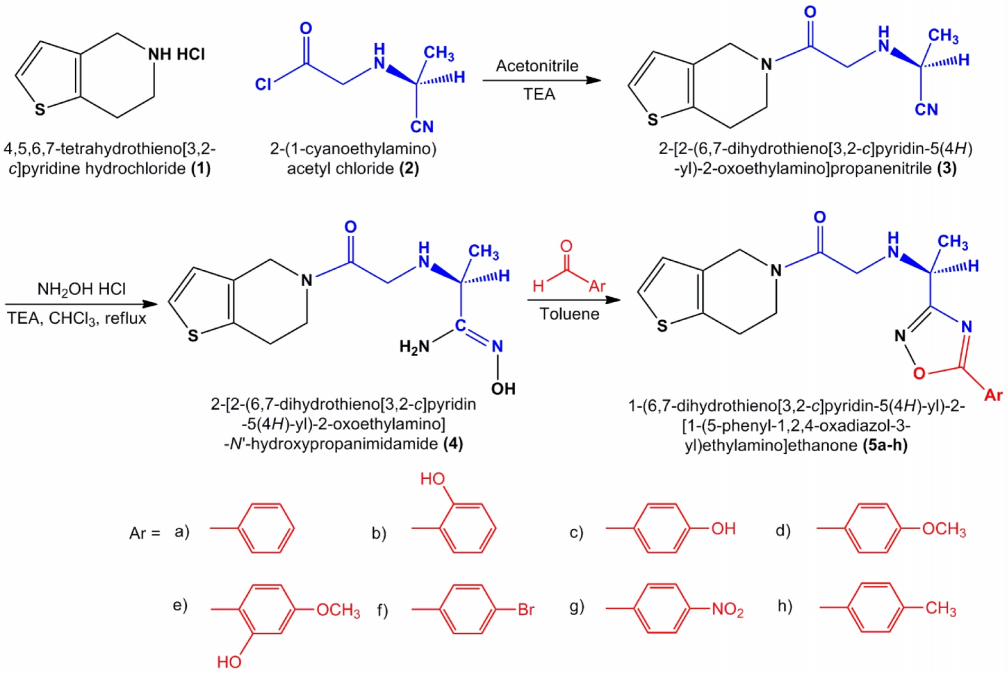


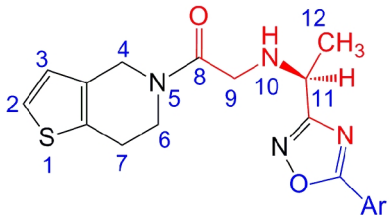
Fig. 1. Synthesis of 1,2,4-oxadiazoles 5a-h

2.2 Synthesis of 2-[2-(6,7-Dihydrothieno[3,2-c]Pyridin-5(4H)-yl)-2-Oxoethyl Amino]-N'-Hydroxypropanimidamide (4)

Amidoximes were prepared by the condensation of carboximidamide **3** (0.01 mole/2.753 g) with hydroxylamine hydrochloride (0.01 mole/0.695 g) in $CHCl_3$ (18 mL), the reaction was performed at room temperature for overnight in the presence of triethylamine (catalytic amount/few drops). After completion of the reaction, the product was filtered and washed with $CHCl_3$ followed by hot water and purified by column chromatography (petroleum ether: chloroform/40:60) then recrystallized from chloroform to give white crystalline solid product designated as **4**. Yield: 82 %; m.p. 172-175ºC; IR (KBr, cm^{-1}): 3480 (O-H, oxime), 3208, 1628 (NH_2), 3084 (C–H str., aromatic), 1672 (C=N, oxime), 1667 (>C=O of amide), 1544 (C=C, asymmetric, str.), 1478–1474 (C=C str. ring), 1227 (C–N str.), 753 (C–H def, aromatic), 728 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.09 (3H, d, J = 6.9Hz, H12), 2.70 (2H, t, J = 6.0Hz, H7), 3.52 (2H, t, J = 6.0Hz, H6), 3.64 (2H, s, H4), 3.86 (2H, J = 7.2, d, H9), 4.34 (1H, m, H11), 6.67 (1H, br, NH), 6.76 (1H, d, J = 7.2 Hz, H3), 7.10 (1H, d, J = 7.2 Hz, H2), 10.78 (1H, s, OH), 11.07 (2H, br, NH_2); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 19.98, 23.47, 38.21, 40.51, 41.32, 47.28, 118.67, 119.09, 130.19, 132.02, 153.00, 173.23. Anal. Calcd. for $C_{12}H_{18}N_4O_2S$: C, 51.04; H, 6.43; N, 19.84; S, 11.36. Found: C, 50.95; H, 6.35; N, 19.76; S, 11.28; LC-MS (m/z) : 282.36.

