

Research Paper

Biological evolution and spectral studies of asymmetrical 3,5-disubstituted-1,2,4-oxadiazoles prepared by one-pot synthesis

ABSTRACT

In present communication, 3,5-disubstituted-1,2,4-oxadiazoles derived from chiral α -amino acid 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 disc 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 are often used in drug discovery, bioisosteric replacements of esters and amides [1], as dipeptide mimetics [2] in a number of pharmacologically important molecules. They can also be found in a number of biologically important drugs, such as muscarinic agonists, [3] serotonergic (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-HT_{1D} (5-hydroxytryptamine) receptors, [8] and histamine H₃ receptors. [9] They shown 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 neutrophil 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 [THTPs] 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 were evaluated for their human phenylethanol-amine N-methyltransferase inhibitory potency and affinity for the α ₂-adrenoceptor. [19]

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

2. MATERIAL 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 ¹H, ¹³C NMR, DEPT-135 spectra were recorded on Bruker 400 MHz instrument using DMSO-d₆ as solvent. The MS-Cl spectrum of were recorded on Shimadzu LC-MS 2010 eV spectrometer in acetonitrile. The melting points were checked by standard open capillary method and are 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 tetra-ethyl amine as base under constant stirring, and the reaction mixture was refluxed for 2.5 h. After completion of the reaction, it was poured in ice-cold water to obtain a light yellow product (2), which was filtered and dried. It was purified by column chromatography and recrystallized from ethanol to give a white crystalline compound (3). Yield: 83 %; m.p. 160-163 °C; Anal. Calcd. for C₁₂H₁₅N₃OS: 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; ¹H-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, 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); ¹³C-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. IR (KBr, cm⁻¹): 3082 (C-H str., aromatic), 1542.5 (C=C, asymmetric, str.), 1478.4-1473.5 (C=C str. ring), 1226 (C-N str.), 752.8 (C-H def, aromatic), 724 (C-S-C str., thiophene), 1665 (>C=O of amide), 3187 (-NH), 2862 (C-H str. aliphatic), 1605 (-CN); LC-MS (m/z) : 249.33.

2.2. Synthesis of 2-((2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-oxoethyl)amino)-N'-hydroxypropanimidamide (4)

Chiral 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 CHCl₃ (18 ml), the reaction was performed at room temp. for over night in presence of try ethyl amine. After completion of the reaction, the product was filtered and washed with CHCl₃ followed by hot water and purified by column chromatography then recrystallized from chloroform to give white crystalline solid (4). Yield: 82 %; m.p. 172-175 °C; Anal. Calcd. for C₁₂H₁₈N₄O₂S: 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; ¹H-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, 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, NH₂); ¹³C-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. IR (KBr, cm⁻¹): 3084 (C-H str., aromatic), 1544 (C=C, asymmetric, str.), 1478-1474 (C=C str. ring), 1227 (C-N str.), 752.7 (C-H def, aromatic), 728 (C-S-C str., thiophene), 1667 (>C=O of amide), 1672 (C=N, oxime), 2723 (O-H, oxime), 3208 (NH₂); 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-(2-(5-phenyl-1,2,4-oxadiazol-3-yl)pyrrolidin-1-yl)ethanone (5a-h)

The chiral amidoxime (0.01 mol/3.084 g) was dissolved in 200 mL of dry toluene, and then aldehyde (0.01mol) was added with a small quantity of paratoluenesulfonic 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. It was recrystallized from chloroform × pet ether (7:3).

