Electronic supplementary materials *Mendeleev Commun.*, 2025, **35**, 537–539

New 7-azacoumarin-3-carboxamide phosphonium salts: cytotoxicity and the Wittig olefination

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Acknowledgments

The authors are grateful to the Collective Spectral-Analytical Center of the Federal Research Center of the Kazan Scientific Center of RAS for technical support of the studies.

Funding

Synthetic (synthesis of compounds **2a, 3a-d**) work was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (grant no. AP23488720). Synthesis of compounds **2b-d** and biological studies were conducted at the Arbuzov Institute of Organic and Physical Chemistry, and were funded by the government assignment for the FRC Kazan Scientific Center of RAS.

Cytotoxicity Assay

Cell lines M-HeLa (epithelioid carcinoma of the cervix. HeLa subline, clone M-HeLa 11), HuTu 80 (adenocarcinoma of the duodenum) were obtained from the collection of the Institute of Cytology of the Russian Academy of Sciences (Russia). Chang liver cells (hepatocyte-like cells) were obtained from the N. F. Gamaleya National Research Center for Epidemiology and Microbiology (Russia).
Cytotoxic activity of phosphonium salts on the 7-azacoumarin-3-carboxamide platform was assessed using the MTT reagent. For this purpose, the cells were incubated for 24 hours with the tested compounds and then stained with an MTT solution at a final concentration of 0.5 mg/ml. After 4 hours, the resulting formazan crystals were dissolved in DMSO. Cells not exposed to the compounds were used as a negative control. 5-Fluorouracil (Sigma-Aldrich, USA) was used as a comparison drug. IC_{50} (half-maximal inhibition concentration) was calculated using the online calculator: MLA - "Quest Graph™ IC_{50} Calculator" (IC50 Calculator. Retrieved June 11, 2024, from: https://www.aatbio.com/tools/ic50-calculator). The selectivity index (SI) of the compounds was determined as the ratio of their IC50 for a normal cell line to the IC_{50} for tumor cells. The selectivity index was calculated relative to the conditionally normal Chang liver cell line.

Experimental Section

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker Avance 400 (Bruker, Billerica, Massachusetts, USA) spectrometer (at the frequencies of 400.05, 100.61, and 161.94 MHz, respectively) and on a Bruker Avance 600 spectrometer (at the frequencies of 600.1, 150.9, and 242.0 MHz, respectively). Values of the chemical shifts for the ^{1}H and ^{13}C nuclei are reported relative to the residual signals of the solvent (DMSO-d_6), and those for the ^{31}P nuclei are given relative to the used standard (H_3PO_4, dP = 0.00). IR spectra were recorded in KBr pellets on a Bruker 3/5 E2_76513 Tensor-27 spectrometer in the range of 400–3600 cm^{-1}. Mass spectra were recorded on an Ultraflex III TOF/TOF Bruker instrument (*p*-nitroaniline as the matrix) and an AmaZon X Bruker instrument. Elemental analysis was performed using a Carlo Erba EA 1108 (Carlo Erba, Cornaredo, Italy) instrument.

Procedures for the Synthesis of Compounds **2a-d**

An equimolar mixture of 7-azacoumarin-3-carboxamide and triphenylphosphine in argon was heated at 150-170 °C for 1-6 hours. After cooling, 10 mL of absolute benzene was added to the reaction mixture and stirred for 3-4 hours at room temperature. The product was isolated as a precipitate by filtration.

{[8-Methyl-2-oxo-3-(*N*-phenylcarbamoyl)-2*H*-pyrano[2,3-*c*]pyridin-5-yl]methyl}-triphenylphosphonium chloride **2a**

The reaction mixture was heated for 1 h at 150 °C. Yield 89%, mp 273-275 °C. IR(KBr), ν, cm^{-1}: 1599 (Ph), 1662 (C(O)-NH), 1728, 1756 (C=O). ^{1}H NMR (DMSO-*d*$_6$), δ ppm: 2.57 (3H, s, CH_3), 5.78 (2H, d, *J* = 15.8 Hz, CH_2), 7.16 (1H, t, *J* = 7.3 Hz, Ph), 7.31 – 7.47 (3H, m, Ph), 7.66 – 7.76 (8H, m, Ph), 7.76 – 7.83 (6H, m, Ph), 7.84 – 7.94 (3H, m, Ph), 8.19 (1H, s, CH_{Py}), 8.27 (1H, d, *J* = 3.1 Hz, CH), 10.48 (1H, s, NH). ^{13}C NMR (DMSO-*d*$_6$), δ, ppm: 19.17; 22.88; 23.27; 117.02; 117.70; 119.78; 119.85; 120.40; 123.73; 124.83; 125.73; 128.79; 129.22; 129.27; 129.42; 130.70; 130.82; 133.64; 134.61; 134.69; 135.79; 138.49; 140.17; 146.54; 147.64; 148.24; 157.83; 159.54. ^{31}P NMR (DMSO-*d*$_6$), δ, ppm: 23.61. Mass spectrum: m/z 555.2 $[M - Cl]^+$. Found, %: C 71.24; H 4.69; Cl 6.10; N 4.87; P 5.36. $C_{35}H_{28}ClN_2O_3P$. Calculated, %: C 71.13; H 4.78; Cl 6.00; N 4.74; P 5.24.

({3-[*N*-(2-Methoxyphenyl)carbamoyl]-8-methyl-2-oxo-2*H*-pyrano[2,3-*c*]pyridin-5-yl}methyl)-triphenylphosphonium chloride **2b**

The reaction mixture was heated for 6 h at 170 °C. Yield 50%, mp 258-261 °C. IR(KBr), ν, cm^{-1}: 1597 (Ph), 1669 (C(O)-NH), 1727 (C=O). ^{1}H NMR (DMSO-*d*$_6$), δ ppm: 2.58 (3H, s, CH_3), 3.92 (3H, s, CH_3), 5.77 (2H, d, *J* = 15.7 Hz, CH_2), 7.00 (1H, ddd, *J* = 8.6, 5.9, 2.9 Hz, Ph), 7.12 – 7.18 (1H, m, Ph), 7.68 – 7.81 (12H, m, Ph + CH_{Py}), 7.82 – 7.91 (3H, m, Ph), 8.28 (1H, d, *J* = 3.1 Hz, Ph), 8.35 (1H, d, *J* = 8.0 Hz, Ph), 8.42 (1H, s, CH), 10.88 (1H, s, NH). ^{13}C NMR (DMSO-*d*$_6$), δ, ppm: 19.68; 23.26; 23.70; 57.14; 110.95; 112.16; 117.51; 118.36; 120.62; 121.71; 123.64; 124.29; 125.80; 127.98; 131.26; 131.28; 135.12; 135.22; 136.22; 142.89; 147.17; 147.25; 148.19; 148.91; 149.57; 158.43; 160.16 ^{31}P NMR (DMSO-*d*$_6$), δ, ppm: 23.77. Mass spectrum: m/z 585.3 $[M - Cl]^+$. Found, %: C 69.74; H 4.78; Cl 5.85; N 4.64; P 5.04. $C_{36}H_{30}ClN_2O_4P$. Calculated, %: C 69.62; H 4.87; Cl 5.71; N 4.51; P 4.99

{[3-(*N*,*N*-Diphenylcarbamoyl)-8-methyl-2-oxo-2*H*-pyrano[2,3-*c*]pyridin-5-yl]methyl}-triphenylphosphonium chloride **2c**

The reaction mixture was heated for 3 h at 150 °C. Yield 39%, mp 204-7 °C dec. IR(KBr), ν, cm^{-1}: 1589 (Ph), 1667 (C(O)-NH), 1742 (C=O). ^{1}H NMR (DMSO-*d*$_6$), δ ppm: 2.42 (3H, s, CH_3), 5.70 (2H, d, *J* = 15.8 Hz, CH_2), 7.27 (10H, br.s, Ph), 7.72 – 7.81 (12H, m, Ph), 7.91 (3H, t, *J* = 7.4 Hz, Ph), 8.07 (1H, s, CH), 8.40 (1H, s, CH_{Py}). ^{13}C NMR (DMSO-*d*$_6$), δ, ppm: 18.98; 22.96; 23.34; 117.08; 117.76; 119.56; 123.40; 127.01; 128.78; 129.70; 130.00; 130.71; 130.81; 134.59; 134.67; 135.91; 138.50; 146.39; 147.26; 155.49; 163.10. ^{31}P NMR (DMSO-*d*$_6$), δ, ppm: 23.58. Mass spectrum: m/z 631.2 $[M - Cl]^+$. Found, %: C 73.74; H 4.78; Cl 5.18; N 4.21;. P 4.59. $C_{41}H_{32}ClN_2O_3P$. Calculated, %: C 73.82; H 4.83; Cl 5.31; N 4.20; P 4.64.

[(3-{*N*-[4-(*N'*-Acetylsulfamoyl)phenyl]carbamoyl}-8-methyl-2-oxo-2*H*-pyrano[2,3-*c*]pyridin-5-yl)methyl]triphenylphosphonium chloride **2d**

The reaction mixture was heated for 5 h at 160 °C. Yield 21%, mp 273-275 °C. IR(KBr), ν, cm^{-1}: 1592 (Ph), 1681 (C(O)-NH), 1725 (C=O). ^{1}H NMR (DMSO-*d*$_6$), δ ppm: 1.94 (3H, s, CH_3), 2.57 (3H, s, CH_3), 5.76 (2H, d, *J* = 15.8 Hz, CH_2), 7.68 – 7.81 (12H, m, Ph), 7.86 (3H, t, *J* = 7.7, Hz, Ph), 7.95 (4H, d, *J* = 7.7, Hz, Ph), 8.18 (1H, s, CH_{Py}), 8.28 (1H, s, CH), 10.91 (1H, s, NH), 12.11 (1H, s, NH). ^{13}C NMR (DMSO-*d*$_6$), δ, ppm: 19.68; 24.24; 117.40; 118.26; 120.65; 126.03; 129.31; 129.69; 129.81; 129.92; 131.24, 131.36; 132.43; 132.52; 133.04; 135.14; 135.23; 136.34; 140.91; 143.47; 147.14; 148.22; 157.82; 160.91; 169.81. ^{31}P NMR (DMSO-*d*$_6$), δ, ppm: 23.22. Mass spectrum: m/z 676.3 $[M - Cl]^+$. Found, %: C 62.33; H 4.28; Cl 5.09; N 5.84; P 4.32; S 4.60. $C_{37}H_{31}ClN_3O_6PS$. Calculated, %: C 62.40; H 4.39; Cl 4.98; N 5.90; P 4.35; S 4.50.

