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Supporting Information

for

Synthesis and HDAC inhibitory activity of pyrimidine-based hydroxamic acids

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Compounds characterization and analytical data; HDAC enzyme activity assay; references; NMR spectra

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Compounds characterization and analytical data

*Ethyl [6-methyl-2-(methylthio)pyrimidin-4-yloxy]acetate (**3**).* White crystals; yield 0.17 g (73%); mp 52–53 °C; ^{1}H NMR ($CDCl_3$): δ 6.37 (s, 1H, CH), 4.87 (s, 2H, OCH_2), 4.23 (q, *J* = 7.2 Hz, 2H, CH_2), 2.47 (s, 3H, SCH_3), 2.38 (s, 3H, CH_3), 1.26 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.4, 168.5 (2), 168.2, 102.1, 62.8, 61.4, 23.9, 14.3, 14.0; HRMS calcd for $C_{10}H_{14}N_2O_3S$: $[M + H]^+$ = 243.0798, found: 243.0797.

*Ethyl [2-(ethylthio)-6-methylpyrimidin-4-yloxy]acetate (**4**).* White crystals; yield 0.18 g (70%); mp 50–52 °C; ^{1}H NMR ($CDCl_3$): δ 6.36 (s, 1H, CH), 4.86 (s, 2H, OCH_2), 4.22 (q, *J* = 7.2 Hz, 2H, CH_2), 3.05 (q, *J* = 7.2 Hz, 2H, SCH_2), 2.37 (s, 3H, CH_3), 1.34 (t, *J* = 7.2 Hz, 3H, CH_3), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.0, 168.6, 168.5, 168.2, 102.1, 62.7, 61.5, 25.2, 23.9, 14.8, 14.3; HRMS calcd for $C_{11}H_{16}N_2O_3S$: $[M + H]^+$ = 257.0954, found: 257.0954.

*Ethyl [6-methyl-2-(methylsulfonyl)pyrimidin-4-yloxy]acetate (**5**)*
A mixture of compound **3** (0.242g, 1 mmol) and oxone (1.54 g, 2.5 mmol) in dry DMF (2 mL) was stirred at 40 °C for 0.5 h, then cooled to room temperature, and poured into ice water. The resultant precipitate collected by filtration, washed with water, and dried to give white solid, yield 0.214 g (78%); mp 83–85 °C; ^{1}H NMR ($CDCl_3$): δ 6.88 (s, 1H, CH), 4.98 (s, 2H, OCH_2), 4.24 (q, *J* = 7.2 Hz, 2H, CH_2), 3.27 (s, 3H, SO_2CH_3), 2.58 (s, 3H, CH_3), 1.28 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.3, 169.7, 167.6, 164.6, 109.9, 63.6, 61.9, 39.1, 24.1, 14.3; HRMS calcd for $C_{10}H_{14}N_2O_5S$: $[M + H]^+$ = 275.0696, found: 275.0691.

*Ethyl [2-(allylamino)-6-methylpyrimidin-4-yloxy]acetate (**6**).* White crystals; yield 0.203 g (81%); mp 108–110 °C; ^{1}H NMR ($CDCl_3$): δ 6.01 (s, 1H, CH), 5.95–5.82 (m, 1H, CH=), 5.41 (very br. s, 1H, NH), 5.20 (d, *J* = 17.2 Hz, 1H, CH_2=), 5.09 (d, *J* = 10.0 Hz, 1H, CH_2=), 4.79 (s, 2H, OCH_2), 4.21 (q, *J* = 7.2 Hz, 2H, CH_2), 4.04–3.91 (m, 2H, NH$\underline{CH_2}$), 2.26 (s, 3H, CH_3), 1.26 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.5, 168.9, 168.6, 161.6, 135.2, 115.8, 96.1, 62.5, 61.3, 43.9, 23.7, 14.3; HRMS calcd for $C_{12}H_{17}N_3O_3$: $[M + H]^+$ = 252.1343, found: 252.1343.

*Ethyl [2-(benzylamino)-6-methylpyrimidin-4-yloxy]acetate (**7**).* White crystals; yield 0.245 g (81%); mp 117–119 °C; ^{1}H NMR ($CDCl_3$): δ 7.36–7.20 (m, 5H, C_6H_5), 6.03 (s, 1H, CH), 5.55 (very br. s, 1H, NH), 4.76 (s, 2H, OCH_2), 4.55 (d, *J* = 6 Hz, 2H, NH$\underline{CH_2}$), 4.13 (q, *J* = 7 Hz, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.21 (t, *J* = 7 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.5, 168.9, 168.8, 161.7, 139.5, 128.7, 127.5, 127.3, 96.3, 62.4, 61.2, 45.5, 23.9, 14.3; HRMS calcd for $C_{16}H_{19}N_3O_3$: $[M + H]^+$ = 302.1499, found: 302.1499.

*Ethyl [2-(dimethylamino)-6-methylpyrimidin-4-yloxy]acetate (**8**).* White crystals; yield 0.168 g (70%); mp 58–60 °C; ^{1}H NMR ($CDCl_3$): δ 5.93 (s, 1H, CH), 4.77 (s, 2H, OCH_2), 4.21 (q, *J* = 7.2 Hz, 2H, CH_2), 3.10 (s, 6H, $N(CH_3)_2$), 2.27 (s, 3H, CH_3), 1.25 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.3, 168.8, 168.6, 161.8, 94.2, 62.4, 61.1, 36.9, 24.3, 14.3; HRMS calcd for $C_{11}H_{17}N_3O_3$: $[M + H]^+$ = 240.1343, found: 240.1343.

*Ethyl [6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yloxy]acetate (**9**).* White crystals; yield 0.241 g (91%); mp 85–87 °C; ^{1}H NMR ($CDCl_3$): δ 5.94 (s, 1H, CH), 4.79 (s, 2H, OCH_2), 4.21 (q, *J* = 7.2

Hz, 2H, CH_2), 3.50 (br. s, 4H, $N(CH_2)_2$), 2.28 (s, 3H, CH_3), 1.98–1.86 (m, 4H, $(CH_2)_2$), 1.25 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.3, 168.8, 168.6, 159.9, 94.2, 62.4, 61.1, 46.6, 25.6, 24.3, 14.3; HRMS calcd for $C_{13}H_{19}N_3O_3$: $[M + H]^+$ = 266.1499, found: 266.1499.

6-Methyluracil ***(10).*** Method **A**. White powder; yield 0.05 g (40%); mp 320 °C (decomp.) (320 °C lit. [1]); ^1H NMR (DMSO-d_6): δ 10.87 (s, 1H, NH); 10.81 (s, 1H, NH); 5.31 (s, 1H, CH), 2.00 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 164.1, 152.9, 151.6, 98.7, 18.2.
Method **B**. White powder; yield 0.07 g (56%) , mp 320 °C (decomp).

Ethyl (6-methyl-2-oxo-1,2-dihydropyrimidin-4-yloxy]acetate ***(11)****.* White powder; R_f 0.5 (chloroform : ethyl acetate : methanol – 4 : 1 : 1); yield 0.155 g (73%); mp 210–212 °C (197 °C lit.[2]); ^1H NMR ($CDCl_3$): δ 12.85 (br. s, 1H, NH), 5.89 (s, 1H, CH), 4.97 (s, 2H, OCH_2), 4.22 (q, J = 7.2 Hz, 2H, CH_2), 2.32 (s, 3H, CH_3), 1.27 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.3, 167.9, 160.3, 157.6, 94.8, 62.6, 61.6, 18.9, 14.2; HRMS calcd for $C_9H_{12}N_2O_4$: $[M + H]^+$ = 213.0870, found: 213.0869.

N-Hydroxy-(6-methyl-2-(methylthio)pyrimidin-4-yloxy]acetamide ***(12)****.* Reaction time 1 h; white solid; yield 0.186 g (81%); mp 182–184 °C; ^1H NMR (DMSO-d_6): δ 10.78 and 10.21 (2s, 1H, NH), 9.29 and 8.97 (2s, 1H, OH), 6.53 (s, 1H, CH), 5.07 and 4.72 (2s, 2H, OCH_2), 2.45 (s, 3H, SCH_3), 2.32 (s, 3H, CH_3) 1.28 (t, *J* = 8 Hz, 3H, CH_3);; ^{13}C NMR (DMSO-d_6): δ 170.2, 168.1 (2), 164.0, 102.1, 62.8, 23.3, 13.4; HRMS calcd for $C_8H_{11}N_3O_3S$: $[M + H]^+$ = 230.0594, found: 230.0594.

[2-(Ethylthio)-6-methylpyrimidin-4-yloxy]-N-hydroxyacetamide ***(13)****.* Reaction time 1 h; white solid; yield 0.177 g (73%); mp 175–176 °C; ^1H NMR (DMSO-d_6): δ 10.76 and 10.21 (2s, 1H, NH), 9.25 and 8.94 (2s, 1H, OH), 6.53 (s, 1H, CH), 5.06 and 4.71 (2s, 2H, OCH_2), 3.04 (q, *J* = 8 Hz, 2H, CH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 169.8, 168.1 (2), 164.0, 102.2, 62.7, 24.4, 23.3, 14.7; HRMS calcd for $C_9H_{13}N_3O_3S$: $[M + H]^+$ = 244.0750, found: 244.0748.

[2-(Allylamino)-6-methylpyrimidin-4-yloxy]-N-hydroxyacetamide ***(14)****.* Reaction time 4 h; white solid; yield 0.162 g (68%); mp 177–179 °C; ^1H NMR (DMSO-d_6): δ 10.71 and 10.12 (2s, 1H, NH), 8.94 (br. s, 1H, OH), 6.02–5.78 (m, 2H, CH, CH=), 5.15 (dd, *J* = 17.2 and 1.2 Hz, 1H, CH_2=), 5.02 (dd, *J* = 10.2 and 1.4 Hz, 1H, CH_2=), 4.94 and 4.62 (2s, 2H, OCH_2), 3.85 (br. s, 2H, $NH\underline{CH_2}$), 2.15 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 168.9, 168.0, 164.4, 161.6, 136.1, 115.0, 94.6, 62.1, 43.1, 23.5; HRMS calcd for $C_{10}H_{14}N_4O_3$: $[M + H]^+$ = 239.1139, found: 239.1140.

[2-(Benzylamino)-6-methylpyrimidin-4-yloxy]-N-hydroxyacetamide ***(15)****.* Reaction time 96 h; white solid; yield 0.176 g (61%); mp 168–170 °C; ^1H NMR (DMSO-d_6): δ 10.71 and 10.15 (2s, 1H, NH), 9.20 and 8.91 (2s, 1H, OH), 7.64 (br. s, 1H, $\underline{NH}CH_2$), 7.37–7.16 (m, 5H, C_6H_5), 5.94 (s, 1H, CH), 5.02 – 4.33 (m, 4H, OCH_2, $NH\underline{CH_2}$), 2.15 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 168.9, 164.4, 161.7, 158.7, 140.6, 128.1, 127.5, 126.5, 94.9, 62.2, 44.0, 23.5; HRMS calcd for $C_{14}H_{16}N_4O_3$: $[M + H]^+$ = 289.1295, found: 289.1295.

[2-(Dimethylamino)-6-methylpyrimidin-4-yloxy]-N-hydroxyacetamide ***(16)****.* Reaction time 1 h; white solid; yield 0.174 g (77%); mp 192–194 °C; ^1H NMR (DMSO-d_6): δ 10.73 and 10.11 (2s, 1H, NH), 9.22 and 8.89 (2s, 1H, OH), 5.93 (s, 1H, CH), 4.98 and 4.62 (2s, 2H, OCH_2), 3.05 (s, 6H, $(CH_3)_2$), 2.19 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 168.5, 167.8, 164.6, 161.3, 93.8, 62.1, 36.4, 23.8; HRMS calcd for $C_9H_{14}N_4O_3$: $[M + H]^+$ = 227.1139, found: 227.1136.

*N-Hydroxy-[6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yloxy]acetamide (**17**).* Reaction time 2 h; white solid; yield 0.176 g (70%); mp 178–180 °C; ^{1}H NMR (DMSO-d_6): δ 10.69 and 10.08 (2s, 1H, NH), 9.17 and 8.87 (2s, 1H, OH), 5.93 (s, 1H, CH), 4.99 and 4.61 (2s, 2H, OCH_2), 3.48–3.38 (m, 4H, $N(CH_2)_2$), 2.18 (s, 3H, CH_3), 1.92–1.83 (m, 4H, $(CH_2)_2$); ^{13}C NMR (DMSO-d_6): δ 168.5, 167.8, 164.6, 159.5, 93.8, 62.1, 46.1, 24.9, 23.7; HRMS calcd for $C_{11}H_{16}N_4O_3$: $[M + H]^+$ = 253.1295, found: 253.1297.

*N-Hydroxy-(2-hydroxy-6-methylpyrimidin-4-yloxy)acetamide (**18**).* Reaction time 1 h; white solid; yield 0.109 g (55%); mp 190–191 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ 11.39 (s, 1H, NH), 10.73 and 10.17 (2s, 1H, NH), 9.27 and 8.94 (2s, 1H, OH), 5.82 (s, 1H, CH), 4.97 and 4.61 (2s, 2H, OCH_2), 2.14 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 171.1, 163.9, 157.2, 156.4, 92.6, 62.3, 18.0; HRMS calcd for $C_7H_9N_3O_4$: $[M + H]^+$ = 200.0666, found: 200.0671.

*Ethyl 4-[6-methyl-2-(methylthio)pyrimidin-4-yloxy]butanoate (**20a**).* Reaction time 4 h; isolated from a mixture of compounds **20a** and **20b**; R_f 0.37 (cyclohexane – ethyl acetate 4:1); colourless oil; yield 0.111 g (41%); ^{1}H NMR ($CDCl_3$): δ 6.18 (s, 1H, CH), 4.37 (t, *J* = 6.4 Hz, 2H, O<u>CH_2</u>CH_2), 4.12 (q, *J* = 7.2 Hz, 2H, O<u>CH_2</u>CH_3), 2.50 (s, 3H, SCH_3), 2.43 (t, *J* = 7.4 Hz, 2H, CH_2CO), 2.33 (s, 3H, CH_3), 2.13–2.00 (m, 2H, CH_2<u>CH_2</u>CH_2), 1.23 (t, *J* = 7.2 Hz, 3H, OCH_2<u>CH_3</u>); ^{13}C NMR ($CDCl_3$): δ 173.1, 171.4, 169.2, 167.7, 102.1, 65.4, 60.6, 30.9, 24.3, 23.7, 14.3, 14.1; HRMS calcd for $C_{12}H_{18}N_2O_3S$: $[M + H]^+$ = 271.1111, found: 271.1108.

*Ethyl 4-[6-methyl-2-(methylthio)-4-oxopyrimidin-3(4H)-yl]butanoate (**20b**).* Reaction time 4 h; isolated from a mixture of compounds **20a** and **20b**; R_f 0.17 (cyclohexane – ethylacetate 4:1); colourless oil; yield 0.084 g (31%); ^{1}H NMR ($CDCl_3$): δ 5.97 (s, 1H, CH), 4.09 (q, *J* = 7.2 Hz, 2H, O<u>CH_2</u>CH_3), 4.03 (t, *J* = 7.6 Hz, 2H, NCH_2), 2.51 (s, 3H, SCH_3), 2.36 (t, *J* = 7.4 Hz, 2H, CH_2CO), 2.16 (s, 3H, CH_3), 2.00 (quintet, *J* = 7.4 Hz, 2H, CH_2<u>CH_2</u>CH_2), 1.21 (t, *J* = 7.2 Hz, 3H, OCH_2<u>CH_3</u>); ^{13}C NMR ($CDCl_3$): δ 172.6, 162.3 (2), 161.4, 107.7, 60.6, 43.3, 31.5, 23.6, 22.8, 15.0, 14.2; HRMS calcd for $C_{12}H_{18}N_2O_3S$: $[M + H]^+$ = 271.1111, found: 271.1117.

*Methyl 6-[6-methyl-2-(methylthio)pyrimidin-4-yloxy]hexanoate (**21a**).* Reaction time 2.75 h; isolated from a mixture of compounds **21a** and **21b**; R_f 0.7 (chloroform – ethyl acetate 4:1); white solid; yield 0.054 g (19%); mp 50 °C; ^{1}H NMR ($CDCl_3$): δ 6.19 (s, 1H, CH), 4.33 (t, *J* = 6.6 Hz, 2H, O<u>CH_2</u>CH_2), 3.66 (s, 3H, OCH_3), 2.52 (s, 3H, SCH_3), 2.38–2.28 (m, 5H, CH_2CO, CH_3), 1.80–1.63 (m, 4H, $2CH_2$), 1.50–1.40 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 174.1, 171.4, 169.4, 167.7, 102.1, 66.2, 51.6, 34.1, 28.6, 25.7, 24.8, 23.8, 14.1; HRMS calcd for $C_{13}H_{20}N_2O_3S$: $[M + H]^+$ = 285.1267, found: 285.1268.

*Methyl 6-[6-methyl-2-(methylthio)-4-oxopyrimidin-3(4H)-yl]hexanoate (**21b**).* Reaction time 2.75 h; isolated from a mixture of compounds **21a** and **21b**; R_f 0.31 (chloroform – ethyl acetate 4:1); white solid; yield 0.026 g (9%); mp 52–54 °C; ^{1}H NMR ($CDCl_3$): δ 6.01 (s, 1H, CH), 3.98 (t, *J* = 8 Hz, 2H, NCH_2), 3.66 (s, 3H, OCH_3), 2.54 (s, 3H, SCH_3), 2.32 (t, *J* = 7.6 Hz, 2H, CH_2CO), 2.20 (s, 3H, CH_3), 1.78–1.63 (m, 4H, $2CH_2$), 1.45–1.35 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 174.1, 162.4, 162.3, 161.3, 107.9, 51.6, 44.0, 34.0, 27.2, 26.5, 24.6, 23.8, 15.1; HRMS calcd for $C_{13}H_{20}N_2O_3S$: $[M + H]^+$ = 285.1267, found: 285.1274.

*Ethyl [6-methyl-2-(methylthio)-5-propylpyrimidin-4-yloxy]acetate (**22a**).* Reaction time 0.5 h; isolated from a mixture of compounds **22a** and **22b**; R_f 0.53 (cyclohexane – ethyl acetate 4:1); white solid; yield 0.1 g (35%); mp 52–54 °C; ^{1}H NMR ($CDCl_3$): δ 4.88 (s, 2H, OCH_2), 4.21 (q, *J* = 7.2 Hz, 2H, $O\underline{CH_2}CH_3$), 2.54 (t, *J* = 7.6 Hz, 2H, CH_2), 2.45 (s, 3H, SCH_3), 2.39 (s, 3H, CH_3), 1.55 (sextet, *J* = 7.6 Hz, 2H, CH_2), 1.25 (t, *J* = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 0.96 (t, *J* = 7.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 168.7, 167.2, 166.2, 165.9, 114.8, 62.9, 61.3, 26.9, 22.0, 21.4, 14.3, 14.2, 14.0; HRMS calcd for $C_{13}H_{20}N_2O_3S$: $[M + H]^+$ = 285.1267, found: 285.1270.

*Ethyl [6-methyl-2-(methylthio)-4-oxo-5-propylpyrimidin-3(4H)-yl]acetate (**22b**).* Reaction time 0.5 h; isolated from a mixture of compounds **22a** and **22b**; R_f 0.39 (cyclohexane – ethyl acetate 4:1); white solid; yield 0.029 g (10%); mp 87–88 °C; ^{1}H NMR ($CDCl_3$): δ 4.77 (s, 2H, NCH_2), 4.23 (q, *J* = 7.2 Hz, 2H, $O\underline{CH_2}CH_3$), 2.55 (s, 3H, SCH_3), 2.45 (t, *J* = 7.6 Hz, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.49 (sextet, *J* = 7.6 Hz, 2H, CH_2), 1.27 (t, *J* = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 0.94 (t, *J* = 7.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 167.0, 162.3, 158.3, 157.3, 119.6, 62.0, 45.2, 28.2, 21.7, 21.6, 14.9, 14.3, 14.2; HRMS calcd for $C_{13}H_{20}N_2O_3S$: $[M + H]^+$ = 285.1267, found: 285.1267.

*Ethyl 4-[6-methyl-2-(methylthio)-5-propylpyrimidin-4-yloxy]butanoate (**23a**).* Reaction time 4 h; isolated from a mixture of compounds **23a** and **23b**; R_f 0.69 (chloroform – ethyl acetate 30:1); colourless oil; yield 0.116 g (37%); ^{1}H NMR ($CDCl_3$): δ 4.37 (t, *J* = 6.4 Hz, 2H, OCH_2), 4.12 (q, *J* = 7.2 Hz, 2H, $O\underline{CH_2}CH_3$), 2.49 (s, 3H, SCH_3), 2.48–2.41 (m, 4H, $2CH_2$), 2.35 (s, 3H, CH_3), 2.08 (quintet, *J* = 6.8 Hz, 2H, $CH_2\underline{CH_2}CH_2$), 1.45 (sextet, *J* = 7.6 Hz, 2H, CH_2), 1.24 (t, *J* = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 0.92 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 173.1, 167.3, 167.0, 165.0, 114.7, 65.4, 60.6, 31.0, 26.8, 24.4, 22.1, 21.3, 14.3, 14.1 (2); HRMS calcd for $C_{15}H_{24}N_2O_3S$: $[M + H]^+$ = 313.1580, found: 313.1580.

*Ethyl 4-[6-methyl-2-(methylthio)-4-oxo-5-propylpyrimidin-3(4H)-yl]butanoate (**23b**).* Reaction time 4 h; isolated from a mixture of compounds **23a** and **23b**; R_f 0.5 (chloroform – ethyl acetate 30:1); white solid; yield 0.1 g (32%); mp 53–55 °C; ^{1}H NMR ($CDCl_3$): δ 4.13 (q, *J* = 7.2 Hz, 2H, $O\underline{CH_2}CH_3$), 4.06 (t, *J* = 7.6 Hz, 2H, NCH_2), 2.53 (s, 3H, SCH_3), 2.46–2.35 (m, 4H, $2CH_2$), 2.24 (s, 3H, CH_3), 2.04 (quintet, *J* = 7.6 Hz, 2H, $CH_2\underline{CH_2}CH_2$), 1.48 (sextet, *J* = 7.6 Hz, 2H, CH_2), 1.25 (t, *J* = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 0.95 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.8, 162.6, 157.8, 157.4, 119.7, 60.7, 43.9, 31.8, 28.3, 23.1, 21.7, 21.5, 14.8, 14.4, 14.3; HRMS calcd for $C_{15}H_{24}N_2O_3S$: $[M + H]^+$ = 313.1580, found: 313.1580.

*Methyl 6-[6-methyl-2-(methylthio)-5-propylpyrimidin-4-yloxy]hexanoate (**24a**).* Reaction time 5 h; isolated from a mixture of compounds **24a** and **24b**; R_f 0.47 (chloroform – ethyl acetate 30:1); colourless oil; yield 0.099 g (30%); ^{1}H NMR ($CDCl_3$): δ 4.37 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.66 (s, 3H, OCH_3), 2.51 (s, 3H, SCH_3), 2.46 (t, *J* = 7.8 Hz, 3H, CH_2CO), 2.36 (s, 3H, CH_3), 2.34 (t, *J* = 7.6 Hz, 2H, CH_2), 1.82–1.65 (m, 4H, $2CH_2$), 1.53–1.41 (m, 4H, $2CH_2$), 0.93 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 174.1, 167.3, 167.2, 164.8, 114.8, 66.2, 51.6, 34.1, 28.7, 26.9, 25.8, 24.8, 22.1, 21.3, 14.2, 14.1; HRMS calcd for $C_{16}H_{26}N_2O_3S$: $[M + H]^+$ = 327.1737, found: 327.1737.

*Methyl 6-[6-methyl-2-(methylthio)-4-oxo-5-propylpyrimidin-3(4H)-yl]hexanoate (**24b**).* Reaction time 5 h; isolated from a mixture of compounds **24a** and **24b**; R_f 0.3 (chloroform – ethyl acetate 30:1); white solid; yield 0.072 g (22%); mp 61–62 °C; ^{1}H NMR ($CDCl_3$): δ 3.98 (t, *J* = 7.8 Hz, 2H, NCH_2), 3.66 (s, 3H, OCH_3), 2.52 (s, 3H, SCH_3), 2.43 (t, *J* = 7.8 Hz, 3H, CH_2CO), 2.32 (t, *J* = 7.4 Hz, 2H, CH_2), 2.24 (s, 3H, CH_3), 1.79–1.60 (m, 4H, $2CH_2$), 1.55–1.35 (m, 4H, $2CH_2$), 0.95 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 174.1, 162.6, 157.7, 157.3, 119.7, 51.6, 44.6, 34.0, 28.3, 27.3, 26.6, 24.6, 21.7, 21.5, 14.8, 14.3; HRMS calcd for $C_{16}H_{26}N_2O_3S$: $[M + H]^+$ = 327.1737, found: 327.1737.

*N-Hydroxy-4-[6-methyl-2-(methylthio)pyrimidin-4-yloxy]butanamide (**25**).* White solid; yield 0.134 g (52%); mp 150–151 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 10.46 and 9.84 (2s, 1H, NH), 9.12 and 8.72 (2s, 1H, OH), 6.46 (s, 1H, CH), 4.27 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.47 (s, 3H, SCH_3), 2.30 (s, 3H, CH_3), 2.08 (t, *J* = 7.4 Hz, 2H, CH_2CO), 1.91 (quintet, *J* = 6.8 Hz, 2H, $CH_2\underline{CH_2}CH_2$); ^{13}C NMR (DMSO-*d*$_6$): δ 170.3, 168.7, 168.4, 167.8, 101.8, 65.6, 28.7, 24.4, 23.3, 13.4; HRMS calcd for $C_{10}H_{15}N_3O_3S$: $[M + H]^+$ = 258.0907, found: 258.0906.

*N-Hydroxy-6-[6-methyl-2-(methylthio)pyrimidin-4-yloxy]hexanamide (**26**).* White solid; yield 0.193 g (68%); mp 112–113 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 10.35 and 9.75 (2s, 1H, NH), 9.01 and 8.68 (2s, 1H, OH), 6.45 (s, 1H, CH), 4.27 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.47 (s, 3H, SCH_3), 2.29 (s, 3H, CH_3), 1.95 (t, *J* = 7.2 Hz, 2H, CH_2CO), 1.68 (quintet, *J* = 7 Hz, 2H, CH_2); 1.53 (quintet, *J* = 7.2 Hz, 2H, CH_2), 1.40–1.27 (m, 2H, CH_2); ^{13}C NMR (DMSO-*d*$_6$): δ 170.3, 169.0, 168.8, 167.8, 101.8, 66.0, 32.2, 27.9, 25.0, 24.8, 23.3, 13.4; HRMS calcd for $C_{12}H_{19}N_3O_3S$: $[M + H]^+$ = 286.1220, found: 286.1223.

*N-Hydroxy-[6-methyl-2-(methylthio)-5-propylpyrimidin-4-yloxy]acetamide (**27**).* White solid; yield 0.230 g (85%); mp 144–146 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 10.76 and 10.18 (2s, 1H, NH), 9.31 and 8.96 (2s, 1H, OH), 5.10 and 4.73 (2s, 2H, OCH_2), 2.55–2.46 (m, 2H, CH_2, overlaps with the residual signal of DMSO-*d*$_6$), 2.44 (s, 3H, SCH_3), 2.34 (s, 3H, CH_3), 1.48 (sextet, *J* = 7.4 Hz, 2H, CH_2), 0.90 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-*d*$_6$): δ 166.3, 166.0, 165.3, 164.3, 114.2, 62.9, 26.1, 21.4, 21.0, 13.9, 13.4; HRMS calcd for $C_{11}H_{17}N_3O_3S$: $[M + H]^+$ = 272.1063, found: 272.1069.

*N-Hydroxy-4-[6-methyl-2-(methylthio)-5-propylpyrimidin-4-yloxy]butanamide (**28**).* White solid; yield 0.275 g (92%); mp 125–127 °C. ^{1}H NMR (DMSO-*d*$_6$): δ 10.42 and 9.84 (2s, 1H, NH), 9.04 and 8.70 (2s, 1H, OH), 4.29 (t, *J* = 6.2 Hz, 2H, OCH_2), 2.48–2.42 (m, 5H, CH_2, SCH_3), 2.33 (s, 3H, CH_3), 2.10 (t, *J* = 7.2 Hz, 2H, CH_2), 1.92 (quintet, *J* = 6.8 Hz, 2H, CH_2), 1.45 (sextet, *J* = 7.4 Hz, 2H, CH_2), 0.89 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-*d*$_6$): δ 168.5, 166.5, 166.4, 164.8, 114.2, 65.5, 28.7, 26.1, 24.5, 21.5, 21.0, 13.8, 13.4; HRMS calcd for $C_{13}H_{21}N_3O_3S$: $[M + H]^+$ = 300.1376, found: 300.1376.

*N-Hydroxy-6-[6-methyl-2-(methylthio)-5-propylpyrimidin-4-yloxy]hexanamide (**29**).* R_f 0.32 (chloroform – ethyl acetate – methanol 14:1:1); yiellowish orange oil; yield 0.249 g (76%); ^{1}H NMR (DMSO-*d*$_6$): δ 10.34 and 9.74 (2 br. s, 1H, NH), 8.69 (very br. s, 1H, OH), 4.29 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.48–2.41 (m, 5H, CH_2, SCH_3), 2.32 (s, 3H, CH_3), 1.95 (t, *J* = 7.4 Hz, 2H, CH_2), 1.69 (quintet, *J* = 7.1 Hz, 2H, CH_2), 1.54 (quintet, *J* = 7.5 Hz, 2H, CH_2), 1.44 (sextet, *J* = 7.5 Hz, 2H, CH_2), 1.36 (quintet, *J* = 7.2 Hz, 2H, CH_2), 0.88 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-*d*$_6$): δ 169.0, 166.6, 166.4, 164.7, 114.1, 66.0, 32.2, 28.0, 26.1, 25.1, 24.8, 21.5, 21.0, 13.8, 13.4; HRMS calcd for $C_{15}H_{25}N_3O_3S$: $[M + H]^+$ = 328.1689, found: 328.1689.

N-Hydroxy-[6-methyl-2-(methylthio)-4-oxo-5-propylpyrimidin-3(4H)-yl]acetamide ***(30)***. White solid; yield 0.141 g (58%); mp 185–187 °C (ethanol-water); ^{1}H NMR (DMSO-d_6): δ 10.78 and 10.39 (2s, 1H, NH), 9.46 and 9.01 (2s, 1H, OH), 4.82 and 4.50 (2s, 2H, NCH_2), 2.50 (s, 3H, SCH_3, overlaps with the residual signal of DMSO-d_6), 2.35 (t, J = 7.2 Hz, 2H, CH_2), 2.24 (s, 3H, CH_3), 1.40 (sextet, J = 7.2 Hz, 2H, CH_2), 0.88 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 162.7, 161.3, 158.0, 157.2, 118.2, 43.8, 27.5, 21.3, 21.1, 14.4, 13.9; HRMS calcd for $C_{11}H_{17}N_3O_3S$: $[M + H]^+$ = 272.1063, found: 272.1066.