2.3 General Procedure for the Synthesis of 1-(6,7-Dihydrothieno[3,2-c] Pyridine-5(4H)-yl)-2-[1-(5-Phenyl-1,2,4-Oxadiazol-3-yl)Ethylamino] Ethanone (5a-h)

The amidoxime **4** (0.01 mol/3.084 g) was dissolved in 200 mL of dry toluene. Various aromatic aldehyde **a-h** (0.01mol) was added to this solution with a small quantity of p-toluenesulfonic acid (PTSA) as a catalyst. The mixture was refluxed for 2-3 h with azeotropic water removal until the amidoxime was consumed (as monitored by thin-layer chromatography). After cooling to room temperature, the solution was washed with water (25 mL). The aqueous layer was extracted with chloroform (3×50 mL). The organic extract was passed through anhydrous $CaCl_2$. Thus obtained solution was filtered, concentrated in vacuum, and the residue obtained was purified by column chromatography on silica gel (5% ethyl acetate × 25% chloroform × 70% pet ether) to afford 1,2,4-oxadiazole derivatives designated as **5a-h**. It was recrystallized from chloroform × pet ether (7:3).



1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-phenyl-1,2,4-oxadiazol-3-yl)ethyl amino]ethanone (5a): Yield: 57 %; m.p. 184-187ºC; IR (KBr, cm^{-1}): 1667 (>C=O of amide), 1607 (C=N, oxadiazole), 1227 (C–N str.), 1220 (C-O, oxadiazole), 912 (N-O, oxadiazole), 752 (C–H def, aromatic), 728 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.28 (3H, d, J=6.4Hz, H12), 2.76 (2H, t, J=5.6Hz, H7), 3.48 (2H, t, J=5.6Hz, H6), 3.73 (2H, s, H4), 3.80 (2H, J=7.2, d, H9), 4.12 (1H, m, H11), 6.02 (1H, br, NH), 6.78 (1H, d, J=7.2 Hz, H3), 7.08 (1H, d, J=7.2 Hz, H2), 7.23-7.56 (5H, m, ArH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 21.43, 24.42, 44.56, 48.26, 48.42, 57.21, 124.43, 125.91, 126.32, 129.14, 132.08, 135.43, 148.64, 152.20, 169.04; Anal. Calcd. for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21; S, 8.70. Found: C, 61.89; H, 5.51; N, 15.17; S, 8.73; LC-MS (m/z): 368.45.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-(2-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)ethylamino]ethanone (5b): Yield: 66 %; m.p. 178-179ºC; IR (KBr, cm^{-1}): 3309 (-O-H str.), 1660 (>C=O of amide), 1602 (C=N, oxadiazole), 1227 (C-O, oxadiazole), 1224 (C–N str.), 906 (N-O, oxadiazole), 750 (C–H def, aromatic), 724 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.26 (3H, d, J=6.4Hz, H12), 2.75 (2H, t, J=5.6Hz, H7), 3.51 (2H, t, J=5.6Hz, H6), 3.72 (2H, s, H4), 3.78 (2H, J=7.2, d, H9), 4.09 (1H, m, H11), 6.13 (1H, br, NH), 6.80 (1H, d, J=7.2 Hz, H3), 7.02 (1H, d, J=7.2 Hz, H2), 7.20-7.55 (4H, m, ArH), 10.18 (1H, s, -OH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 22.47, 25.40, 43.36, 48.56, 49.40, 56.28, 125.47, 126.90, 126.72, 129.44, 132.28, 136.43, 148.60, 153.20, 169.14; Anal. Calcd. for $C_{19}H_{20}N_4O_3S$: C, 59.36; H, 5.24; N, 14.57; S, 8.34. Found: C, 59.40; H, 5.21; N, 14.53; S, 8.37; LC-MS (m/z): 384.45.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)ethylamino]ethanone (5c): Yield: 75 %; m.p. 192-195ºC; IR (KBr, cm^{-1}): 3285 (-O-H str.), 1662 (>C=O of amide), 1611 (C=N, oxadiazole), 1229 (C–N str.), 1217 (C-O, oxadiazole), 908 (N-O, oxadiazole), 753 (C–H def, aromatic), 726 (C–S–C str., thiophene);