Procedures for the Synthesis of Compounds **3a-d**

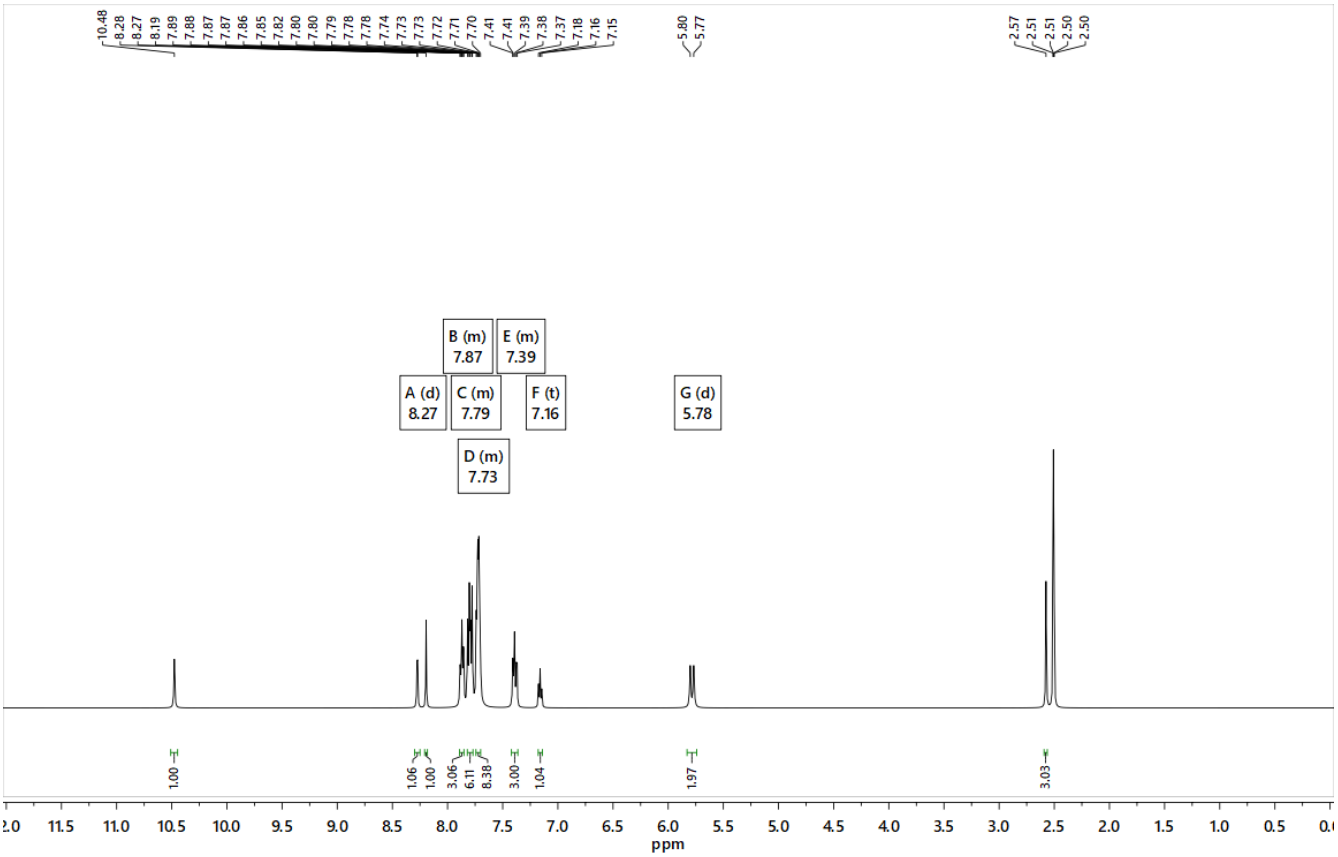
To a suspension of phosphonium salt **2a** cooled to 0 °C in 2 mL of absolute ethanol in a stream of argon, equimolar amounts of potassium *tert*-butoxide and, after 0.5 h, the corresponding aromatic aldehyde were added. The next day, the formed precipitate was separated and washed successively with 10 mL of hot water, 10 mL of benzene, and 10 mL of ether, then it was dried under vacuum.

(*Z*)-8-Methyl-2-oxo-*N*-phenyl-5-styryl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamide **3a**
Yield of **3a** 31%, mp 174-178 °C. IR(KBr), ν, cm^{-1}: 1598 (Ph), 1670 (C(O)-NH), 1719 (C=O). ^{1}H NMR (DMSO-d_6), δ ppm: 2.62 (3H, s, CH_3), 6.97 (1H, d, *J* = 12.2 Hz, CH), 7.06 (1H, d, *J* = 12.2 Hz, CH), 7.11 – 7.24 (5H, m, Ph), 7.39 (3H, q, *J* = 8.1. 7.2 Hz, Ph), 7.70 (2H, d, *J* = 8.0 Hz, Ph), 8.17 (1H, s, CH), 8.60 (1H, s, CH_{Py}), 10.60 (1H, s, NH). ^{13}C NMR (DMSO-d_6), δ, ppm: 19.51; 120.73; 120.94; 121.00; 122.27; 123.15; 125.49; 126.20; 128.36; 128.93; 129.53; 129.68; 129.87; 130.00; 136.31; 136.49; 138.84; 142.67; 144.53; 146.48; 148.10; 159.62; 160.39. Mass spectrum: m/z 383.2 $[M + H]^+$. Found, %: C 75.43; H 4.78; N 7.42. $C_{24}H_{18}N_2O_3$. Calculated, %: C 75.38; H 4.74; N 7.33.

(*Z*)-5-(2-Hydroxystyryl)-8-methyl-2-oxo-*N*-phenyl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamide **3b**
Yield of **3b** 15%, mp 162-164 °C. IR(KBr), ν, cm^{-1}: 1598 (Ph), 1676 (C(O)-NH), 1721 (C=O). ^{1}H NMR (DMSO-d_6), δ ppm: 2.50 (3H, s, CH_3), 6.52 (1H, d, *J* = 7.7 Hz, CH), 6.76 (1H, d, *J* = 7.5 Hz, CH), 6.86 (1H. d, *J* = 8.2 Hz, Ph), 6.92 (1H. d, *J* = 12.6 Hz, Ph), 7.02 (1H, t, *J* = 7.2 Hz, Ph), 7.09 (1H. d, *J* = 11.8 Hz, Ph), 7.15 (1H, t, *J* = 7.4 Hz, Ph), 7.38 (2H, t, *J* = 7.8 Hz, Ph), 7.70 (2H, t, *J* = 8.2 Hz, Ph), 8.15 (1H, s, CH), 8.64 (1H, s, CH_{Py}), 9.81 (1H, br.s, OH), 10.59 (1H, s, NH). ^{13}C NMR (DMSO-d_6), δ, ppm: 19.44; 116.86; 119.62; 120.96; 122.00; 122.09; 123.44; 125.46; 125.93; 129.99; 130.25; 130.32; 130.41; 132.52; 138.86; 142.79; 144.59; 145.95; 147.98; 156.68; 159.63; 160.42. Mass spectrum: m/z 399.2 $[M + H]^+$. Found, %: C 72.19; H 4.68; N 6.97. $C_{24}H_{18}N_2O_4$. Calculated, %: C 72.35; H 4.55; N 7.03