N-Hydroxy-4-[6-methyl-2-(methylthio)-4-oxo-5-propylpyrimidin-3(4H)-yl]butanamide ***(31)***. Yield 0.188 g (63%); mp 133–135 °C. ^{1}H NMR (DMSO-d_6): δ): δ 10.41 and 9.83 (2s, 1H, NH), 9.04 and 8.72 (2s, 1H, OH), 3.93 (t, J = 7.6 Hz, 2H, NCH_2), 2.51 (s, 3H, SCH_3, overlaps with the residual signal of DMSO-d_6), 2.35 (t, J = 7.6 Hz, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.01 (t, J = 7.6 Hz, 2H, CH_2),), 1.81 (quintet, J = 7.6 Hz, 2H, CH_2), 1.40 (sextet, J = 7.4 Hz, 2H, CH_2), 0.88 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 168.1, 161.3, 157.3, 157.0, 118.4, 43.6, 29.8, 27.5, 23.5, 21.2, 21.1, 14.2, 13.9; HRMS calcd for $C_{13}H_{21}N_3O_3S$: $[M + H]^+$ = 300.1376, found: 300.1375.

HDAC enzyme activity assay

Recombinant HDAC4 and 8 were produced as described in [3]. Serial dilutions of the inhibitor in the assay buffer (25 mM Tris-HCl, pH 8.0, 75 mM KCl, 0.001% Pluronic F-127) were incubated with HDAC in a black 96-well microtiter half-area plate (Greiner) for 60 min at 30 °C. Subsequently the reaction was started by the addition of 20 µM Boc-Lys(trifluoroacetyl)-AMC (Bachem) as a substrate. After incubation for 60 min at 30 °C, the reaction was stopped by the addition of 1.7 µM SATFMK. The deacetylated substrate was transformed into a fluorescent product by the addition of 0.4 mg/mL trypsin (Applichem). The release of AMC was followed in a microplate reader (PheraStar Plus, BMG Labtech) at 450 nm (λ_{Ex} = 350 nm) and correlated to enzyme activity. Dose–response curves were produced with GraphPad Prism and fitted to a four parameters logistic function to obtain IC_{50} values [4].

$$EA = E_0 + \frac{(E_{max} - E_0)}{1 + 10^{(\log(IC_{50}) - x) \cdot h}}$$

in which EA is the enzyme activity at a given inhibitor concentration x; E_{max} and E_0 are the enzyme activities determined at zero and complete inhibition, respectively; IC_{50} represents the inhibitor concentration at which half the enzyme molecules are inhibited; h is the slope of the curve.

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1. Gut, J.; Morávek, J.; Párkányi, C.; Prystaš, M.; Škoda, J.; Šorm, F. *Collect. Czech. Chem. Commun*. **1959**, *24*, 3154–3162. doi: 10.1135/cccc19593154
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3. Jänsch, N.; Meyners, C.; Muth, M.; Kopranovic, A.; Witt, O.; Oehme, I.; Meyer-Almes, F.-J. *Redox Biol*. **2019**, *20*, 60–67. doi: 10.1016/j.redox.2018.09.013
4. Volund, A. *Biometrics*. **1978**, *34*, 357. doi: 10.2307/2530598

Copies of NMR spectra

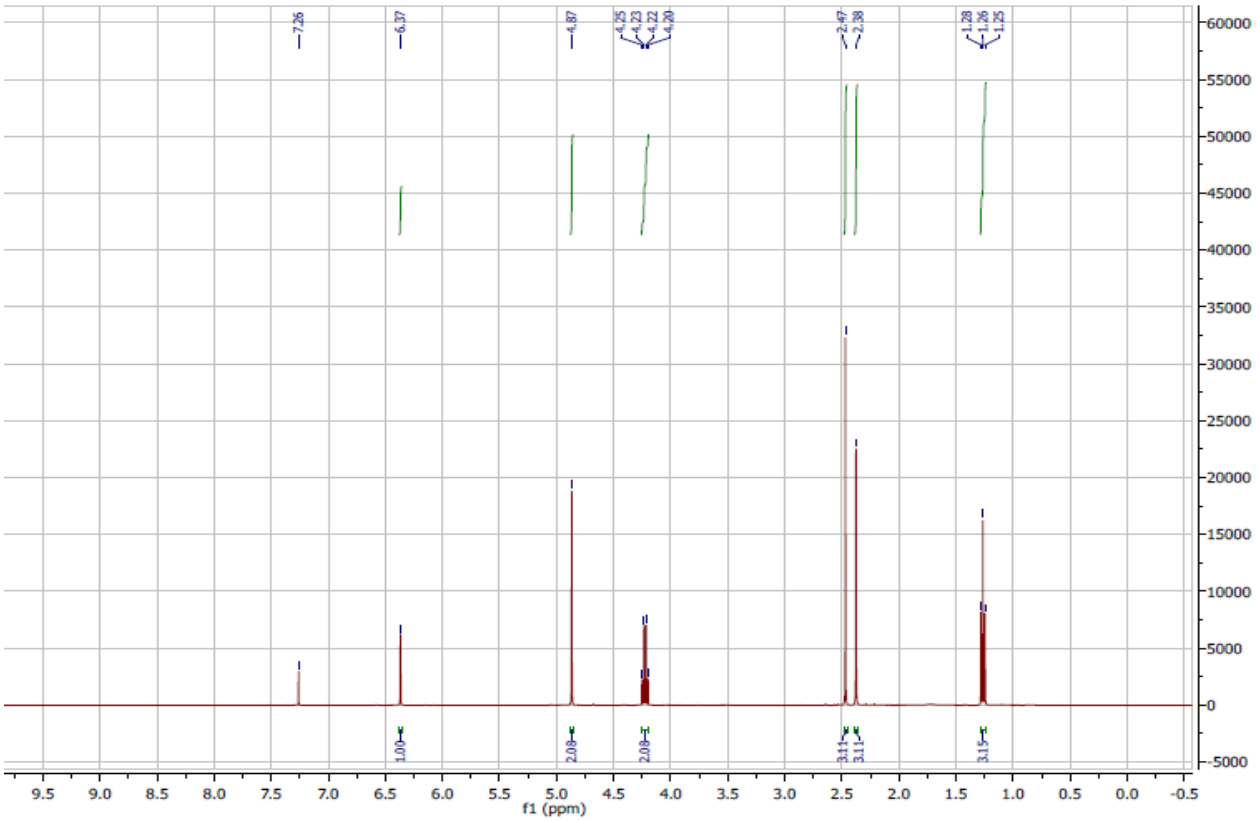

Figure S1: ^{1}H NMR spectrum of compound **3** ($CDCl_3$).

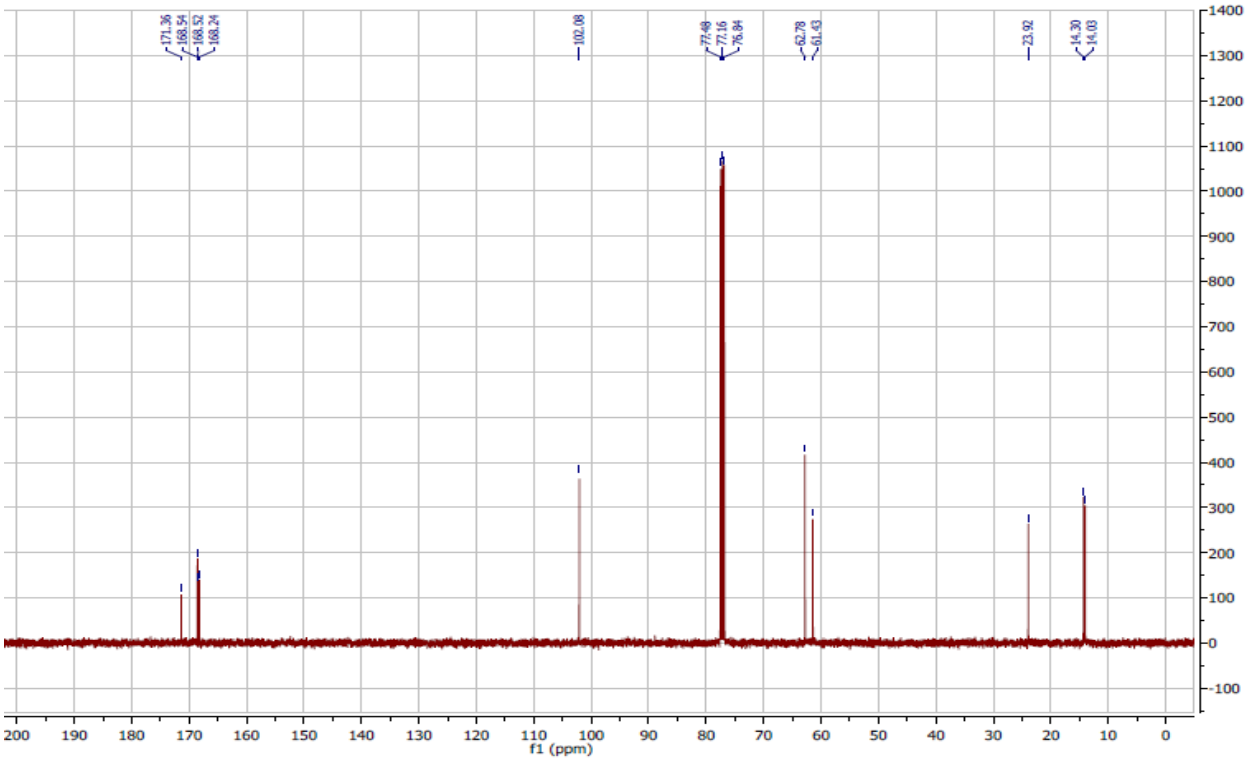

Figure S2: ^{13}C NMR spectrum of compound **3** ($CDCl_3$).

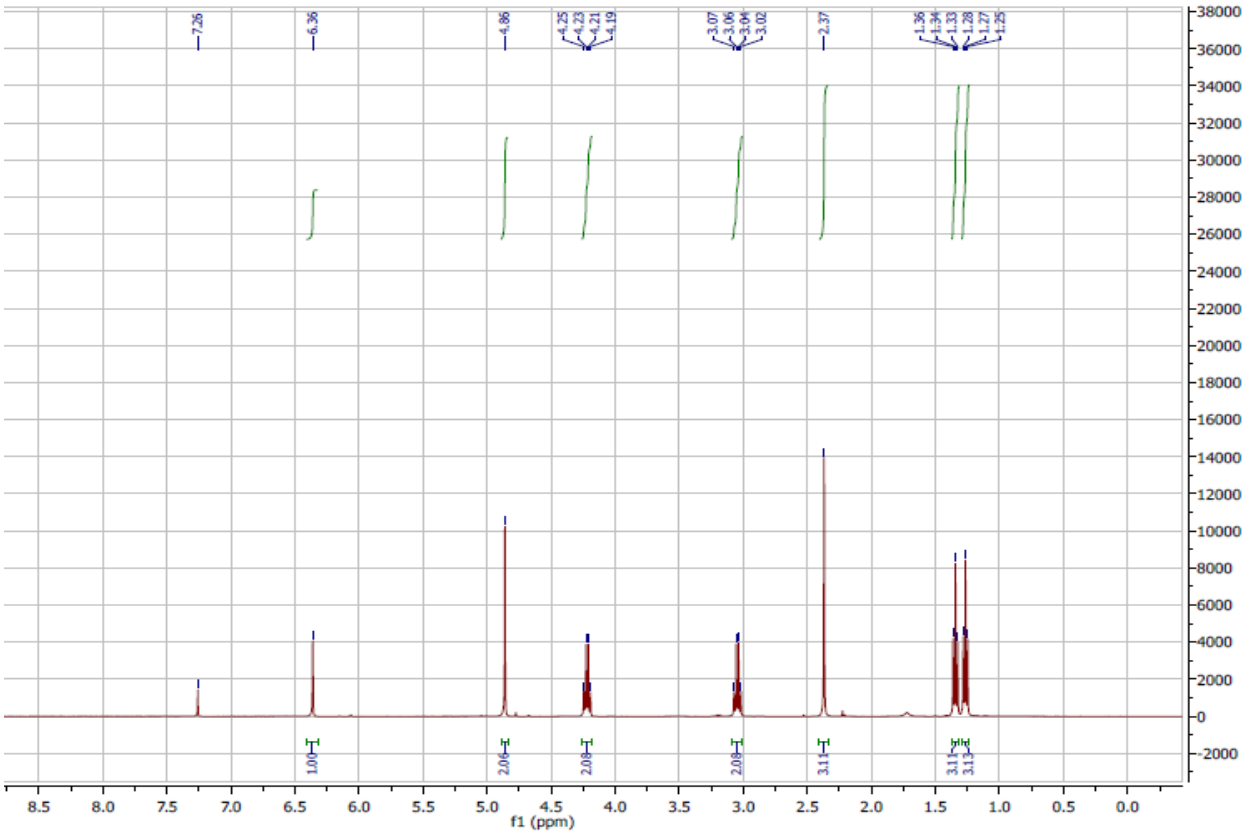

Figure S3: ^{1}H NMR spectrum of compound **4** ($CDCl_3$).

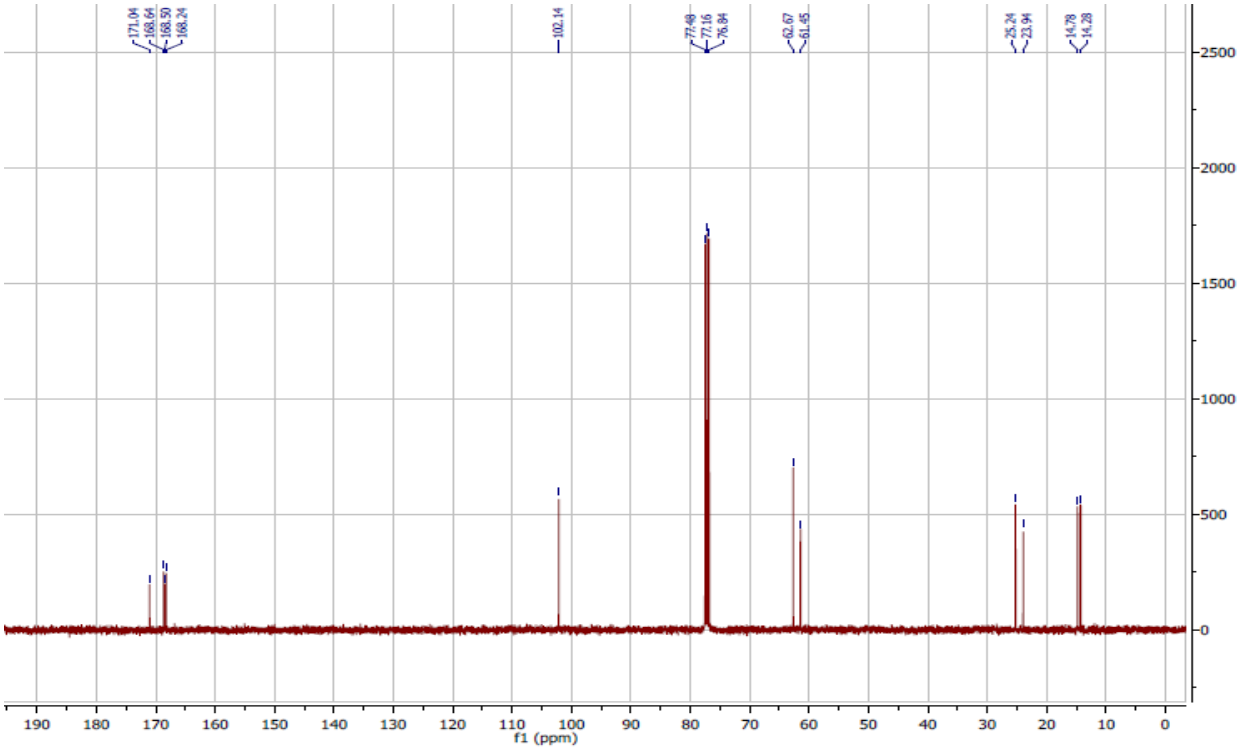

Figure S4: ^{13}C NMR spectrum of compound **4** ($CDCl_3$).

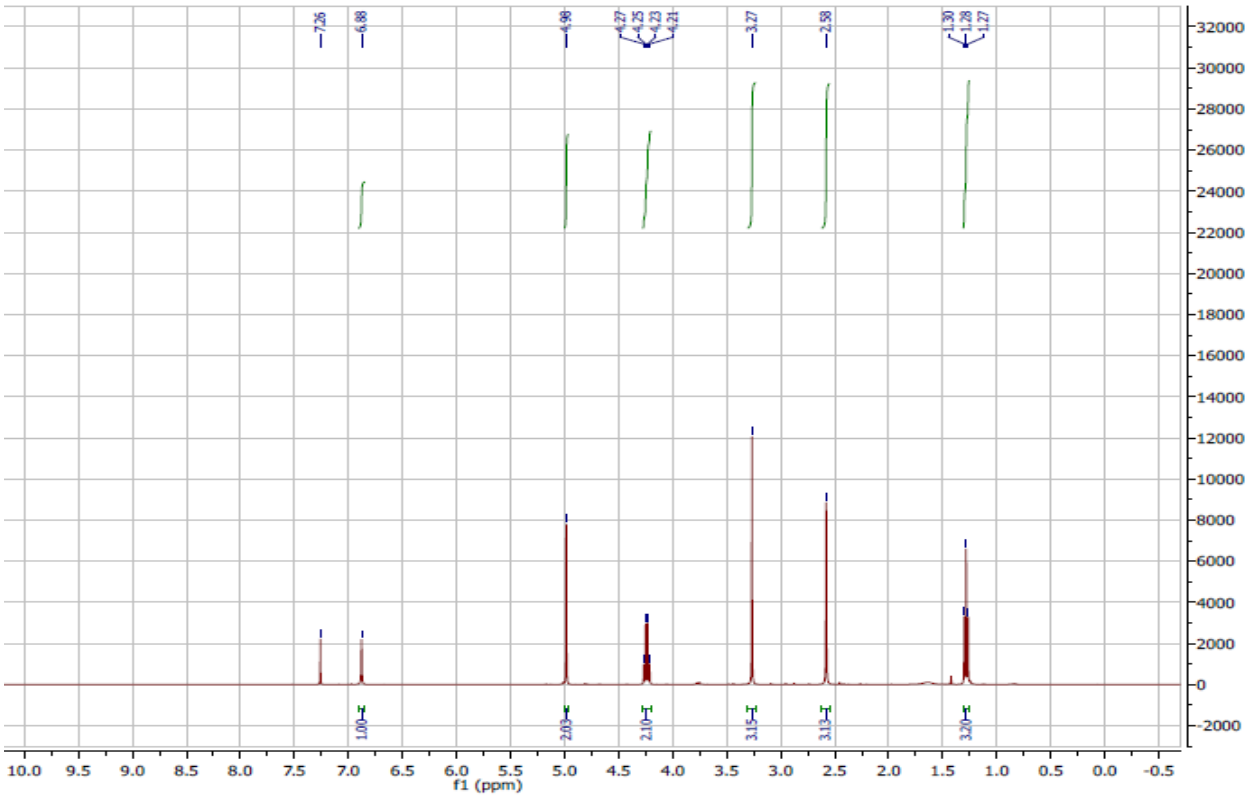

Figure S5: ^{1}H NMR spectrum of compound **5** ($CDCl_3$).

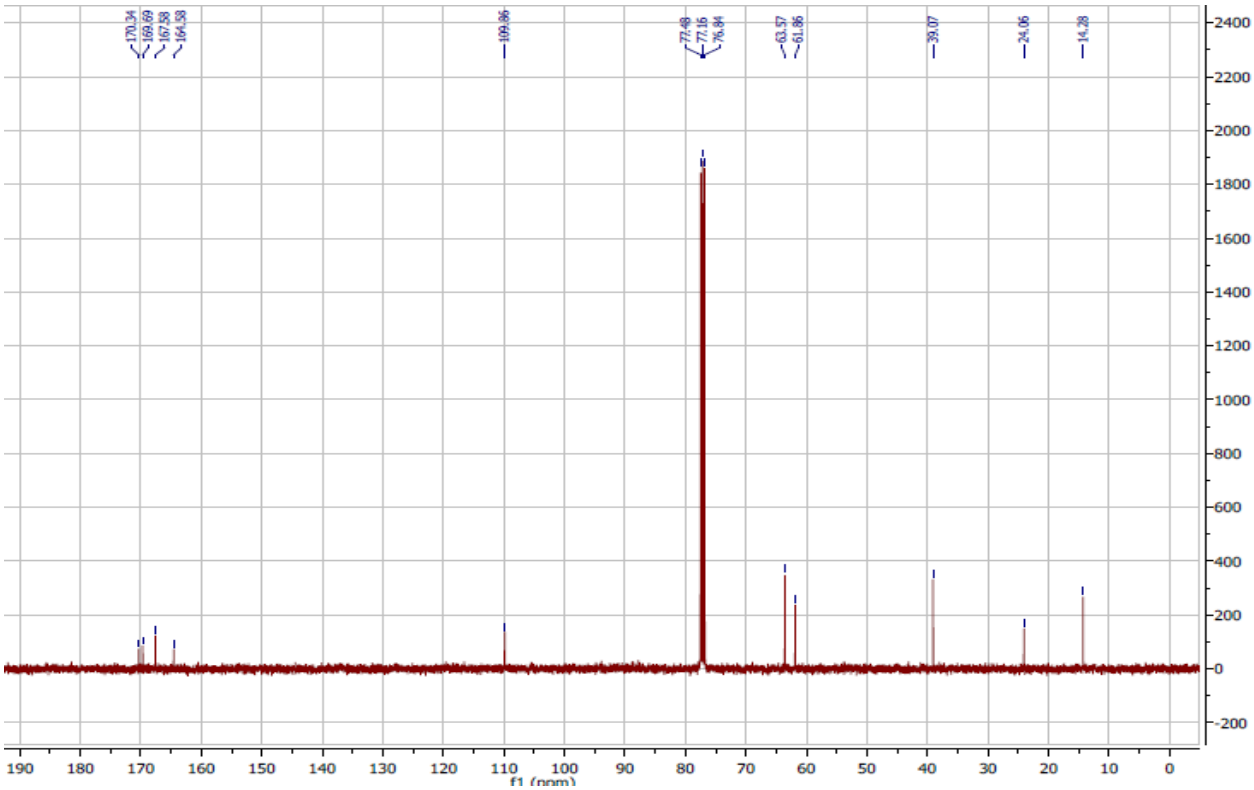

Figure S6: ^{13}C NMR spectrum of compound **5** ($CDCl_3$).

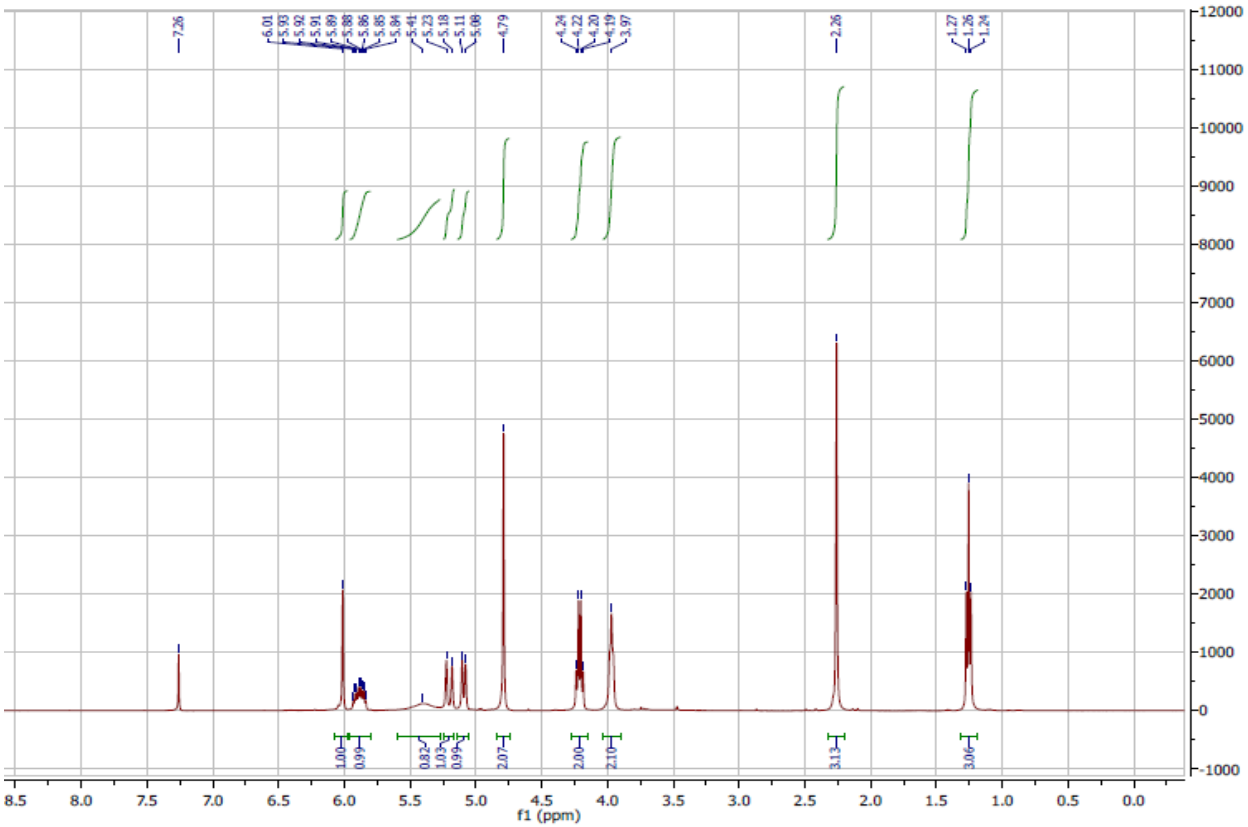

Figure S7: ^{1}H NMR spectrum of compound **6** ($CDCl_3$).

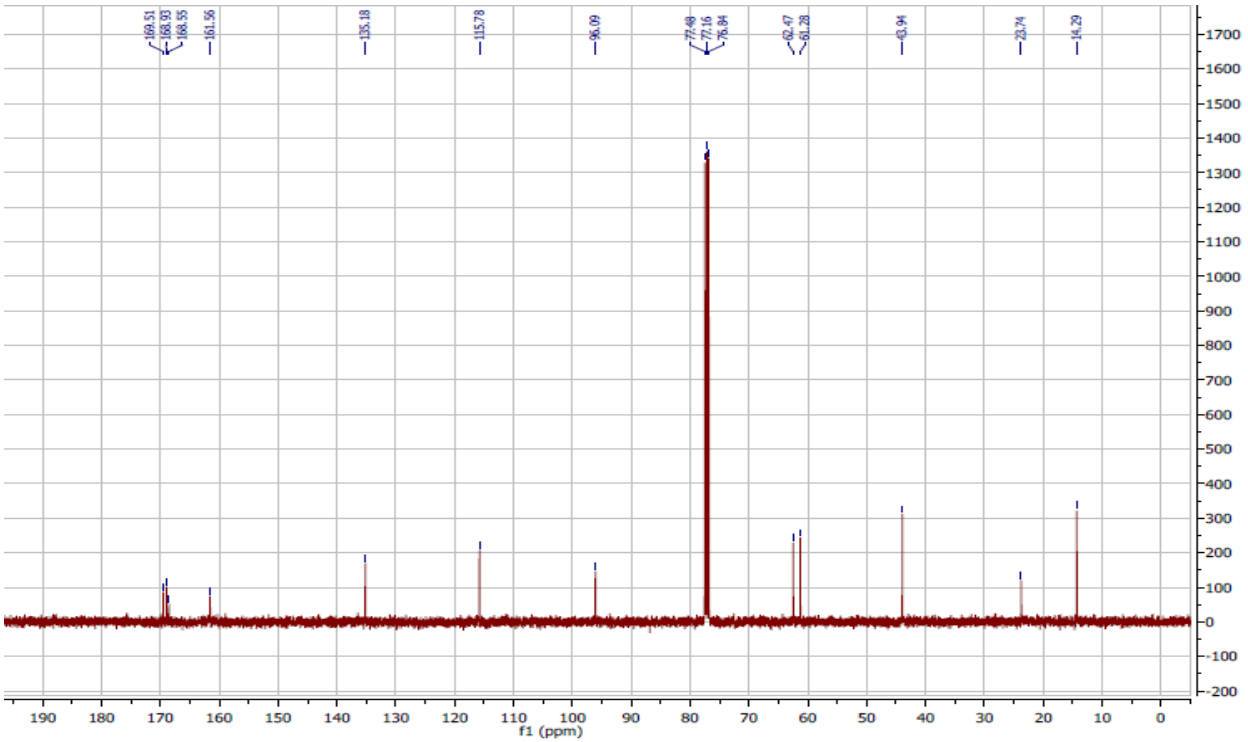

Figure S8: ^{13}C NMR spectrum of compound **6** ($CDCl_3$).