^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.28 (3H, d, J=6.4Hz, H12), 2.77 (2H, t, J=5.6Hz, H7), 3.48 (2H, t, J=5.6Hz, H6), 3.70 (2H, s, H4), 3.80 (2H, J=7.2, d, H9), 4.12 (1H, m, H11), 6.02 (1H, br, NH), 6.78 (1H, d, J=7.2 Hz, H3), 7.08 (1H, d, J=7.2 Hz, H2), 7.20-7.55 (5H, m, ArH), 10.24 (1H, s, -OH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 22.23, 24.40, 45.51, 46.85, 48.53, 57.36, 125.43, 125.98, 126.12, 129.24, 132.28, 135.17, 148.27, 151.20, 170.02; Anal. Calcd. for $C_{19}H_{20}N_4O_3S$: C, 59.36; H, 5.24; N, 14.57; S, 8.34. Found: C, 59.39; H, 5.28; N, 14.61; S, 8.30; LC-MS (m/z): 384.45.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-(4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)ethylamino]ethanone (5d): Yield: 70 %; m.p. 176-178ºC; IR (KBr, cm^{-1}): 3326 (-O-H str.), 1660 (>C=O of amide), 1613 (C=N, oxadiazole), 1233 (C–N str.), 1214 (C-O, oxadiazole), 911 (N-O, oxadiazole), 750 (C–H def, aromatic), 724 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.23 (3H, d, J = 6.4Hz, H12), 2.70 (2H, t, J=5.6Hz, H7), 3.41 (2H, t, J=5.6Hz, H6), 3.70 (2H, s, H4), 3.82 (2H, J=7.2, d, H9), 3.92 (3H, s, -OCH_3), 4.23 (1H, m, H11), 6.12 (1H, br, NH), 6.78 (1H, d, J=7.2 Hz, H3), 7.08 (1H, d, J=7.2 Hz, H2), 7.23-7.56 (5H, m, ArH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 21.13, 24.52, 44.57, 48.20, 48.52, 57.36, 124.30, 125.87, 126.12, 129.34, 132.08, 135.52, 148.44, 153.00, 169.21; Anal. Calcd. for $C_{20}H_{22}N_4O_3S$: C, 60.28; H, 5.56; N, 14.06; S, 8.05. Found: C C, 60.31; H, 5.52; N, 14.02; S, 8.11; LC-MS (m/z): 398.48.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-(2-hydroxy-4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)ethylamino]ethanone (5e): Yield: 74 %; m.p. 224-228ºC; IR (KBr, cm^{-1}): 1664 (>C=O of amide), 1617 (C=N, oxadiazole), 1231 (C–N str.), 1214 (C-O, oxadiazole), 903 (N-O, oxadiazole), 757 (C–H def, aromatic), 729 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.29 (3H, d, J = 6.4Hz, H12), 2.77 (2H, t, J = 5.6Hz, H7), 3.42 (2H, t, J = 5.6Hz, H6), 3.76 (2H, s, H4), 3.84 (2H, J = 7.2, d, H9), 3.92 (3H, s, -OCH_3), 4.23 (1H, m, H11), 6.09 (1H, br, NH), 6.78 (1H, d, J = 7.2 Hz, H3), 7.12 (1H, d, J = 7.2 Hz, H2), 7.25-7.50 (5H, m, ArH), 10.36 (1H, s, -OH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 23.43, 25.42, 45.56, 48.26, 49.42, 58.21, 125.43, 126.91, 127.32, 128.14, 133.08, 136.47, 148.24, 150.21, 168.87; Anal. Calcd. for $C_{20}H_{22}N_4O_4S$: C, 57.96; H, 5.35; N, 13.52; S, 7.74. Found: C, 57.92; H, 5.38; N, 13.49; S, 7.70; LC-MS (m/z): 414.48.

