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(*E*)-3-Arylidene-4-diazopyrrolidine-2,5-diones conveniently elaborated into cytotoxic compounds bearing primary sulfonamide and Michael acceptor moieties

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Contents:

Experimental section

All commercial reagents were used without purification. NMR spectra were recorded using a Bruker Avance III spectrometer (^{1}H: 400.13 MHz; ^{13}C: 100.61 MHz; the residual solvent peaks were used as internal standards: δ = 7.26 and 2.50 for ^{1}H in $CDCl_3$ and DMSO-d_6, respectively, δ = 39.52 and 77.16 for ^{13}C in DMSO-d_6 and $CDCl_3$, respectively). Mass spectra were recorded using a Bruker microTOF spectrometer (ionization by electrospray, positive ions detection). Melting points were determined in open capillary tubes on Stuart SMP50 Automatic Melting Point Apparatus.

General experimental procedures

Diazo compounds were prepared according to our previously reported method[2,6,7], all commercial reagents were used without additional purification. Mass spectra were recorded with a Bruker Maxis HRMS-ESI-qTOF spectrometer (electrospray ionization mode). NMR spectroscopic data were recorded with Bruker Avance 400 spectrometer (400.13 MHz for ^{1}H, 100.61 MHz for ^{13}C and 376.50 MHz for ^{19}F) and were referenced to residual solvent proton peaks and solvent carbon peaks.

*General procedure for the preparation of **3a-o***

To a stirred solution of the corresponding diazo compound **1** (0.5 mmol) in toluene/1,4-dioxane (4:3) mixture was added 3- or 4-aminobenzenesulfonamide (0.5 mmol) and catalyst $Rh_2(OAc)_4$ (1 mol%). The emission of N_2 was observed, and the precipitate was formed. The mixture was stirred at 110 °C for 1 hour. The precipitate was filtered off, washed with hexane and dried in air to afford compounds **3b**, **3d**, **3e**, **3j-e**, **3o**. Compounds **3a**, **3c**, **3f**, **3m** were soluble in the reaction mixture, which was concentrated *in vacuo*, crystallized in hexane and filtered off. Compound **3f** was additionally purified by recrystallization from MeOH.

(E)-4-((4-Benzylidene-1-(4-methoxyphenyl)-2,5-dioxopyrrolidin-3-yl)amino)benzenesulfonamide (**3a**). Yield 112 mg (77%). Light-yellow solid; m.p. 248.1-250.1 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.76 (d, *J* = 2.0 Hz, 1H, NH), 7.65 (d, *J* = 7.1 Hz, 2H, ArH), 7.57 (d, *J* = 8.8 Hz, 2H, ArH), 7.42 – 7.31 (m, 3H, ArH), 7.31 – 7.24 (m, 2H, ArH), 7.12 – 7.04 (m, 2H, ArH), 7.00 (s, 2H, NH_2), 6.95 (d, *J* = 9.1 Hz, 1H, -CH=), 6.84 (d, *J* = 8.2 Hz, 2H, ArH), 5.84 (dd, *J* = 9.2, 1.9 Hz, 1H, CH), 3.81 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 174.12, 168.75, 159.02, 150.01, 136.49, 132.82, 131.84, 131.26, 130.41, 128.60, 128.11, 127.35, 126.29, 124.70, 114.23, 112.04, 55.40, 52.32. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_5S+H]^+$ 464,1275, found 464,1275.

(E)-3-((4-Benzylidene-1-(4-methoxyphenyl)-2,5-dioxopyrrolidin-3-yl)amino)benzenesulfonamide (**3b**). Yield 191 mg (88%). Light-yellow solid; m.p. 156.0-157.9 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.76 (d, *J* = 2.0 Hz, 1H, NH), 7.69 (d, *J* = 6.9 Hz, 2H, ArH), 7.43 – 7.26 (m, 6H, ArH), 7.23 (s, 2H, NH_2), 7.16 (s, 1H, ArH), 7.13 – 7.04 (m, 3H, ArH), 6.98 (d, *J* = 7.7 Hz, 1H, ArH), 6.81 (d, *J* = 9.2 Hz, 1H, -CH=), 5.75 (dd, *J* = 9.1, 2.0 Hz, 1H, CH), 3.82 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 174.29, 168.80, 159.02, 147.34, 144.83, 136.45, 132.91, 131.33, 130.36, 129.59, 128.62, 128.15, 126.54, 124.76, 115.47, 114.22, 114.09, 109.97, 55.40, 52.68. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_5S+H]^+$ 464,1275, found 464,1273.

(E)-4-((4-Benzylidene-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3c**). Yield 115 mg (77%). Light-yellow solid; m.p. 252.0-253.9 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.78 (d, *J* = 2.1 Hz, 1H, NH), 7.66 (d, *J* = 7.1 Hz, 2H, ArH), 7.60 – 7.51 (m, 4H, ArH), 7.49 – 7.43 (m, 1H, ArH), 7.42 – 7.30 (m, 5H, ArH), 7.00 (s, 2H, NH_2), 6.97 (d, *J* = 9.1 Hz, 1H, -CH=), 6.85 (d, *J* = 8.3 Hz, 2H, ArH), 5.87 (dd, *J* = 9.2, 2.0 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.94, 168.54, 149.99, 136.73, 132.79, 132.20, 131.90, 131.30, 130.47, 129.02, 128.62, 128.53, 127.37, 126.92, 126.23, 112.07, 52.42. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{23}H_{19}N_3O_4S+H]^+$ 434,1169, found 434,1169.

(E)-4-((4-Benzylidene-2,5-dioxo-1-(4-(trifluoromethyl)phenyl)pyrrolidin-3-yl)amino)benzenesulfonamide (**3d**). Yield 164 mg (78%). Light-yellow solid; m.p. 258.0-260.0 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.95 (d, *J* = 8.5 Hz, 2H, ArH), 7.83 (d, *J* = 2.0 Hz, 1H, NH), 7.67 (t, *J* = 7.9 Hz, 4H, ArH), 7.59 (d, *J* = 8.7 Hz, 2H, ArH), 7.45 – 7.33 (m, 3H, ArH), 7.02 (s, 2H, NH_2), 6.95 (d, *J* = 9.0 Hz, 1H, -CH=), 6.88 (d, *J* = 8.2 Hz, 2H, ArH), 5.88 (dd, *J* = 9.1, 1.8 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.60, 168.19, 149.88, 137.25, 135.79, 132.72, 132.07, 131.37, 130.62, 129.14 - 128.26 (m), 128.67, 127.67, 127.40, 126.29 - 126.06

(m), 125.96, 123.94 (q, *J* = 272.3 Hz), 112.19, 52.52. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.07. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{18}FN_3O_4S+H]^+$ 502,1043, found 502,1048.

(E)-4-((1-(4-Methoxyphenyl)-2,5-dioxo-4-(thiophen-2-ylmethylidene)pyrrolidin-3-yl)amino)benzenesulfonamide (**3e**). Yield 182 mg (82%). Beige solid; m.p. 227.0-228.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 2.3 Hz, 1H, NH), 7.85 (d, *J* = 2.0 Hz, 1H, thiophene), 7.64 – 7.57 (m, 3H, ArH + thiophene), 7.55 (t, *J* = 7.6 Hz, 2H, ArH), 7.49 – 7.43 (m, 1H, ArH), 7.40 – 7.32 (m, 3H, ArH+thiophene), 7.06 (d, *J* = 8.8 Hz, 1H, -CH=), 7.01 (s, 2H, NH_2), 6.91 (d, *J* = 8.4 Hz, 2H, ArH), 5.72 (dd, *J* = 9.0, 1.9 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.86, 168.77, 149.90, 135.43, 133.27, 132.24, 131.99, 130.59, 128.99, 128.46, 128.23, 127.61, 127.44, 126.92, 123.68, 112.19, 52.16. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{21}H_{17}N_3O_4S_2+H]^+$ 470,0844, found 470,0846.

(E)-4-((2,5-Dioxo-4-(thiophen-3-ylmethylidene)-1-(4-trifluoromethylphenyl)pyrrolidin-3-yl)amino)benzenesulfonamide (**3f**). Yield 100 mg (48%). Beige amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, *J* = 2.3 Hz, 1H, NH), 7.94 (d, *J* = 8.5 Hz, 2H, ArH), 7.89 (d, *J* = 1.9 Hz, 1H, thiophen), 7.68 – 7.58 (m, 5H, ArH + thiophen), 7.35 (d, *J* = 4.8 Hz, 1H, -CH=), 7.03 (d, *J* = 4.8 Hz, 3H, NH_2 + thiophen), 6.93 (d, *J* = 8.3 Hz, 2H, ArH), 5.73 (dd, *J* = 9.2, 1.8 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.51, 168.40, 149.79, 135.82, 135.37, 133.58, 132.15, 131.08, 128.59 (d, *J* = 32.2 Hz), 128.21, 127.70, 127.63, 127.46, 126.13 (q, *J* = 3.5 Hz), 123.94 (q, *J* = 272.2Hz), 123.38, 112.29, 52.25. ^{19}F NMR (376 MHz, DMSO-d_6) δ -56.31. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{22}H_{16}F_3N_3O_4S_2+H]^+$ 508,0607, found 508,0607.

(E)-3-((4-(2-Methoxybenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3g**). Yield 96 mg (45%). Beige amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, *J* = 2.2 Hz, 1H, NH), 7.56 (dd, *J* = 16.5, 8.2 Hz, 3H, ArH), 7.46 (dd, *J* = 8.4, 6.4 Hz, 1H, ArH), 7.43 – 7.34 (m, 3H, ArH), 7.28 (t, *J* = 7.9 Hz, 1H, ArH), 7.22 (s, 2H, NH_2), 7.11 (s, 1H, ArH), 7.07 (d, *J* = 8.0 Hz, 2H, ArH), 6.90 (d, *J* = 7.6 Hz, 1H, ArH), 6.83 (t, *J* = 7.5 Hz, 1H, ArH), 6.68 (d, *J* = 9.1 Hz, 1H, -CH=), 5.78 (dd, *J* = 10.0, 3.0 Hz, 1H, CH), 3.86 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.28, 168.59, 158.25, 147.43, 144.75, 132.30, 132.24, 130.62, 130.57, 129.48, 128.46, 126.98, 125.97, 121.21, 120.18, 115.37, 113.93, 111.40, 109.86, 79.18, 55.77, 53.06. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_5S+H]^+$ 464,1275, found 464,1272.

(E)-4-((4-(2-Methoxybenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3h**). Yield 164 mg (78%). Light-yellow solid; m.p. 219.9-221.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, *J* = 2.3 Hz, 1H, NH), 7.54 (q, *J* = 7.4 Hz, 5H, ArH), 7.49 – 7.44 (m, 1H,

ArH), 7.41 – 7.34 (m, 3H, ArH), 7.07 (d, *J* = 8.1 Hz, 1H, -CH=), 6.98 (s, 2H, NH_2), 6.87 – 6.73 (m, 4H, ArH), 5.88 (dd, *J* = 9.1, 2.3 Hz, 1H, CH), 3.86 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 174.11, 168.53, 158.26, 153.17, 150.07, 132.24, 131.68, 130.68, 130.42, 129.00, 128.48, 127.26, 126.94, 125.70, 121.11, 120.13, 111.93, 111.41, 55.78, 52.73. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_5S+H]^+$ 464,1275, found 464,1271.

(E)-4-((4-(4-Methoxybenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3i**). Yield 113 mg (78%). Light-yellow solid; m.p. 244.1-246.0 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.74 (d, *J* = 1.9 Hz, 1H, NH), 7.64 (d, *J* = 8.8 Hz, 2H, ArH), 7.61 – 7.55 (m, 2H, ArH), 7.53 (d, *J* = 7.8 Hz, 2H, ArH), 7.49 – 7.43 (m, 1H, ArH), 7.40 – 7.33 (m, 2H, ArH), 7.00 (s, 2H, NH_2), 6.98 (d, 1H, -CH=), 6.90 (t, *J* = 9.2 Hz, 4H, ArH), 5.82 – 5.77 (dd, 1H, CH), 3.78 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.98, 168.77, 161.11, 150.02, 136.72, 133.51, 132.29, 131.87, 128.97, 128.41, 127.35, 126.92, 125.45, 123.01, 114.22, 112.20, 55.44, 52.34. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_5S+H]^+$ 464,1275, found 464,1275.