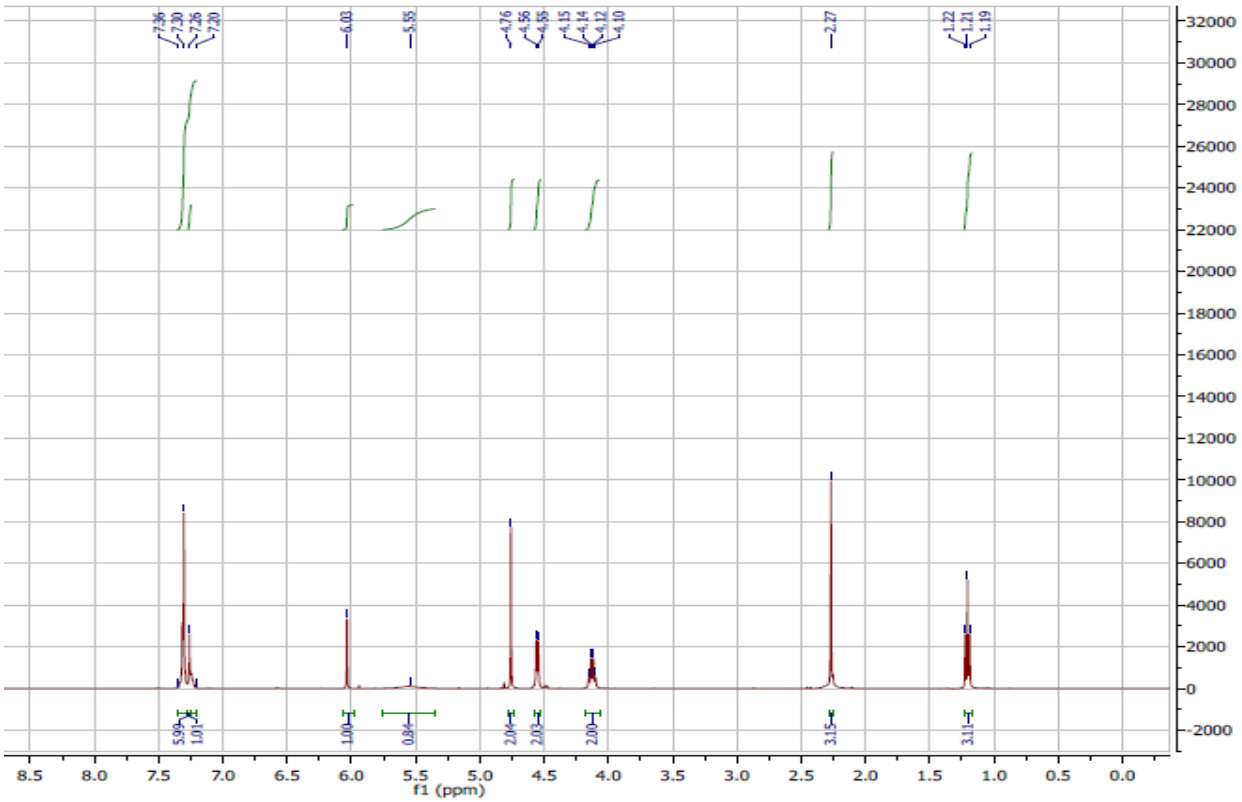

Figure S9: ^{1}H NMR spectrum of compound **7** ($CDCl_3$).

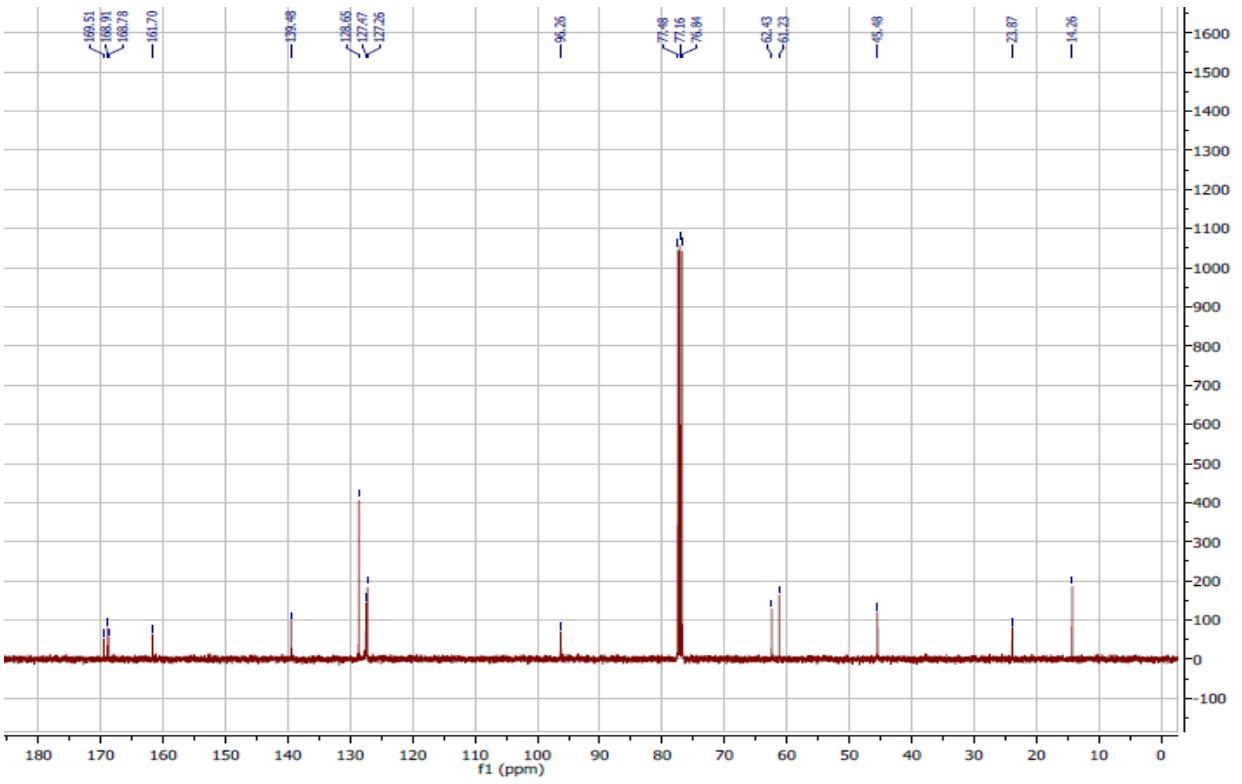

Figure S10: ^{13}C NMR spectrum of compound **7** ($CDCl_3$).

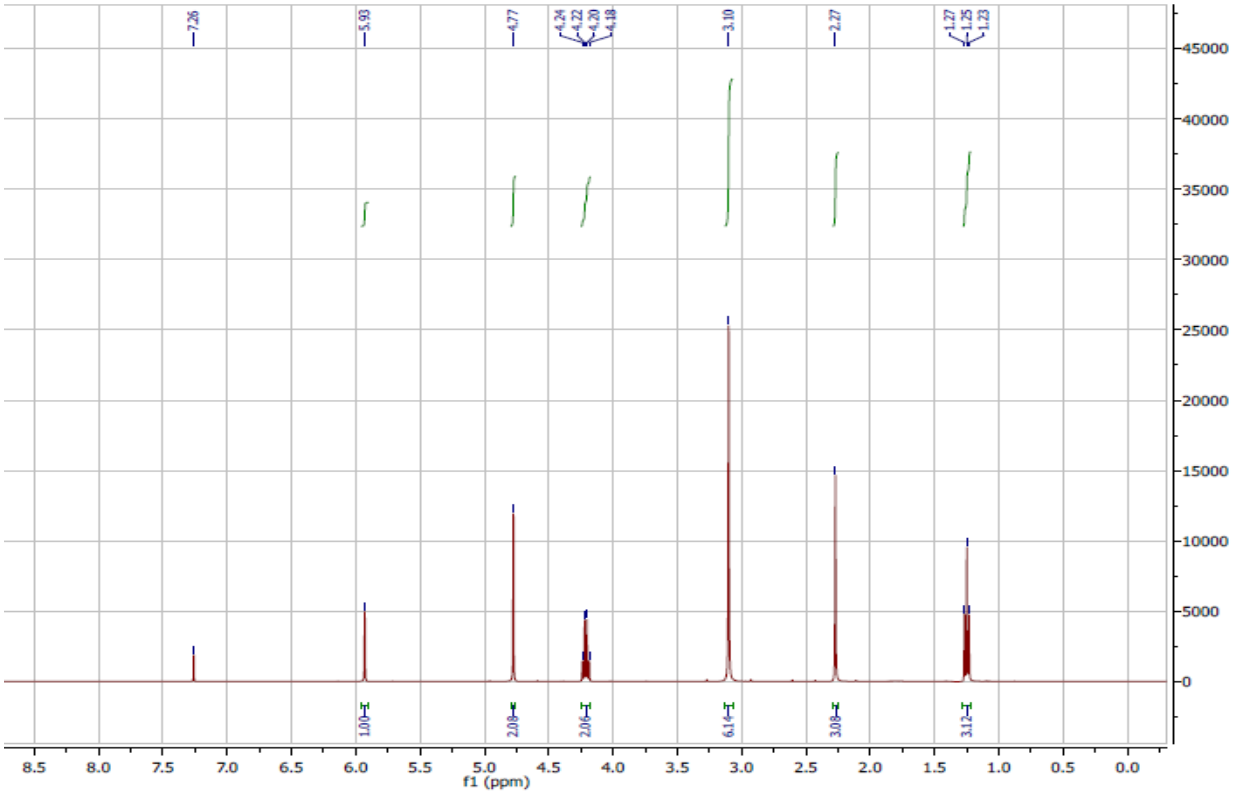

Figure S11: ^{1}H NMR spectrum of compound **8** ($CDCl_3$).

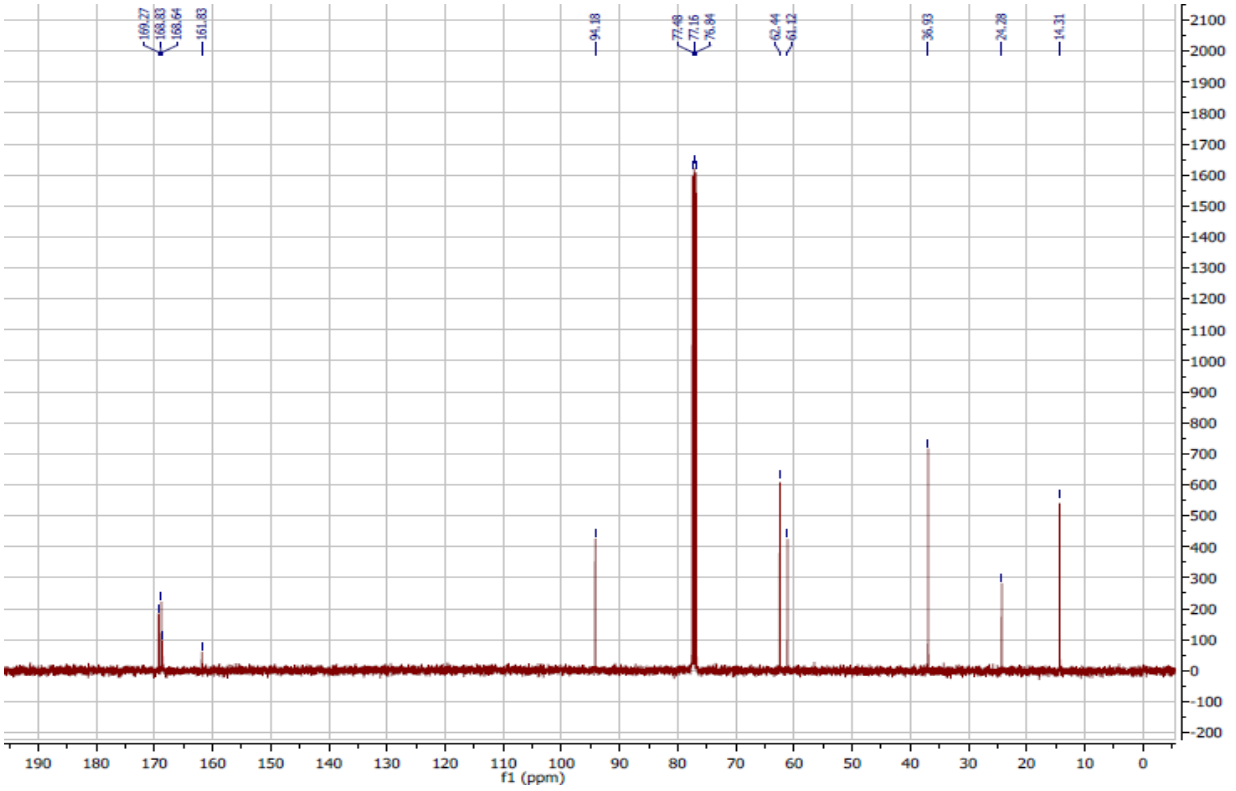

Figure S12: ^{13}C NMR spectrum of compound **8** ($CDCl_3$).

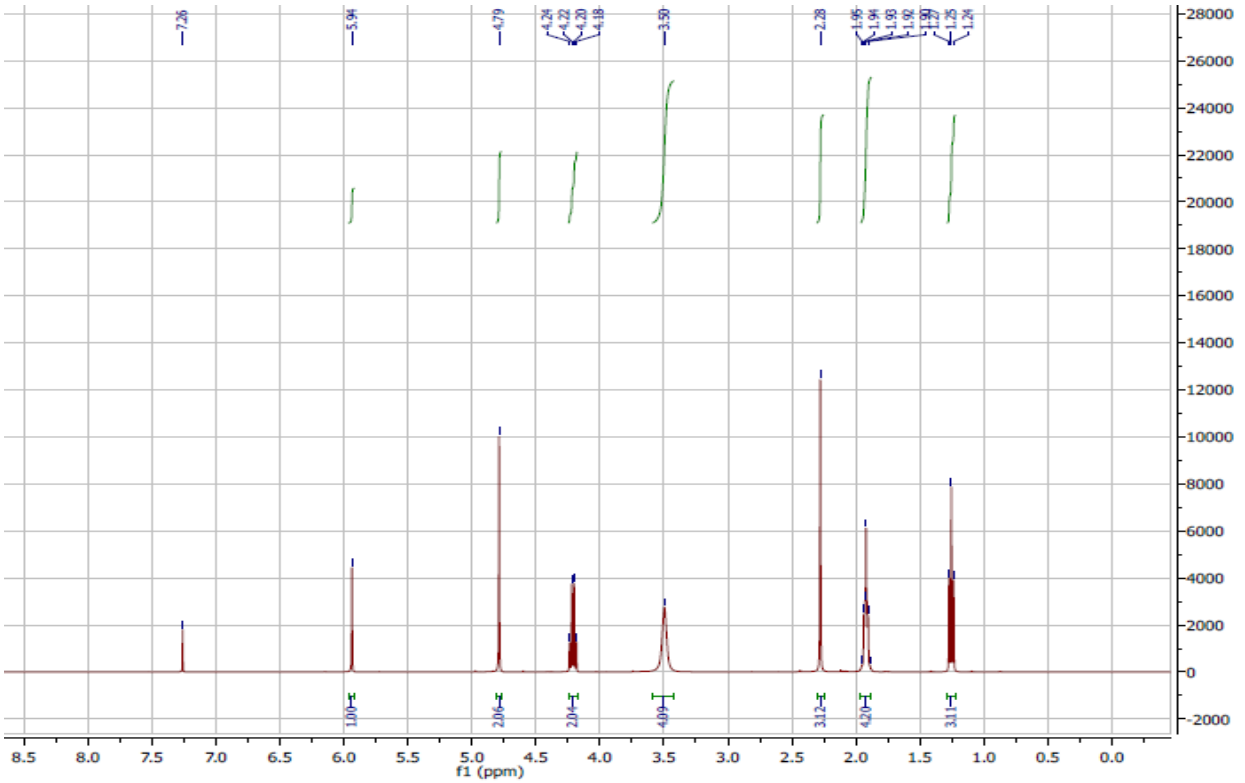

Figure S13: ^{1}H NMR spectrum of compound **9** ($CDCl_3$).

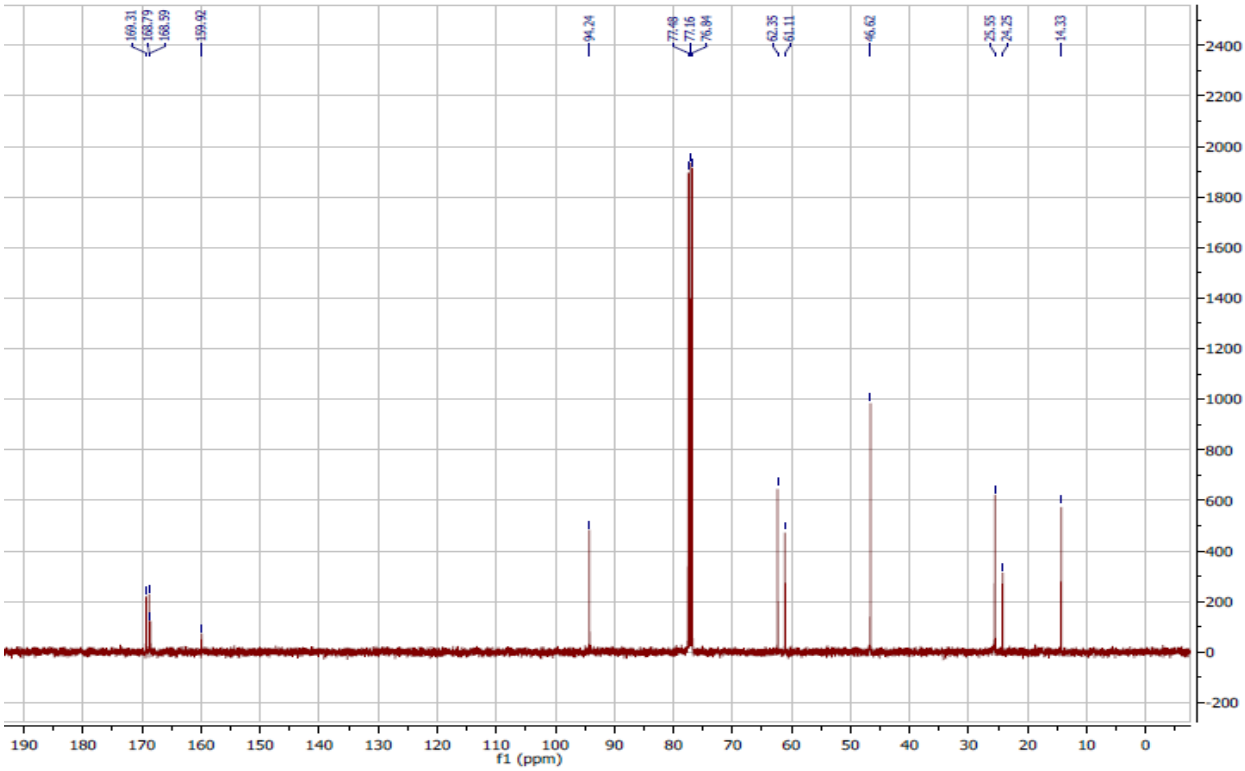

Figure S14: ^{13}C NMR spectrum of compound **9** ($CDCl_3$).

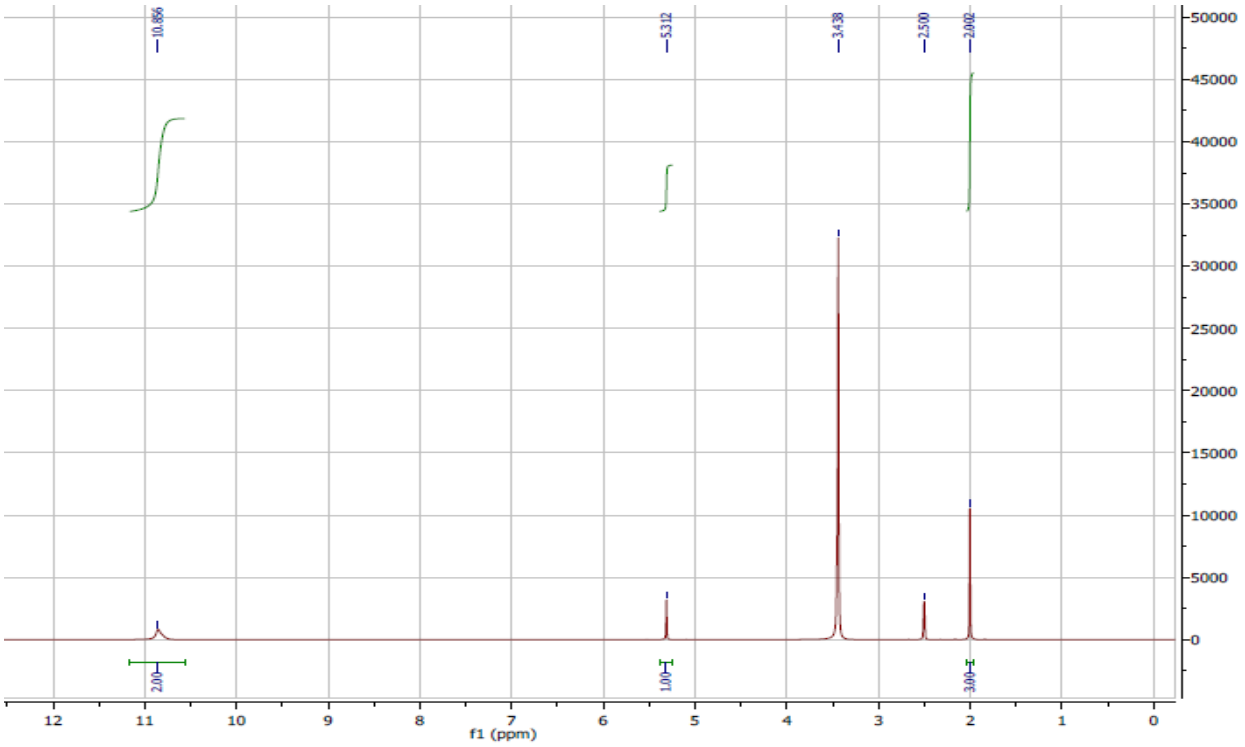

Figure S15: ^{1}H NMR spectrum of compound **10** (DMSO-d_6).

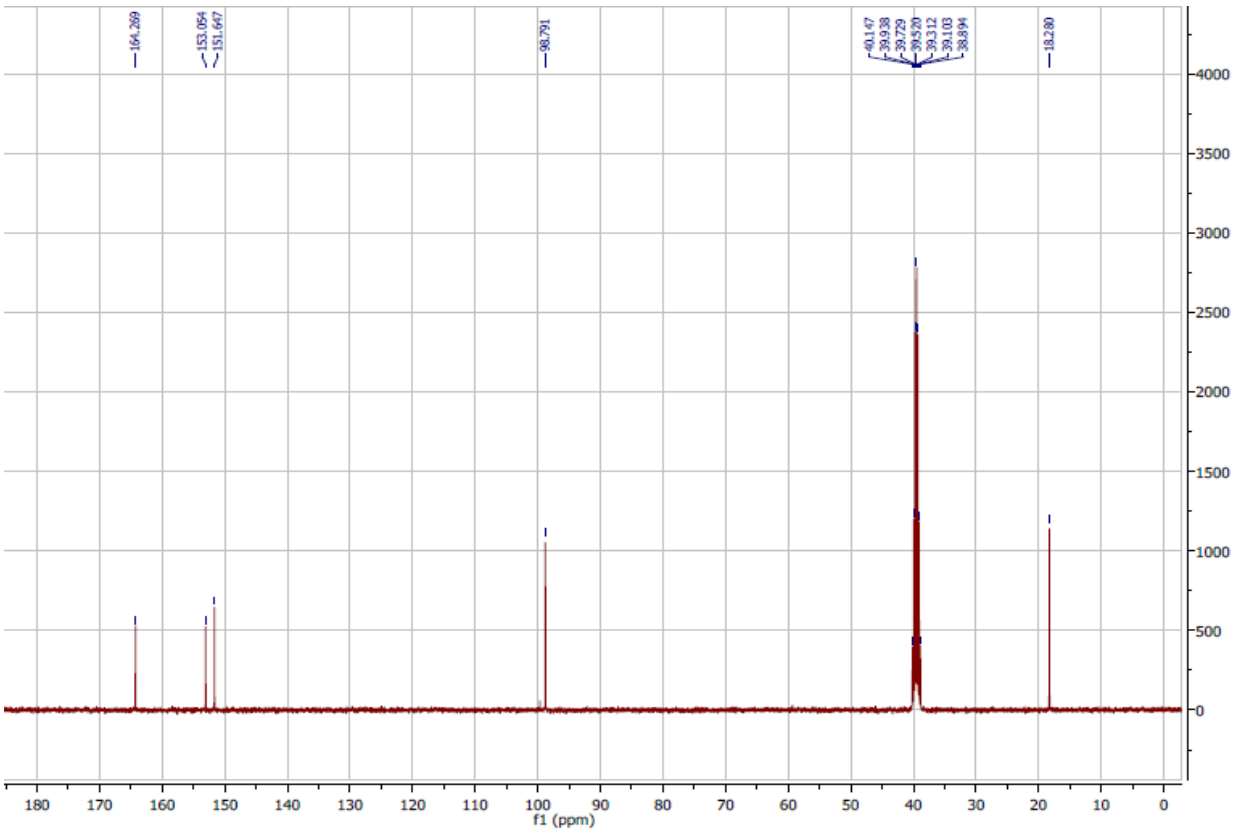

Figure S16: ^{13}C NMR spectrum of compound **10** (DMSO-d_6).

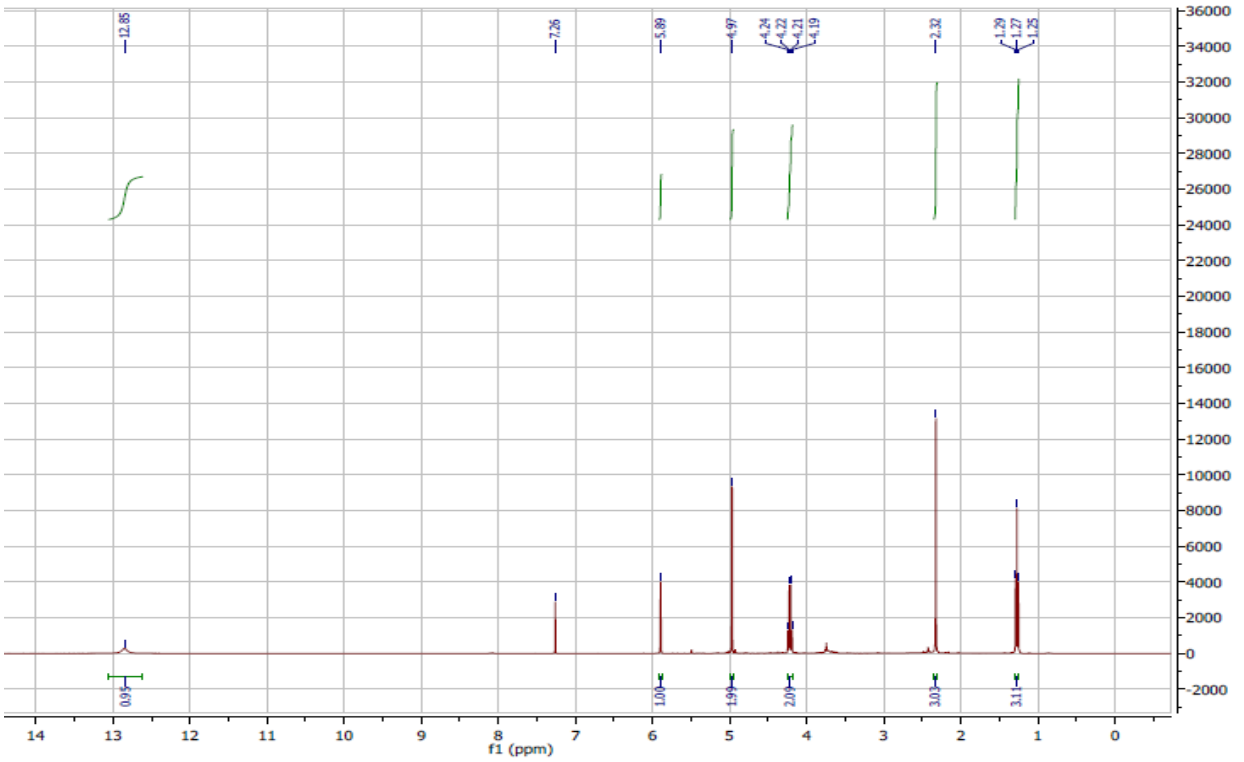

Figure S17: ^{1}H NMR spectrum of compound **11** ($CDCl_3$).

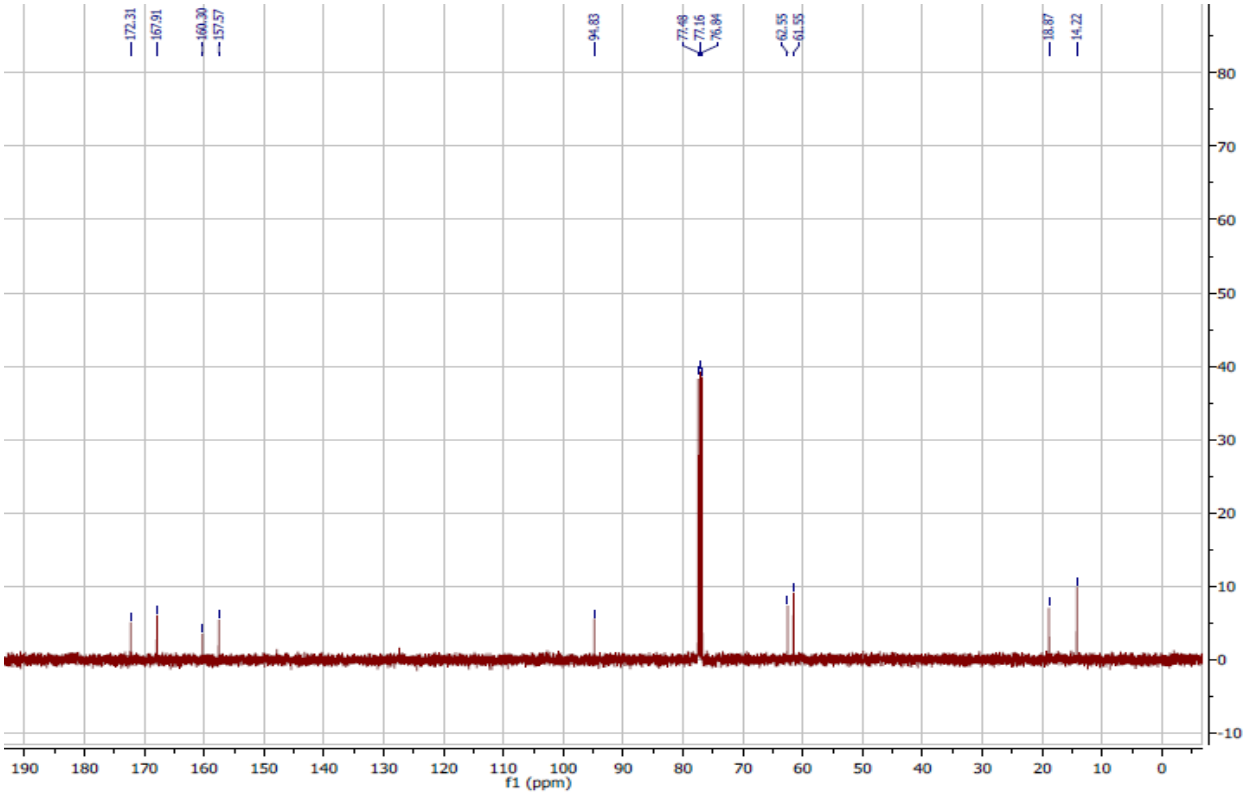

Figure S18: ^{13}C NMR spectrum of compound **11** ($CDCl_3$).