2-[1-(5-(4-bromophenyl)-1,2,4-oxadiazol-3-yl)ethylamino)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl]ethanone (5f): Yield: 62 %; m.p. 179-182ºC; IR (KBr, cm^{-1}): 1664 (>C=O of amide), 1614 (C=N, oxadiazole), 1223 (C-O, oxadiazole), 1216 (C–N str.), 1019 (C-Br str.), 923 (N-O, oxadiazole), 748 (C–H def, aromatic), 727 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.29 (3H, d, J=6.4Hz, H12), 2.76 (2H, t, J=5.6Hz, H7), 3.48 (2H, t, J=5.6Hz, H6), 3.73 (2H, s, H4), 3.80 (2H, J=7.2, d, H9), 4.12 (1H, m, H11), 6.07 (1H, br, NH), 6.78 (1H, d, J=7.2 Hz, H3), 7.13 (1H, d, J=7.2 Hz, H2), 7.30-7.65 (5H, m, ArH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 20.42, 23.42, 44.56, 48.21, 48.42, 57.08, 125.43, 125.87, 126.30, 129.17, 132.00, 135.44, 148.34, 152.22, 169.14; Anal. Calcd. for $C_{19}H_{19}BrN_4O_2S$: C, 51.01; H, 4.28; Br, 17.86; N, 12.52; S, 7.17. Found: C, 51.06; H, 4.31; Br, 17.88; N, 12.48; S, 7.21; LC-MS (m/z): 447.35.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-(4-nitrophenyl)-1,2,4-oxadiazol-3-yl)ethylamino]ethanone (5g): Yield: 53 %; m.p. 204-206ºC; IR (KBr, cm^{-1}): 1658 (>C=O of amide), 1608 (C=N, oxadiazole), 1221 (C–N str.), 1216 (C-O, oxadiazole), 904 (N-O, oxadiazole), 754 (C–H def, aromatic), 729 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.22 (3H, d, J=6.4Hz, H12), 2.71 (2H, t, J=5.6Hz, H7), 3.43 (2H, t, J=5.6Hz, H6), 3.71 (2H, s, H4), 3.82 (2H, J=7.2, d, H9), 4.12 (1H, m, H11), 6.06 (1H, br, NH), 6.78 (1H, d, J=7.2 Hz, H3), 7.02 (1H, d, J=7.2 Hz, H2), 7.25-7.45 (4H, m, ArH); ^{13}C-

NMR (400 MHz, DMSO-d6), δ (ppm): 21.09, 24.57, 44.56, 48.26, 49.42, 57.34, 124.09, 125.72, 126.32, 129.21, 132.19, 135.81, 148.27, 152.00, 169.21; Anal. Calcd. for $C_{19}H_{19}N_5O_4S$: C, 55.19; H, 4.63; N, 16.94; S, 7.76. Found: C, 55.23; H, 4.66; N, 16.97; S, 7.79; LC-MS (m/z): 413.45.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-(p-tolyl)-1,2,4-oxadiazol-3-yl)ethyl amino]ethanone (5h): Yield: 66 %; m.p. 187-190ºC; IR (vmax, KBr, cm^{-1}): 1658 (>C=O of amide), 1607 (C=N, oxadiazole), 1235 (C–N str.), 1220 (C-O, oxadiazole), 913 (N-O, oxadiazole), 757 (C–H def, aromatic), 728 (C–S–C str., thiophene); ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm): 1.28 (3H, d, J=6.4Hz, H12), 1.42 (3H, s, $-CH_3$), 2.76 (2H, t, J=5.6Hz, H7), 3.48 (2H, t, J=5.6Hz, H6), 3.73 (2H, s, H4), 3.80 (2H, J=7.2, d, H9), 4.12 (1H, m, H11), 6.02 (1H, br, NH), 6.78 (1H, d, J=7.2 Hz, H3), 7.08 (1H, d, J=7.2 Hz, H2), 7.23-7.56 (5H, m, ArH); ^{13}C-NMR (400 MHz, DMSO-d6), δ (ppm): 24.43, 27.42, 42.56, 47.26, 48.42, 51.21, 125.43, 125.91, 127.32, 130.14, 134.08, 135.43, 149.64, 153.21, 170.04; Anal. Calcd. for $C_{20}H_{22}N_4O_2S$: C, 62.80; H, 5.80; N, 14.65; S, 8.38. Found: C, 62.76; H, 5.83; N, 14.62; S, 8.34; LC-MS (m/z): 382.448.