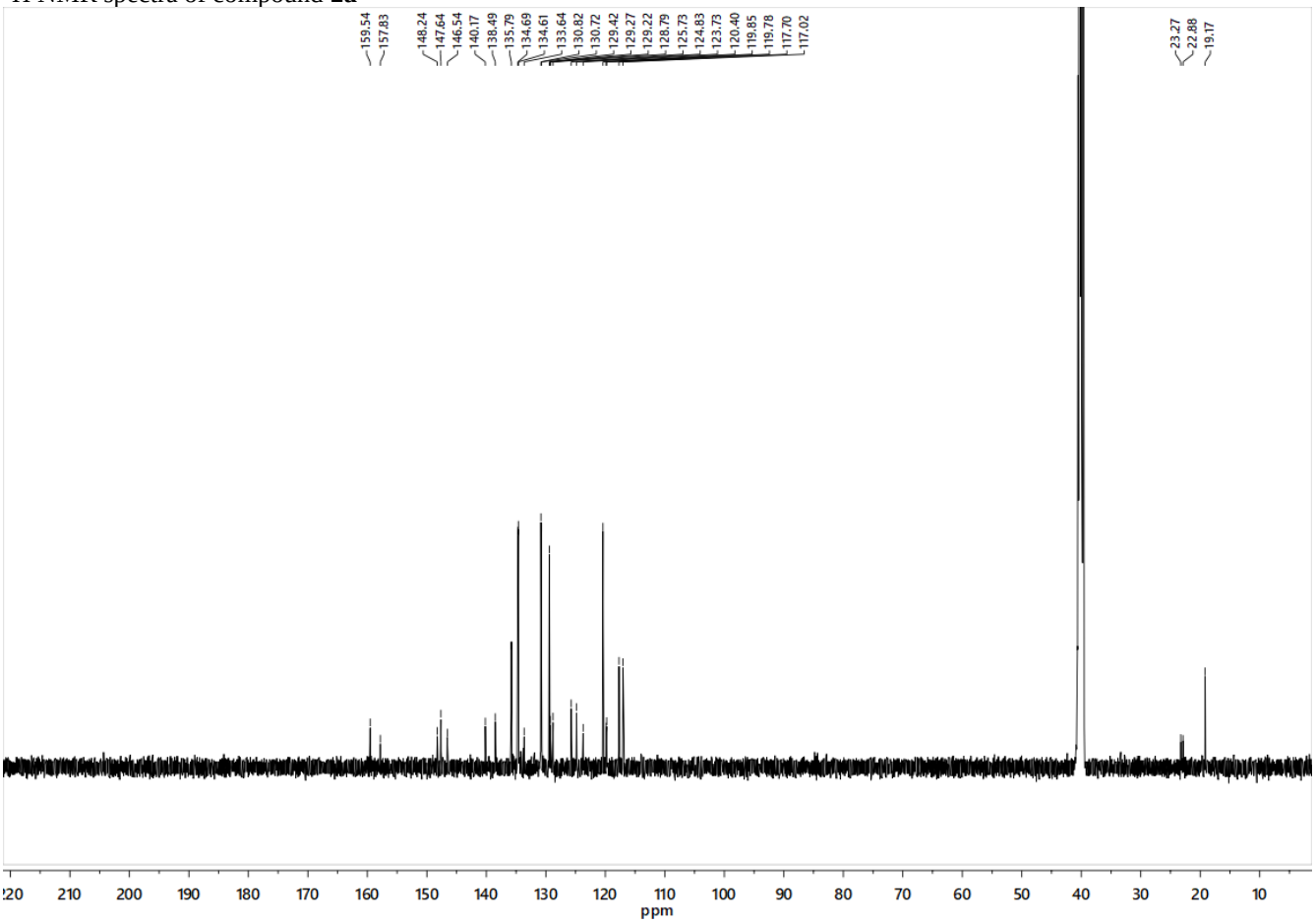
(*Z*)-5-(2-Hydroxy-5-nitrostyryl)-8-methyl-2-oxo-*N*-phenyl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamide **3c**
Yield of **3c** 26%, mp dec. IR(KBr), ν, cm^{-1}: 1597 (Ph), 1674 (C(O)-NH), 1729 (C=O). Спектр ^{1}H NMR (DMSO-d_6), δ ppm: 2.61 (3H, s, CH_3), 6.98 (1H, d, *J* = 9.0 Hz, Ph), 7.05 (1H, d, *J* = 12.2 Hz, CH), 7.11 (1H, d, *J* = 12.2 Hz, CH), 7.16 (1H, t, *J* = 7.4 Hz, Ph), 7.39 (2H, t, *J* = 7.9 Hz, Ph), 7.68(1H, d, *J* = 2.9, Ph), 7.70 (2H, d, *J* = 7.3 Hz, Ph),7.94 (1H, dd, *J* = 9.1, 2.9, Ph), 8.20 (1H, s, CH), 8.60 (1H, s, CH_{Py}), 10.58 (1H, s, NH). ^{13}C NMR (DMSO-d_6), δ, ppm: 18.94; 116.99; 120.40; 121.43; 123.73; 123.93; 124.92; 125.85; 125.91; 129.09; 129.47; 130.24; 138.37; 138.54; 141.79; 143.80; 145.96; 147.46; 158.87;159.96. Mass spectrum: m/z 444.2 $[M + H]^+$. Found, %: C 65.14; H 3.69; N 9.56. $C_{24}H_{17}ClN_3O_6$. Calculated, %: C 65.01; H 3.86; N 9.48.

(*Z*)-5-(5-Chloro-2-hydroxystyryl)-8-methyl-2-oxo-*N*-phenyl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamide **3d**
Yield of **3d** 36%, mp 289-293 °C. IR(KBr), ν, cm^{-1}: 1597 (Ph), 1673 (C(O)-NH), 1730 (C=O). ^{1}H NMR (DMSO-d_6), δ ppm: 2.60 (3H, s, CH_3), 6.81 (1H, s, Ph), 6.88 (1H, d, *J* = 8.7 Hz, CH), 6.94 – 7.04 (2H. m, Ph), 7.07 (1H, d, *J* = 8.9 Hz, CH), 7.16 (1H, s, Ph), 7.39 (2H, t, *J* = 7.9 Hz, Ph), 7.71 (2H, d, *J* = 8.4 Hz, Ph), 8.14 (1H, s, CH), 8.64 (1H, s, CH_{Py}), 10.18 (1H, br.s, OH), 10.60 (1H, s, NH). ^{13}C NMR (DMSO-d_6), δ, ppm: 19.47; 118.48; 120.76; 120.96; 122.16; 122.96; 123.62; 125.40; 125.46; 126.17; 129.52; 129.79; 130.00; 131.18; 138.89; 142.56; 144.39; 146.24; 147.96; 155.59; 160.50. Mass spectrum: m/z 433.2 $[M + H]^+$. Found, %: C 66.73; H 4.07; Cl 8.10; N 6.58. $C_{24}H_{17}ClN_2O_4$. Calculated, %: C 66.60; H 3.96; Cl 8.19; N 6.47.

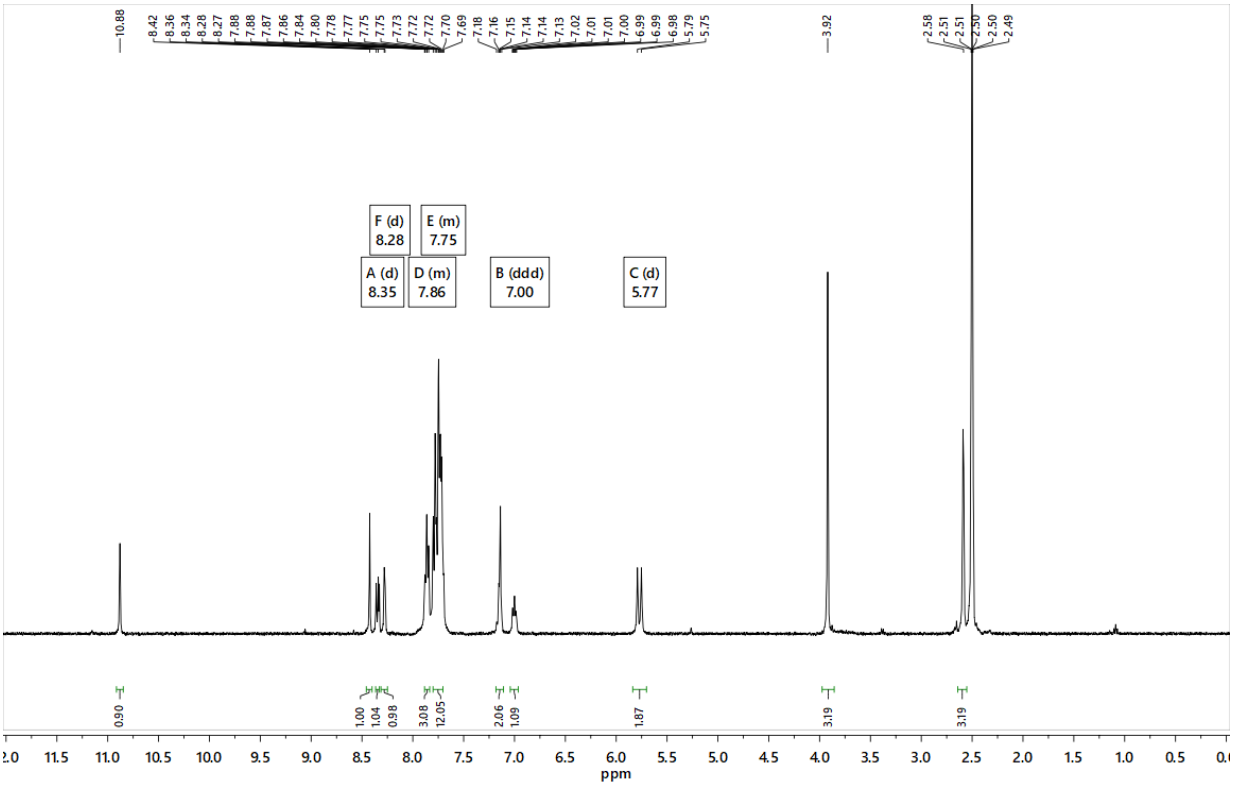


^{1}H NMR (DMSO-d_6) δ 8.27 (d, J = 3.1 Hz, 1H), 7.94 – 7.84 (m, 3H), 7.83 – 7.76 (m, 6H), 7.76 – 7.66 (m, 8H), 7.47 – 7.31 (m, 3H), 7.16 (t, J = 7.3 Hz, 1H), 5.78 (d, J = 15.8 Hz, 2H).