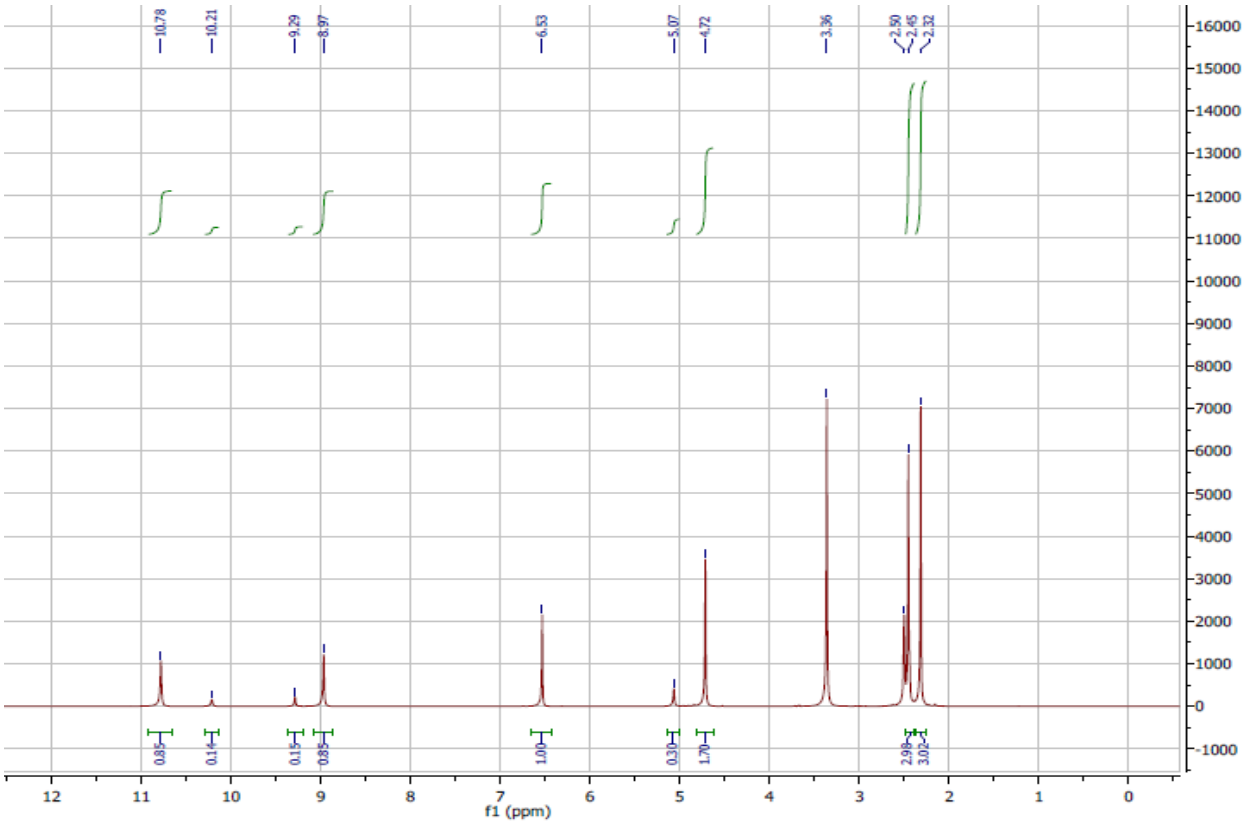

Figure S19: ^{1}H NMR spectrum of compound **12** (DMSO-d_6).

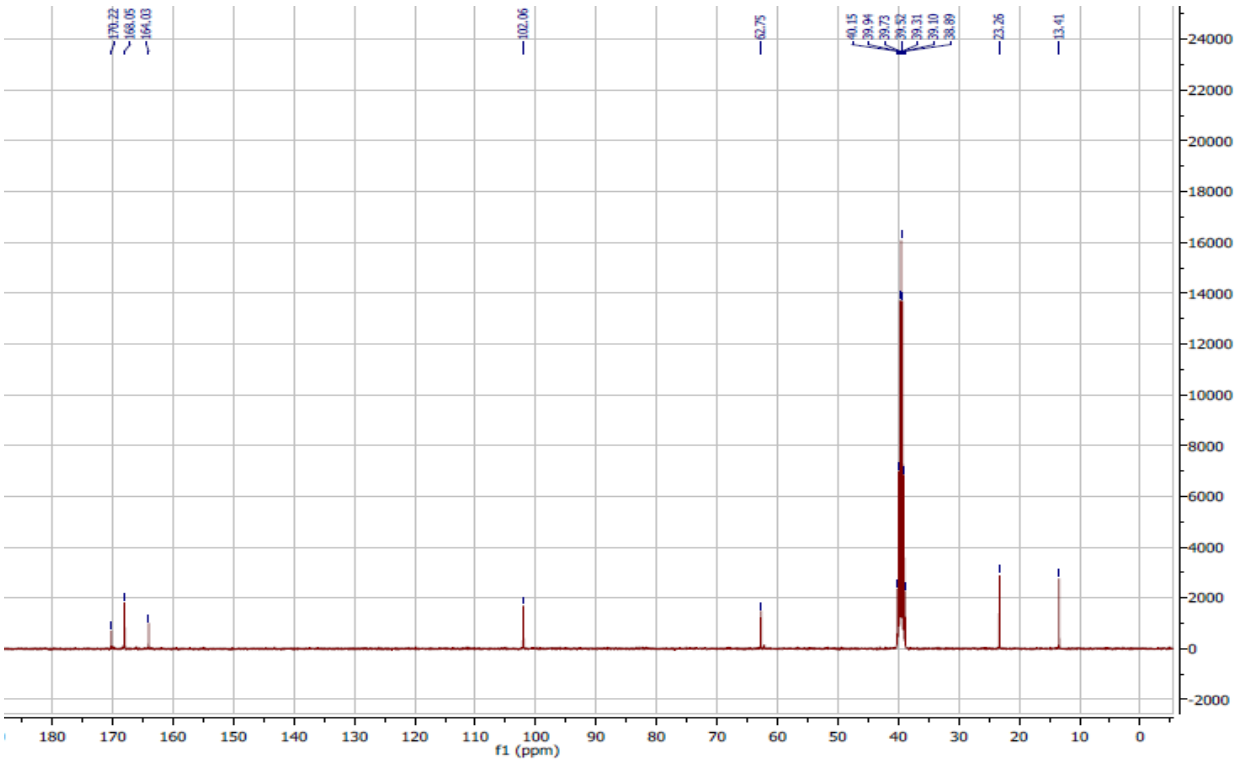

Figure S20: ^{13}C NMR spectrum of compound **12** (DMSO-d_6).

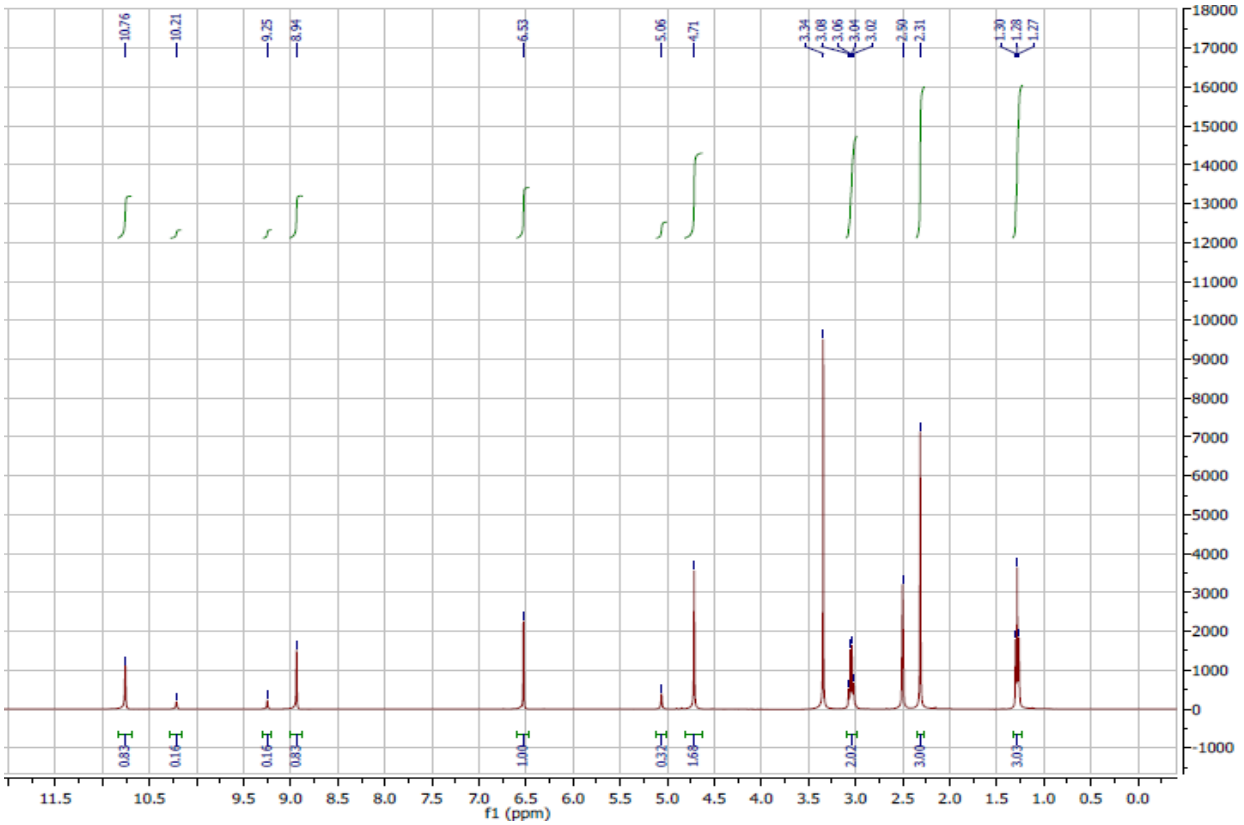

Figure S21: ^{1}H NMR spectrum of compound **13** (DMSO-d_6).

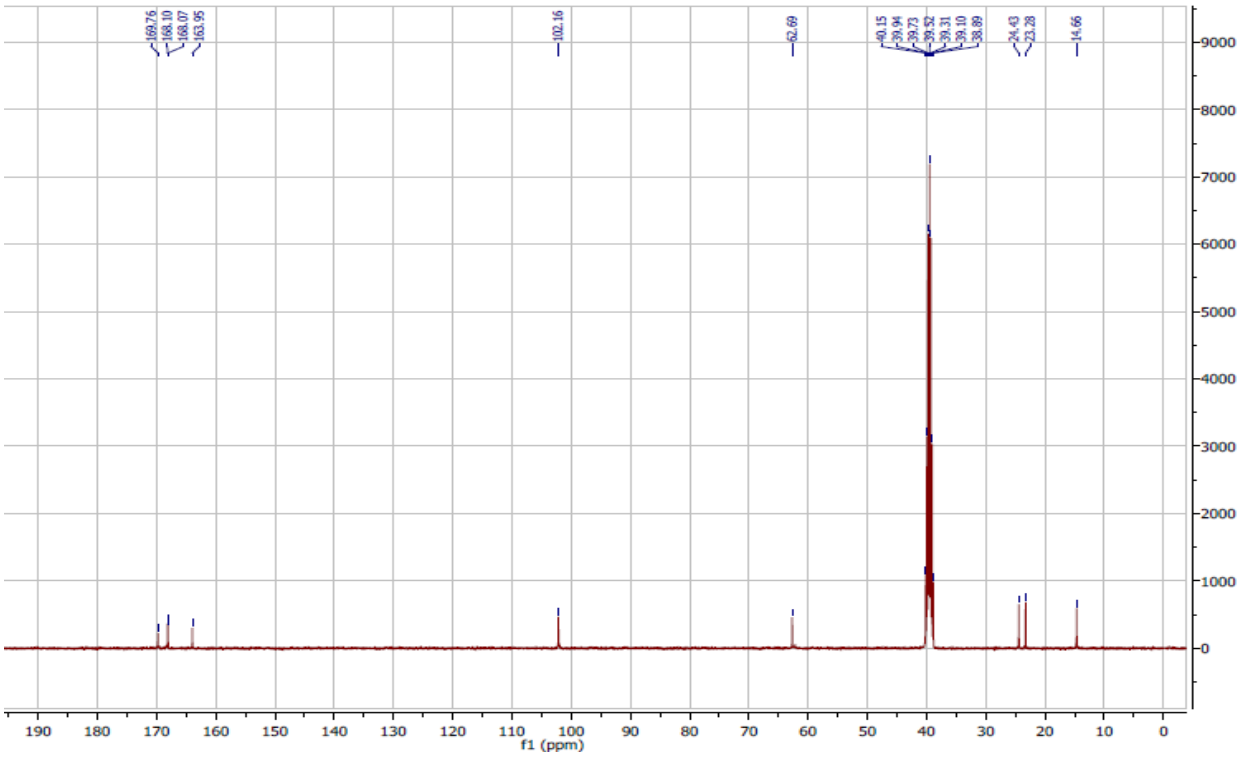

Figure S22: ^{13}C NMR spectrum of compound **13** (DMSO-d_6).

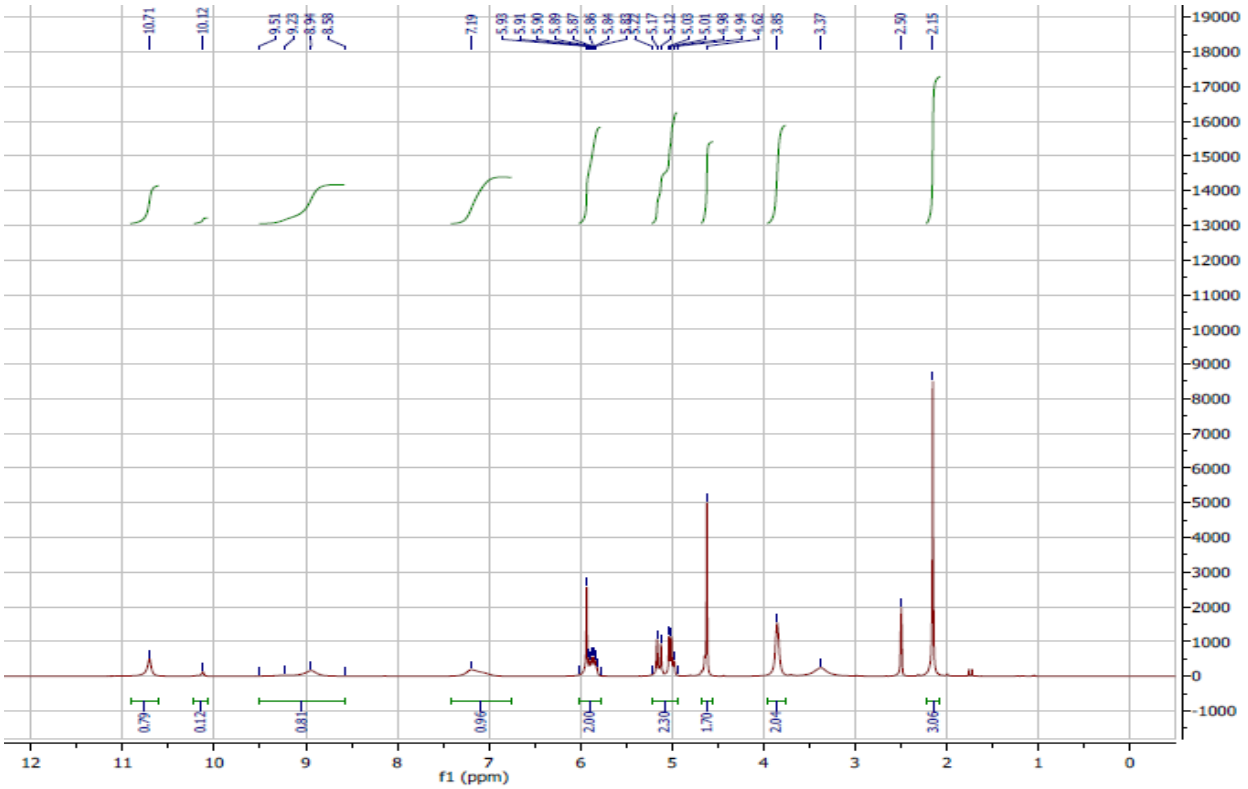

Figure S23: ^{1}H NMR spectrum of compound **14** (DMSO-d_6).

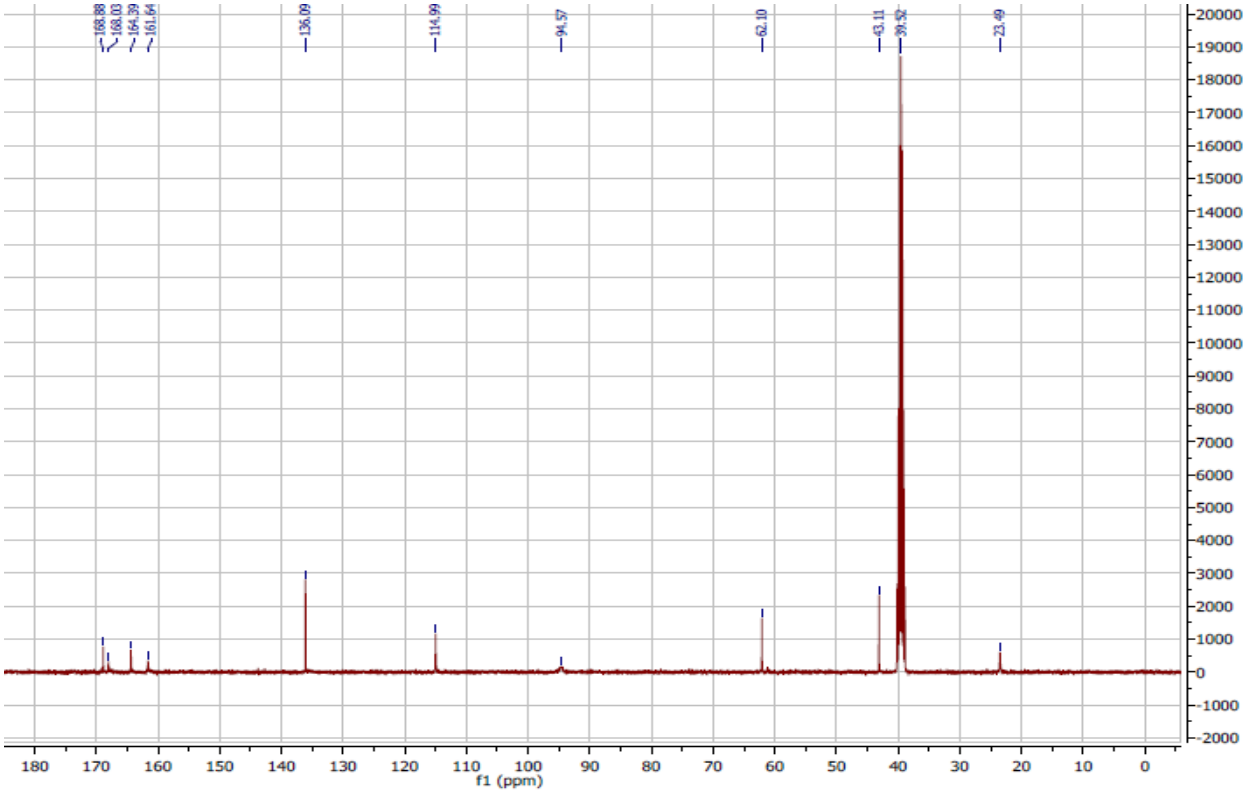

Figure S24: ^{13}C NMR spectrum of compound **14** (DMSO-d_6).

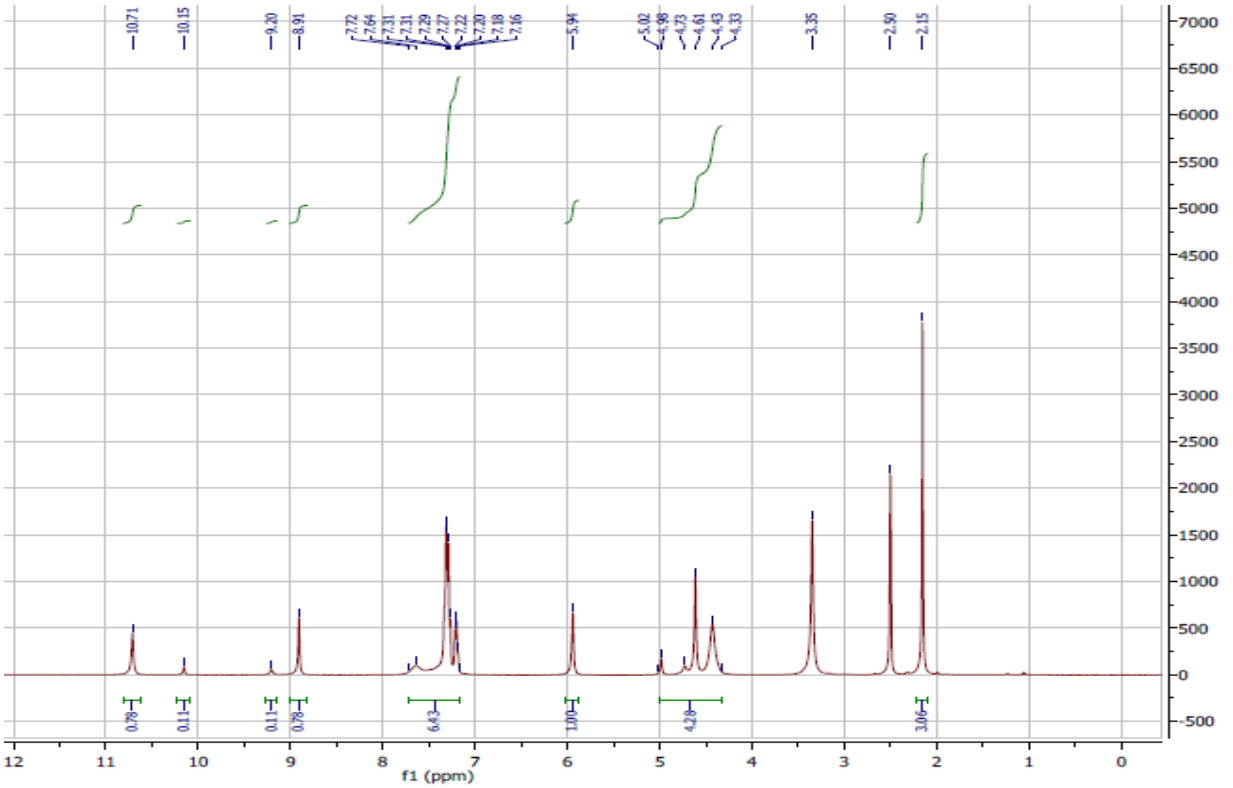

Figure S25: ^{1}H NMR spectrum of compound **15** (DMSO-d_6).

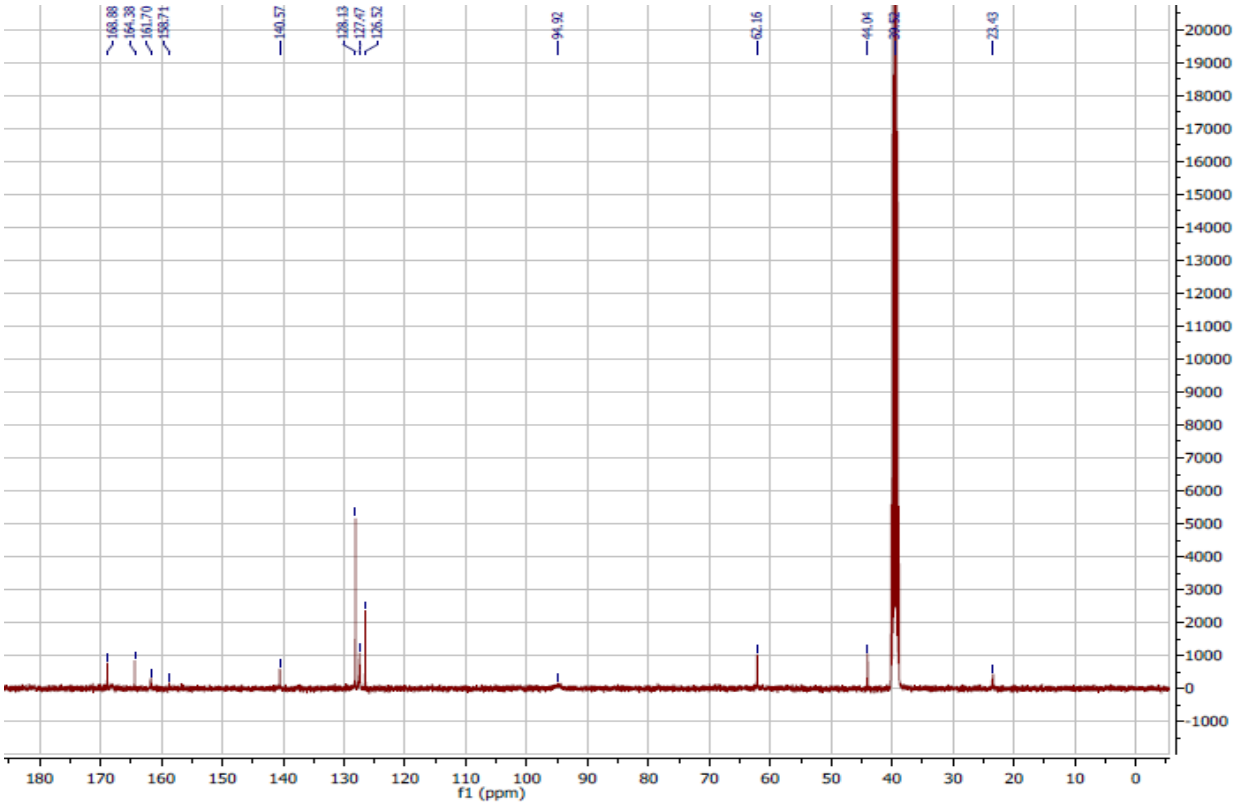

Figure S26: ^{13}C NMR spectrum of compound **15** (DMSO-d_6).

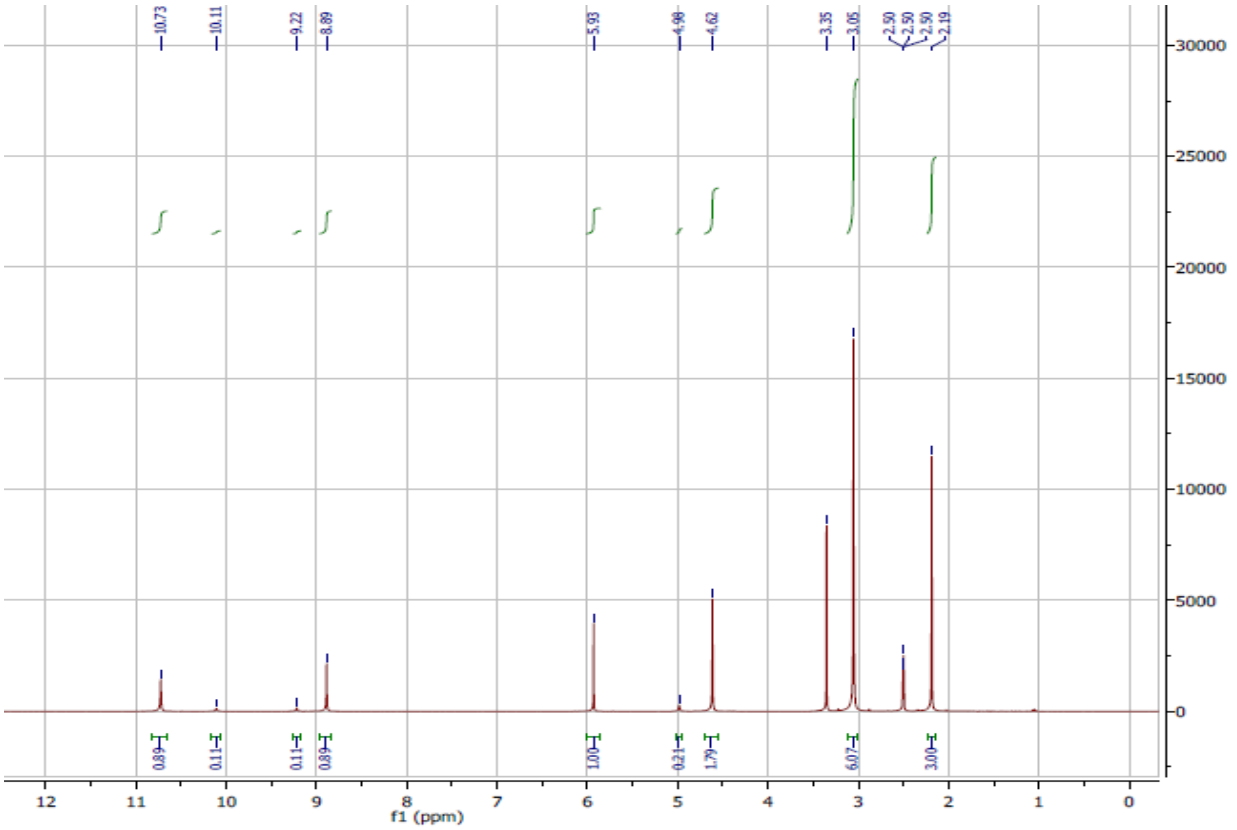

Figure S27: ^{1}H NMR spectrum of compound **16** (DMSO-d_6).

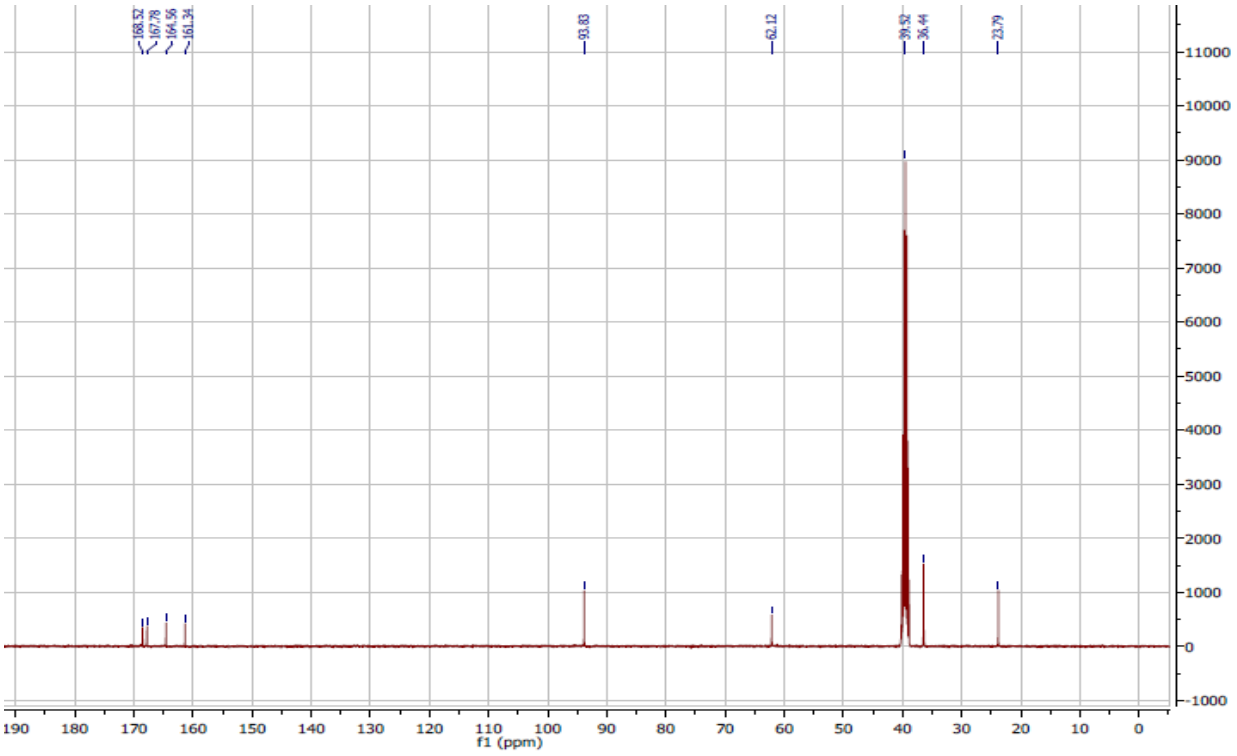

Figure S28: ^{13}C NMR spectrum of compound **16** (DMSO-d_6).