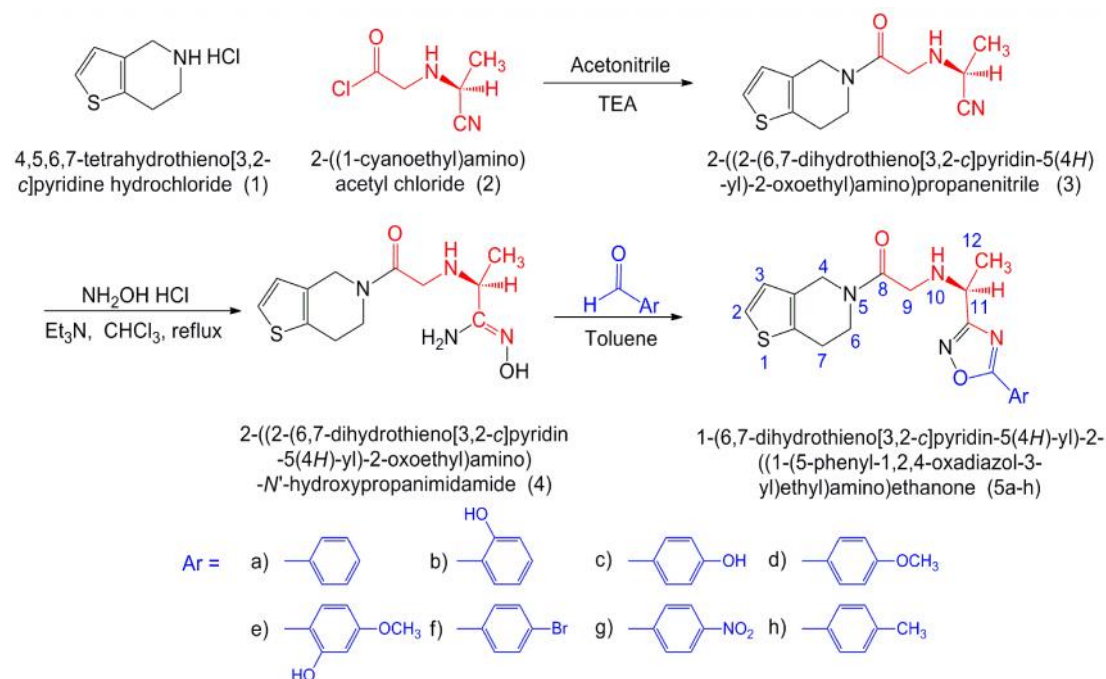


Figure 1: Synthesis of chiral 1,2,4-oxadiazoles 5a-h

The condensation of amidoxime 4 with aromatic aldehydes refluxed in 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 imine derivative 6. Then this intermediate is cyclized to 4,5-dihydro-1,2,4-oxadiazole derivative 7, which is finally oxidized under the reaction conditions to produce 1,2,4-oxadiazole (Figure 2).

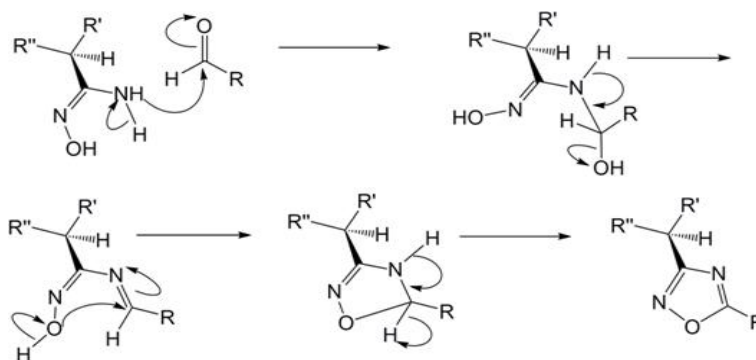


Figure 2: Proposed mechanism for the synthesis of 1,2,4-oxadiazole

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. 184-187 °C; 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; IR (KBr, cm^{-1}): 1227 (C–N str.), 752 (C–H def, aromatic), 728 (C–S–C str., thiophene), 912 (N–O, oxadiazole), 1220 (C–O, oxadiazole), 1607 (C=N, oxadiazole), 1667 (>C=O of amide); LC-MS (m/z) : 368.45; ^1H -NMR (400 MHz, DMSO- d_6), 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, 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- d_6), 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.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-((1-(5-(2-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)ethyl)amino)ethanone (5b): Yield: 66 %; m.p. 178-179 °C; 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; IR (KBr, cm^{-1}): 1224 (C–N str.), 750 (C–H def, aromatic), 724 (C–S–C str., thiophene), 906 (N–O, oxadiazole), 3309 (–O–H str.), 1227 (C–O, oxadiazole), 1602 (C=N, oxadiazole), 1660 (>C=O of amide); LC-MS (m/z) : 384.45; ^1H -NMR (400 MHz, DMSO- d_6), 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, 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- d_6), 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.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-((1-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)ethyl)amino)ethanone (5c): Yield: 75 %; m.p. 192-195 °C; 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; IR (KBr, cm^{-1}): 1229 (C–N str.), 753 (C–H def, aromatic), 726 (C–S–C str., thiophene), 908 (N–O, oxadiazole), 1217 (C–O, oxadiazole), 1611 (C=N, oxadiazole), 1662 (>C=O of amide), 3285 (–O–H str.); LC-MS (m/z) : 384.45; ^1H -NMR (400 MHz, DMSO- d_6), 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, 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- d_6), 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.