^{1}H NMR spectra of compound **2a**

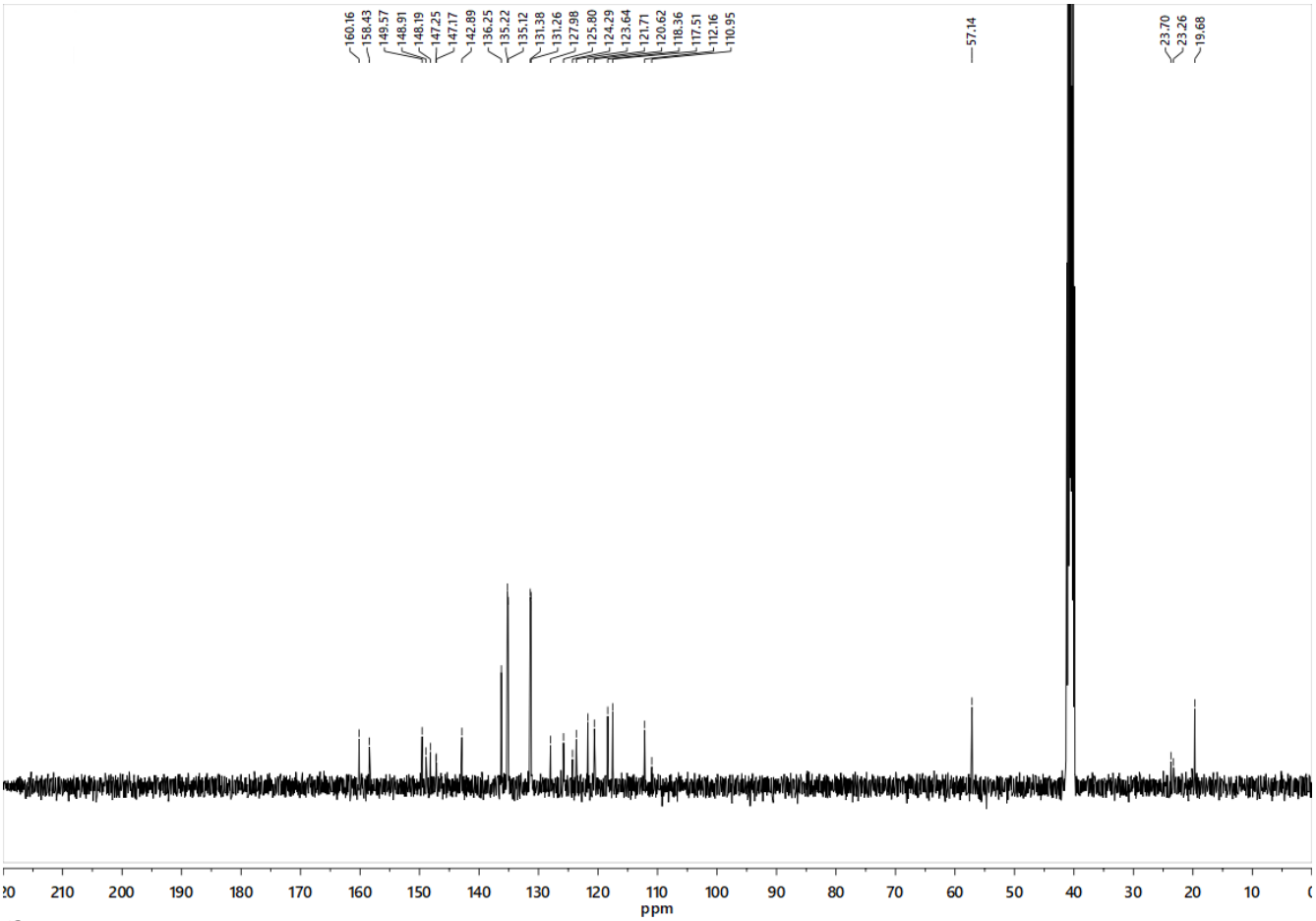


^{13}C NMR spectra of compound **2a**

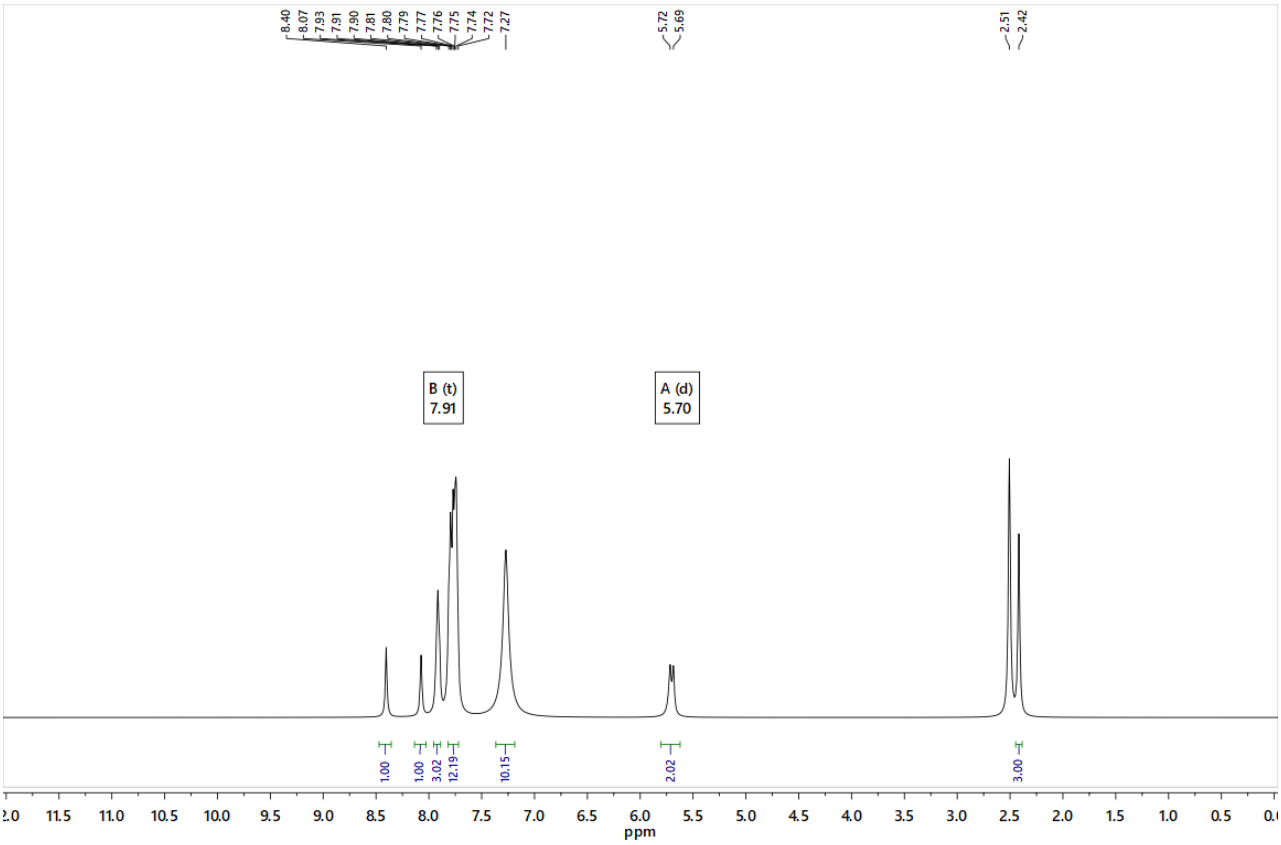


^{1}H NMR (DMSO-*d*$_6$) δ 8.35 (d, *J* = 8.0 Hz, 1H), 8.28 (d, *J* = 3.1 Hz, 1H), 7.91 – 7.82 (m, 3H), 7.81 – 7.68 (m, 12H), 7.00 (ddd, *J* = 8.6, 5.9, 2.9 Hz, 1H), 5.77 (d, *J* = 15.7 Hz, 2H).