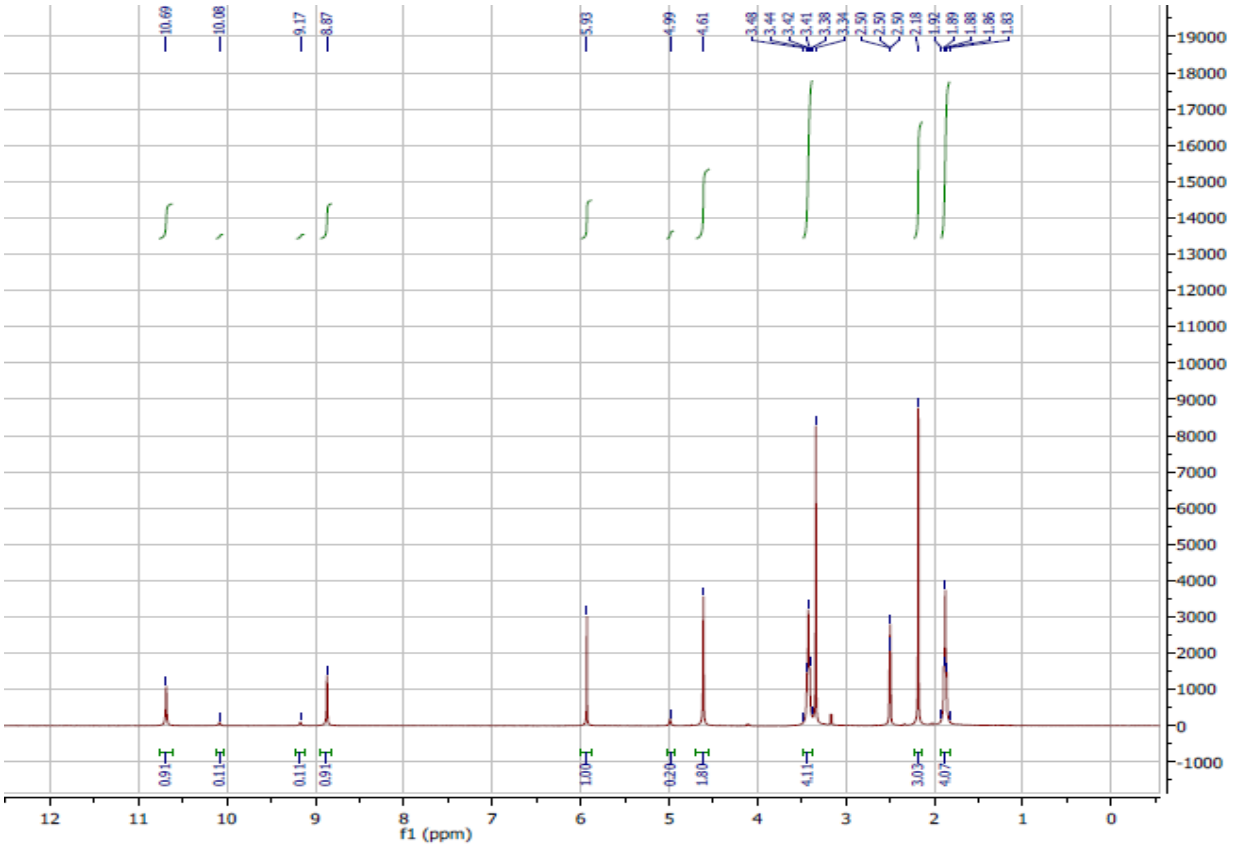

Figure S29: ^{1}H NMR spectrum of compound **17** (DMSO-d_6).

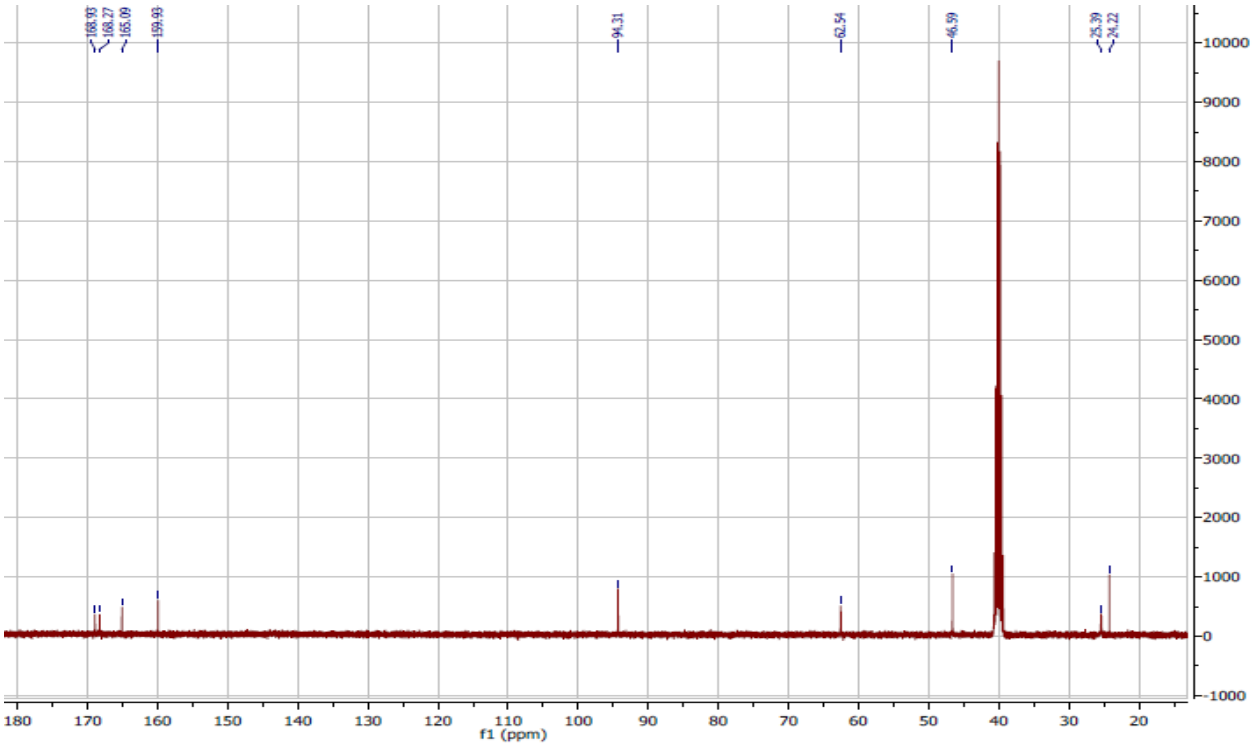

Figure S30: ^{13}C NMR spectrum of compound **17** (DMSO-d_6).

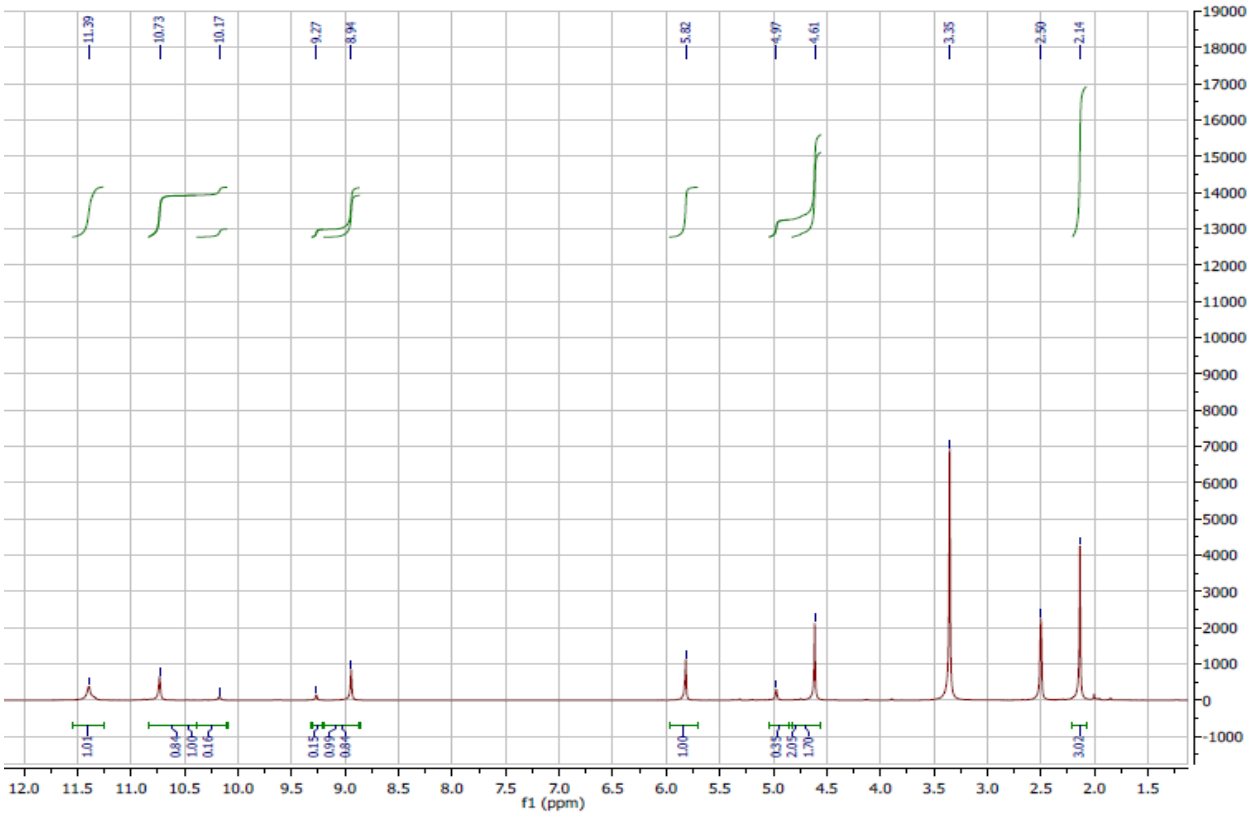

Figure S31: ^{1}H NMR spectrum of compound **18** (DMSO-d_6).

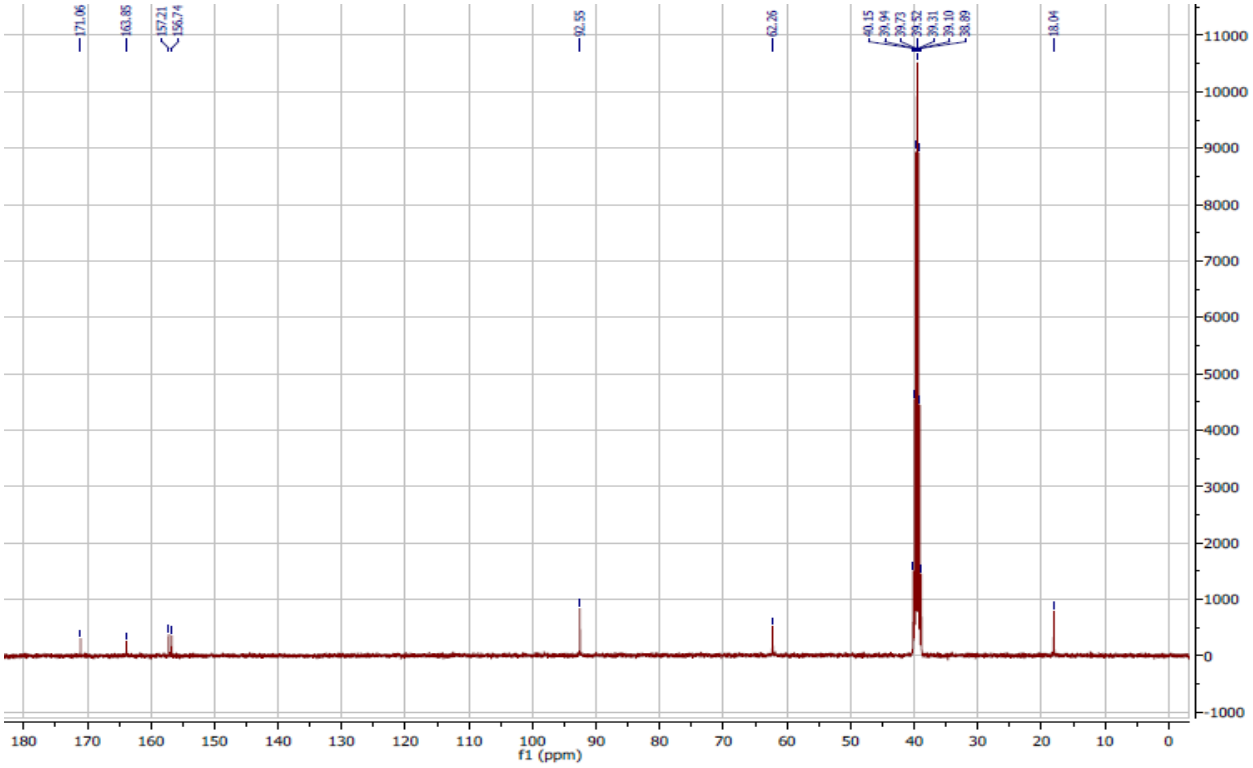

Figure S32: ^{13}C NMR spectrum of compound **18** (DMSO-d_6).

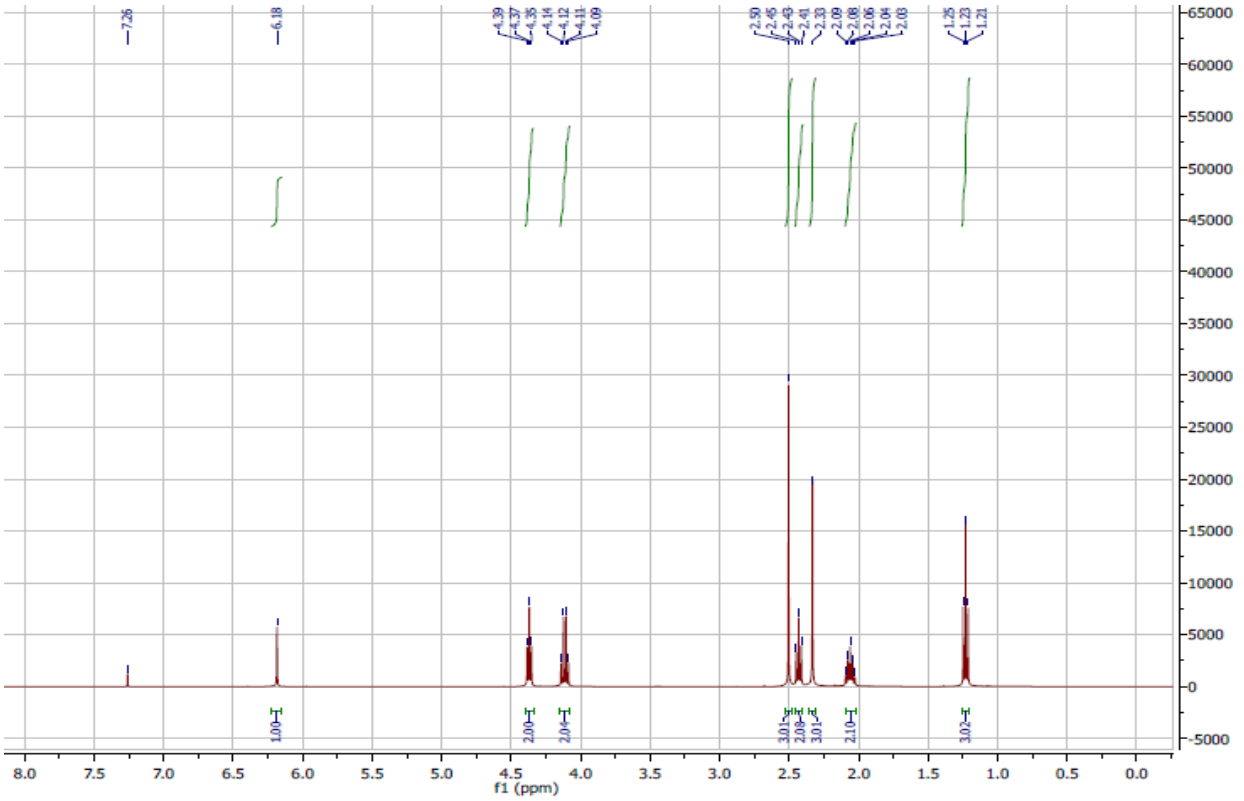

Figure S33: ^{1}H NMR spectrum of compound **20a** ($CDCl_3$).

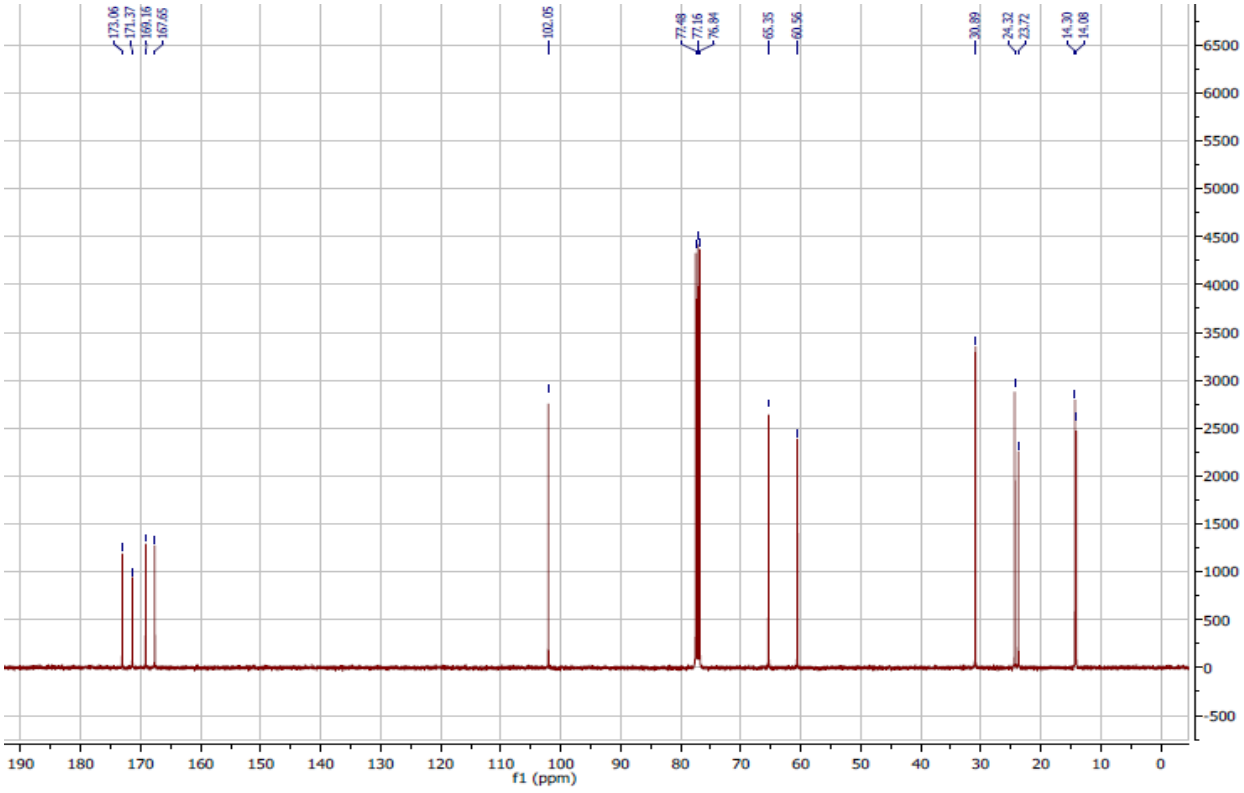

Figure S34: ^{13}C NMR spectrum of compound **20a** ($CDCl_3$).

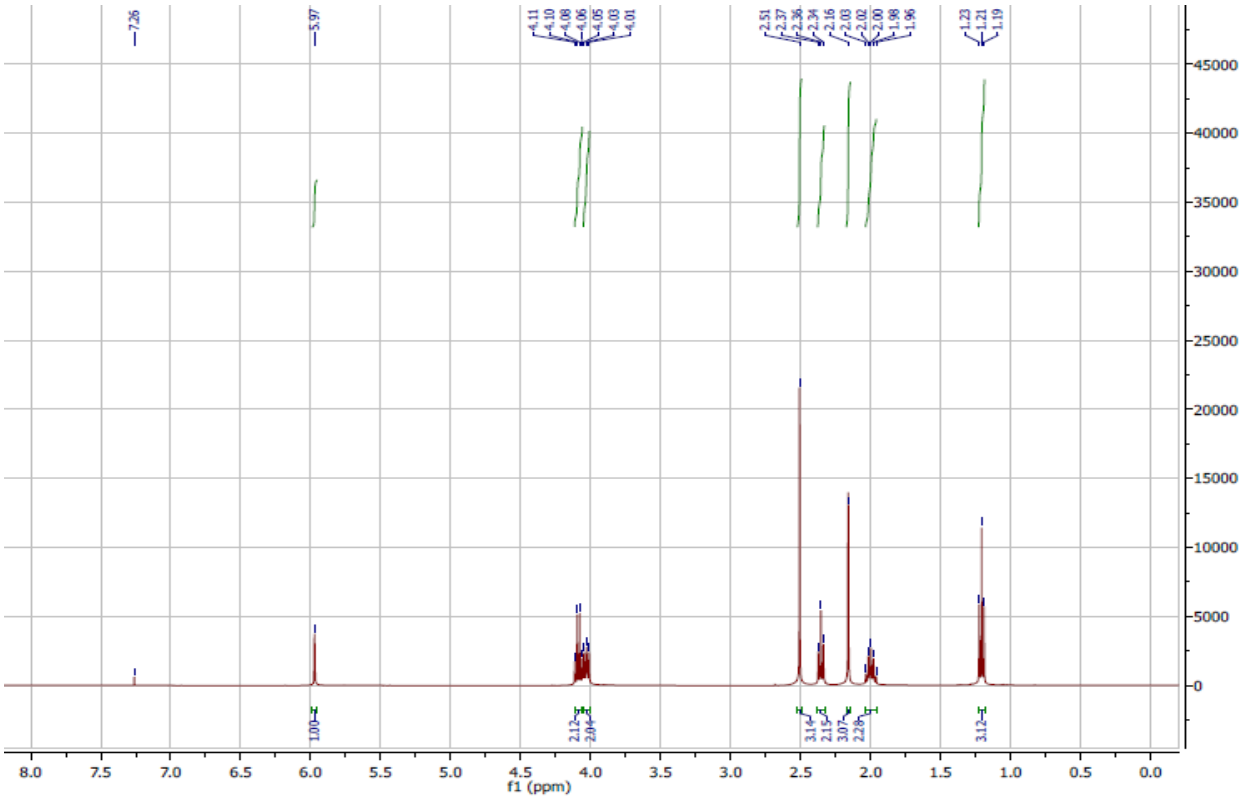

Figure S35: ^{1}H NMR spectrum of compound **20b** ($CDCl_3$).

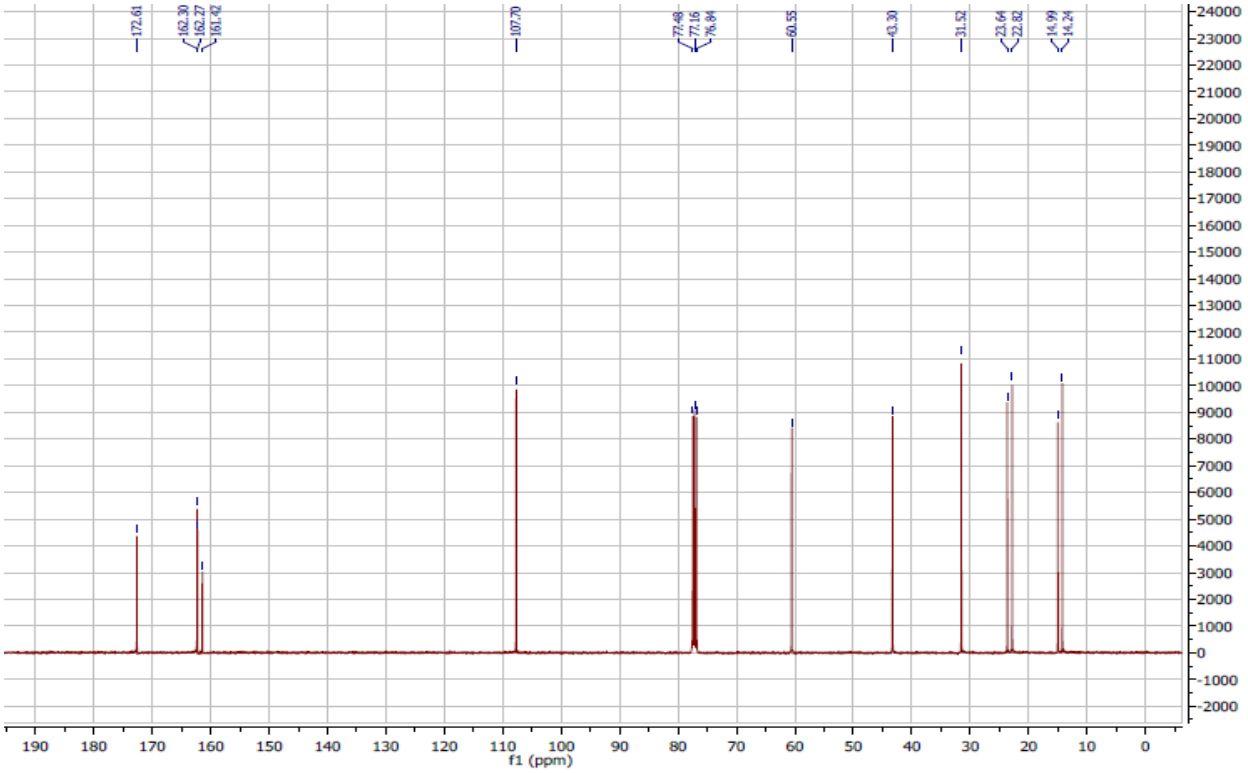

Figure S36: ^{13}C NMR spectrum of compound **20b** ($CDCl_3$).

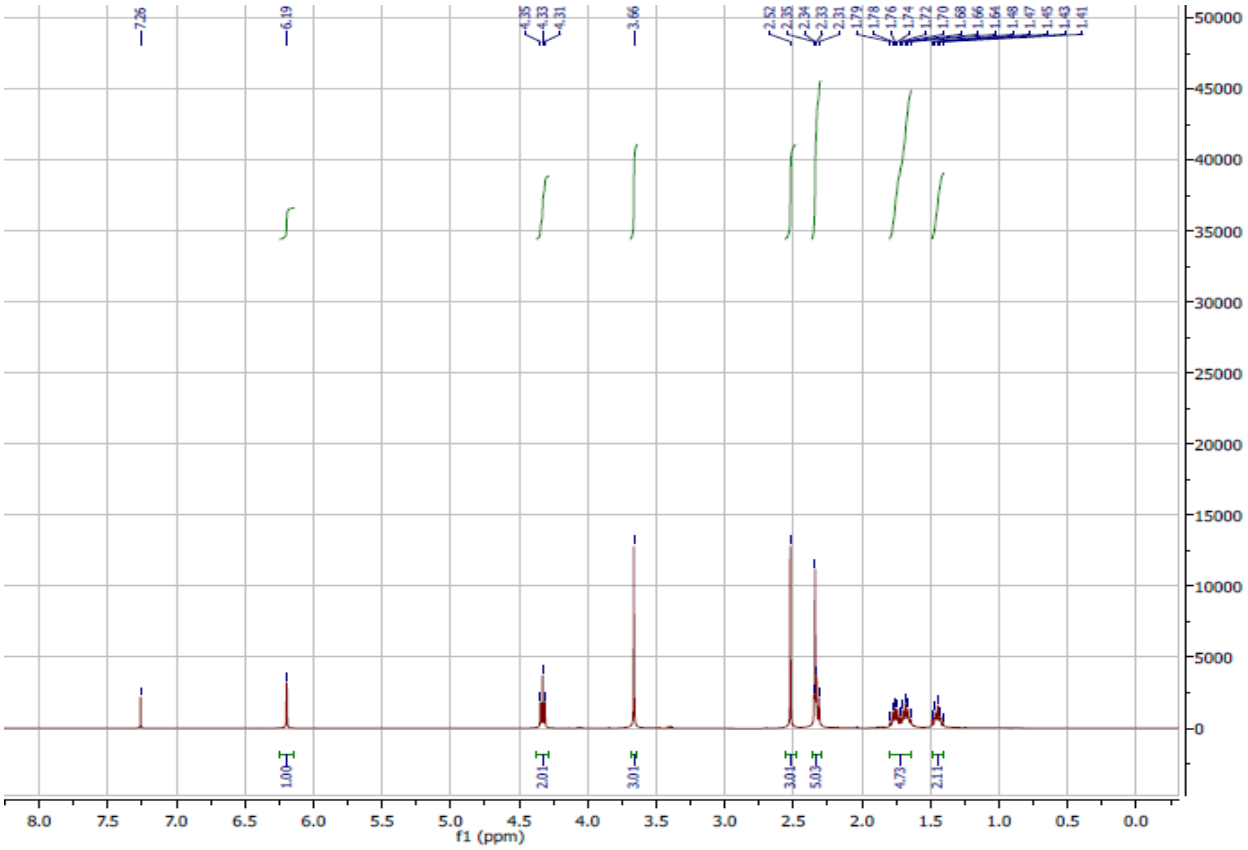

Figure S37: ^{1}H NMR spectrum of compound **21a** ($CDCl_3$).