(E)-4-((4-(4-Methylbenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3j**). Yield 108 mg (73%). Light-yellow solid; m.p. 252.5-254.5 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.75 (d, *J* = 2.0 Hz, 1H, NH), 7.56 (dt, *J* = 10.3, 8.3 Hz, 6H, ArH), 7.49 – 7.43 (m, 1H, ArH), 7.40 – 7.35 (m, 2H, ArH), 7.17 (d, *J* = 8.0 Hz, 2H, ArH), 7.01 (s, 2H, NH_2), 6.98 (d, 1H, -CH=), 6.87 (d, *J* = 8.3 Hz, 2H, ArH), 5.84 (dd, *J* = 9.1, 1.9 Hz, 1H, CH), 2.31 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.97, 168.65, 150.01, 140.78, 136.78, 132.24, 131.85, 131.43, 130.07, 129.30, 129.00, 128.47, 127.37, 126.92, 124.99, 112.06, 52.37, 20.99. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_4S+H]^+$ 448,1326, found 448,1325.

(E)-4-((4-(3-Methylbenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3k**). Yield 177 mg (80%). Light-yellow solid; m.p. 237.0-239.0 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.73 (d, *J* = 2.2 Hz, 1H, NH), 7.62 – 7.51 (m, 4H, ArH), 7.50 – 7.33 (m, 5H, ArH), 7.23 (dt, *J* = 15.8, 7.6 Hz, 2H, ArH), 6.99 (s, 2H, NH_2), 6.95 (d, *J* = 9.1 Hz, 1H, -CH=), 6.86 (d, *J* = 8.3 Hz, 2H, ArH), 5.85 (dd, *J* = 9.1, 2.3 Hz, 1H, CH), 2.08 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 174.06, 168.58, 150.16, 137.82, 136.81, 132.66, 132.22, 131.91, 131.62, 131.13, 129.01, 128.89, 128.50, 127.37, 126.91, 126.10, 112.40, 111.84, 52.48, 20.58. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_4S+H]^+$ 448,1326, found 448,1325.

(E)-4-((4-(4-Chlorobenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3l**). Yield 103 mg (71%). Light-yellow solid; m.p. 225.9-227.4 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.80 (d, *J* = 2.3 Hz, 1H, NH), 7.72 – 7.66 (m, 2H, ArH), 7.61 – 7.52 (m, 4H, ArH),

7.51 – 7.44 (m, 1H, ArH), 7.43 – 7.36 (m, 4H, ArH), 7.01 (s, 2H, NH_2), 6.96 (d, *J* = 9.2 Hz, 1H, -CH=), 6.83 (d, *J* = 8.4 Hz, 2H, ArH), 5.89 (dd, *J* = 9.2, 2.4 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.81, 168.33, 149.84, 135.27, 135.10, 132.86, 132.14, 132.05, 131.73, 129.04, 128.60, 128.57, 127.38, 127.04, 126.91, 112.16, 52.44. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{23}H_{18}ClN_3O_4S+H]^+$ 468.0779, found 468.0779.

(E)-4-((4-(4-Chlorobenzylidene)-2,5-dioxo-1-(p-tolyl)pyrrolidin-3-yl)amino)benzenesulfonamide (**3m**). Yield 140 mg (59%). Light-yellow solid; m.p. 226.1-228.0 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.77 (d, *J* = 2.0 Hz, 1H, NH), 7.67 (d, *J* = 8.6 Hz, 2H, ArH), 7.57 (d, *J* = 8.7 Hz, 2H, ArH), 7.40 (d, *J* = 8.5 Hz, 2H, ArH), 7.34 (d, *J* = 8.3 Hz, 2H, ArH), 7.24 (d, *J* = 8.3 Hz, 2H, ArH), 6.99 (s, 2H, NH_2), 6.94 (d, *J* = 9.0 Hz, 1H, -CH=), 6.82 (d, *J* = 8.2 Hz, 2H, ArH), 5.86 (dd, *J* = 9.2, 1.9 Hz, 1H, CH), 2.37 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO- *d*$_6$) δ 173.89, 168.41, 149.85, 138.13, 135.14, 135.07, 132.84, 132.01, 131.75, 129.53, 129.49, 128.59, 127.37, 127.08, 126.67, 112.15, 52.38, 20.78. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{20}ClN_3O_4S+H]^+$ 482,0936, found 482,0937.

(E)-3-((4-(4-Chlorobenzylidene)-2,5-dioxo-1-(p-tolyl)pyrrolidin-3-yl)amino)benzenesulfonamide (**3n**). Yield 156 mg (66%). Light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.76 (d, *J* = 1.7 Hz, 1H, NH), 7.70 (d, *J* = 8.5 Hz, 2H, ArH), 7.41 (d, *J* = 8.5 Hz, 2H, ArH), 7.36 – 7.27 (m, 3H, ArH), 7.24 (m, 4H, ArH + NH_2), 7.12 (dd, *J* = 16.6, 8.9 Hz, 2H, ArH), 6.95 (d, *J* = 6.7 Hz, 1H, -CH=), 6.79 (d, *J* = 9.1 Hz, 1H, ArH), 5.81 – 5.74 (m, 1H, CH), 2.37 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 174.51, 168.91, 147.69, 145.34, 138.56, 135.56, 135.49, 133.34, 132.31, 130.06, 129.92, 129.06, 127.85, 127.16, 116.16, 114.73, 110.38, 53.20, 21.23. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{20}ClN_3O_4S+H]^+$ 482,0936, found 482,0935.

(E)-4-((4-(4-Fluorobenzylidene)-2,5-dioxo-1-phenylpyrrolidin-3-yl)amino)benzenesulfonamide (**3o**). Yield 186 mg (85%). Light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.81 (d, *J* = 2.1 Hz, 1H, NH), 7.74 (dd, *J* = 8.8, 5.6 Hz, 2H, ArH), 7.56 (dd, *J* = 14.1, 8.3 Hz, 4H, ArH), 7.50 – 7.43 (m, 1H, ArH), 7.40 – 7.35 (m, 2H, ArH), 7.20 (t, *J* = 8.8 Hz, 2H, ArH), 7.00 (s, 2H, NH_2), 6.96 (d, *J* = 9.0 Hz, 1H, -CH=), 6.85 (d, *J* = 8.2 Hz, 2H, ArH), 5.87 (dd, *J* = 9.2, 1.9 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.87, 168.47, 163.03 (d, *J* = 250.4 Hz), 149.88, 135.56, 133.77, 133.70, 132.18, 132.03, 129.52, 129.03, 128.54, 128.22, 127.37, 126.92, 125.89, 115.71 (d, *J* = 21.7 Hz), 112.20. ^{19}F NMR (376 MHz, DMSO-*d*$_6$) δ -109.04. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{23}H_{18}FN_3O_4S+H]^+$ 452,1075, found 452,1072.

*General procedure for the preparation of **2a-e***

Products **3** were dissolved in DMF, and the mixture was stirred for 12 hours at 100 °C. After that it was poured into 50 ml of distilled water and extracted with EtOAc (3×30 ml). The solvent was removed *in vacuo*. The compounds were purified by column chromatography on silica gel using chloroform/methanol as an eluent.

(E)-4-((4-(2-Methoxybenzyl)-2,5-dioxo-1-phenyl-2,5-dihydro-1H-pyrrol-3-yl)amino)-benzenesulfonamide (**2a**). Yield 36 mg (42%). Green amorphous solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.51 (s, 1H, NH), 7.62 (d, *J* = 8.5 Hz, 2H, ArH), 7.52 – 7.46 (m, 2H, ArH), 7.43 – 7.35 (m, 3H, ArH), 7.24 (s, 2H, NH_2), 7.10 (dd, *J* = 12.8, 8.9 Hz, 3H, ArH), 6.92 – 6.86 (m, 1H, ArH), 6.81 – 6.71 (m, 2H, ArH), 3.62 (s, 3H, OMe), 3.51 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 171.81, 167.20, 157.07, 142.78, 139.61, 138.80, 132.55, 129.28, 129.07, 127.81, 127.76, 126.93, 126.62, 125.81, 121.54, 120.45, 110.64, 106.11, 55.56, 23.64. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_5S+H]^+$ 464,1275, found 464,1275.

(E)-4-((4-(3-Methylbenzyl)-2,5-dioxo-1-phenyl-2,5-dihydro-1H-pyrrol-3-yl)amino)-benzenesulfonamide (**2b**). Yield 41 mg (53%). Yellow solid; m.p. 173.0-174.6 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.60 (s, 1H, NH), 7.73 (d, *J* = 8.6 Hz, 2H, ArH), 7.52 – 7.47 (m, 2H, ArH), 7.39 (dd, *J* = 15.7, 7.3 Hz, 3H, ArH), 7.29 (s, 2H, NH_2), 7.17 (d, *J* = 8.6 Hz, 2H, ArH), 7.01 (t, *J* = 7.5 Hz, 1H, ArH), 6.88 (d, *J* = 7.4 Hz, 1H, ArH), 6.62 (d, *J* = 7.5 Hz, 1H, ArH), 6.49 (s, 1H, ArH), 3.53 (s, 2H, CH_2), 2.14 (s, 3H, Me). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 171.96, 167.22, 142.52, 139.51, 139.34, 138.60, 137.77, 132.52, 129.28, 129.11, 128.61, 127.80, 127.01, 126.93, 126.77, 125.41, 122.48, 106.16, 28.82, 21.40. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{21}N_3O_4S+H]^+$ 448,1326, found 448,1330.

(E)-4-((4-(4-Chlorobenzyl)-2,5-dioxo-1-phenyl-2,5-dihydro-1H-pyrrol-3-yl)amino)-benzenesulfonamide (**2c**) Yield 44 mg (55%). Yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.65 (s, 1H, NH), 7.70 (d, *J* = 8.6 Hz, 2H, ArH), 7.53 – 7.46 (m, 2H, ArH), 7.44 – 7.36 (m, 3H, ArH), 7.31 (s, 2H, NH_2), 7.14 (dd, *J* = 12.5, 8.5 Hz, 4H, ArH), 6.80 (d, *J* = 8.4 Hz, 2H, ArH), 3.55 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 171.93, 167.09, 142.37, 139.97, 139.49, 137.60, 132.52, 130.92, 130.20, 129.27, 128.54, 127.81, 126.95, 126.74, 122.58, 104.99, 28.29. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{23}H_{18}ClN_3O_4S+H]^+$ 468,0779, found 468,0772.

(E)-4-((4-(4-Fluorobenzyl)-2,5-dioxo-1-phenyl-2,5-dihydro-1H-pyrrol-3-yl)amino)-benzenesulfonamide (**2d**). Yield 41 mg (53%). Yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.63 (s, 1H, NH), 7.69 (d, *J* = 8.6 Hz, 2H, ArH), 7.53 – 7.46 (m, 2H, ArH), 7.39 (dd, *J* = 16.3, 7.6 Hz, 3H, ArH), 7.30 (s, 2H, NH_2), 7.12 (d, *J* = 8.6 Hz, 2H, ArH), 6.92 (t, *J* = 8.9 Hz, 2H, ArH), 6.80 (dd, *J* = 8.3, 5.8 Hz, 2H, ArH), 3.55 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 171.51, 166.68, 161.48, 159.55, 141.95, 139.38, 138.94, 134.09 (d, *J* = 2.9 Hz), 132.08, 129.64 (d, *J* = 8.0 Hz), 128.81, 126.50, 126.26, 122.03, 114.84 (d, *J* = 21.1 Hz), 104.92,

31.32. ^{19}F NMR (376 MHz, DMSO) δ -117.26. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{23}H_{18}FN_3O_4S+H]^+$ 452,1075, found 452,1077.