^{1}H NMR spectra of compound **2b**

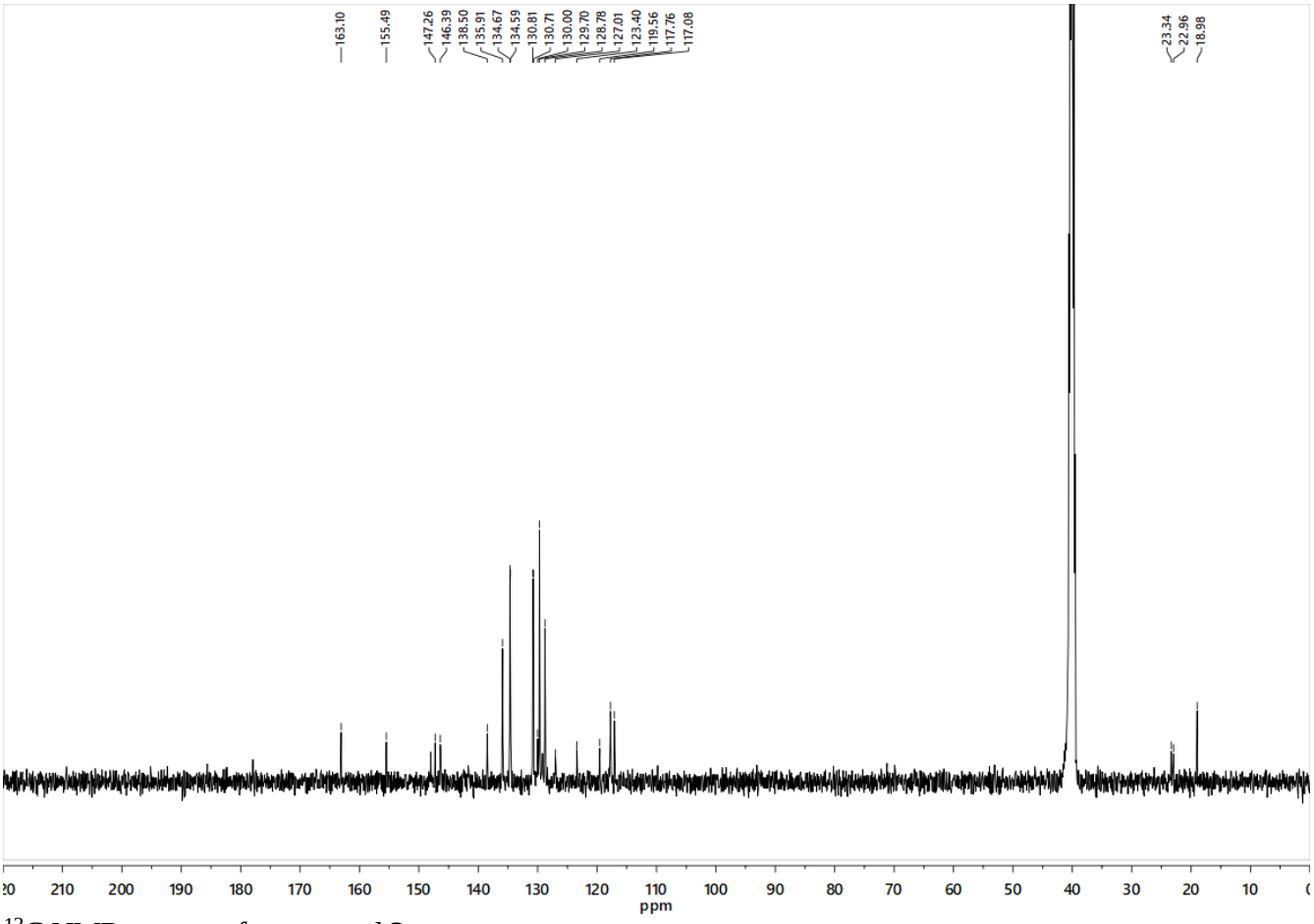


^{13}C NMR spectra of compound **2b**

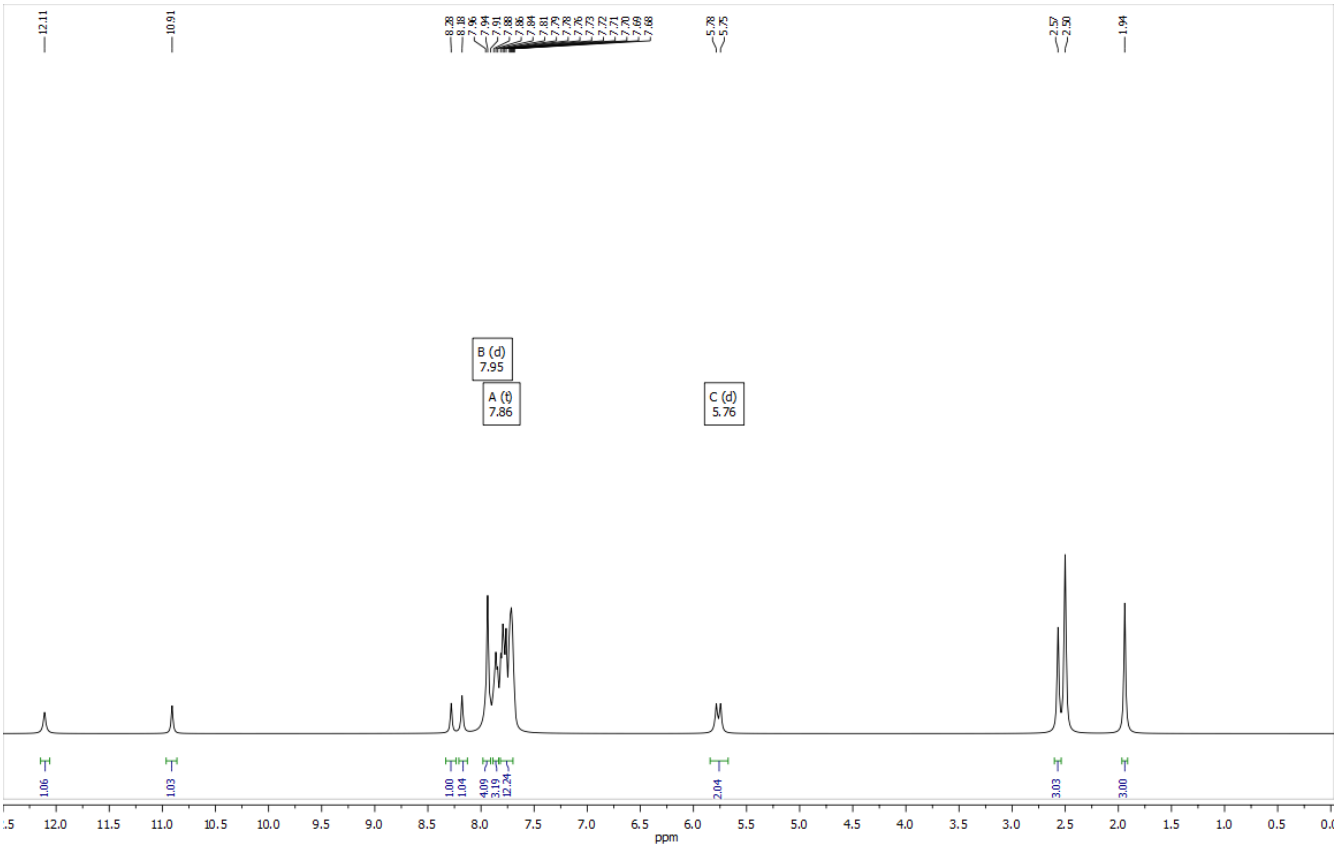


^{1}H NMR (DMSO-d_6) δ 7.91 (t, J = 7.4 Hz, 3H), 5.70 (d, J = 15.8 Hz, 2H).