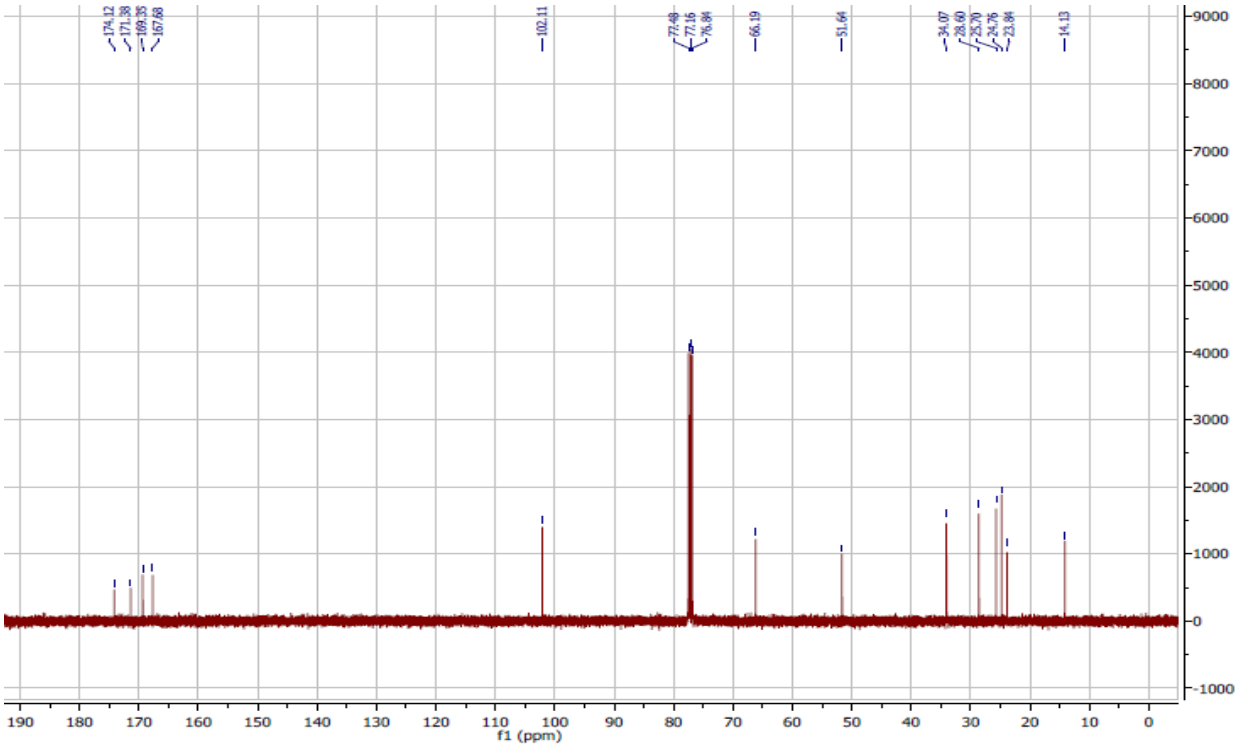

Figure S38: ^{13}C NMR spectrum of compound **21a** ($CDCl_3$).

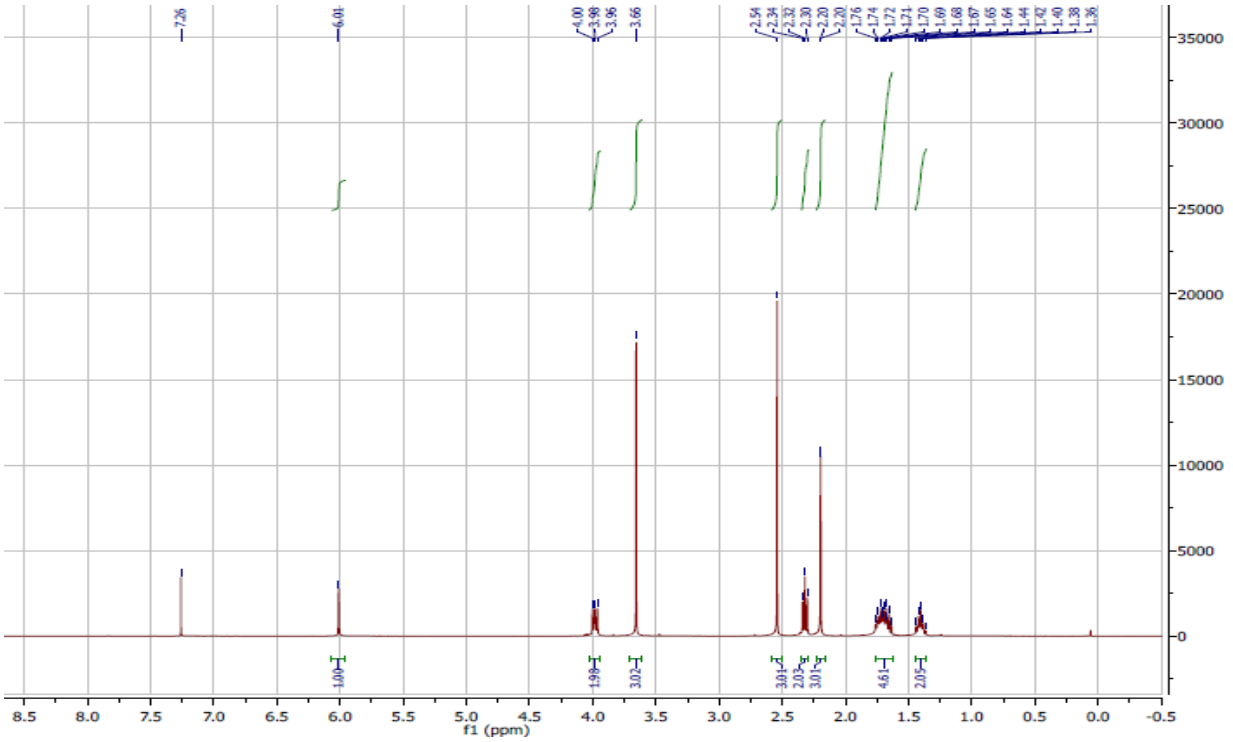

Figure S39: ^{1}H NMR spectrum of compound **21b** ($CDCl_3$).

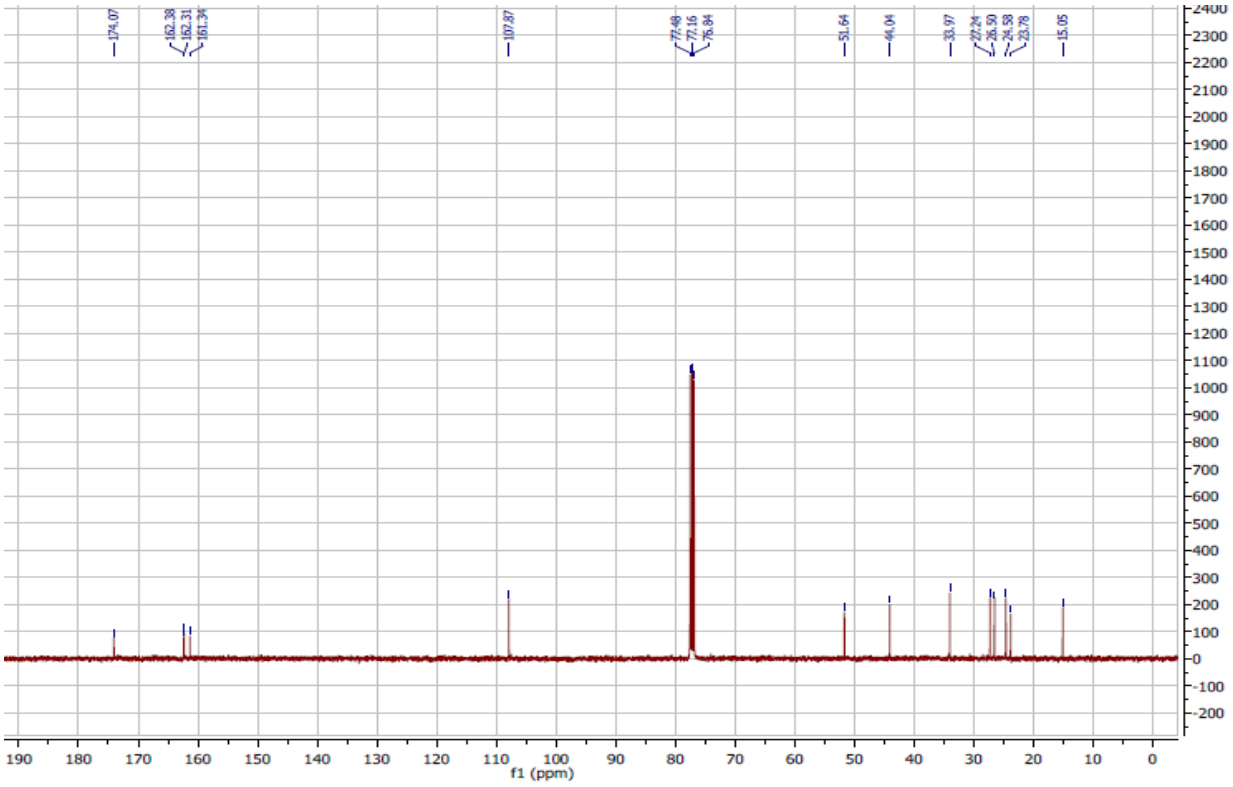

Figure S40: ^{13}C NMR spectrum of compound **21a** ($CDCl_3$).

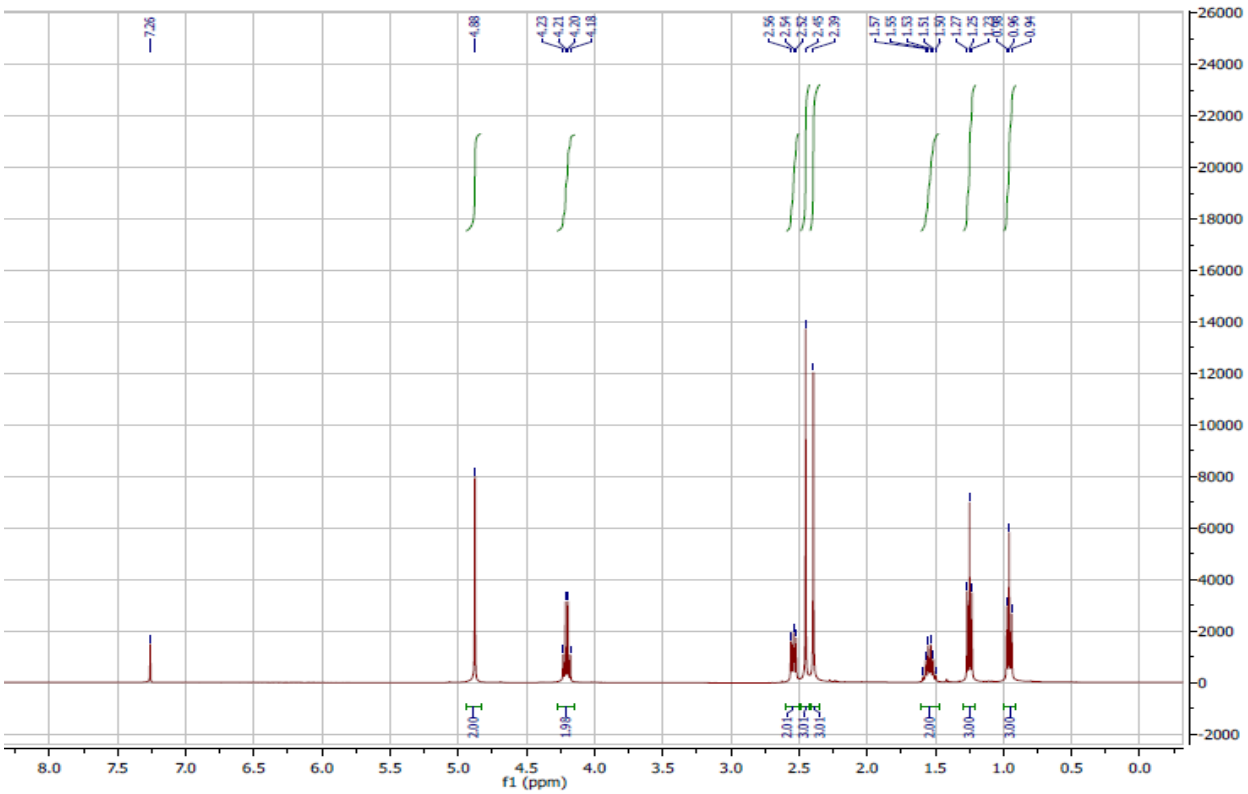

Figure S41: ^{1}H NMR spectrum of compound **22a** ($CDCl_3$).

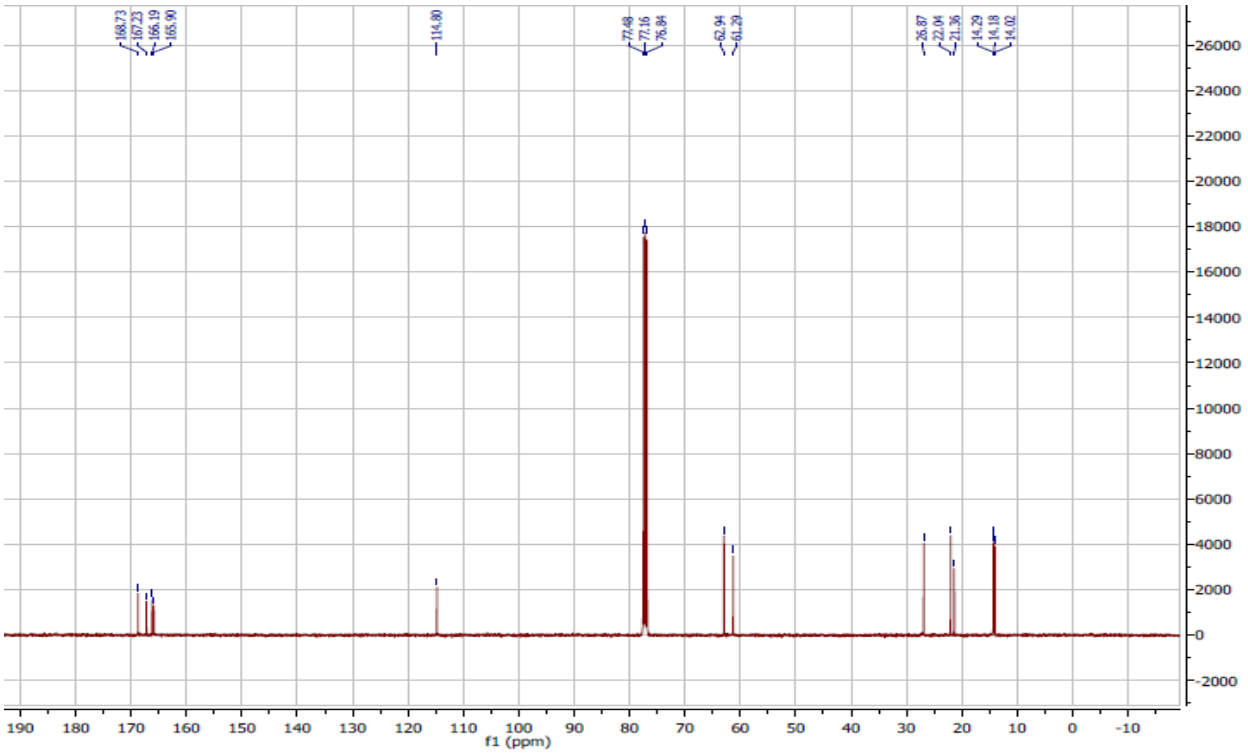

Figure S42: ^{13}C NMR spectrum of compound **22a** ($CDCl_3$).

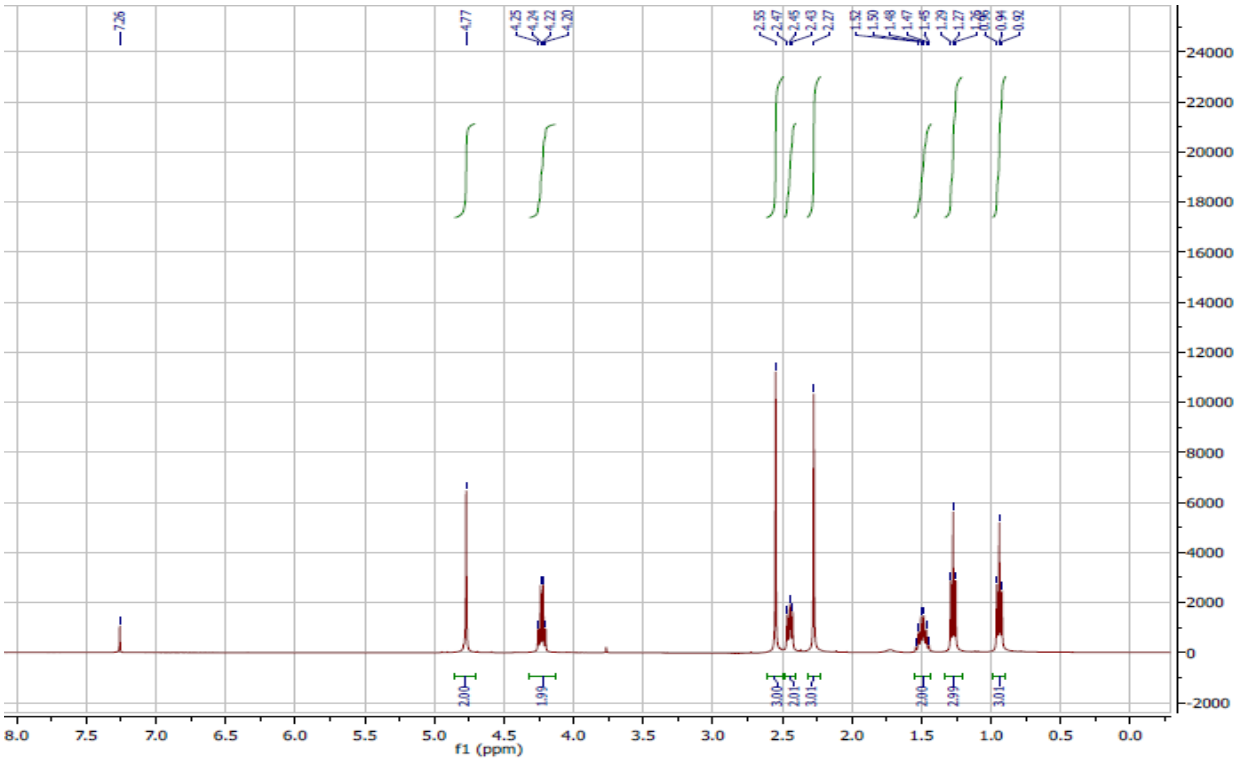

Figure S43: ^{1}H NMR spectrum of compound **22b** ($CDCl_3$).

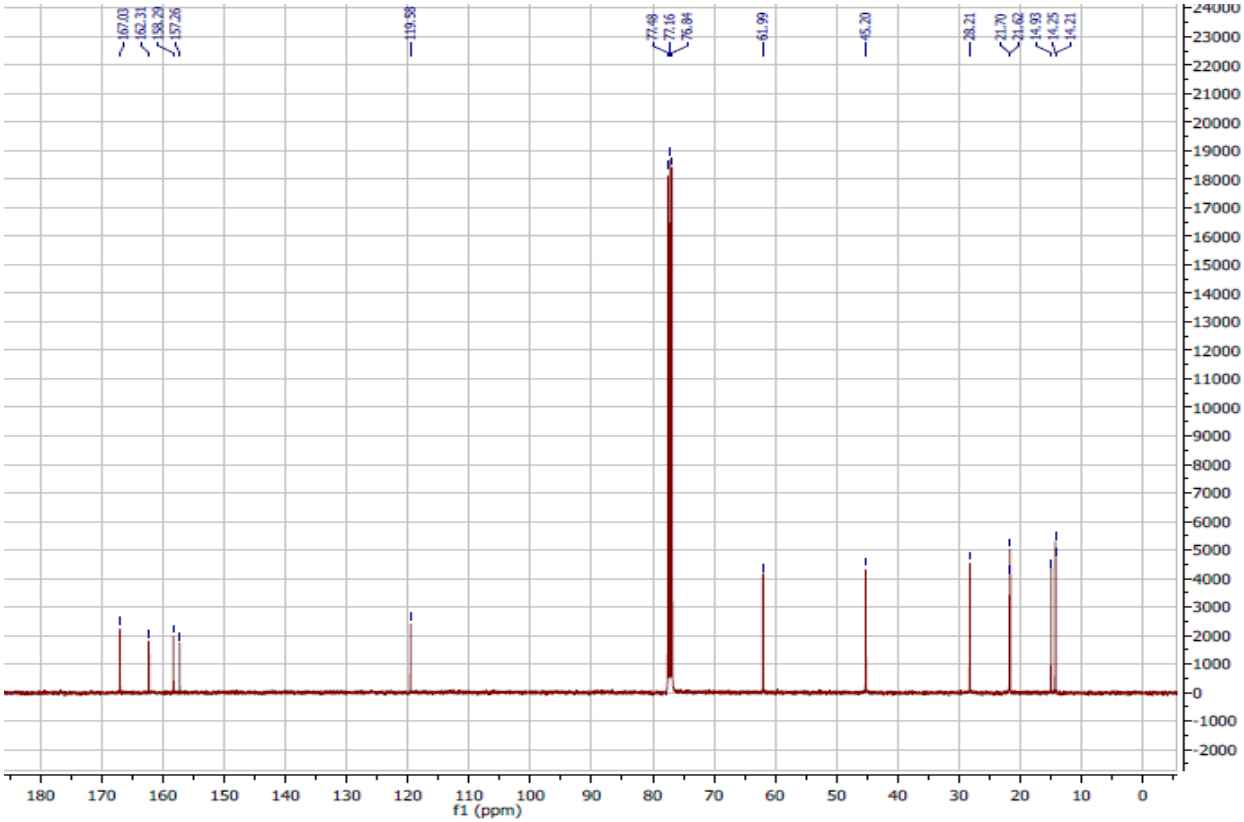

Figure S44: ^{13}C NMR spectrum of compound **22b** ($CDCl_3$).

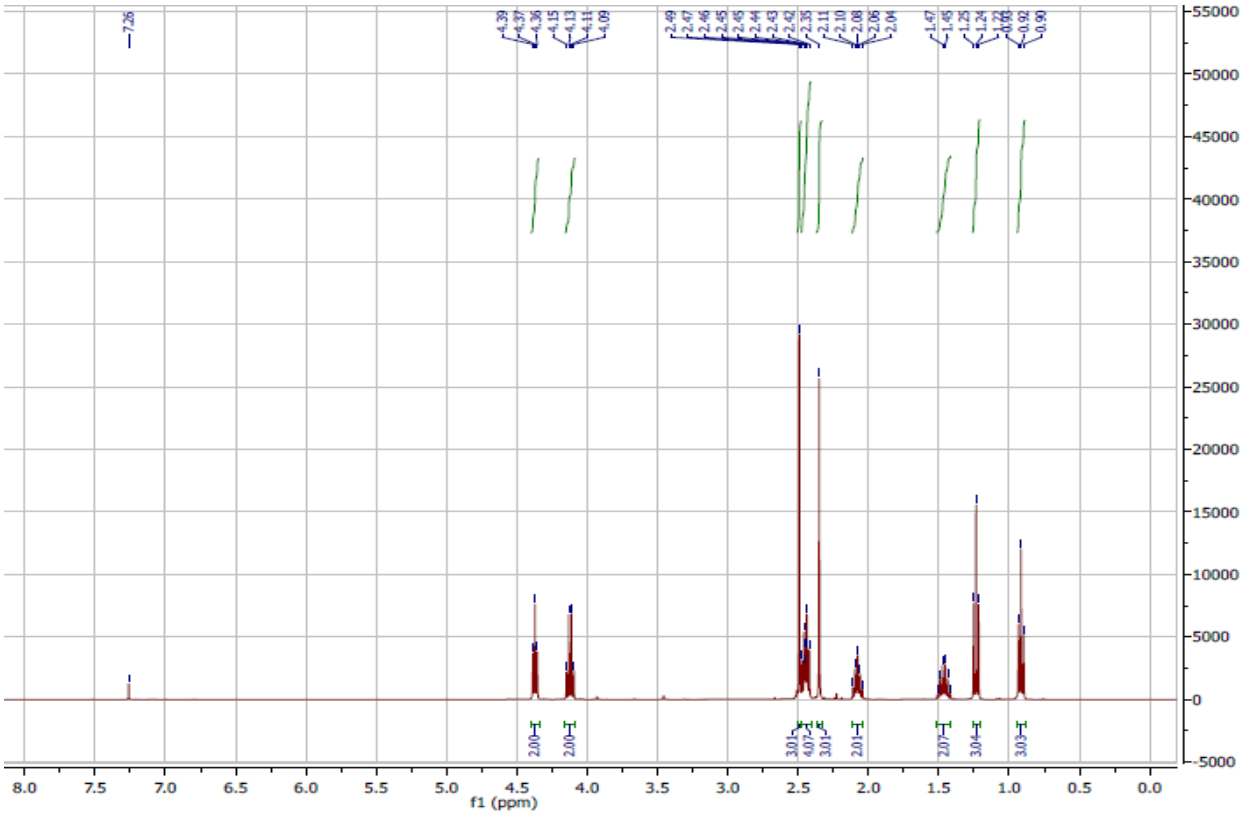

Figure S45: ^{1}H NMR spectrum of compound **23a** ($CDCl_3$).

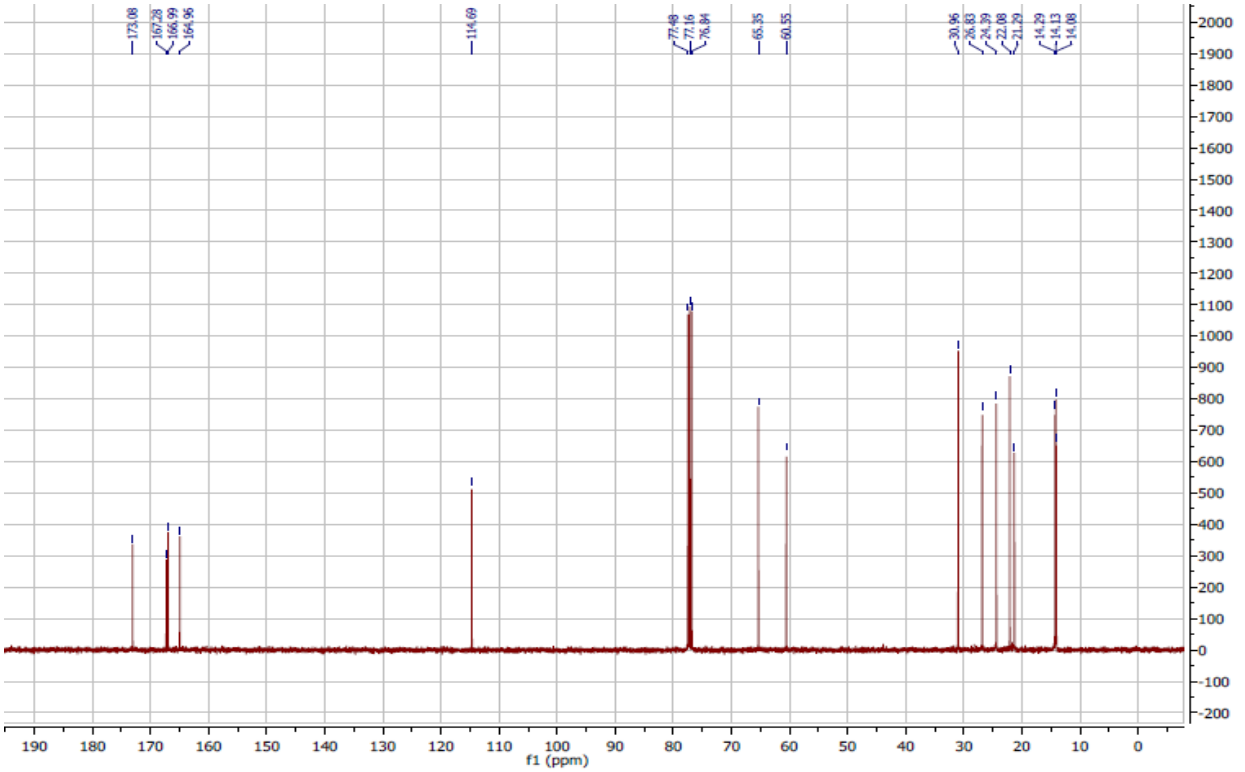

Figure S46: ^{13}C NMR spectrum of compound **23a** ($CDCl_3$).

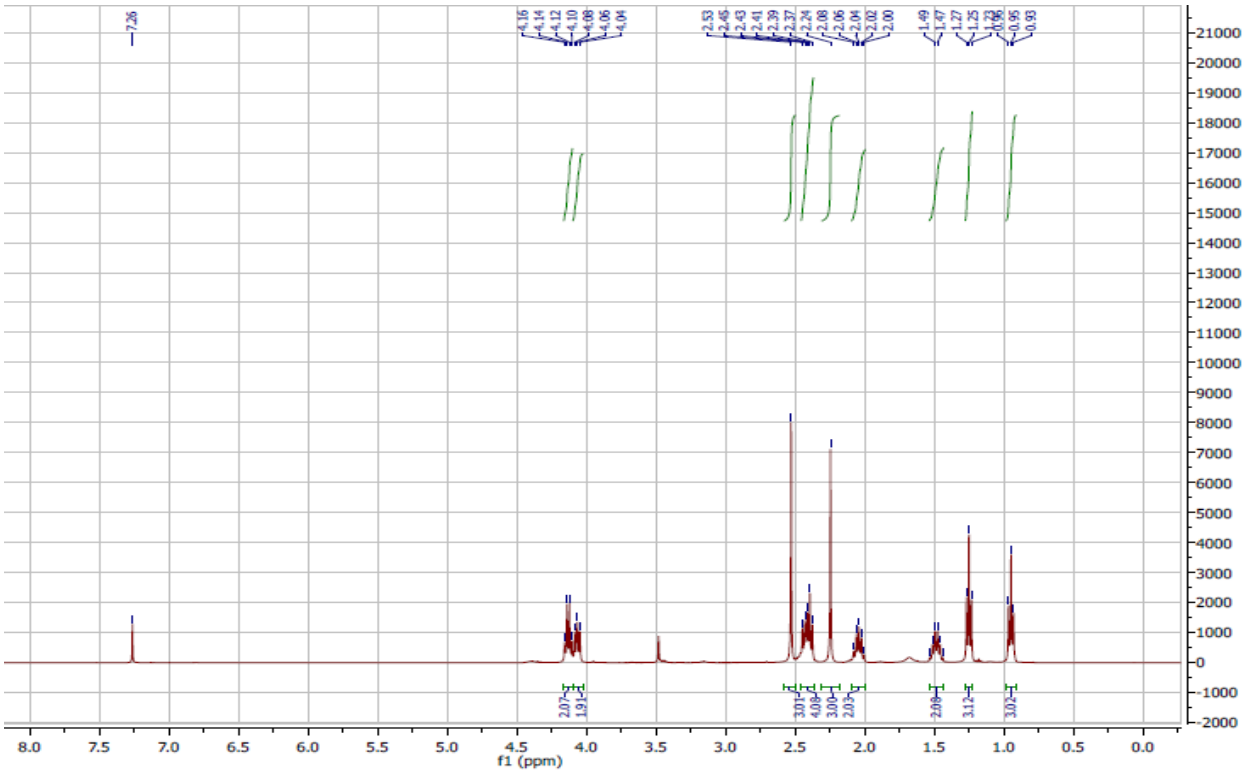

Figure S47: ^{1}H NMR spectrum of compound **23b** ($CDCl_3$).