(E)-4-((4-Benzyl-2,5-dioxo-1-(4-(trifluoromethyl)phenyl)-2,5-dihydro-1H-pyrrol-3-yl)amino)-benzenesulfonamide (**2e**). Yield 25 mg (36%). Yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.70 (s, 1H, NH), 7.88 (d, *J* = 8.4 Hz, 2H, ArH), 7.70 (dd, *J* = 8.5, 4.0 Hz, 4H, ArH), 7.29 (s, 2H, NH_2), 7.20 – 7.05 (m, 5H, ArH), 6.84 (d, *J* = 6.9 Hz, 2H, ArH), 3.59 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 170.92, 166.25, 141.94, 139.61, 138.98, 138.00, 135.78, 128.25, 127.95, 126.37, 126.31, 125.91 (m, *J* = 3.1 Hz), 124.11 (q, *J* = 272.0 Hz), 122.02, 112.42, 105.86, 28.40. ^{19}F NMR (376 MHz, DMSO) δ -60.84. HRMS (ESI) *m/z* $[M+H]^+$ calculated for $[C_{24}H_{18}F_3N_3O_4S+H]^+$ 502,1043, found 502,1049.

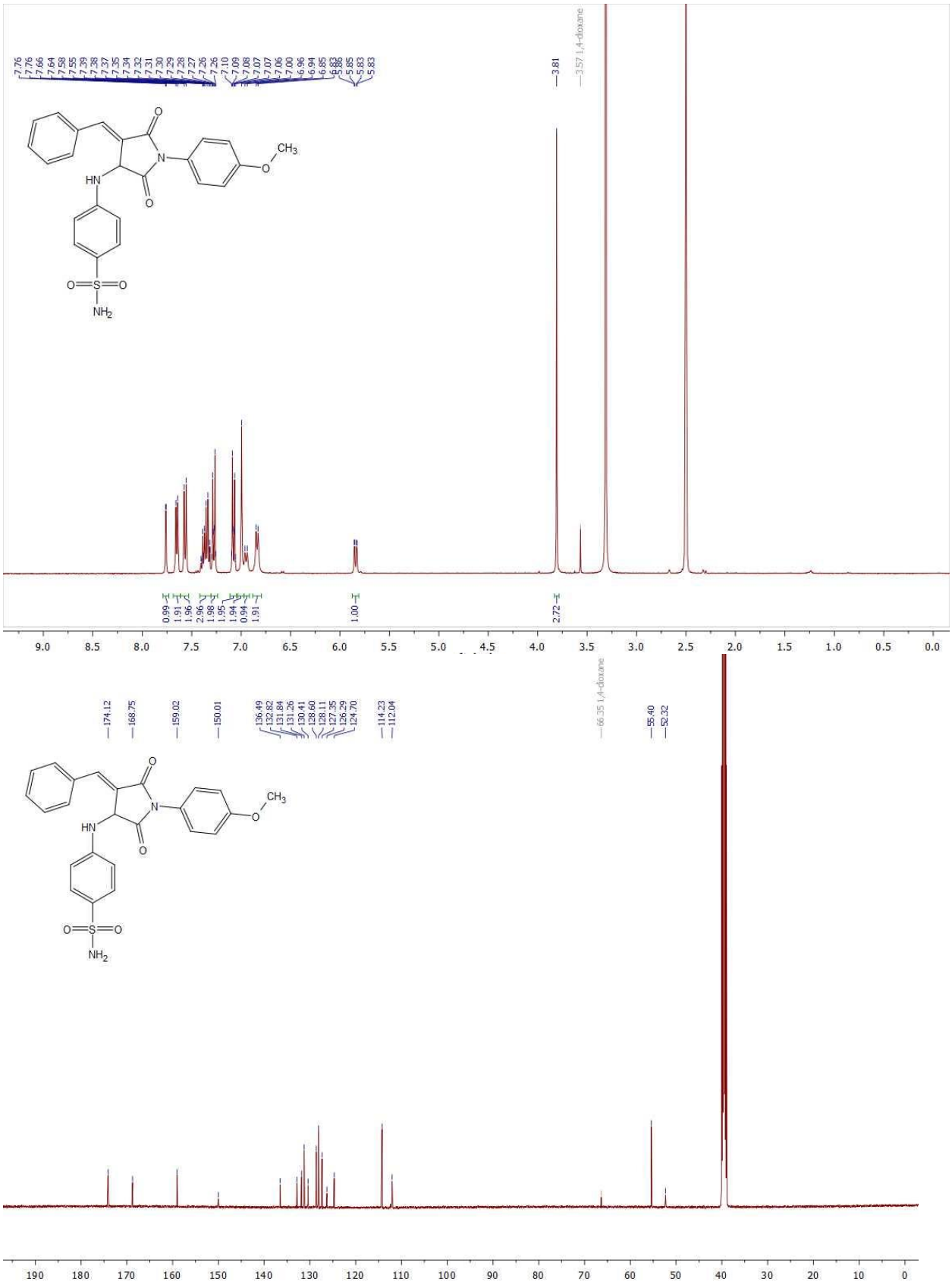
Cell culture

HCT 116 colon cancer cells were purchased from the ATCC and maintained in McCoy's 5A (Gibco, UK) supplemented with 10% fetal bovine serum (FBS, Gibco, UK), penicillin (100 UI mL^{-1}), streptomycin (100 mg mL^{-1}) and GlutaMax (2.00 mM, Gibco, UK) under a humidified atmosphere of 95% air/5% CO_2 at 37 °C. Subconfluent monolayers, in the log growth phase, were harvested by a brief treatment with TrypLE Express solution (Gibco, UK) in phosphate buffered saline (PBS, Capricorn Scientific, Germany) and washed three times in serum-free PBS. The number of viable cells was determined by trypan blue exclusion.

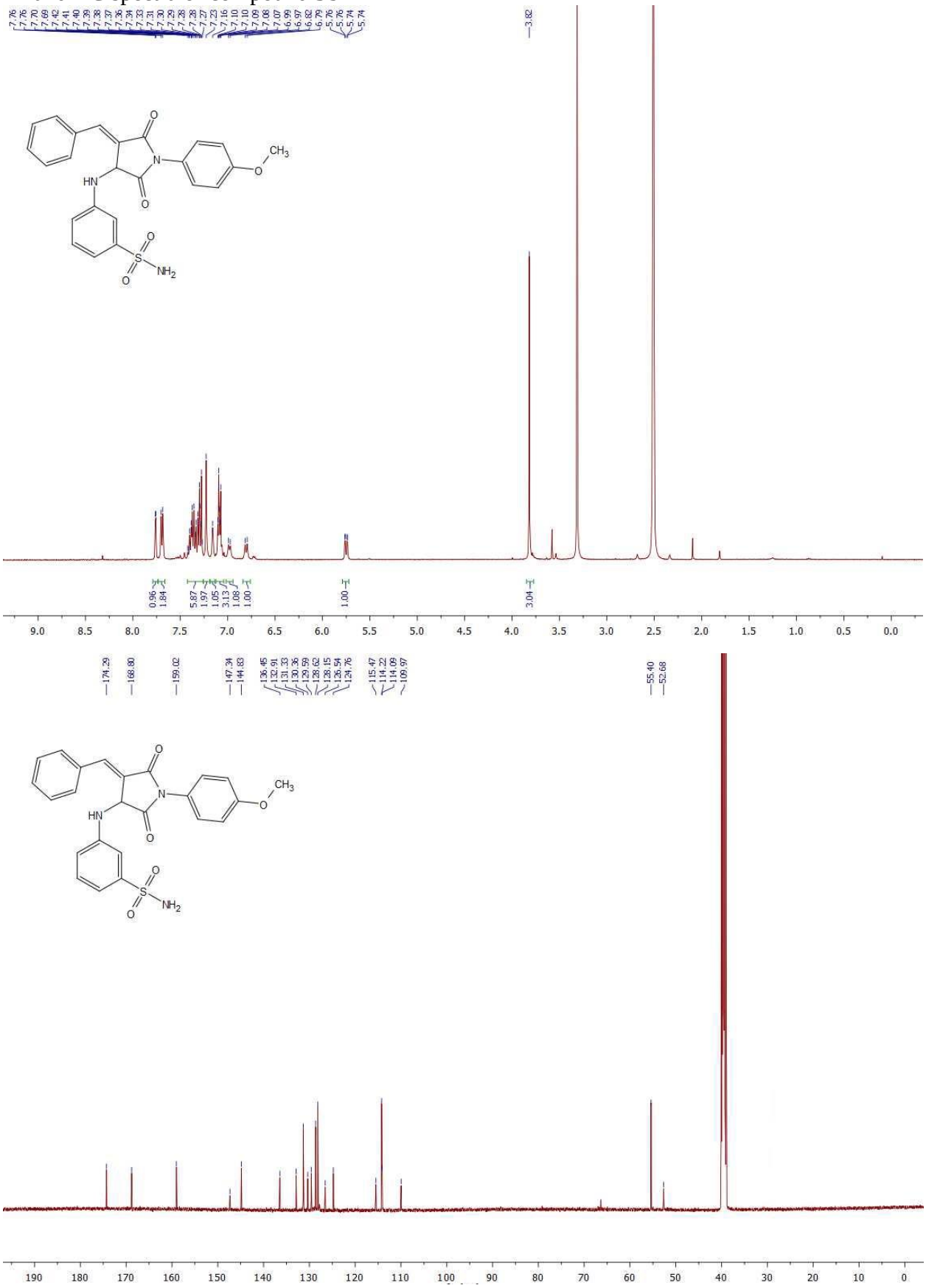
MTT assay

The effects of the synthesized compounds **3a-o** and **2a-e** on cell viability were determined using the MTT colorimetric test. HCT 116 cells were diluted with the growth medium containing 100 μM $CoCl_2$ for chemically induced hypoxia to 3.5×10^4 cells per mL and the aliquots (7×10^3 cells per 200 μL) were placed in individual wells in 96-multiplates (Eppendorf, Germany) and incubated for 24 h. The next day the cells were then treated with synthesized compounds separately at 100 μM concentration and incubated for 72 h at 37 °C in 5% CO_2 atmosphere. Each compound was tested in triplicate. After incubation, the cells were then treated with 40 μL MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg ml^{-1} in PBS) and incubated 4 h. After an additional 4 h incubation, the medium with MTT was removed and DMSO (150 μl) was added to dissolve the crystals formazan. The plates were shaken for 10 min. The optical density of each well was determined at 560 nm using a microplate reader GloMax Multi+ (Promega, USA). Each of the tested compounds was evaluated for the antiproliferative action in three separate experiments.