^{1}H NMR spectra of compound **2c**

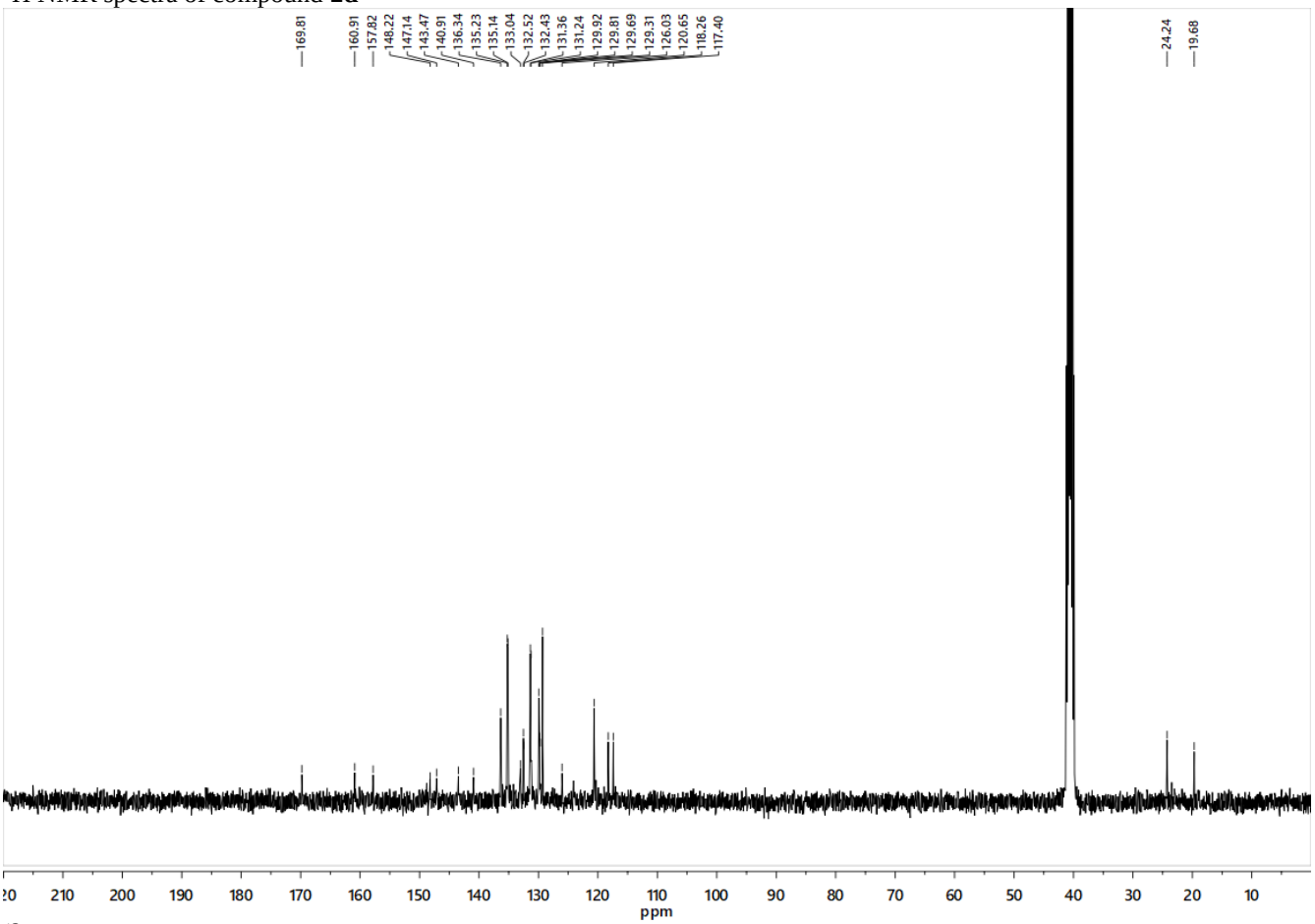


^{13}C NMR spectra of compound **2c**

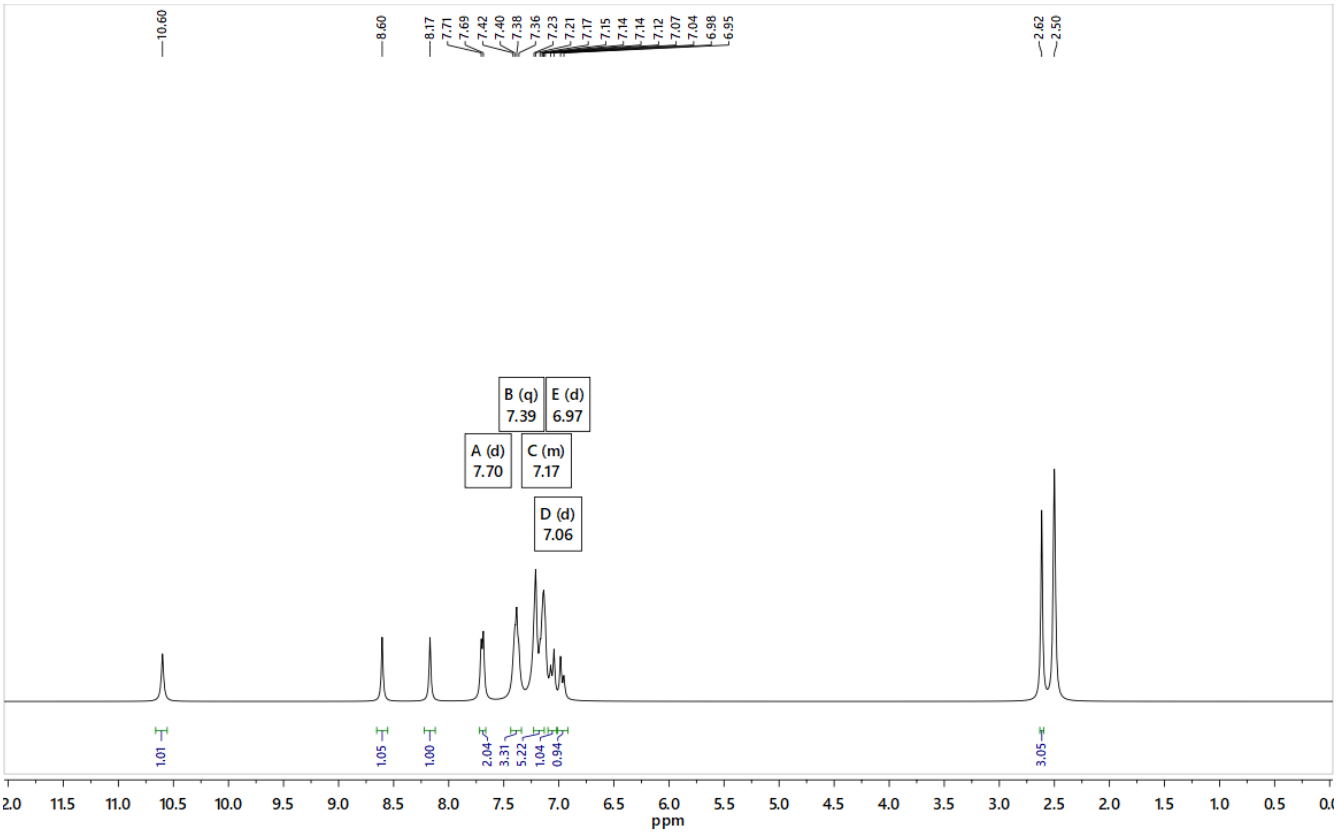


^{1}H NMR (DMSO-d_6) δ 7.95 (d, J = 7.7 Hz, 4H), 7.86 (t, J = 7.7 Hz, 3H), 5.76 (d, J = 15.8 Hz, 2H).

^{1}H NMR spectra of compound **2d**

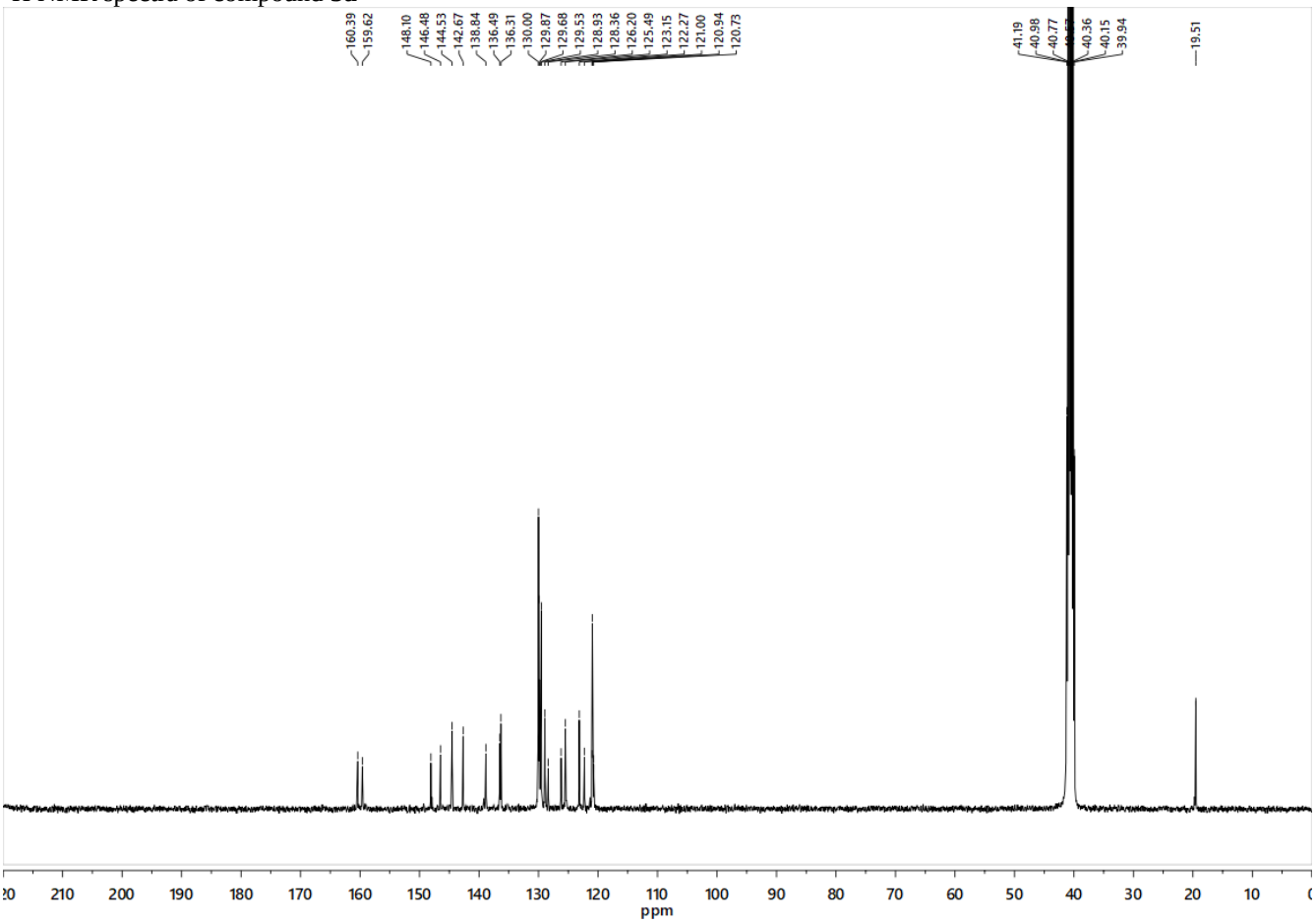


^{13}C NMR spectra of compound **2d**

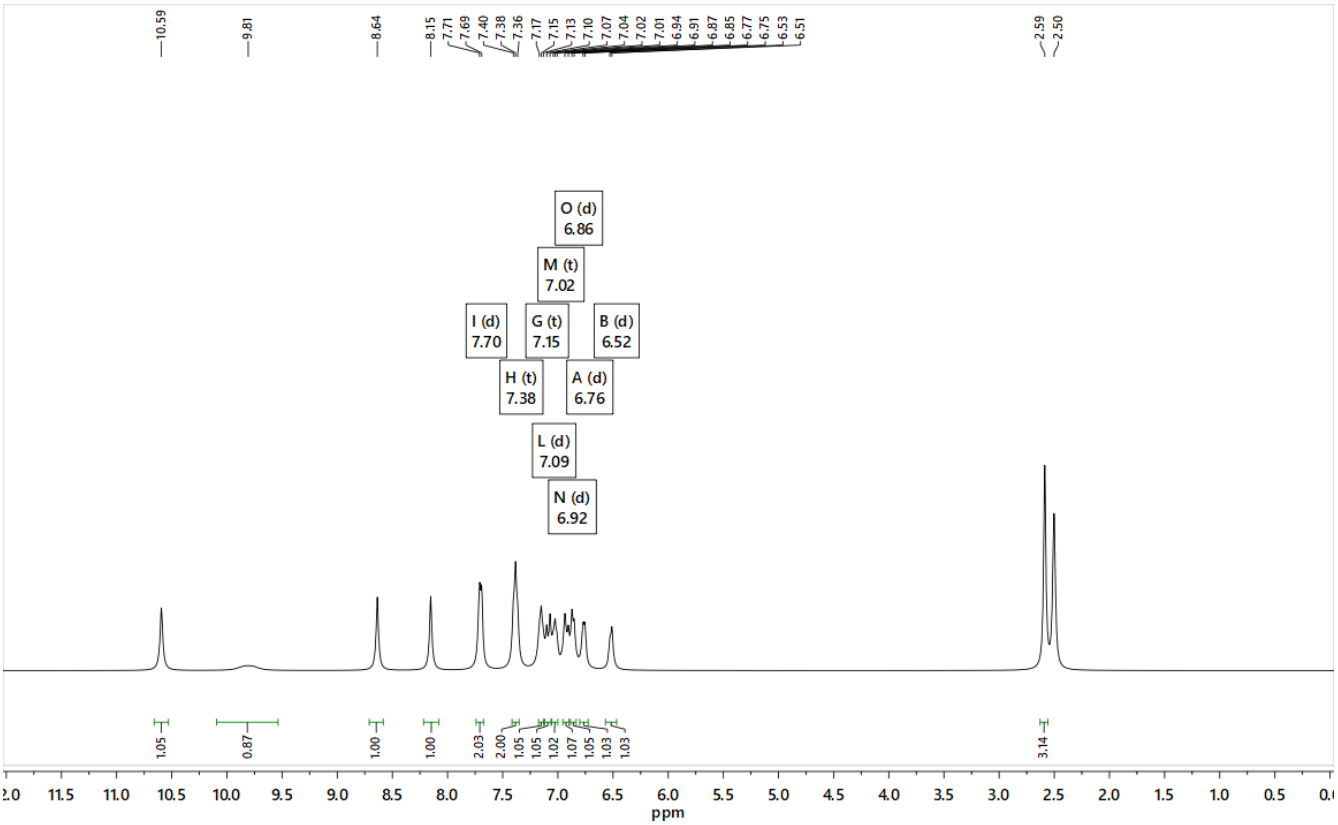


^{1}H NMR (DMSO-d_6) δ 7.70 (d, J = 8.0 Hz, 2H), 7.39 (q, J = 8.1, 7.2 Hz, 3H), 7.24 – 7.11 (m,5H), 7.06 (d, J = 12.2 Hz, 1H), 6.97 (d, J = 12.2 Hz, 1H).