2.4 Antimicrobial Activity

Compounds **5a–h** were screened for *in vitro* antibacterial activity against Gram-positive bacterial strains (*Bacillus subtilis* (MTCC 121) [BS] and *Staphylococcus aureus* (ATCC 29213) [SA]) and Gram-negative bacterial strains (*Escherichia coli* (ATCC 25922) [EC] and *Salmonella typhimurium* (MTCC 733) [ST]) utilizing the agar diffusion assay [20,21]. The wells were dug in the media with the help of a sterile metallic borer. Recommended concentration (100 µl) of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells were supplemented with DMSO and reference antibacterial drug. DMSO served as negative control. Ampicillin and Nalidixic acid were used as positive controls for Gram positive bacteria and Gram negative bacteria respectively. The plates were incubated immediately at 37ºC for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

Compounds **5a–h** were also examined for antifungal activity against different fungal strains, i.e. *Candida albicans* (MTCC 1637) [CA] and *Aspergillus flavus* (AIIMS) [AF]. The antifungal drug, Amphotericin B was used as a positive control. Antifungal screening for compounds **5a–h** and positive control was performed at a recommended concentration. The fungal strains were grown and maintained on potato dextrose agar plates. The cultures of the fungi were purified by single spore isolation technique. Each compound **5a–h** in DMSO solution was prepared for testing against spore germination of each fungus. The fungal culture plates were inoculated and incubated at 25± 2ºC for 48 h. The plates were then observed and the diameters of the zone of inhibition (in mm) were measured. The percentage inhibition for fungi was calculated after five days using the formula given below:

$$\text{Percentage of inhibition} = 100(X-Y) / X$$

Where, X = Area of colony in control plate.
Y = Area of colony in test plate.

The twofold serial dilution technique [22] was followed to determine the Minimum Inhibitory Concentration (MIC) of the compounds which were found significantly active against preliminary tested microorganism. The tested compounds were dissolved in DMSO and then diluted with culture medium (Mueller–Hinton agar medium for the bacteria and Sabouraud liquid medium for the fungi), at the recommended concentration. The final amount applied was of 104 CFU/plate for the bacteria and 103 CFU/tube for the fungi. The MIC values were read after incubation at 35ºC for a period of 24 h (bacteria) and 48 h (fungi). The lowest concentration of the test substance that completely inhibited growth of the microorganism was recorded as the MIC, expressed in μg mL^{-1}. All experiments were performed in triplicate for an average.

3. RESULTS AND DISCUSSION

3.1 Synthesis of Compounds

The ^{1}H-NMR spectrum showed doublet at 1.18 and a broad singlet at 6.70 δ (ppm) indicating the presence of methyl and amine groups in compound **3**. Furthermore the FT-IR bands at 3187, 2862 and 2205 cm^{-1} confirmed the presence of secondary amine, methyl and nitrile groups respectively, which clearly shown that the 2-(2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-oxoethylamino)propanenitrile 3 was successfully prepared from the corresponding 2-(1-cyanoethylamino)acetyl chloride by a simple treatment with THTP. The physicochemical parameters and ^{13}C-NMR spectral data were also in favored of the proposed structure of compound **3**.