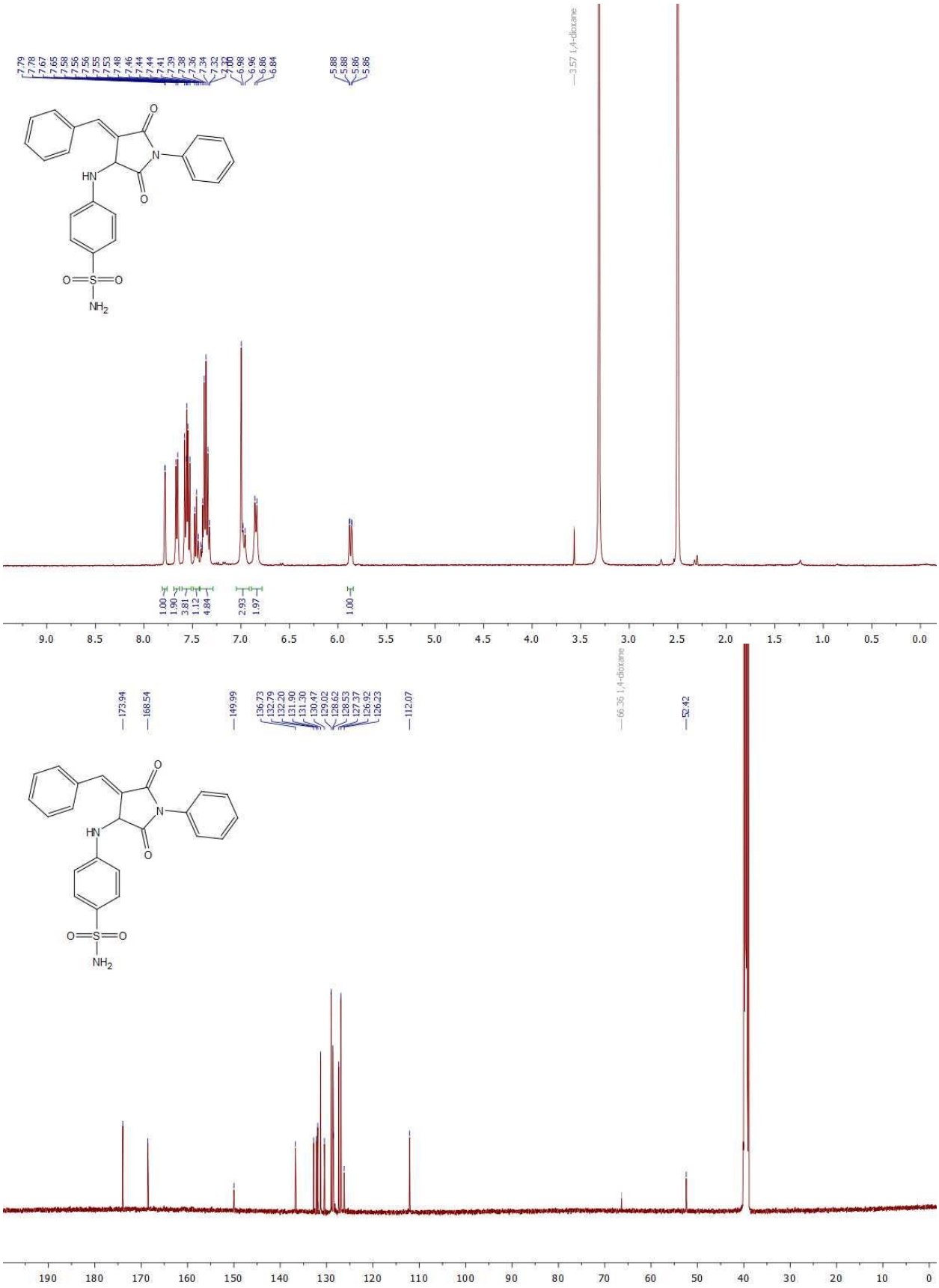
^{1}H and ^{13}C spectra of compound **3a**



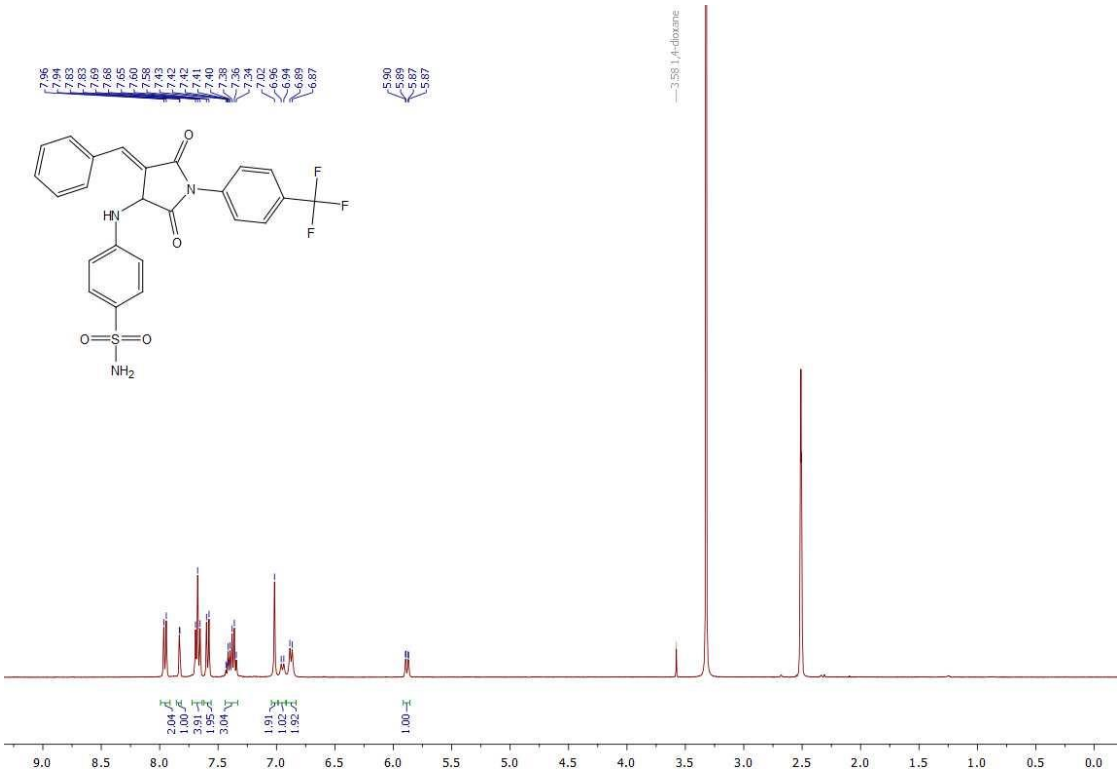
^{1}H and ^{13}C spectra of compound **3b**

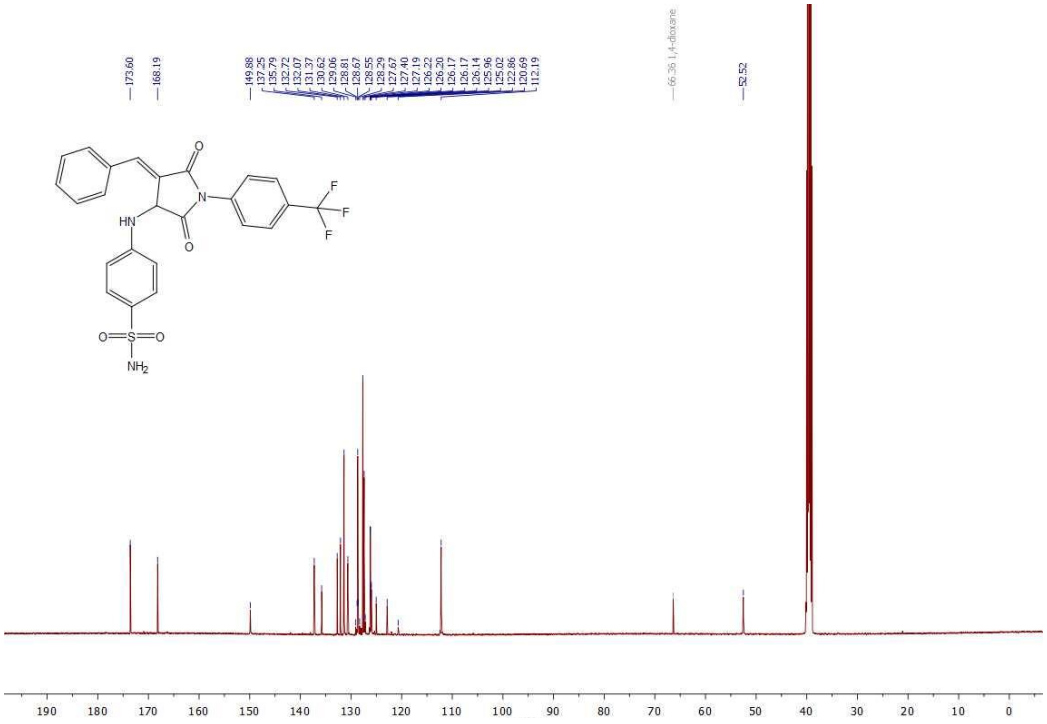


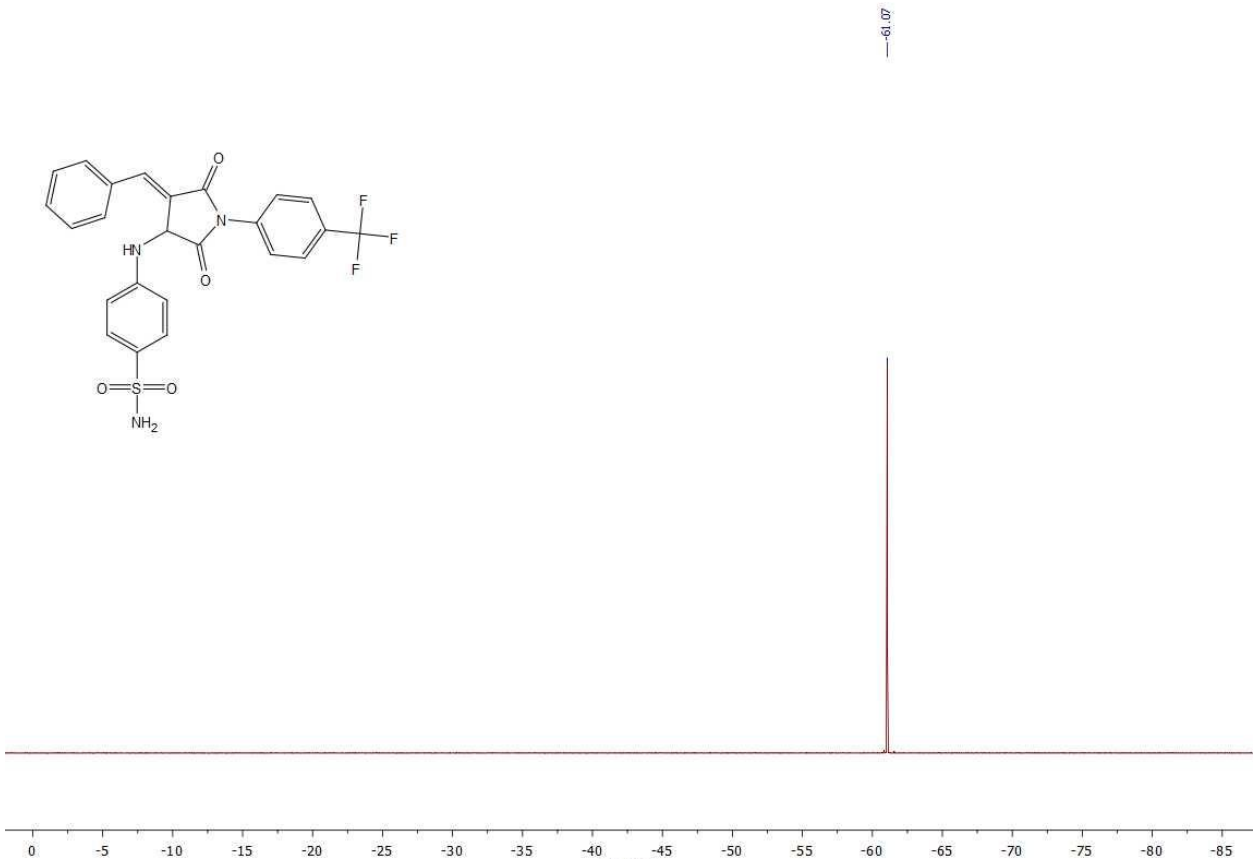
^{1}H and ^{13}C spectra of compound **3c**