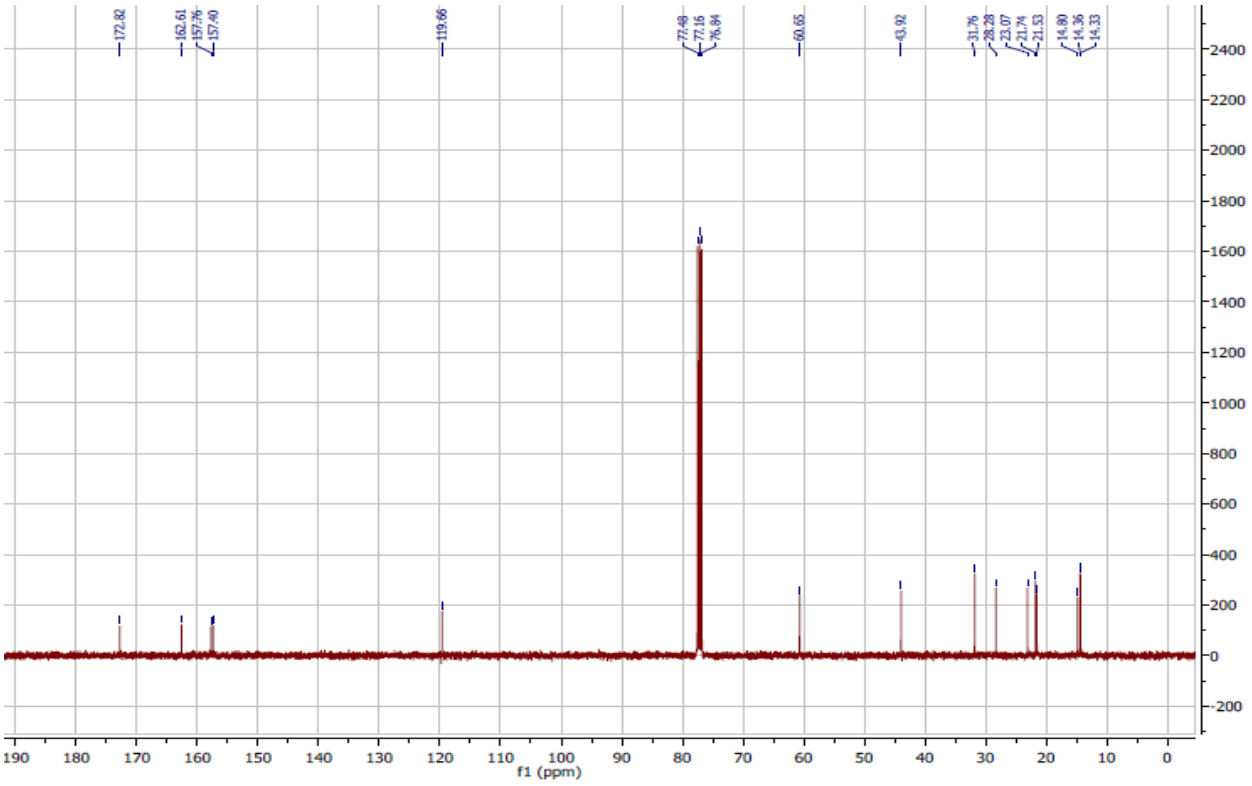

Figure S48: ^{13}C NMR spectrum of compound **23b** ($CDCl_3$).

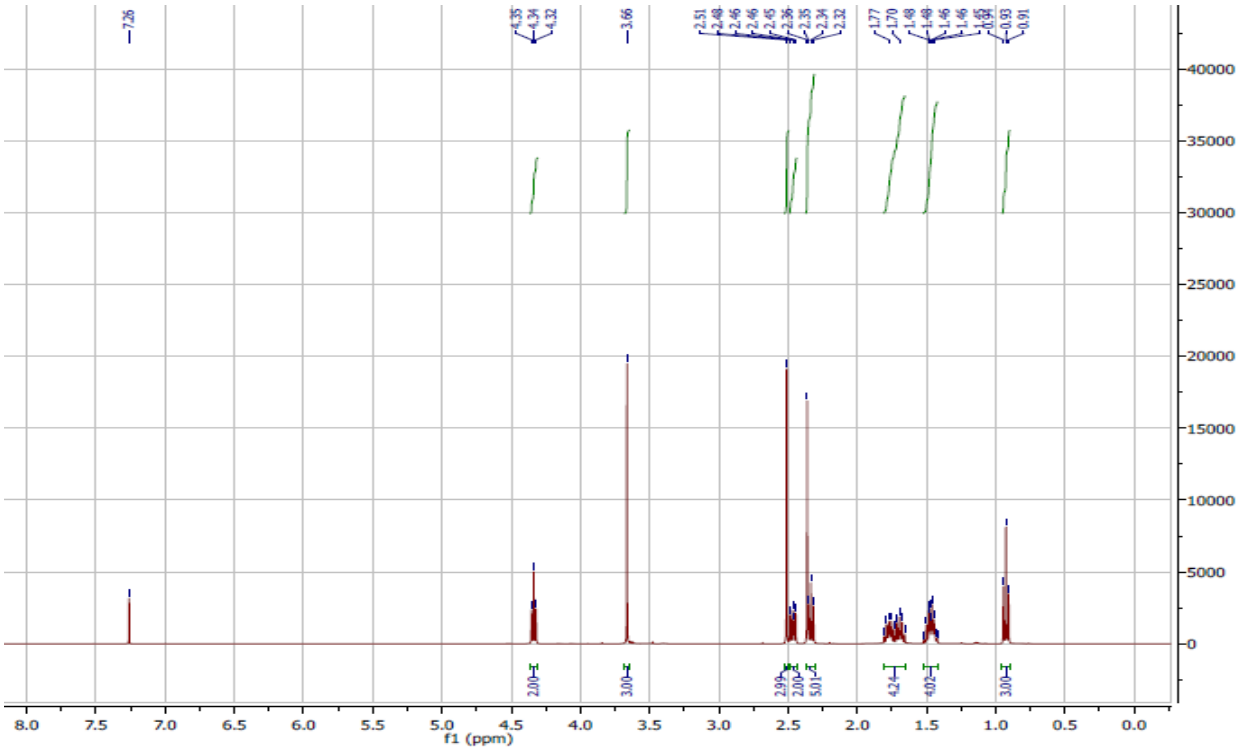

Figure S49: ^{1}H NMR spectrum of compound **24a** ($CDCl_3$).

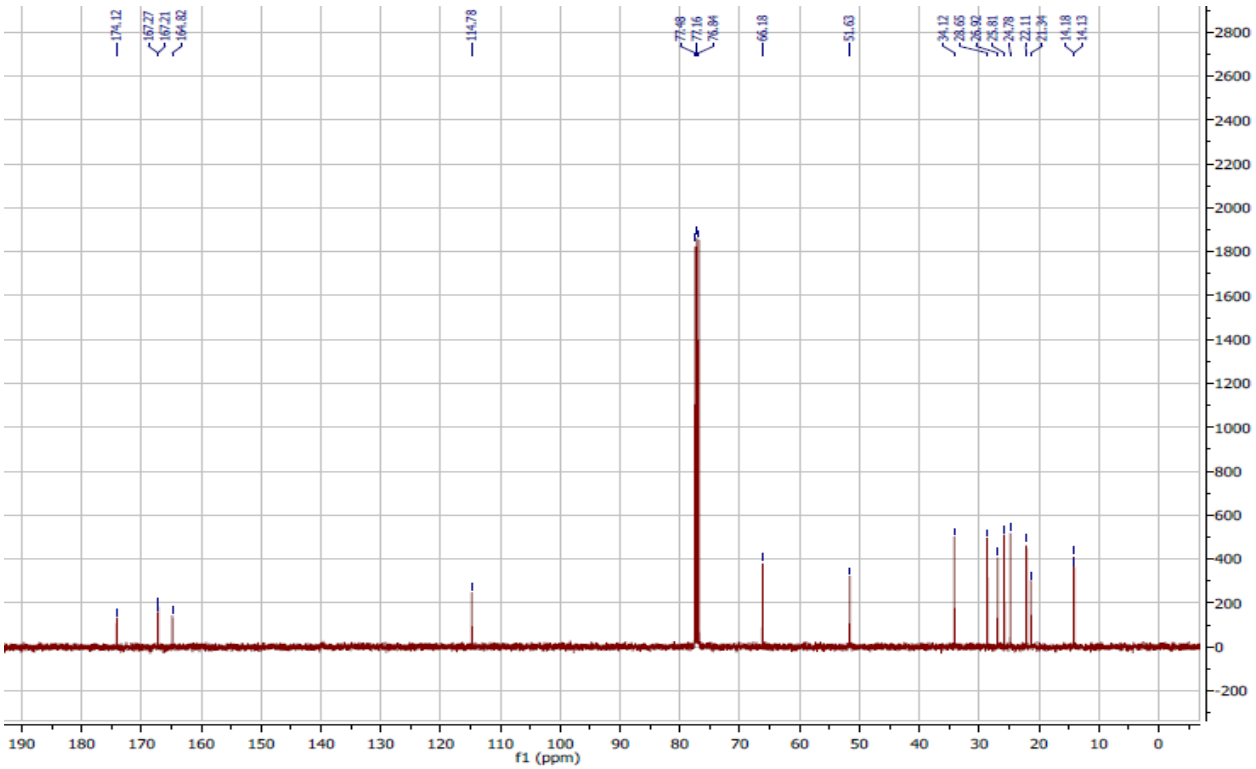

Figure S50: ^{13}C NMR spectrum of compound **24a** ($CDCl_3$).

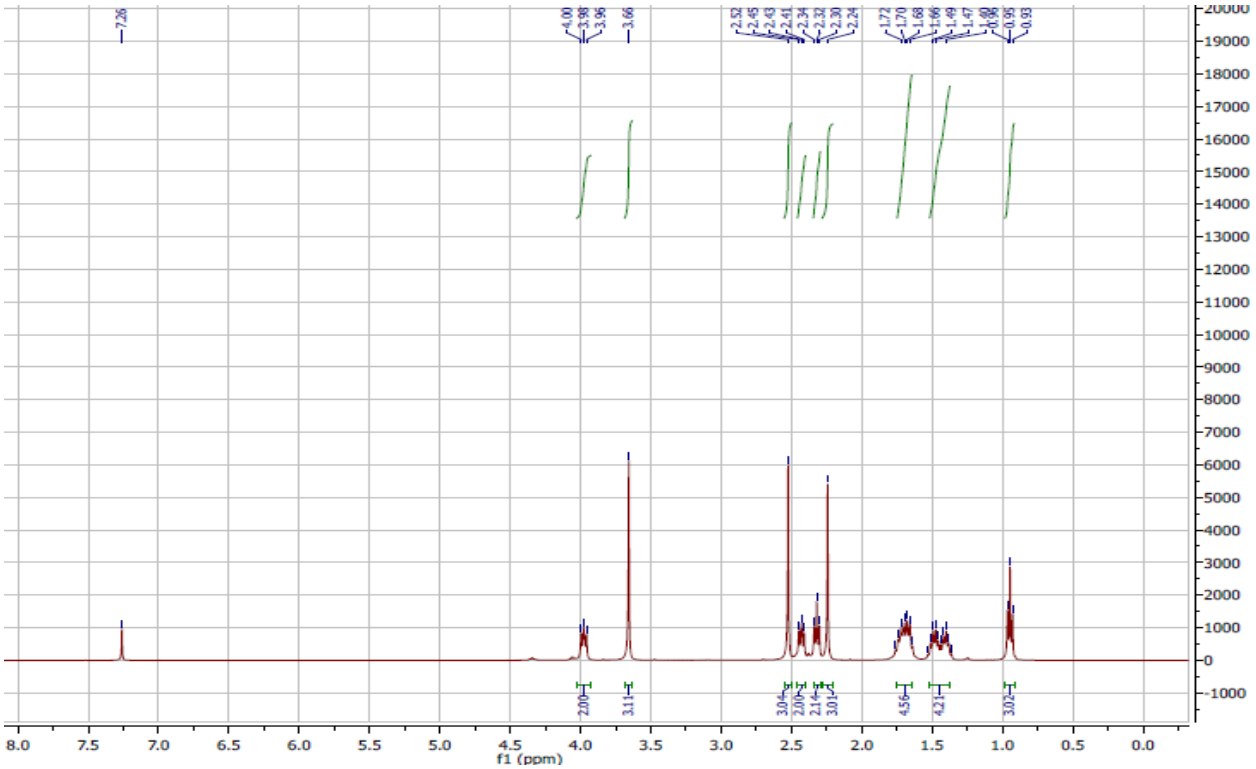

Figure S51: ^{1}H NMR spectrum of compound **24b** ($CDCl_3$).

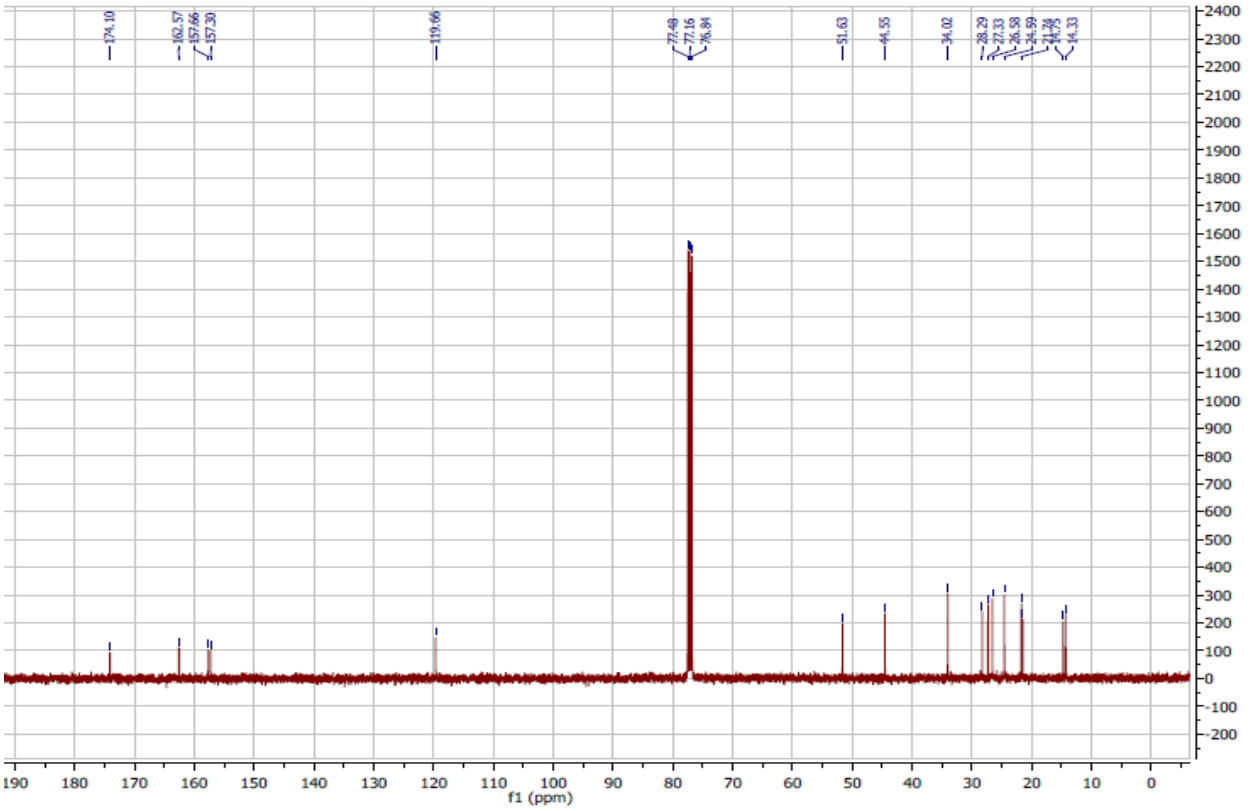

Figure S52: ^{13}C NMR spectrum of compound **24b** ($CDCl_3$).

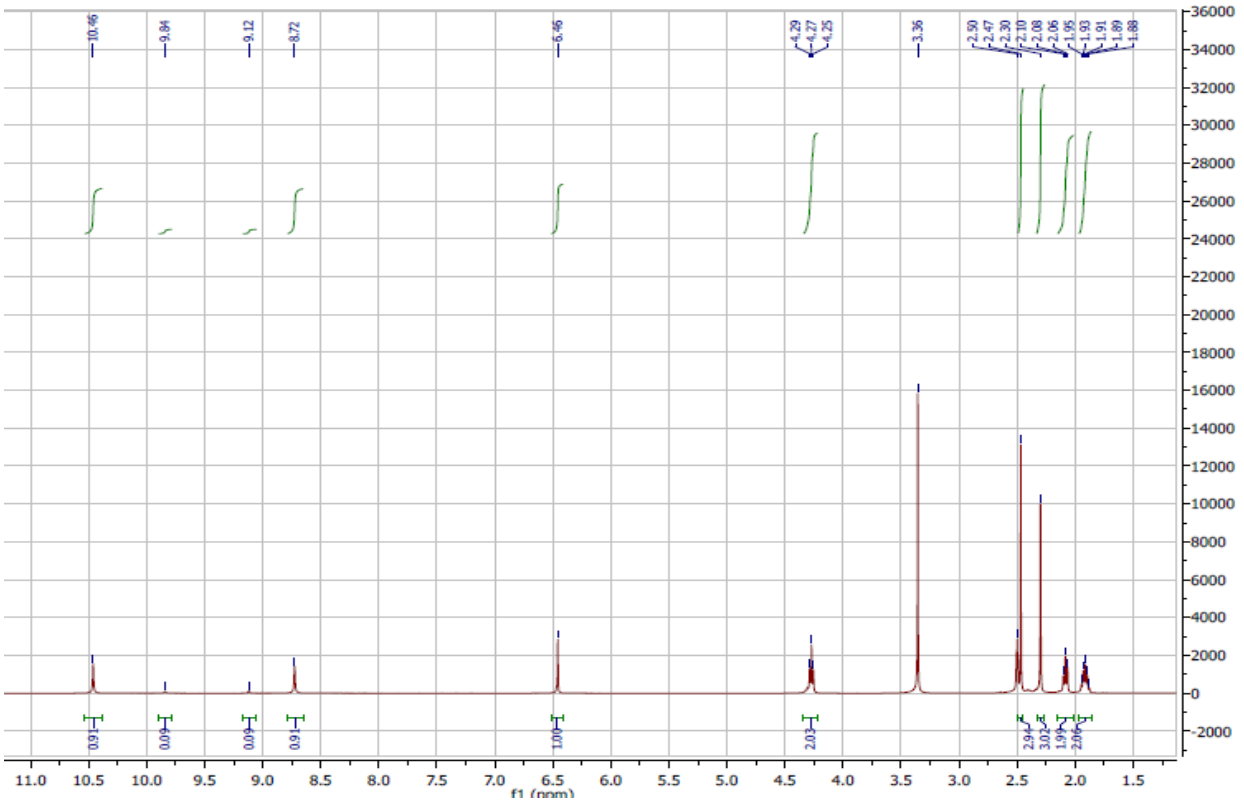

Figure S53: ^{1}H NMR spectrum of compound **25** (DMSO-d_6).

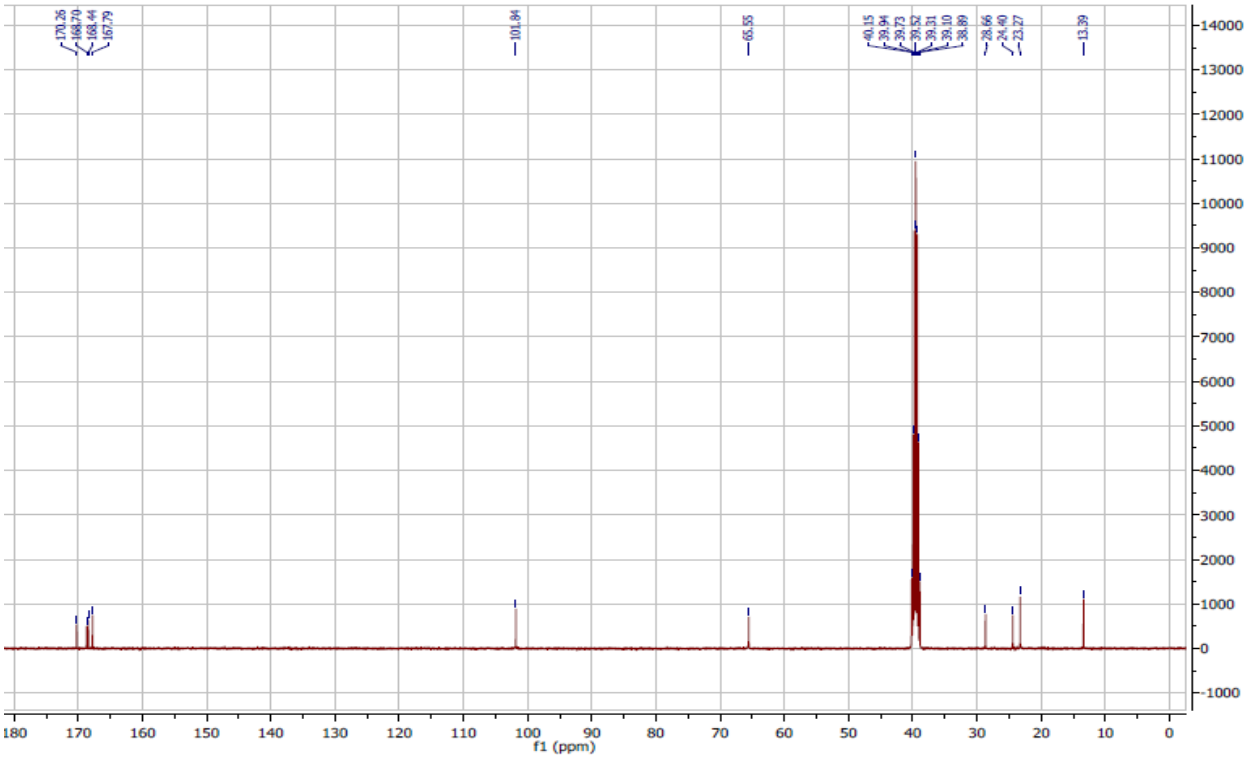

Figure S54: ^{13}C NMR spectrum of compound **25** (DMSO-d_6).

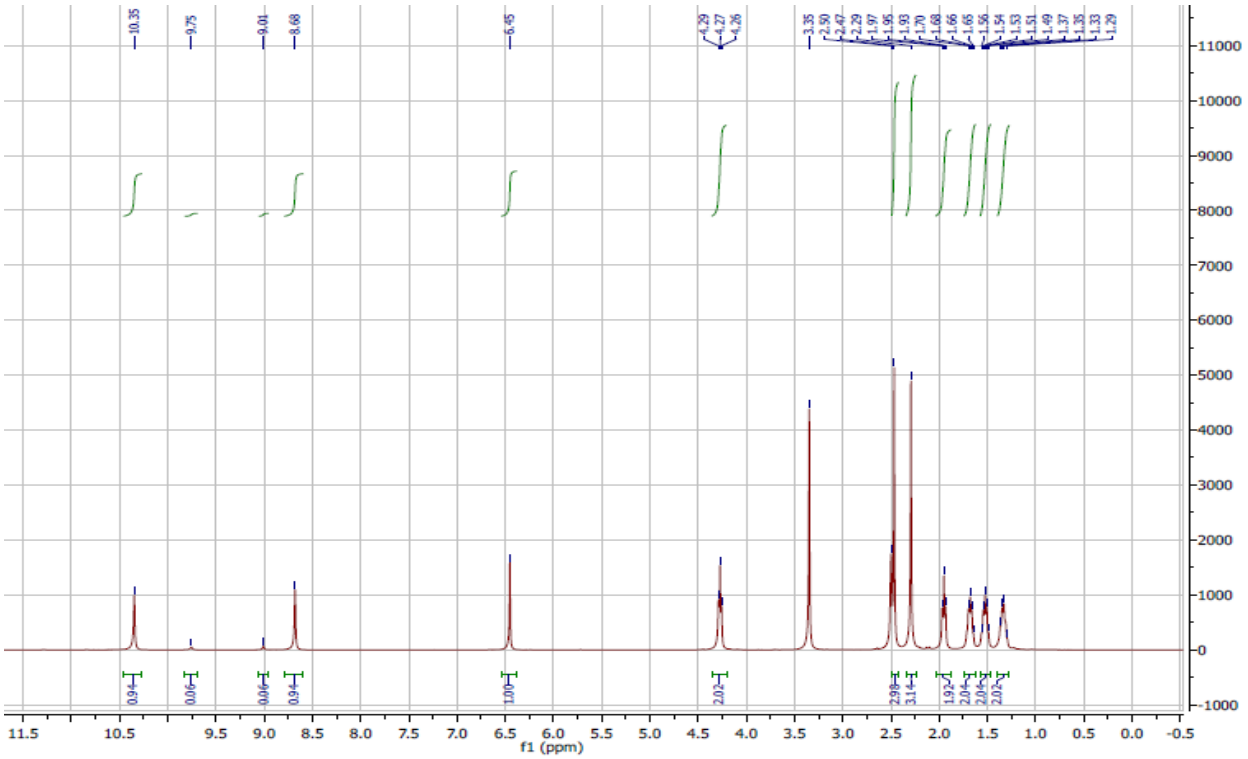

Figure S55: ^{1}H NMR spectrum of compound **26** (DMSO-d_6).

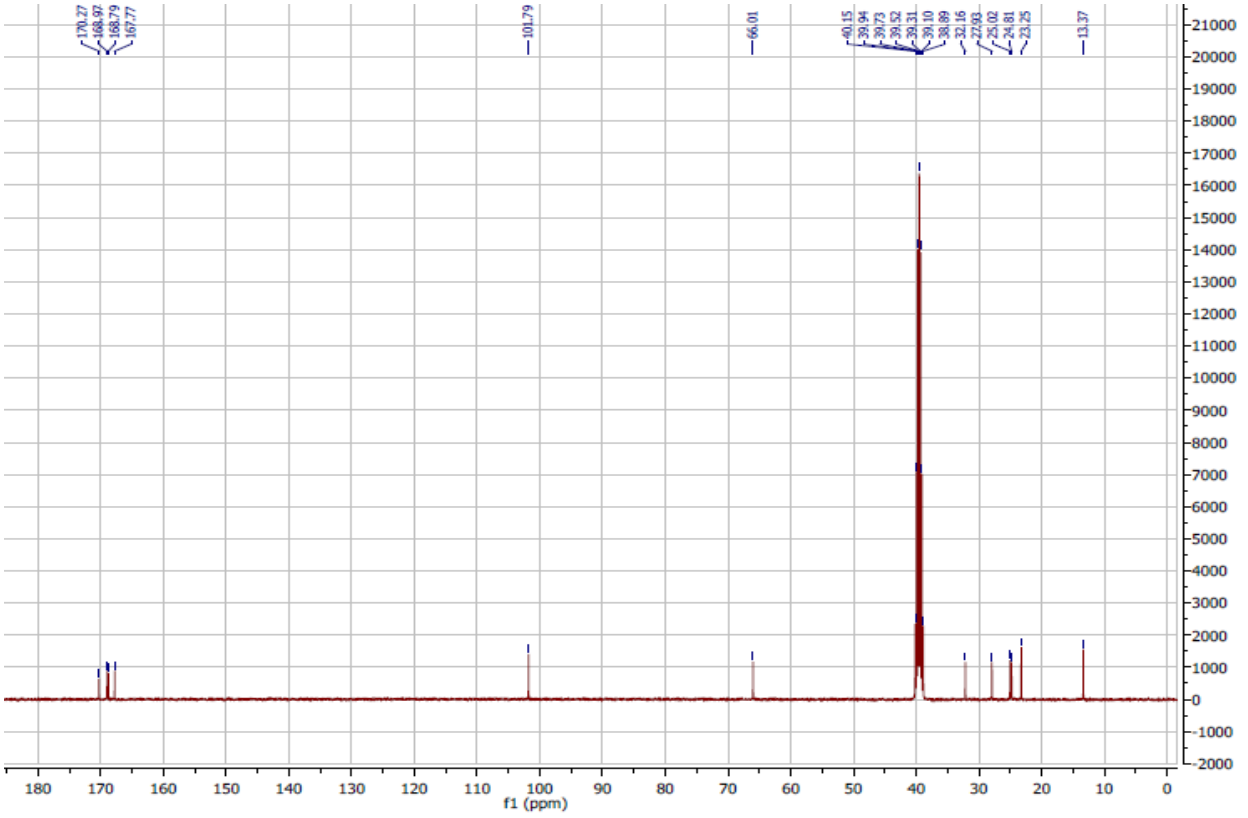

Figure S56: ^{13}C NMR spectrum of compound **26** (DMSO-d_6).