The reaction mixture of compound **3** with one equivalent of hydroxylamine hydrochloride and few drops of triethylamine was kept overnight at room temperature to produced amidoxime 4. While comparing the ^{1}H-NMR data of compound **3** and 4, two additional peaks corresponding to hydroxyl and free amine groups were observed at 10.78 and 11.07 δ (ppm). FT-IR bands at 1672, 3480 and 3208, The N-H absorption should appear around 3300 in IR cm^{-1} representing C=N, O-H and $–NH_2$ groups respectively in compound 4. The physicochemical parameters and ^{13}C-NMR spectral data were also in support of the proposed structure of compound 4 as shown in Fig. 1.

The condensation of this amidoxime with various aromatic aldehyde in toluene for 3-4 h to affords the 1,2,4-oxadiazoles 5a-h with good yields. Significant differences were observed while comparing the ^{1}H-NMR spectrum of compound 4 and **5**, the peaks due to free amine and hydroxyl groups were disappeared from the compounds 5a-h (Fig. 3). In the FT-IR spectrum the bands due to free amine and hydroxyl groups were absent and additional bands at about 915 (N-O), 1220 (C-O), 1605 cm^{-1} (C=N) were found due to the formation of oxadiazole ring. The physicochemical parameters and ^{13}C-NMR spectral data were also confirmed the proposed structure of compound 5a-h. To be able to verify the existence of methylene group, we have carried out DEPT-135 (Fig. 4) of compound **5a** additionally. The expected structures were thus clearly verified by the spectroscopic and elemental analyses which indicated moreover the absence of any detectable impurity, particularly of reagents used to prepare compound 5a–h.

The condensation of amidoxime 4 with aromatic aldehydes refluxed in the presence of toluene gave the corresponding 1,2,4-oxadiazoles. Mechanistically, it is reasonable to assume that first amidoxime is condensed with aldehyde to form in situ the intermediate imine derivative. Then this intermediate is cyclized to 4,5-dihydro-1,2,4-oxadiazole

derivative, which is finally oxidized under the reaction conditions to produce 1,2,4-oxadiazole (Fig. 2).