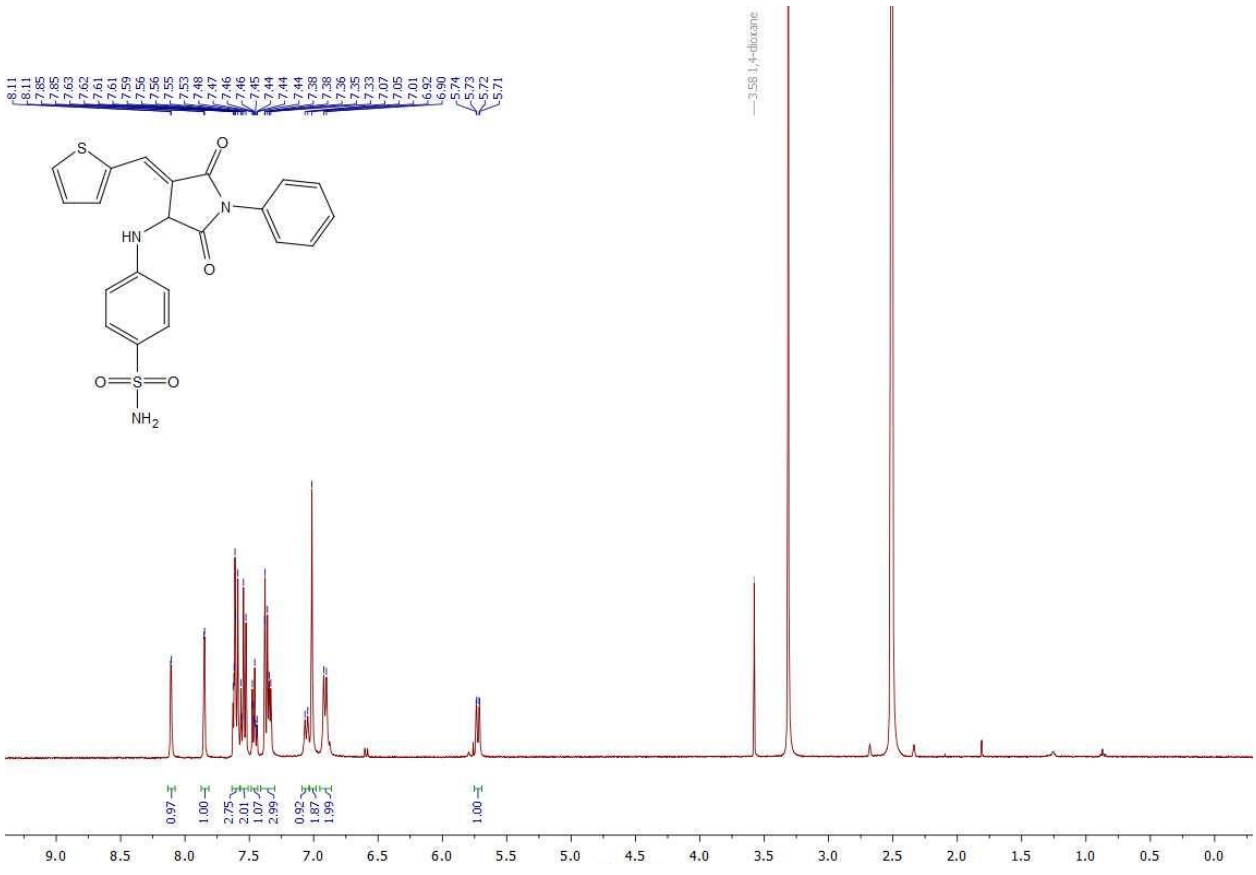
^{1}H, ^{13}C and ^{19}F spectra of compound **3d**

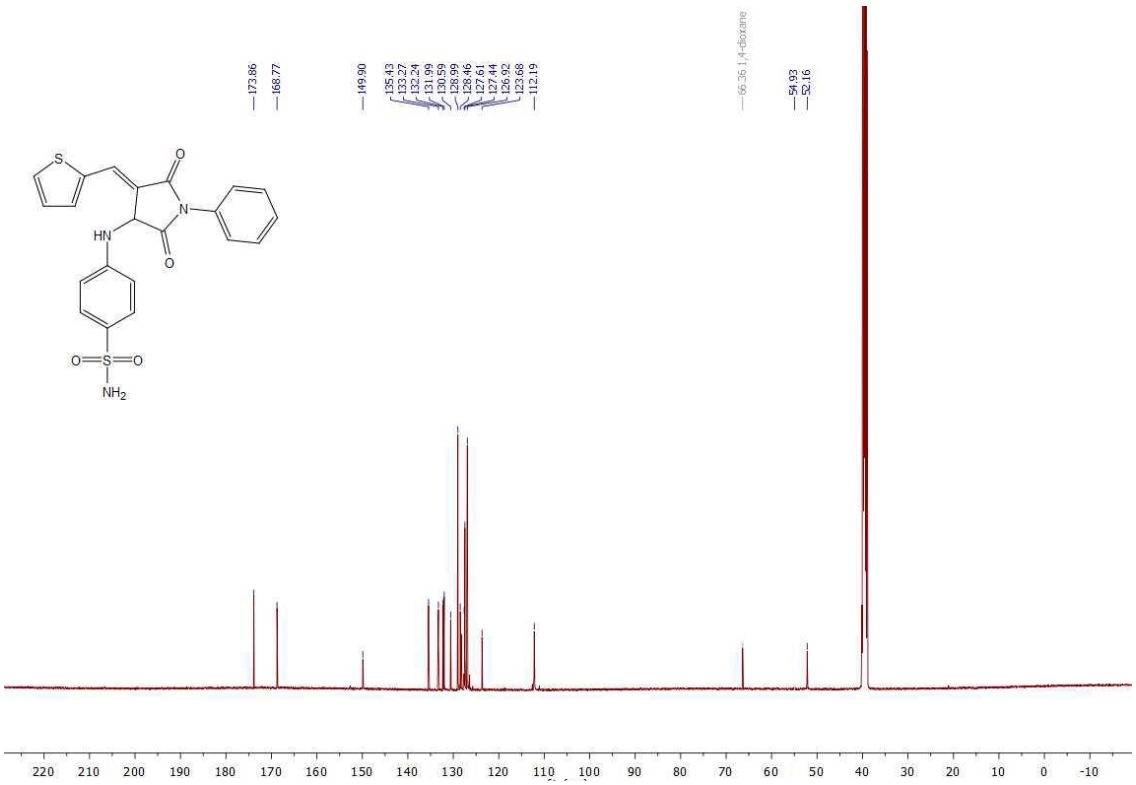




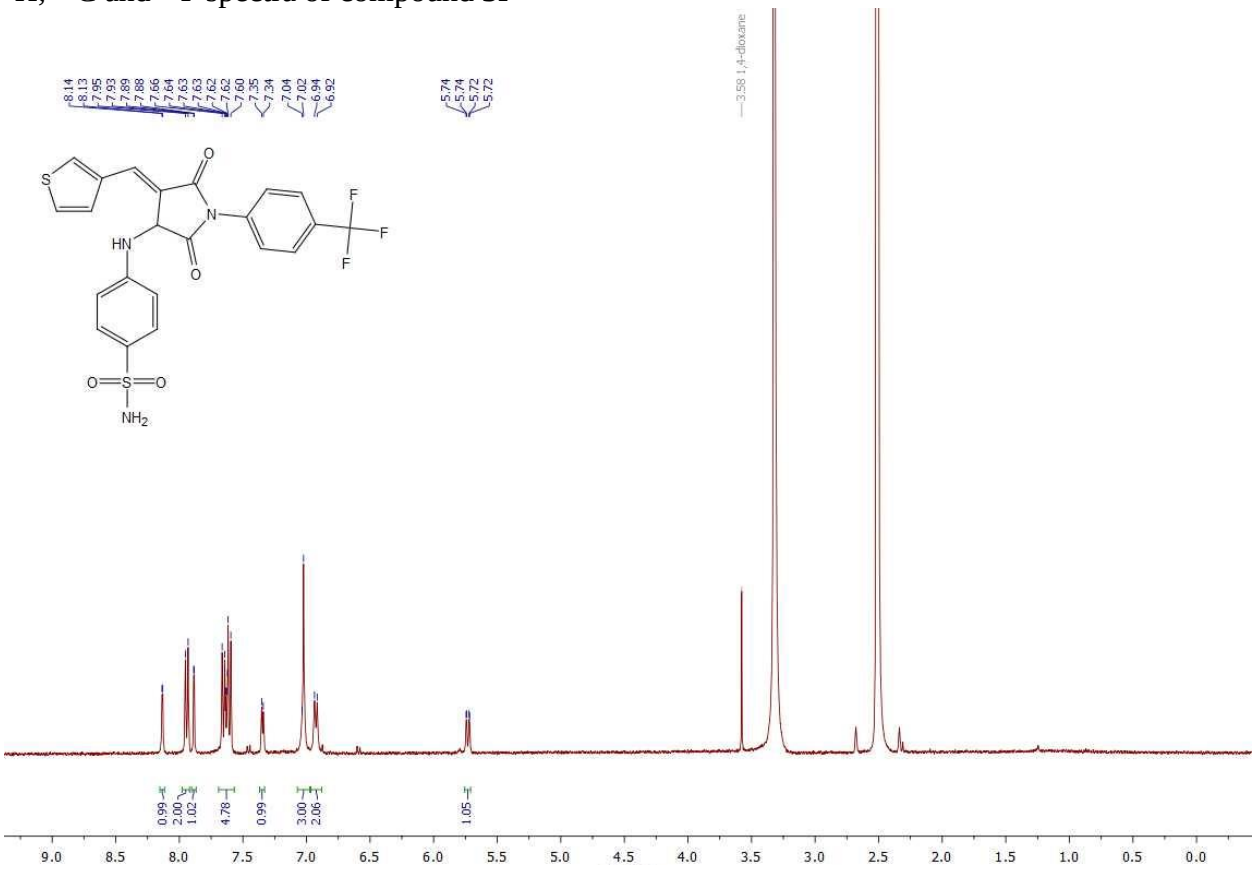


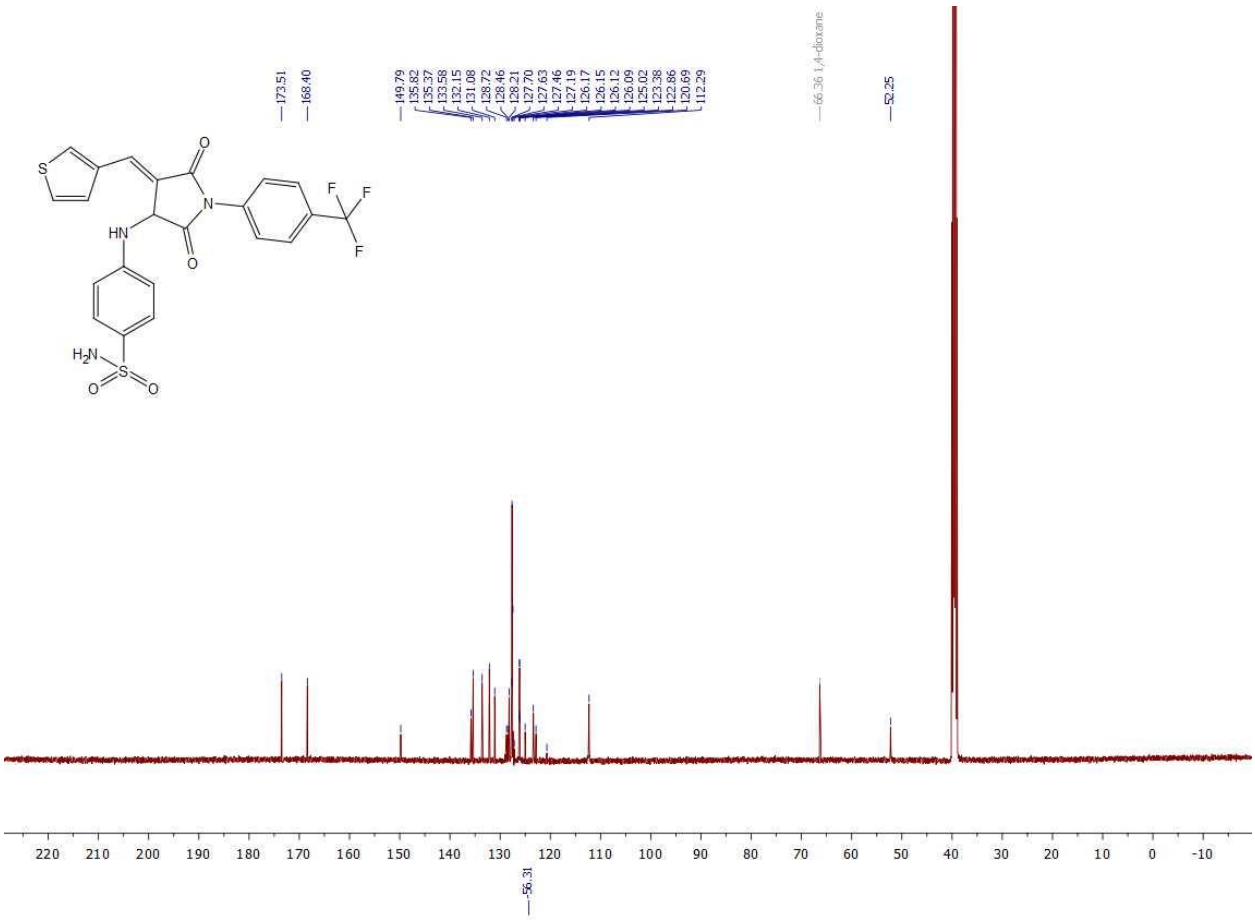
^{1}H and ^{13}C spectra of compound **3e**