^{1}H NMR spectra of compound **3a**

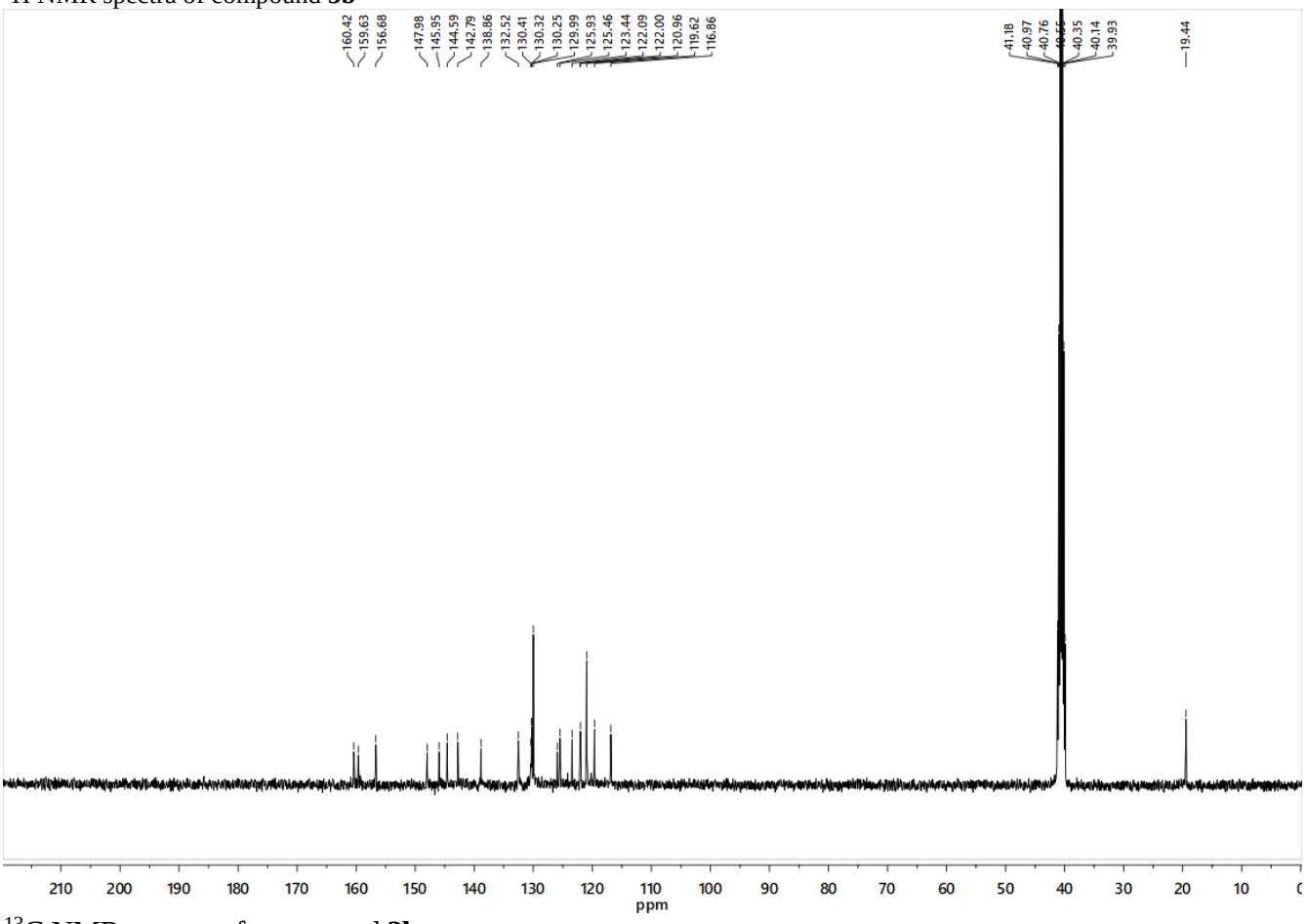


^{13}C NMR spectra of compound **3a**

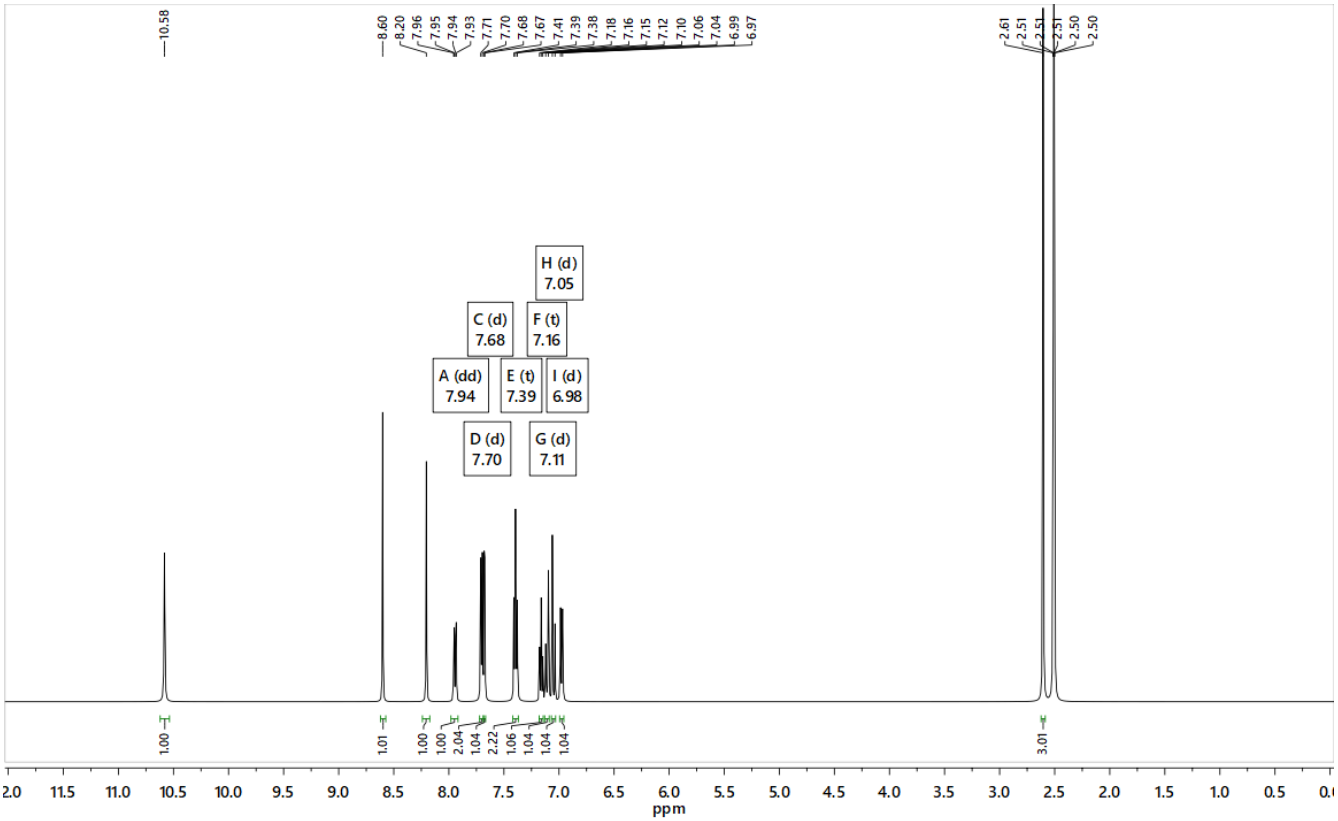


^{1}H NMR (DMSO-d_6) δ 7.70 (d, J = 8.2 Hz, 2H), 7.38 (t, J = 7.8 Hz, 2H), 7.15 (t, J = 7.4 Hz, 1H), 7.09 (d, J = 11.8 Hz, 1H), 7.02 (t, J = 7.2 Hz, 1H), 6.92 (d, J = 12.6 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 7.5 Hz, 1H), 6.52 (d, J = 7.7 Hz, 1H).