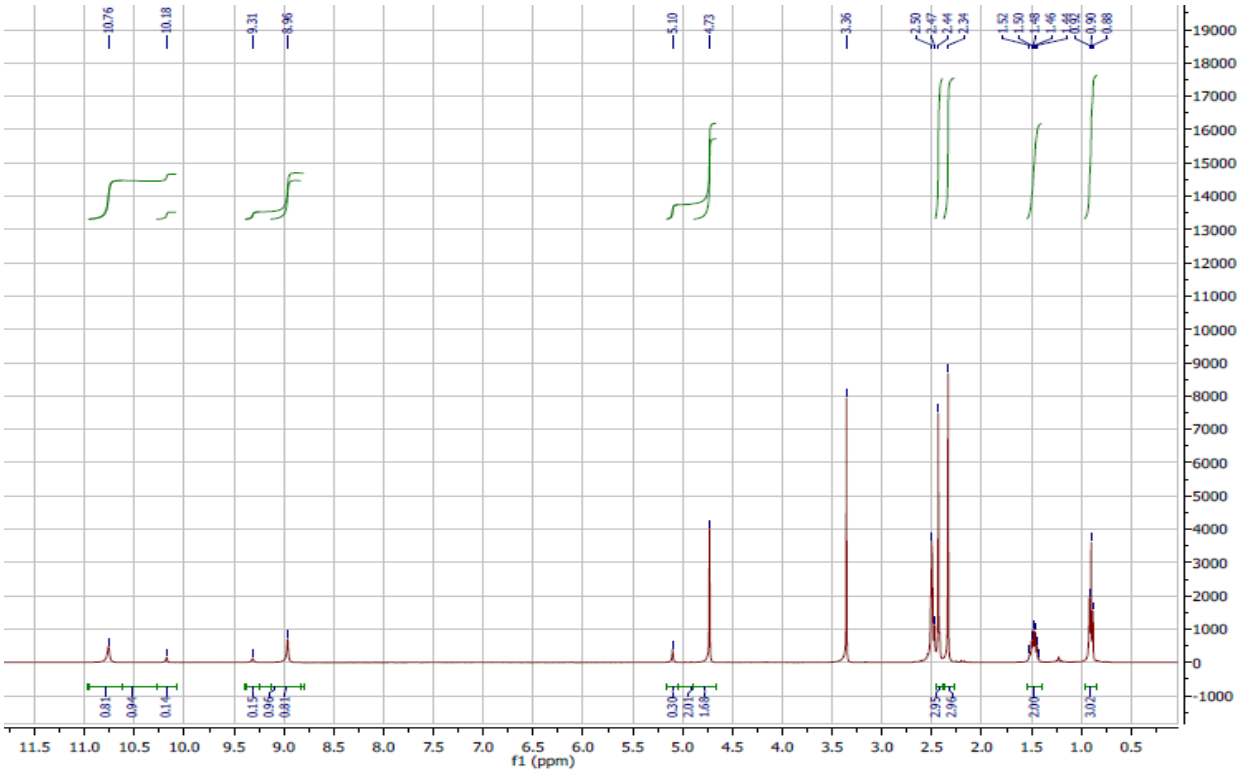

Figure S57: ^{1}H NMR spectrum of compound **27** (DMSO-d_6).

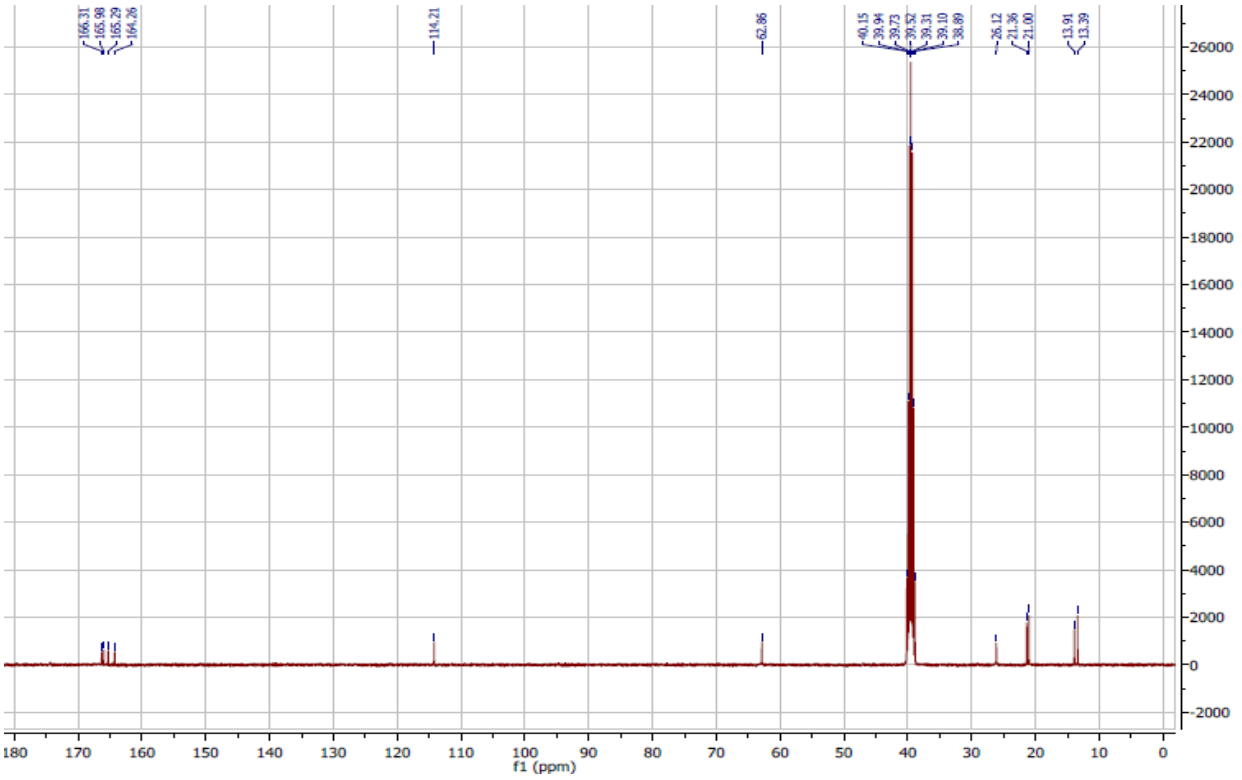

Figure S58: ^{13}C NMR spectrum of compound **27** (DMSO-d_6).

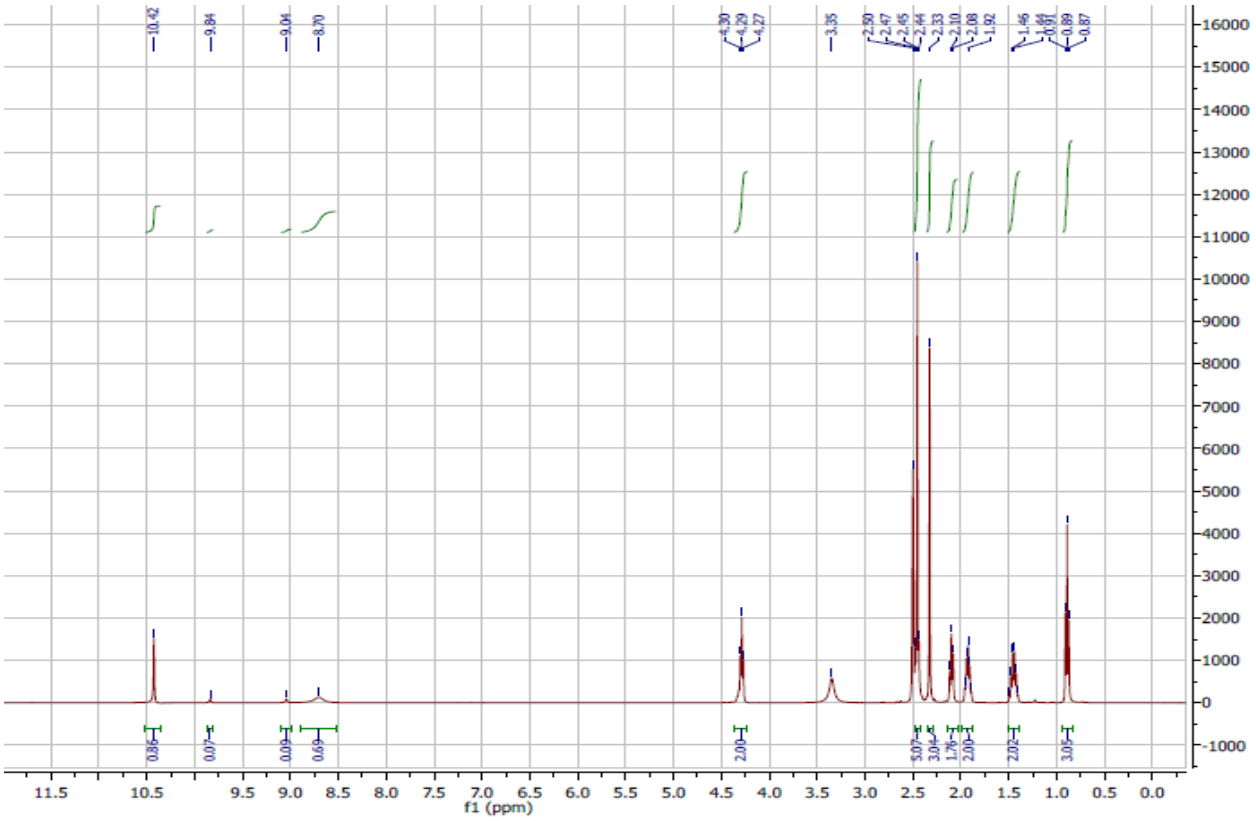

Figure S59: ^{1}H NMR spectrum of compound **28** (DMSO-d_6).

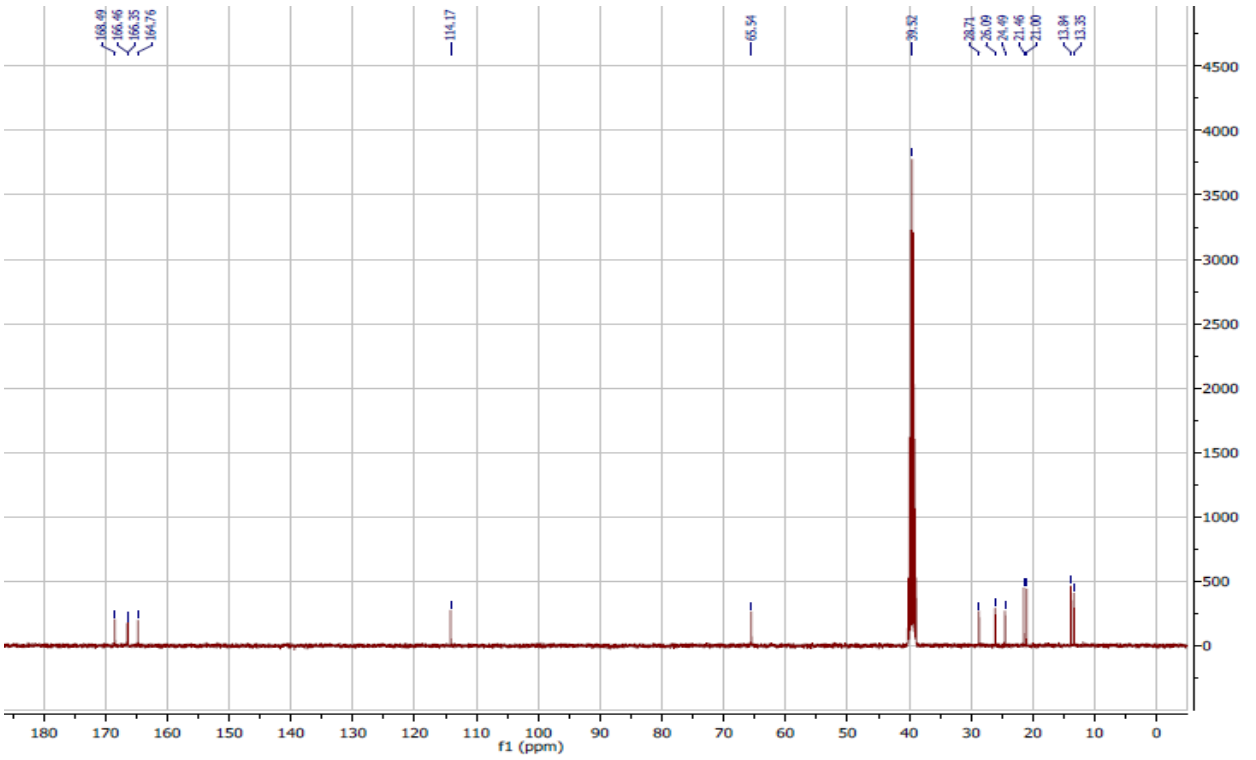

Figure S60: ^{13}C NMR spectrum of compound **28** (DMSO-d_6).

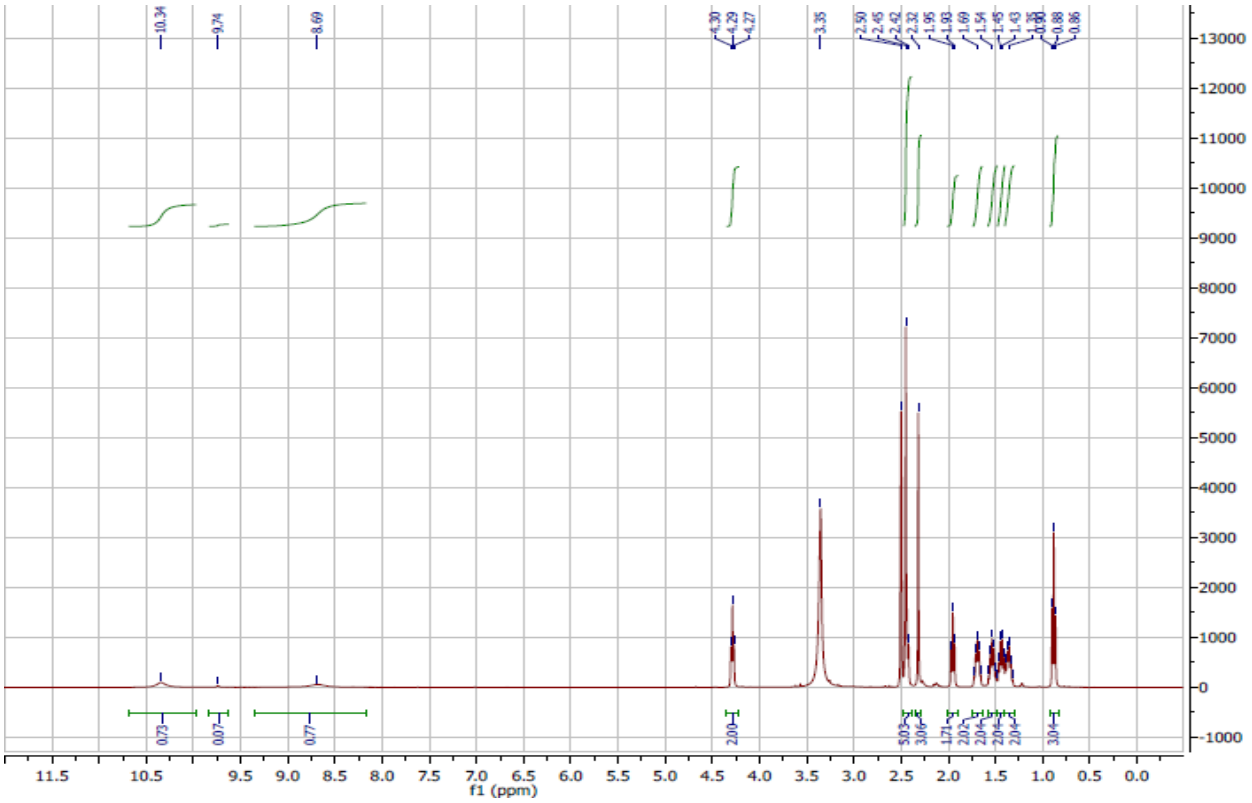

Figure S61: ^{1}H NMR spectrum of compound **29** (DMSO-d_6).

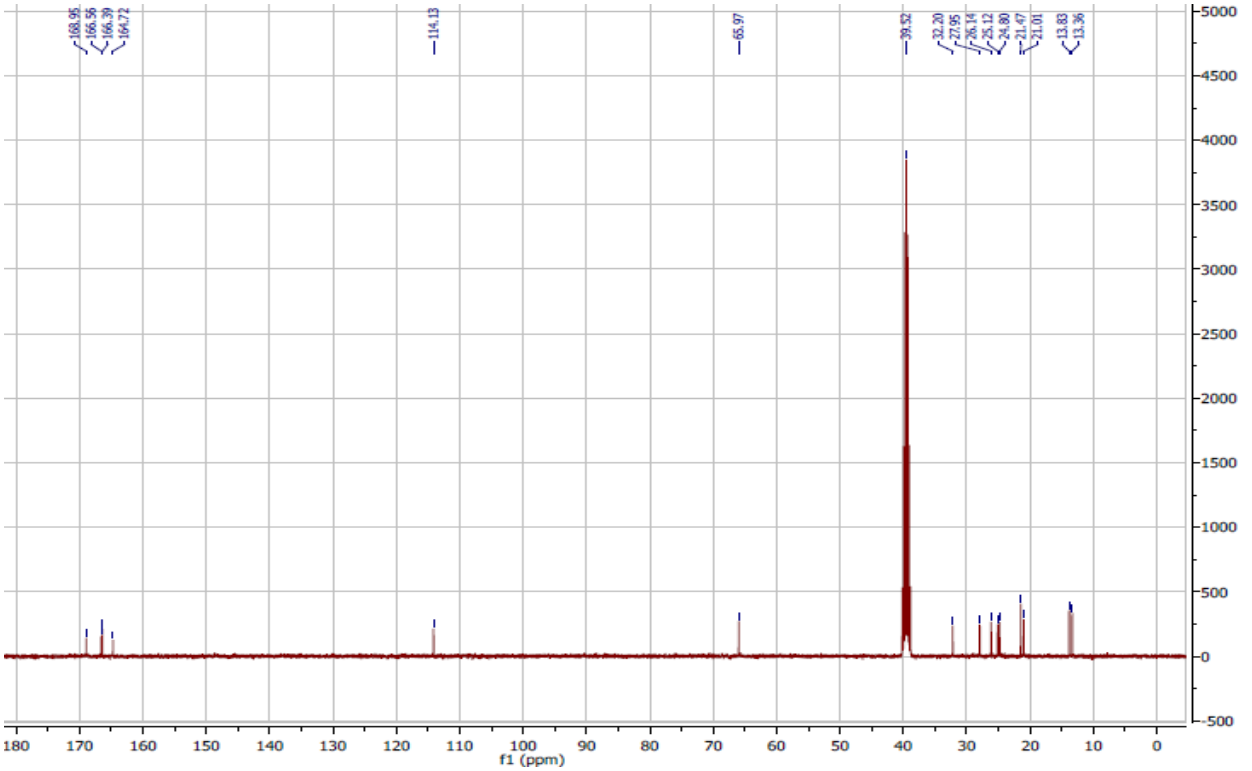

Figure S62: ^{13}C NMR spectrum of compound **29** (DMSO-d_6).

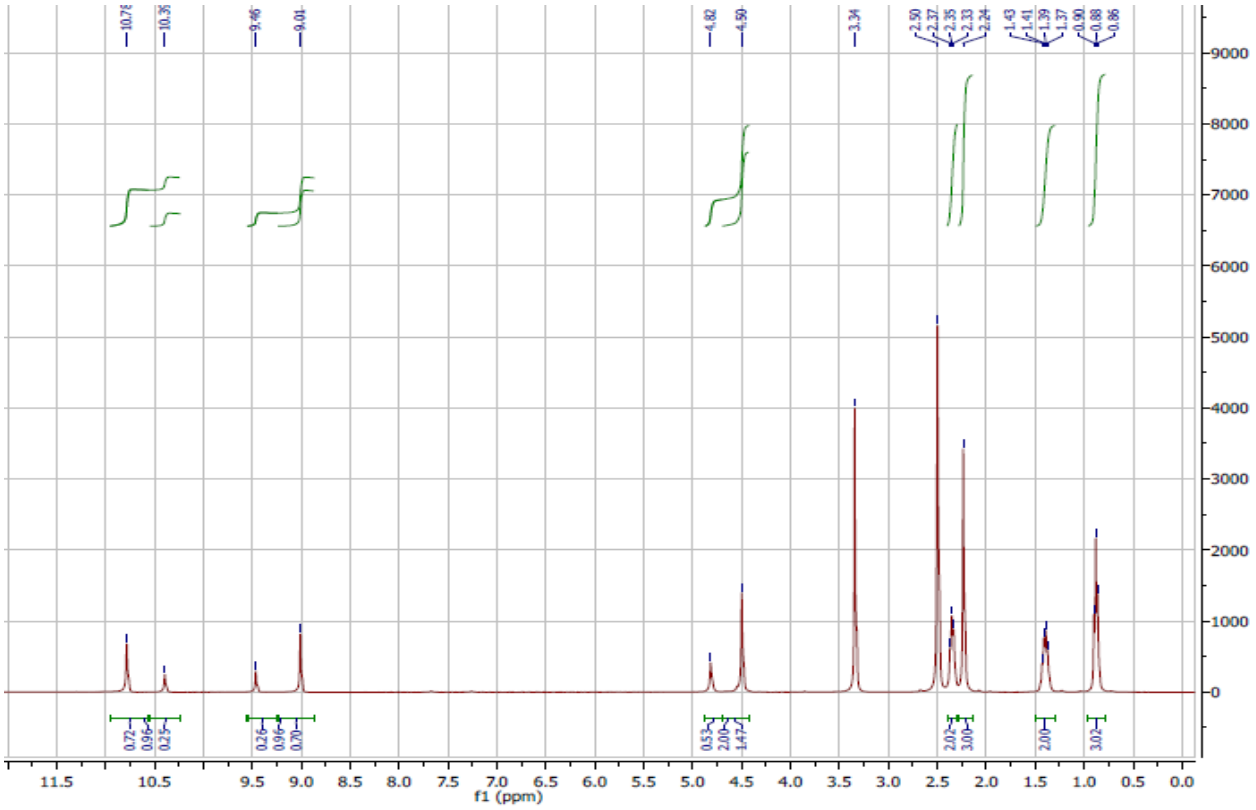

Figure S63: ^{1}H NMR spectrum of compound **30** (DMSO-d_6).

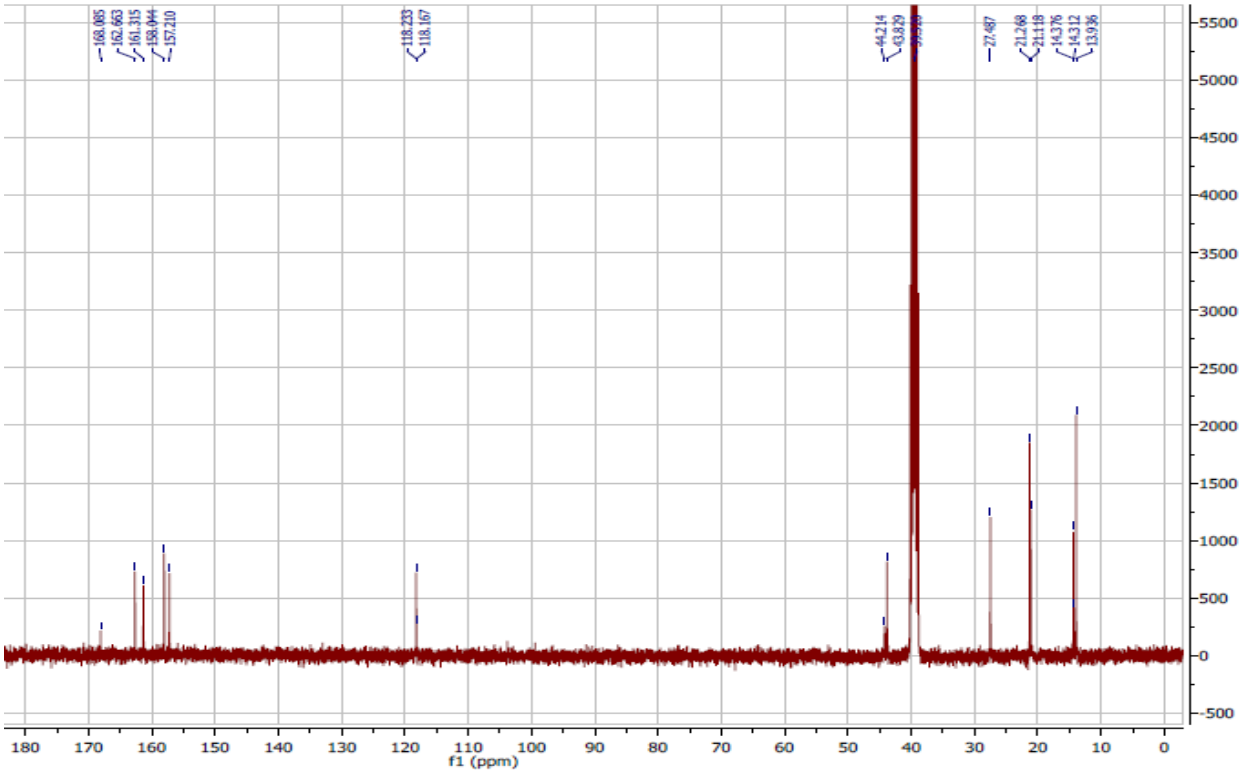

Figure S64: ^{13}C NMR spectrum of compound **30** (DMSO-d_6).

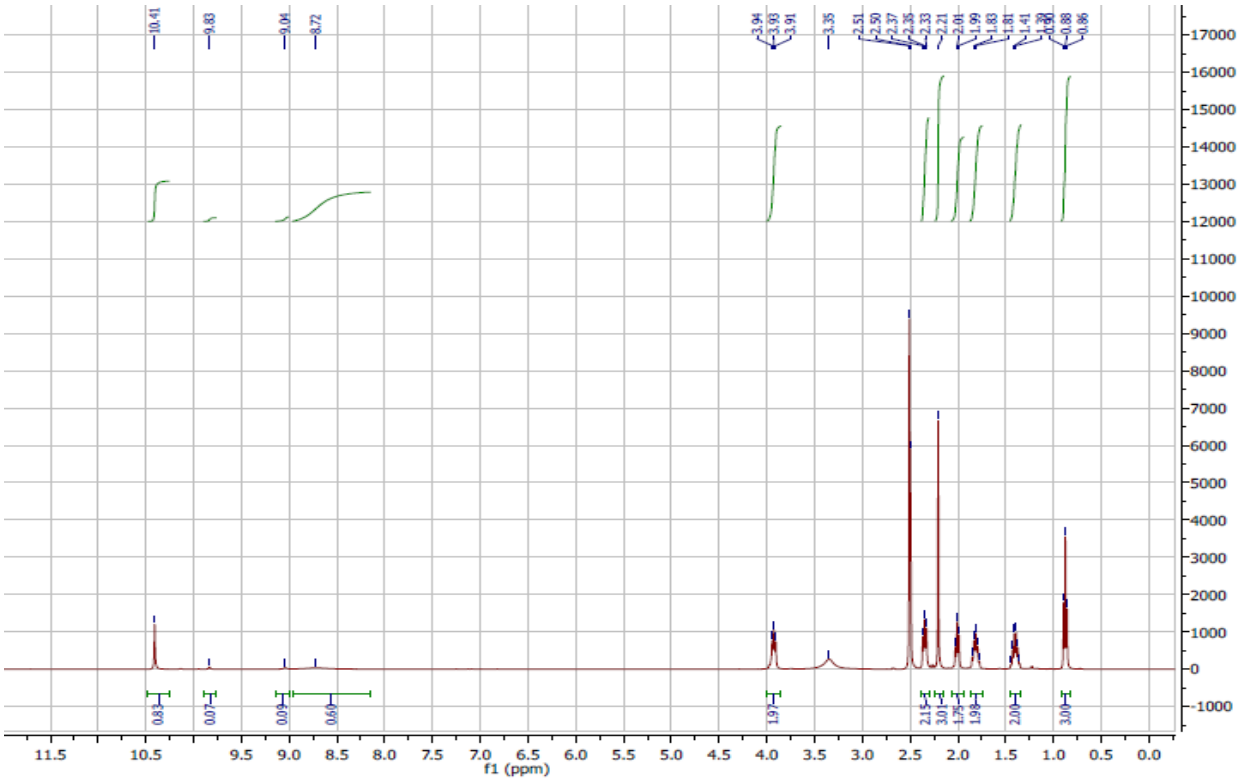

Figure S65: ^{1}H NMR spectrum of compound **31** (DMSO-d_6).

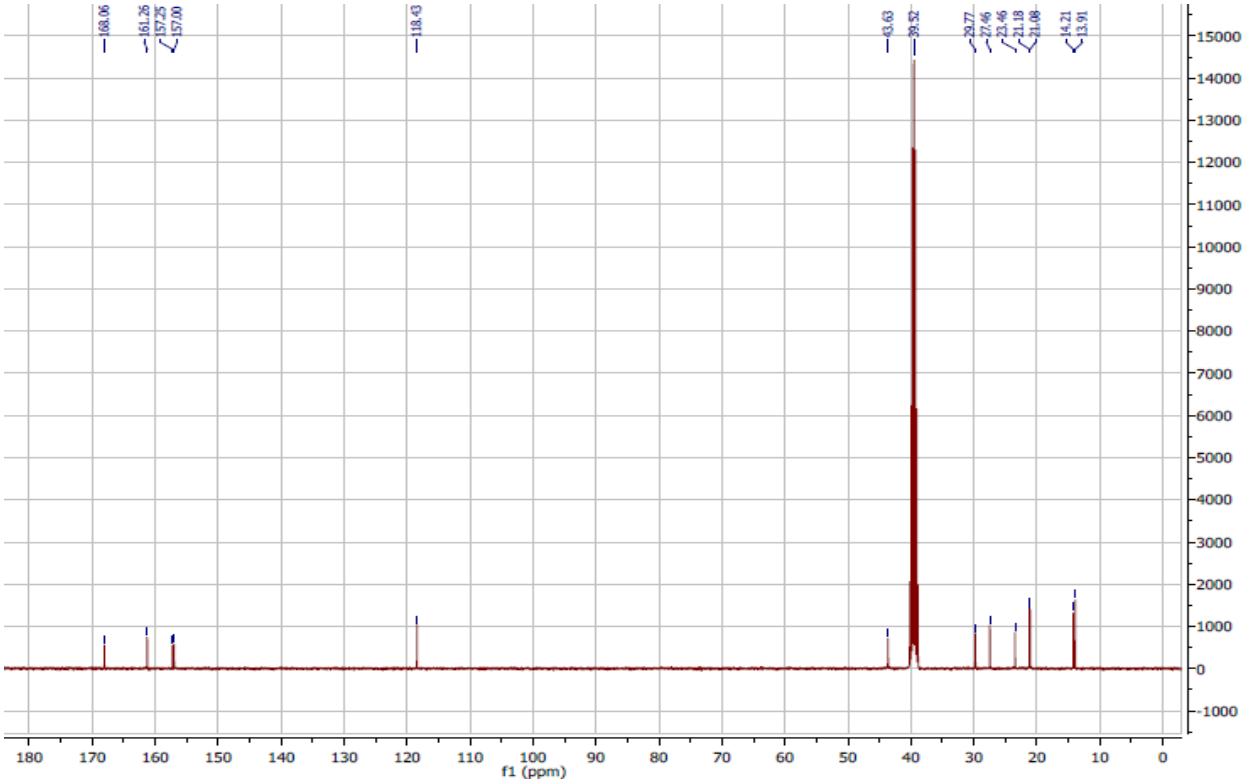

Figure S66: ^{13}C NMR spectrum of compound **31** (DMSO-d_6).