^{1}H, ^{13}C and ^{19}F spectra of compound **3f**

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127.46
127.19
126.17
126.15
126.12
126.09
125.02
123.38
122.86
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112.29
66.36 1,4-dioxane
52.25
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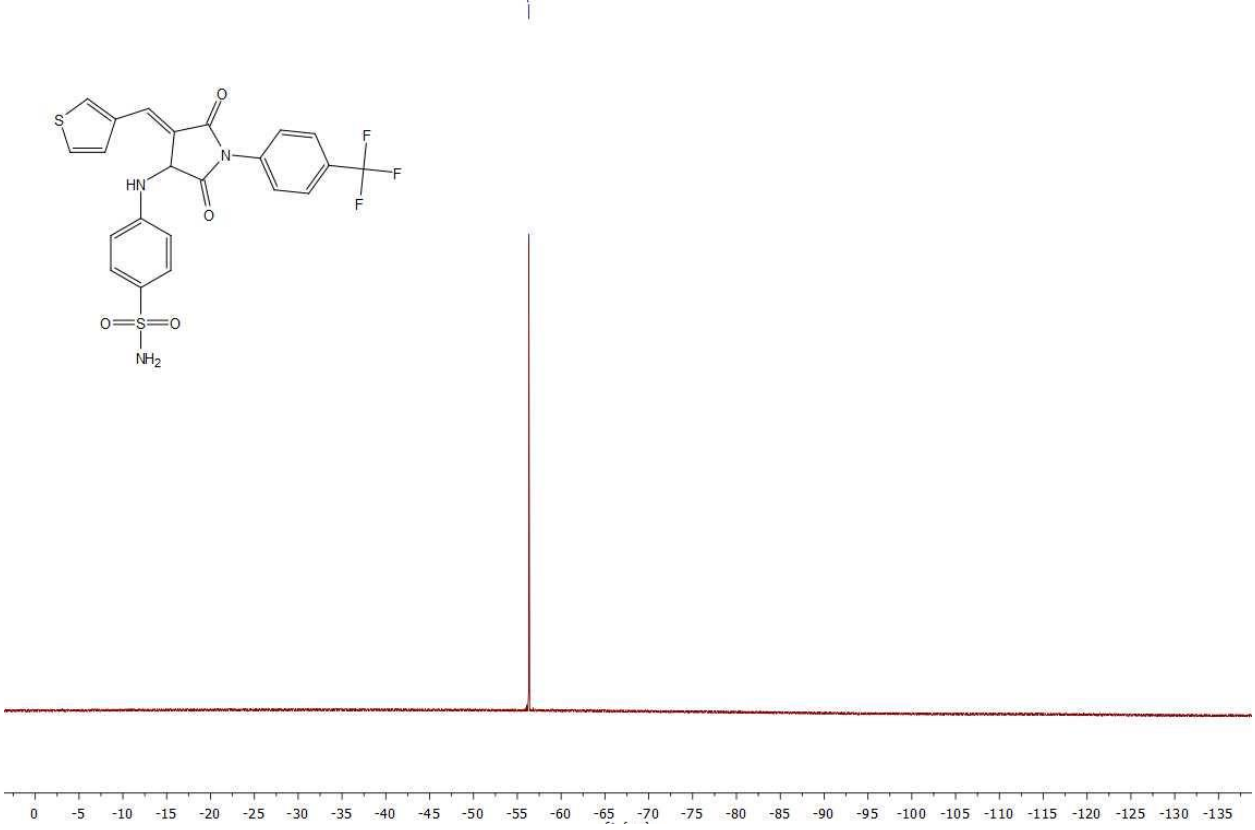
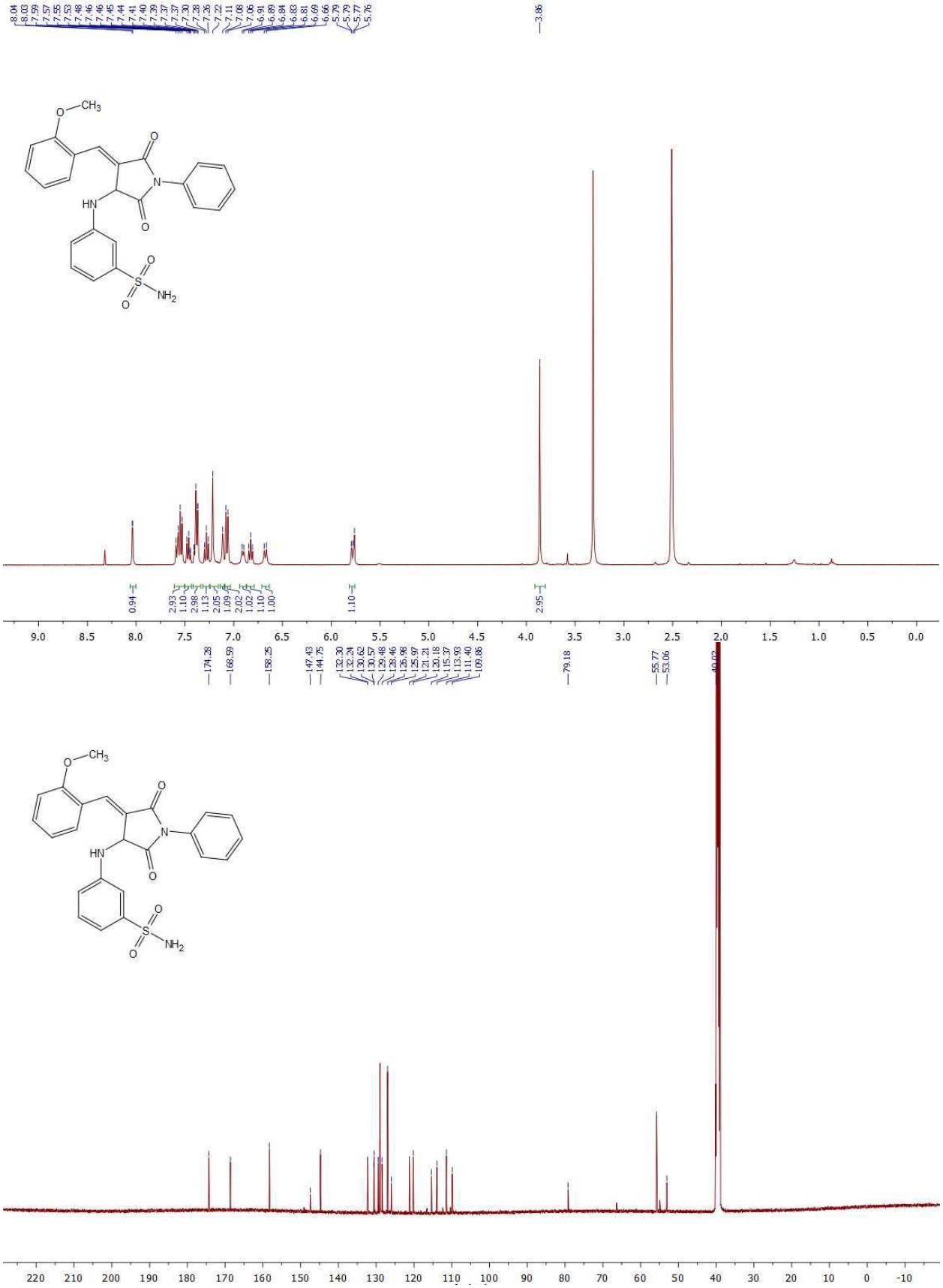
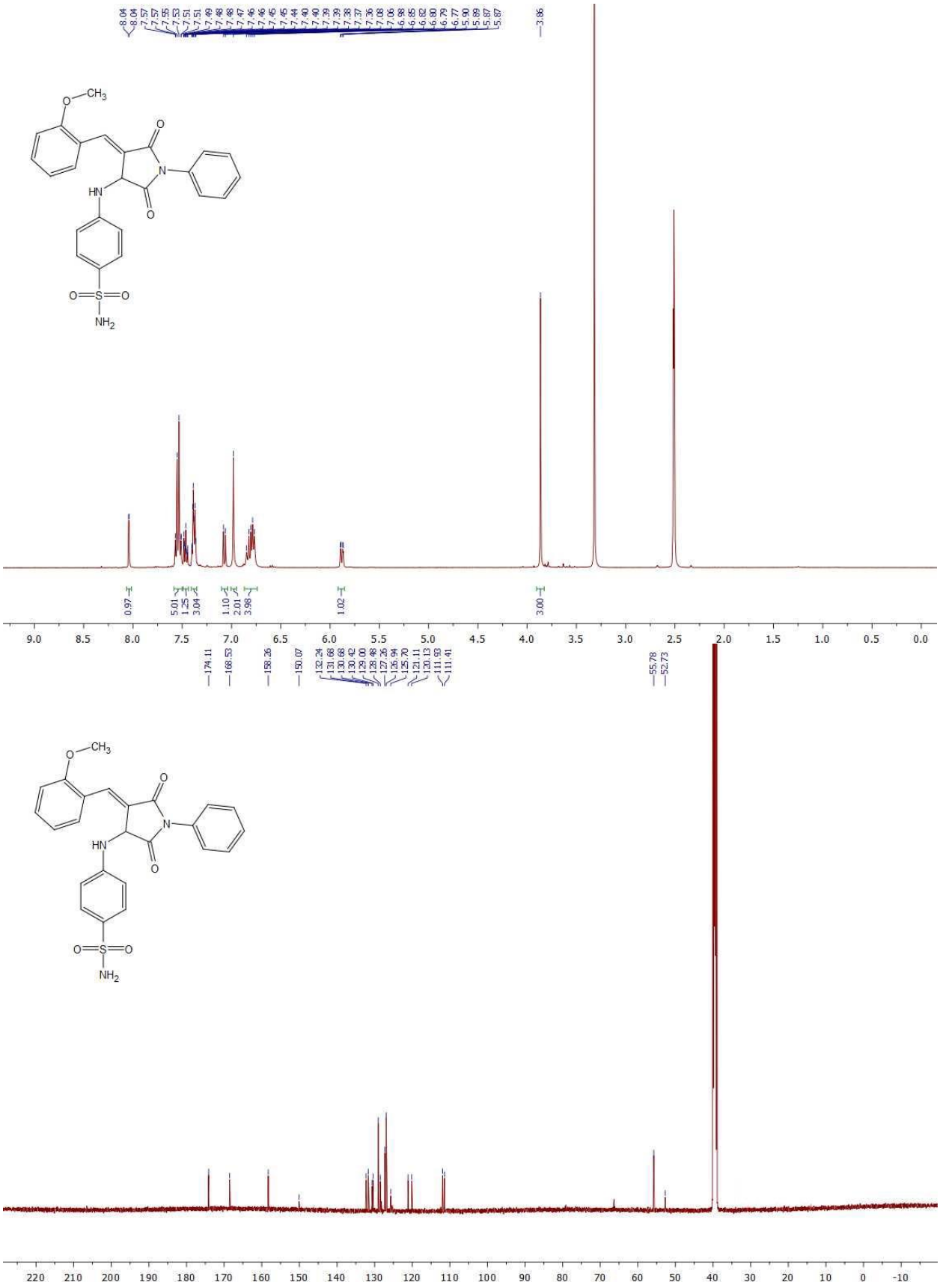