^{1}H NMR spectra of compound **3b**

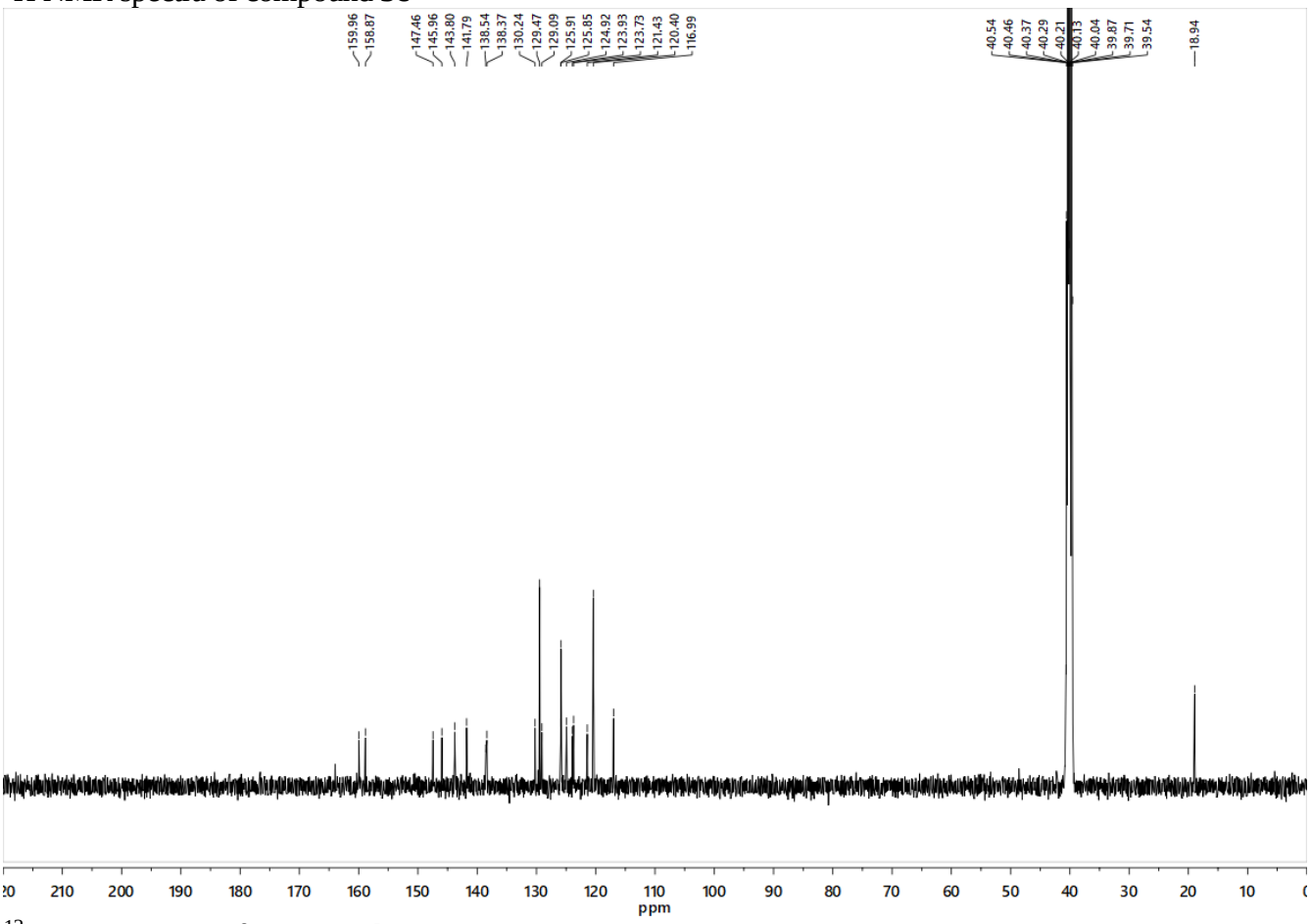


^{13}C NMR spectra of compound **3b**

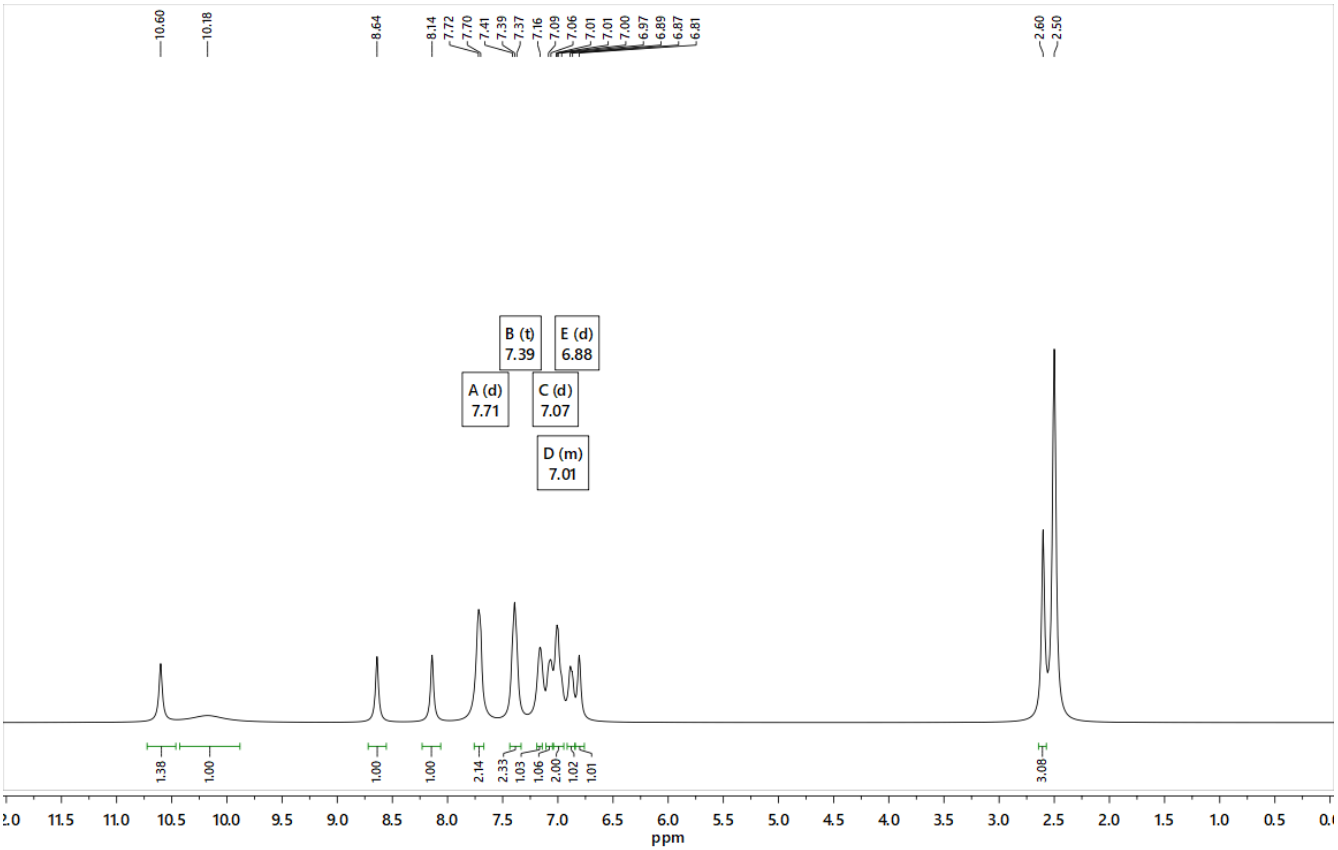


^{1}H NMR (DMSO-d_6) δ 7.94 (dd, J = 9.1, 2.9 Hz, 1H), 7.70 (d, J = 7.3 Hz, 2H), 7.68 (d, J = 2.9 Hz, 1H), 7.39 (t, J = 7.9 Hz, 2H), 7.16 (t, J = 7.4 Hz, 1H), 7.11 (d, J = 12.2 Hz, 1H), 7.05 (d, J = 12.2 Hz, 1H), 6.98 (d, J = 9.0 Hz, 1H).

^{1}H NMR spectra of compound **3c**

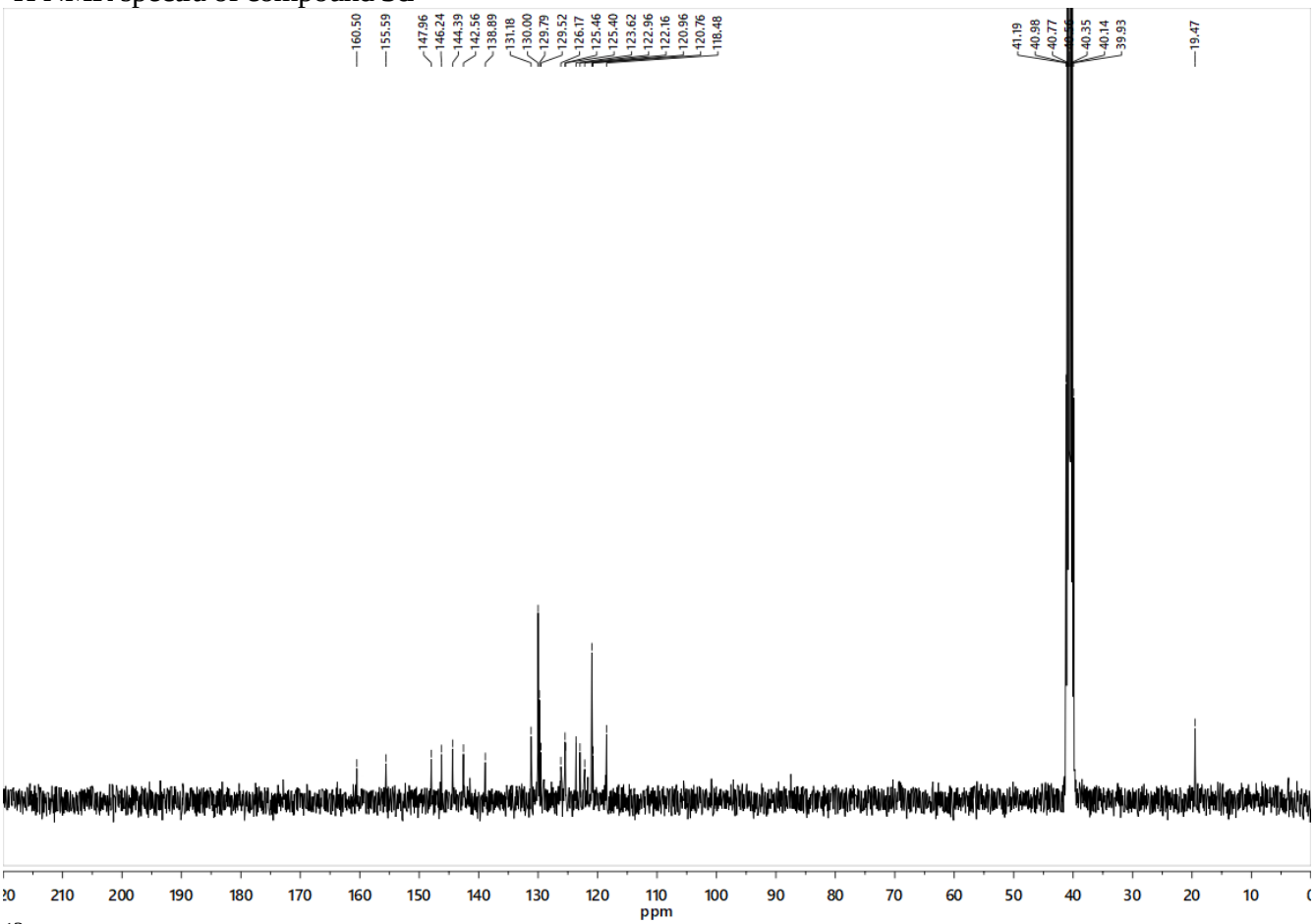


^{13}C NMR spectra of compound **3c**



^{1}H NMR (DMSO-d_6) δ 7.71 (d, J = 8.4 Hz, 2H), 7.39 (t, J = 7.9 Hz, 2H), 7.07 (d, J = 8.9 Hz, 1H), 7.04 – 6.94 (m, 2H), 6.88 (d, J = 8.7 Hz, 1H).

^{1}H NMR spectra of compound **3d**



^{13}C NMR spectra of compound **3d**