1-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-((1-(5-(4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)ethyl)amino)ethanone (5d): Yield: 70 %; m.p. 176-178 °C; Anal. Calcd. for $C_{20}H_{22}N_4O_3S$: C, 60.28; H, 5.56; N, 14.06; S, 8.05. Found: C, 60.31; H, 5.52; N, 14.02; S,

142 8.11; IR (KBr, cm^{-1}): 1233 (C–N str.), 750 (C–H def, aromatic), 724 (C–S–C str., thiophene),
143 911 (N–O, oxadiazole), 3326 (–O–H str.), 1214 (C–O, oxadiazole), 1613 (C=N, oxadiazole),
144 1660 ($>\text{C}=\text{O}$ of amide); LC-MS (m/z): 398.48; ^1H -NMR (400 MHz, DMSO- d_6), δ (ppm):
145 1.23 (3H, d, $J = 6.4\text{Hz}$, H12), 2.70 (2H, t, $J = 5.6\text{Hz}$, H7), 3.41 (2H, t, $J = 5.6\text{Hz}$, H6), 3.70
146 (2H, s, H4), 3.82 (2H, d, H9), 3.92 (3H, s, $-\text{OCH}_3$), 4.23 (1H, m, H11), 6.12 (1H, br, NH), 6.78
147 (1H, d, $J = 7.2\text{ Hz}$, H3), 7.08 (1H, d, $J = 7.2\text{ Hz}$, H2), 7.23–7.56 (5H, m, ArH); ^{13}C -NMR (400
148 MHz, DMSO- d_6), δ (ppm): 21.13, 24.52, 44.57, 48.20, 48.52, 57.36, 124.30, 125.87, 126.12,
149 129.34, 132.08, 135.52, 148.44, 153.00, 169.21.

150 **1-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-((1-(5-(2-hydroxy-4-methoxyphenyl)-1,2,4**
151 **-oxadiazol-3-yl)ethyl)amino)ethanone (5e)**: Yield: 74 %; m.p. 224–228 °C; Anal. Calcd. for
152 $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_4\text{S}$: C, 57.96; H, 5.35; N, 13.52; S, 7.74. Found: C, 57.92; H, 5.38; N, 13.49; S,
153 7.70; IR (KBr, cm^{-1}): 1231 (C–N str.), 757 (C–H def, aromatic), 729 (C–S–C str., thiophene),
154 903 (N–O, oxadiazole), 1214 (C–O, oxadiazole), 1617 (C=N, oxadiazole), 1664 ($>\text{C}=\text{O}$ of
155 amide); LC-MS (m/z): 414.48; ^1H -NMR (400 MHz, DMSO- d_6), δ (ppm): 1.29 (3H, d, $J =$
156 6.4Hz , H12), 2.77 (2H, t, $J = 5.6\text{Hz}$, H7), 3.42 (2H, t, $J = 5.6\text{Hz}$, H6), 3.76 (2H, s, H4), 3.84
157 (2H, d, H9), 3.92 (3H, s, $-\text{OCH}_3$), 4.23 (1H, m, H11), 6.09 (1H, br, NH), 6.78 (1H, d, $J = 7.2$
158 Hz, H3), 7.12 (1H, d, $J = 7.2\text{ Hz}$, H2), 7.25–7.50 (5H, m, ArH), 10.36 (1H, s, $-\text{OH}$); ^{13}C -NMR
159 (400 MHz, DMSO- d_6), δ (ppm): 23.43, 25.42, 45.56, 48.26, 49.42, 58.21, 125.43, 126.91,
160 127.32, 128.14, 133.08, 136.47, 148.24, 150.21, 168.87.