56.31
0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135

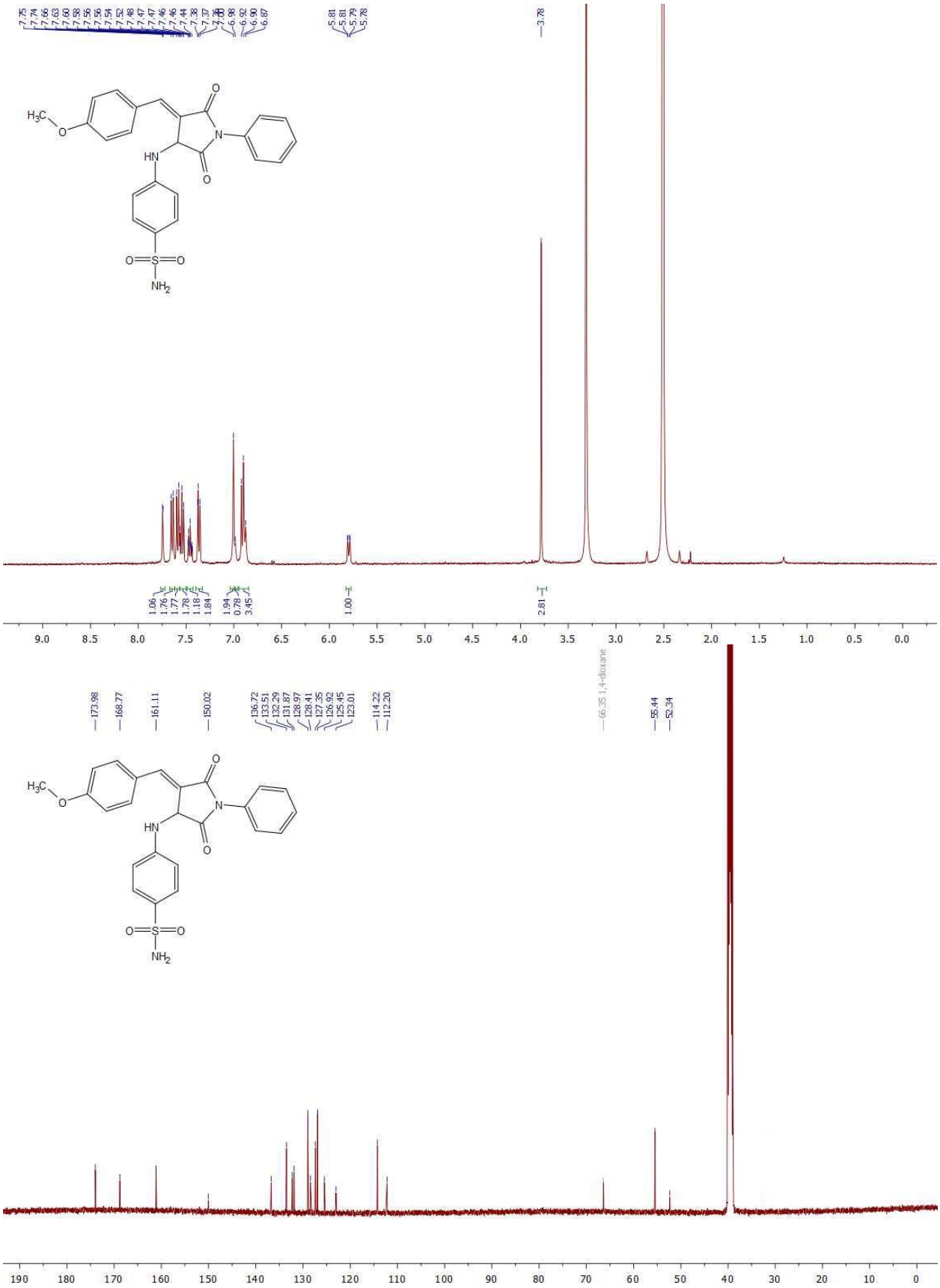
^{1}H and ^{13}C spectra of compound **3g**



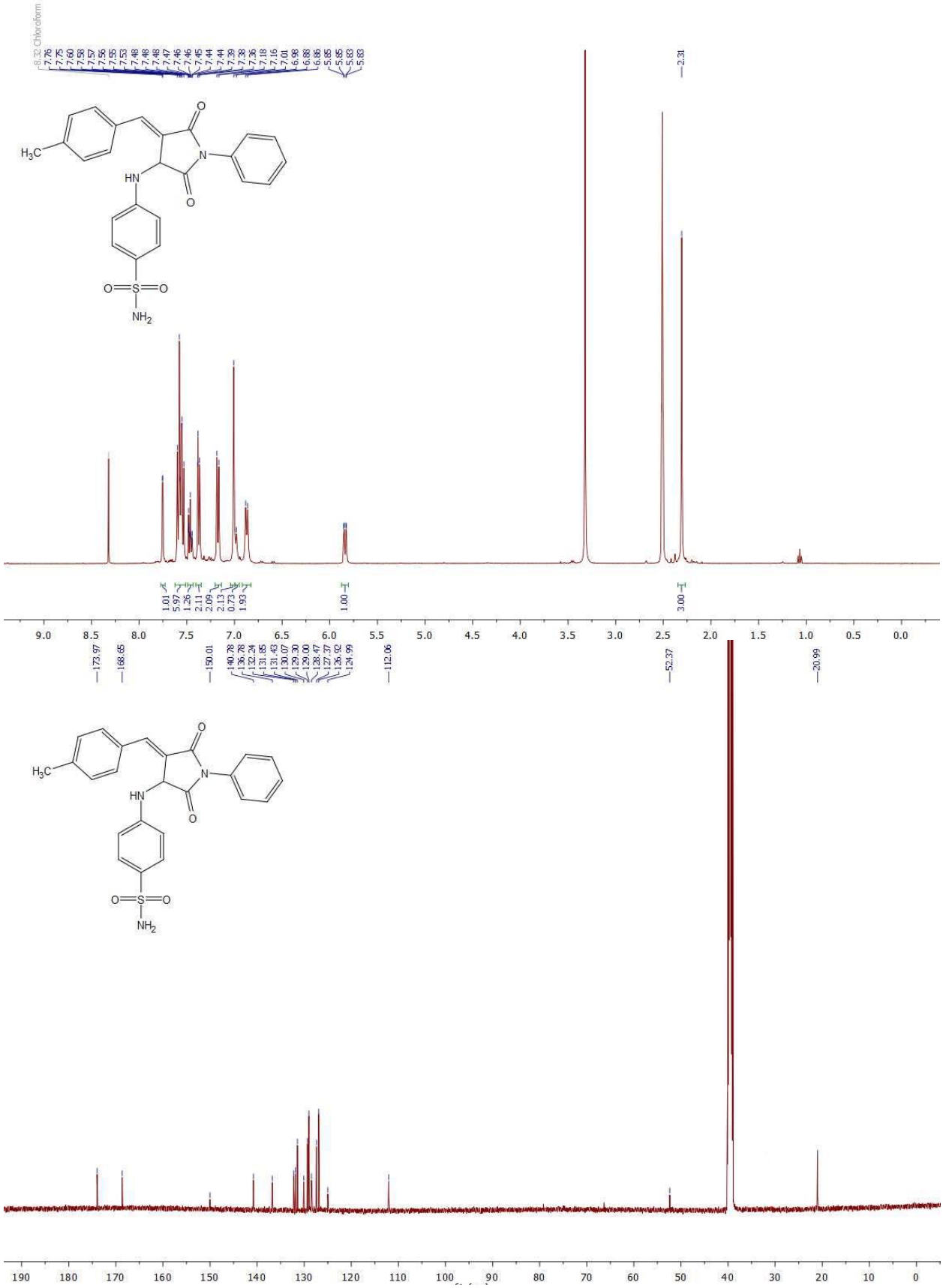
^{1}H and ^{13}C spectra of compound **3h**



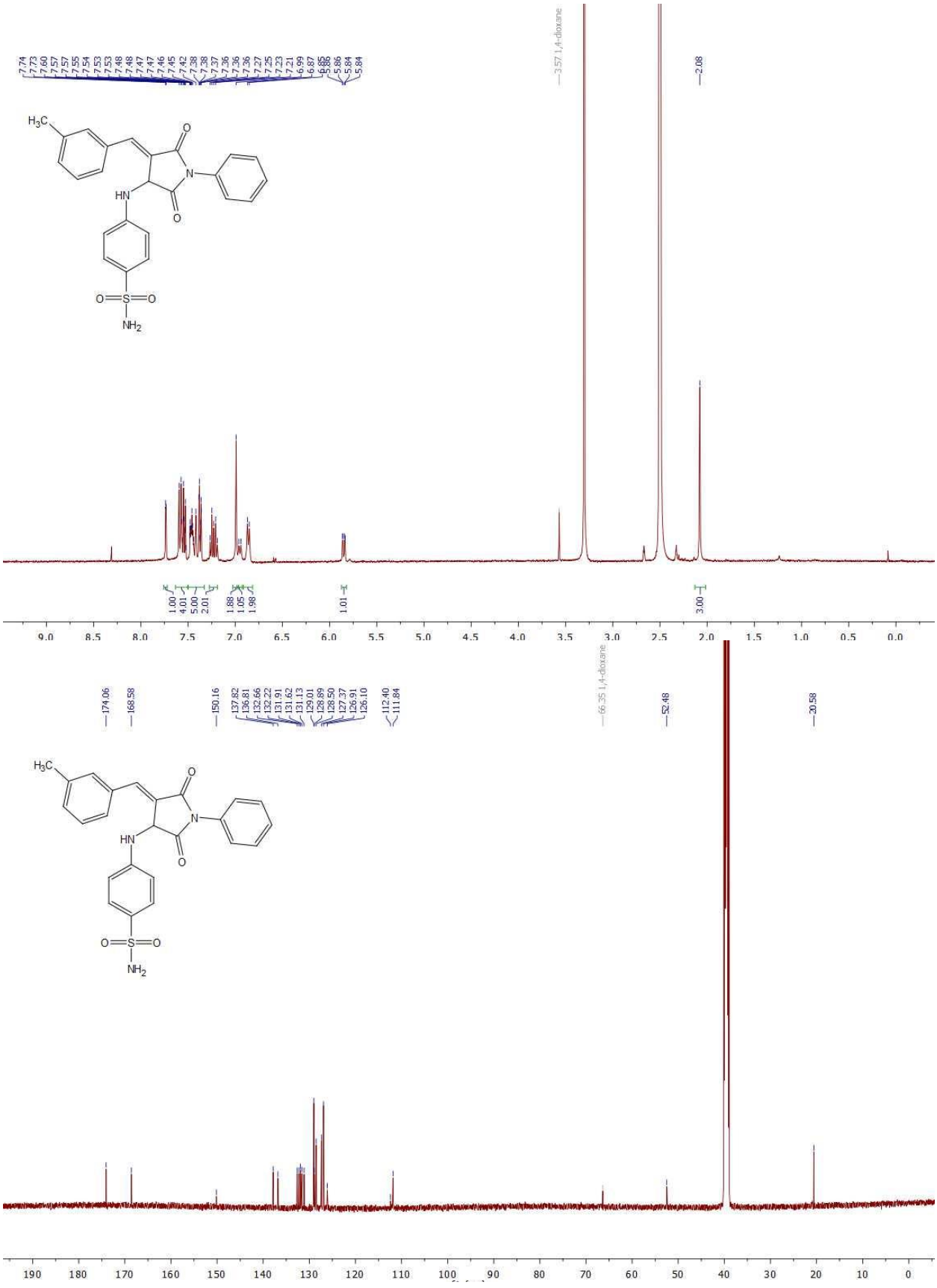
^{1}H and ^{13}C spectra of compound **3i**