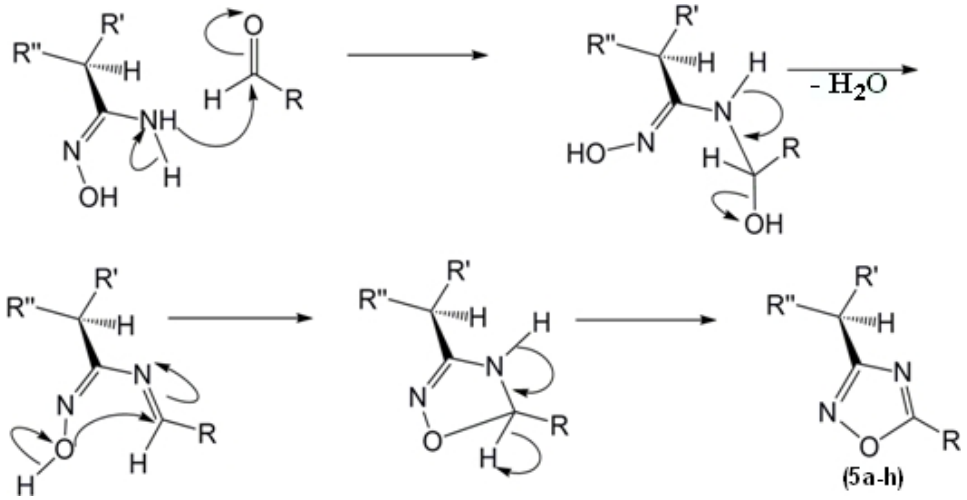


Fig. 2. Proposed mechanism for the synthesis of 1,2,4-oxadiazole

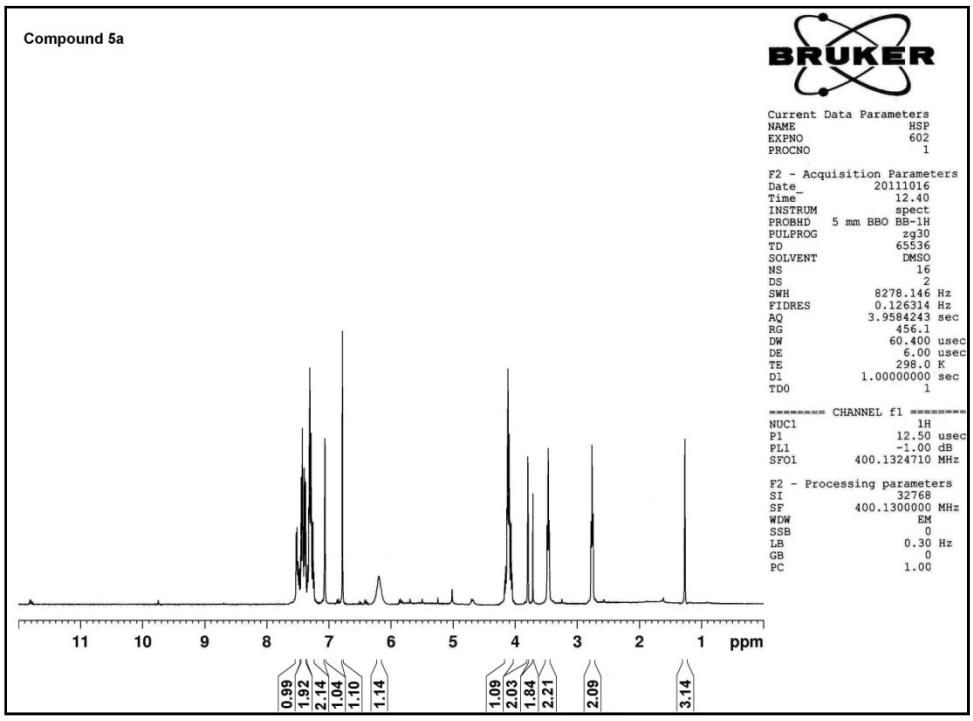


Fig. 3. 1H-NMR of compound 5a

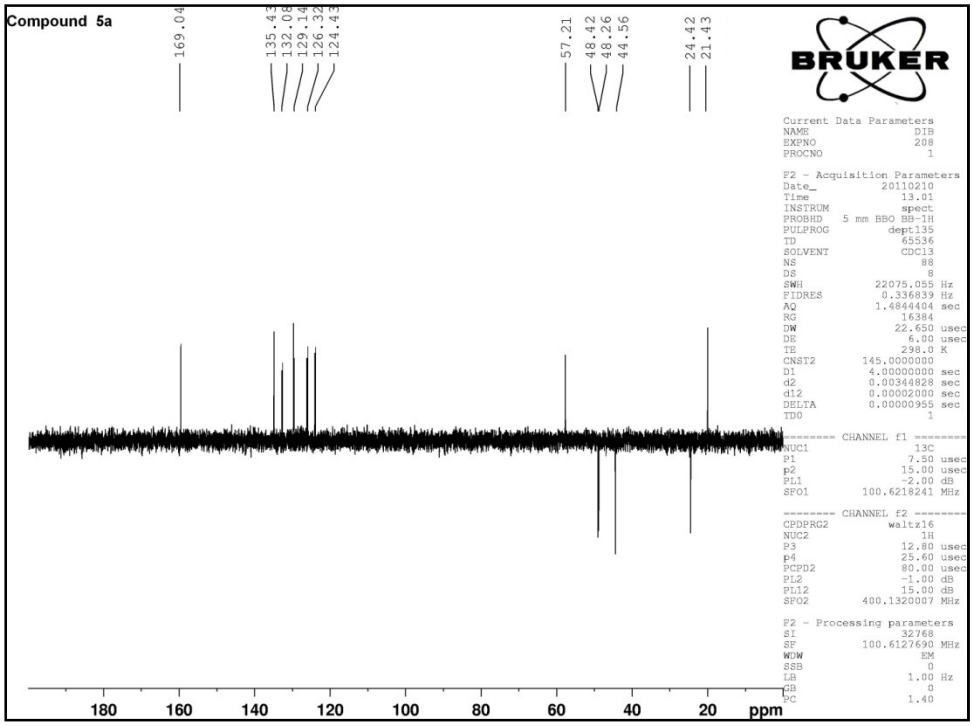


Fig. 4. DEPT-135 of compound 5a

3.2 Antimicrobial Activity

3.2.1 Antibacterial activity

The results of the preliminary testing of the antibacterial activity of the final compounds are given in Table 1. The results revealed that the majority of the synthesized compounds show varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram positive bacteria was higher than against the Gram-negative bacteria. The oxadiazole derivatives 5a-h displayed the highest activity as compare to compounds 3 and 4. Compounds 5g, and 5e showed excellent activity against the Gram-positive bacteria this might be due to the presence hydroxyl, methoxy and nitro groups. Compounds 5a, 5b and 5c were found very less active (less than 10 zone of inhibition) against all the Gram negative bacteria.

Based on preliminary result of tested compound, selective compounds 5d-g were studied for their minimum inhibitory concentration. The values of the MIC against the microorganism susceptible in the preliminary test are reported in Table 2. The results showed significant inhibitory effects, majority of the tested compounds were shown MIC values of 2-32 μg mL^{-1}. This class of compounds presented high activity against Gram positive bacteria and showed good antibacterial activity against all these microorganisms. The compound 5g was found more active than Ampicillin and Nalidixic Acid against *S. Aureus* and *E. coli* respectively due to the presence of strong electron withdrawing nitro group.

3.2.2 Antifungal activity