161 **2-((1-(5-(4-bromophenyl)-1,2,4-oxadiazol-3-yl)ethyl)amino)-1-(6,7-dihydrothieno[3,2-*c*]**
162 **pyridin-5(4*H*)-yl)ethanone (5f)**: Yield: 62 %; m.p. 179–182 °C; Anal. Calcd. for
163 $\text{C}_{19}\text{H}_{19}\text{BrN}_4\text{O}_2\text{S}$: C, 51.01; H, 4.28; Br, 17.86; N, 12.52; S, 7.17. Found: C, 51.06; H, 4.31; Br,
164 17.88; N, 12.48; S, 7.21; IR (KBr, cm^{-1}): 1216 (C–N str.), 748 (C–H def, aromatic), 727 (C–
165 S–C str., thiophene), 923 (N–O, oxadiazole), 1223 (C–O, oxadiazole), 1614 (C=N,
166 oxadiazole), 1664 ($>\text{C}=\text{O}$ of amide), 1019 (C–Br str.); LC-MS (m/z): 447.35; ^1H -NMR (400
167 MHz, DMSO- d_6), δ (ppm): 1.29 (3H, d, $J = 6.4\text{Hz}$, H12), 2.76 (2H, t, $J = 5.6\text{Hz}$, H7), 3.48
168 (2H, t, $J = 5.6\text{Hz}$, H6), 3.73 (2H, s, H4), 3.80 (2H, d, H9), 4.12 (1H, m, H11), 6.07 (1H, br,
169 NH), 6.78 (1H, d, $J = 7.2\text{ Hz}$, H3), 7.13 (1H, d, $J = 7.2\text{ Hz}$, H2), 7.30–7.65 (5H, m, ArH); ^{13}C -
170 NMR (400 MHz, DMSO- d_6), δ (ppm): 20.42, 23.42, 44.56, 48.21, 48.42, 57.08, 125.43,
171 125.87, 126.30, 129.17, 132.00, 135.44, 148.34, 152.22, 169.14.

172 **1-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-((1-(5-(4-nitrophenyl)-1,2,4-oxadiazol-3-**
173 **yl)ethyl)amino)ethanone (5g)**: Yield: 53 %; m.p. 204–206 °C; Anal. Calcd. for $\text{C}_{19}\text{H}_{19}\text{N}_5\text{O}_4\text{S}$:
174 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; IR (KBr,
175 cm^{-1}): 1221 (C–N str.), 754 (C–H def, aromatic), 729 (C–S–C str., thiophene), 904 (N–O,
176 oxadiazole), 1216 (C–O, oxadiazole), 1608 (C=N, oxadiazole), 1658 ($>\text{C}=\text{O}$ of amide); LC-
177 MS (m/z): 413.45; ^1H -NMR (400 MHz, DMSO- d_6), δ (ppm): 1.22 (3H, d, $J = 6.4\text{Hz}$, H12),
178 2.71 (2H, t, $J = 5.6\text{Hz}$, H7), 3.43 (2H, t, $J = 5.6\text{Hz}$, H6), 3.71 (2H, s, H4), 3.82 (2H, d, H9),
179 4.12 (1H, m, H11), 6.06 (1H, br, NH), 6.78 (1H, d, $J = 7.2\text{ Hz}$, H3), 7.02 (1H, d, $J = 7.2\text{ Hz}$,
180 H2), 7.25–7.45 (4H, m, ArH); ^{13}C -NMR (400 MHz, DMSO- d_6), δ (ppm): 21.09, 24.57, 44.56,
181 48.26, 49.42, 57.34, 124.09, 125.72, 126.32, 129.21, 132.19, 135.81, 148.27, 152.00,
182 169.21.

183 **1-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)-2-((1-(5-(*p*-tolyl)-1,2,4-oxadiazol-3-yl)ethyl)**
184 **amino)ethanone (5h)**: Yield: 66 %; m.p. 187–190 °C; Anal. Calcd. for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_2\text{S}$: C,
185 62.80; H, 5.80; N, 14.65; S, 8.38. Found: C, 62.76; H, 5.83; N, 14.62; S, 8.34; IR (ν_{max} , KBr,
186 cm^{-1}): 1235 (C–N str.), 757 (C–H def, aromatic), 728 (C–S–C str., thiophene), 913 (N–O,
187 oxadiazole), 1220 (C–O, oxadiazole), 1607 (C=N, oxadiazole), 1658 ($>\text{C}=\text{O}$ of amide); LC-
188 MS (m/z): 382.448; ^1H -NMR (400 MHz, DMSO- d_6), δ (ppm): 1.28 (3H, d, $J = 6.4\text{Hz}$, H12),
189 1.42 (3H, s, $-\text{CH}_3$), 2.76 (2H, t, $J = 5.6\text{Hz}$, H7), 3.48 (2H, t, $J = 5.6\text{Hz}$, H6), 3.73 (2H, s, H4),
190 3.80 (2H, d, H9), 4.12 (1H, m, H11), 6.02 (1H, br, NH), 6.78 (1H, d, $J = 7.2\text{ Hz}$, H3), 7.08

(1H, d, J = 7.2 Hz, H2), 7.23-7.56 (5H, m, ArH); ¹³C-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.