The in vitro antifungal activities of the derivatives of 1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-[1-(5-phenyl-1,2,4-oxadiazol-3-yl) ethylamino]ethanone 5a-h and of Amphotericin B as a reference drug on three fungi species are given in Tables 1 and 2. Some of the compounds tested were endowed with a good activity against Candida albicans. Compounds with a para-substitution in the benzene ring were also found to be potent against all species (inhibition zone 10–32 mm) with medium activity. All the other tested compounds exhibited insignificant chemotherapeutical activity against the tested microorganism.

Table 1. Antimicrobial activities of 1,2,4-oxadiazoles (Inhibitory zone diameter/mm)

Sample	Mean zone inhibition (in mm)[a]					
	Gram positive bacteria		Gram negative bacteria		Fungal	
	BS	SA	EC	ST	CA	AF
5a	--	11	--	--	--	--
5b	--	14	--	--	10	--
5c	13	23	--	--	12	--
5d	16	28	--	--	17	12
5e	18	29	15	12	19	18
5f	16	30	--	10	20	17
5g	20	31	25	12	21	19
5h	10	--	--	--	--	10
Ampicillin	24	30	nt	nt	nt	nt
Nalidixic Acid	nt	nt	23	18	nt	nt
Amphotericin B	nt	nt	nt	nt	34	28

"--" indicates no sensitivity or mean inhibition zone diameter less than 10 mm. "nt" not tested. [a] Values are mean (n=3)

Table 2. Antibacterial and antifungal activities of 1,2,4-oxadiazoles (MIC/µgmL^{-1})

Sample	Minimum inhibitory concentration (µg ml^{-1})					
	Gram positive bacteria		Gram negative bacteria		Fungal	
	BS	SA	EC	ST	CA	AF
5d	16	8	16	32	32	16
5e	8	4	8	16	8	16
5f	8	16	8	16	16	8
5g	4	2	4	8	2	8
Ampicillin	2	4	nt	nt	nt	nt
Nalidixic acid	nt	nt	4	2	nt	nt
Amphotericin B	nt	nt	nt	nt	4	≤ 1

"--" indicates no sensitivity. "nt" not tested.

4. CONCLUSION

In summary, a convenient one-pot method for the preparation of novel, high yielding, biologically active chiral 3,5-disubstituted-1,2,4-oxadiazoles have been developed using

chiral amidoxime, derived from inexpensive and readily available amino acid. The synthesized compounds were screened for their antibacterial and antifungal activities against a spectrum of microbial organisms. With regards to the structure-activity relationship of the oxadiazoles derivatives, the group with a substituent on para-position exhibited enhanced antimicrobial activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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