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 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 37°C 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 compounds (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± 2°C 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 tests 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, within the range 128–1 µg mL⁻¹. 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 35 °C 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 ¹H-NMR spectrum shown doublets 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 1605 cm⁻¹ confirmed the presence of secondary amine, methyl and nitrile

groups respectively. Which clearly shown that the chiral 2-(2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-oxoethylamino)propanenitrile **3** was successfully prepared from the corresponding optically pure 2-(1-cyanoethylamino)acetyl chloride by a simple treatment with THTPs. The physicochemical parameters and ^{13}C -NMR spectral data were also in favored of the proposed structure of compound **3**.

The reaction of compound **3** with 1 equivalent of hydroxylamine hydrochloride and 2 equivalents of triethylamine overnight at room temperature produced amidoxime **4**. While comparing the ^1H -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, 2723 and 3208 cm^{-1} representing C=N, O-H and $-\text{NH}_2$ groups 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 figure 1.

The condensation of this amidoxime with various aliphatic and aromatic aldehyde in toluene for 3-4 h affords the chiral 1,2,4-oxadiazoles **5a-h** in good chemical yields. Significant differences were observed while comparing the ^1H -NMR spectrum of compound **4** and **5**, i.e. the peaks due to free amine and hydroxyl groups were disappeared from the compounds **5a-h** (Figure 3). From the FT-IR spectrum a band due to free amine and hydroxyl groups were disappeared and additional bands at about 915 (N-O), 1220 (C-O), 1605 cm^{-1} (C=N) were found due to 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 (Figure 4) of compound **5a** additionally. The expected structures were thus clearly verified by the spectroscopic and elemental analysis which indicated moreover the absence of any detectable impurity, particularly of reagents used to prepare compound **5a-h**.

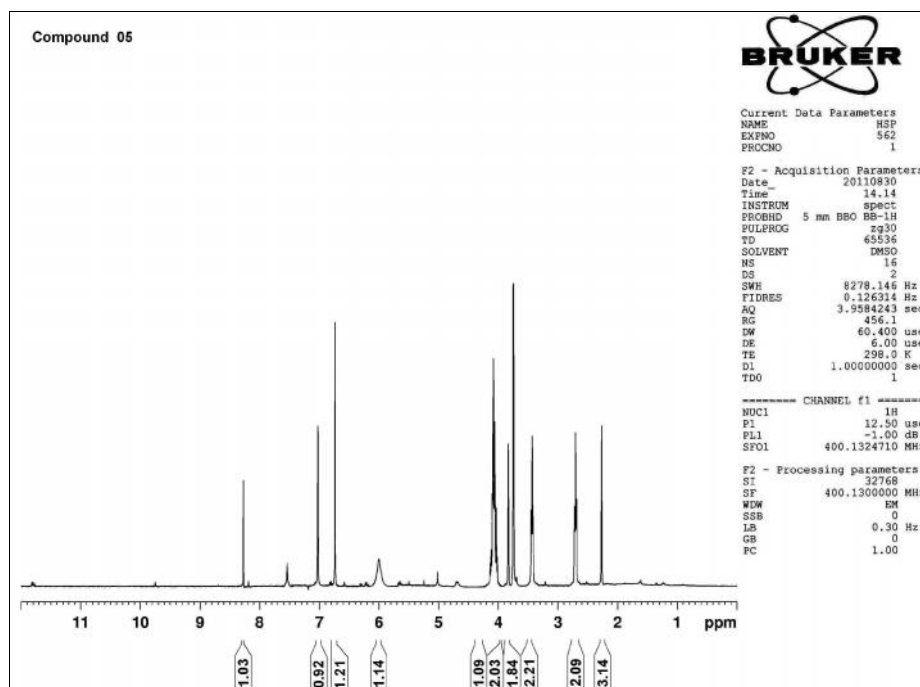


Figure 3: ^1H -NMR of compound **5a**

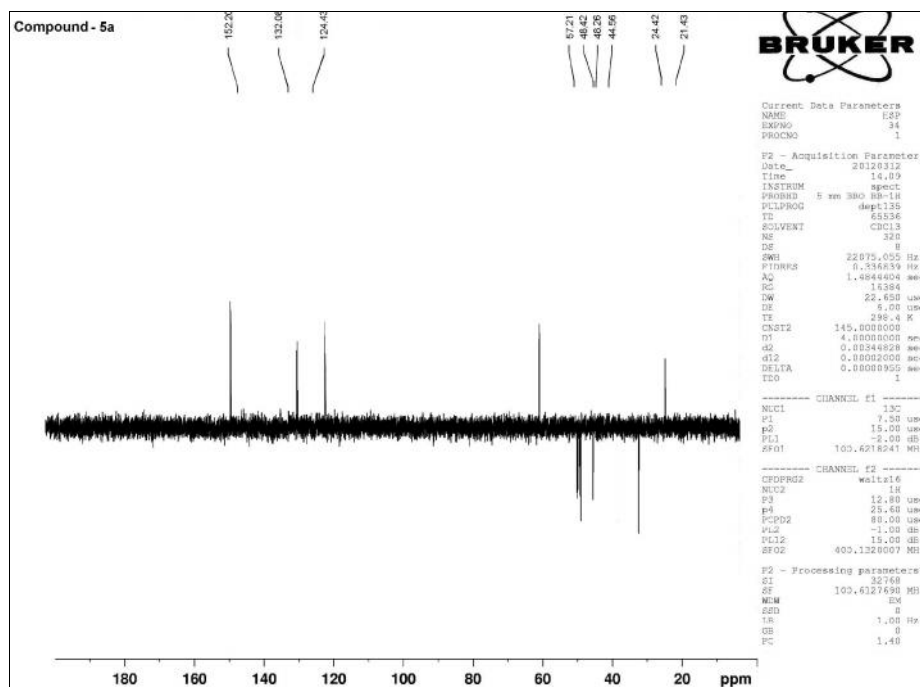


Figure 4: DEPT-135 of compound 5a

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. Compounds 5g, and 5e showed excellent activity against the Gram-positive bacteria. Compounds 5a, 5b and 5c were found very less active (less than 10) against all the Gram negative bacteria. Based on preliminary result of tested compound, selectively compounds 5d, 5e, 5f and fg 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, with the majority of the compounds tested having MIC values of 2-32 $\mu\text{g mL}^{-1}$. 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.

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-(2-(5-phenyl-1,2,4-oxadiazol-3-yl)pyrrolidin-1-yl)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

293 compounds exhibited insignificant chemotherapeutical activity against the tested
294 microorganism.

295

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

Sample	<i>Mean zone inhibition (in mm)^a</i>					
	<i>Gram positive bacteria</i>		<i>Gram negative bacteria</i>		<i>Fungal</i>	
	<i>BS</i>	<i>SA</i>	<i>EC</i>	<i>ST</i>	<i>CA</i>	<i>AF</i>
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

297 “--” indicates no sensitivity or mean inhibition zone diameter less than 10 mm. “nt” not tested. ^a Values are mean (n=3)

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Table 2: Antibacterial and antifungal activities of 1,2,4-oxadiazoles (MIC/ $\mu\text{g mL}^{-1}$)

Sample	<i>Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$)</i>					
	<i>Gram positive bacteria</i>		<i>Gram negative bacteria</i>		<i>Fungal</i>	
	<i>BS</i>	<i>SA</i>	<i>EC</i>	<i>ST</i>	<i>CA</i>	<i>AF</i>
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.

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305 4. CONCLUSION

306 In summary, a convenient one-pot method for the preparation of novel, high yielding,
307 biologically active chiral 3,5-disubstituted-1,2,4-oxadiazoles have been developed using
308 chiral amidoxime, derived from inexpensive and readily available amino acid. The
309 synthesized compounds were screened for their antibacterial and antifungal activities
310 against a spectrum of microbial organisms. With regards to the structure-activity relationship
311 of the oxadiazoles derivatives, the group with a substituent on para-position exhibited
312 enhanced antimicrobial activity.

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317 COMPETING INTERESTS

318
319 Authors have declared that no competing interests exist.
320

321 AUTHORS' CONTRIBUTIONS

322
323 This work was carried out in collaboration with all authors. All the authors read and
324 approved the final manuscript.
325

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