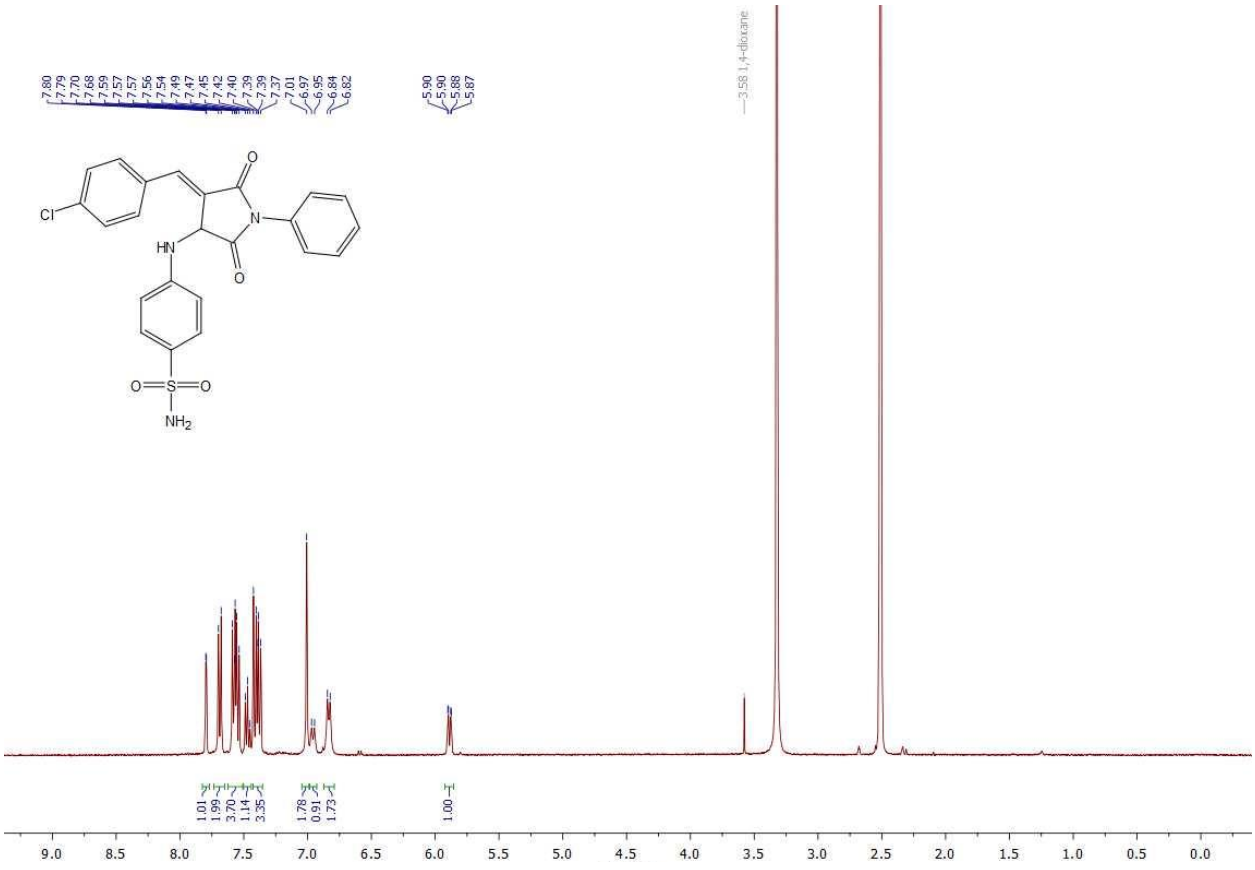
^{1}H and ^{13}C spectra of compound **3j**

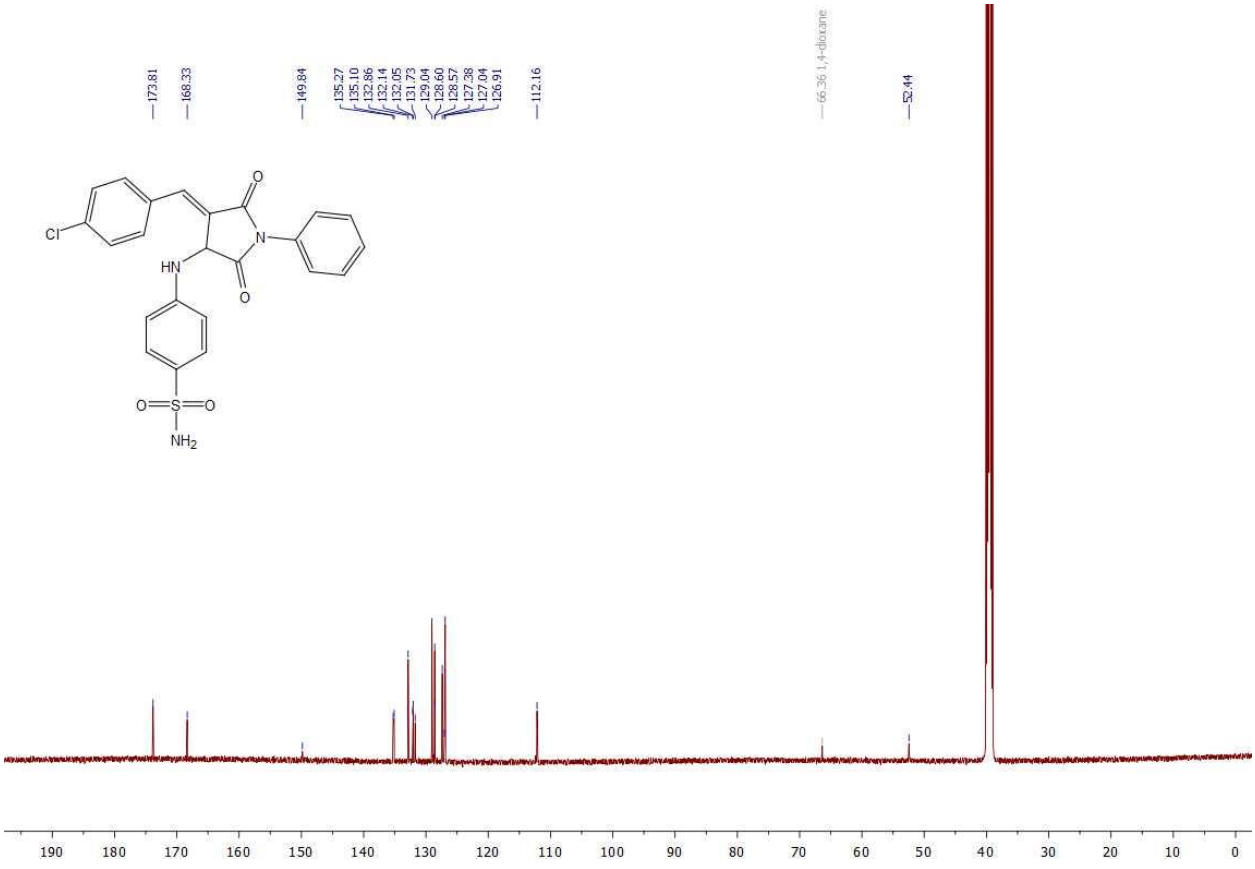


^{1}H and ^{13}C spectra of compound **3k**

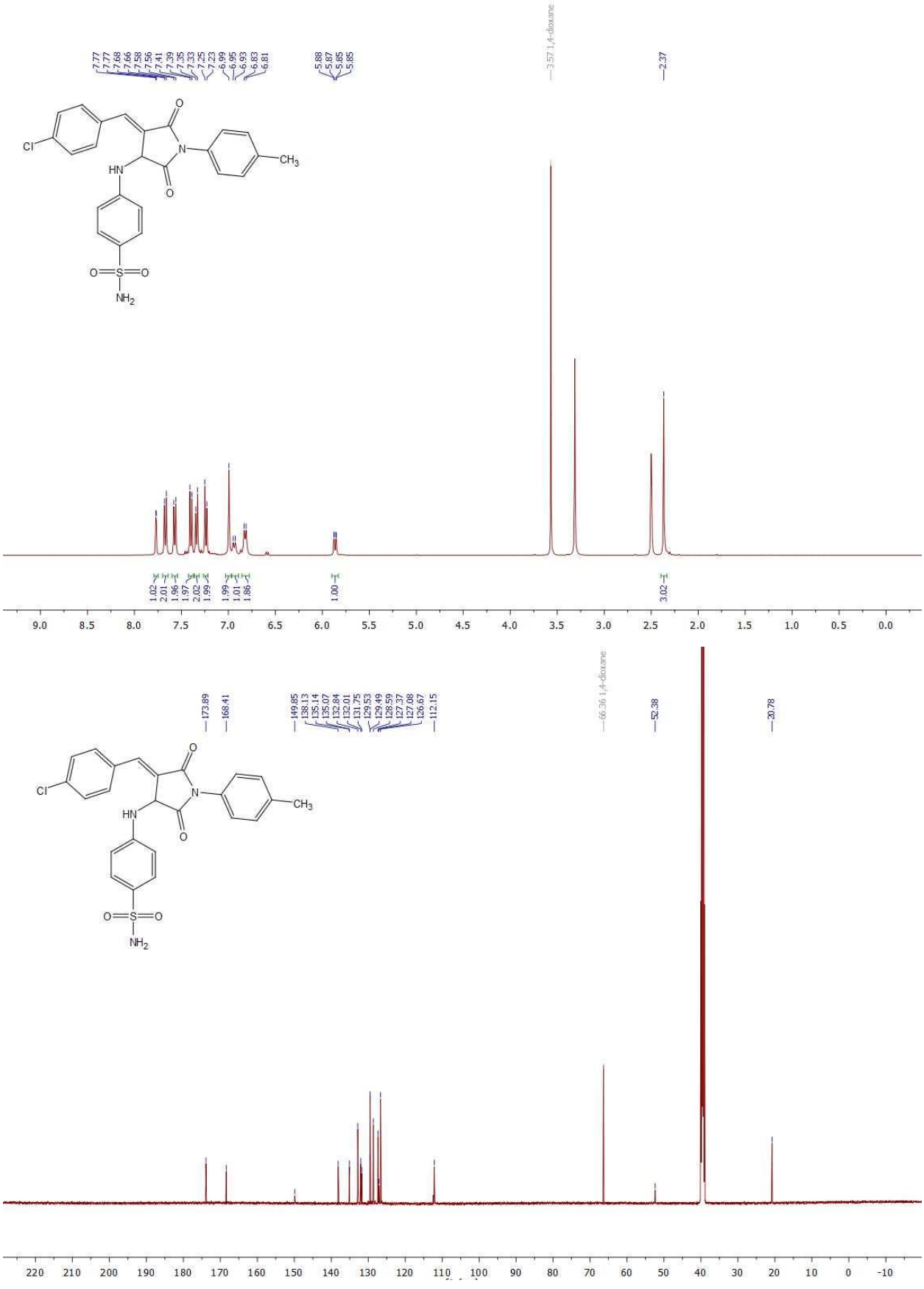


^{1}H and ^{13}C spectra of compound **3l**

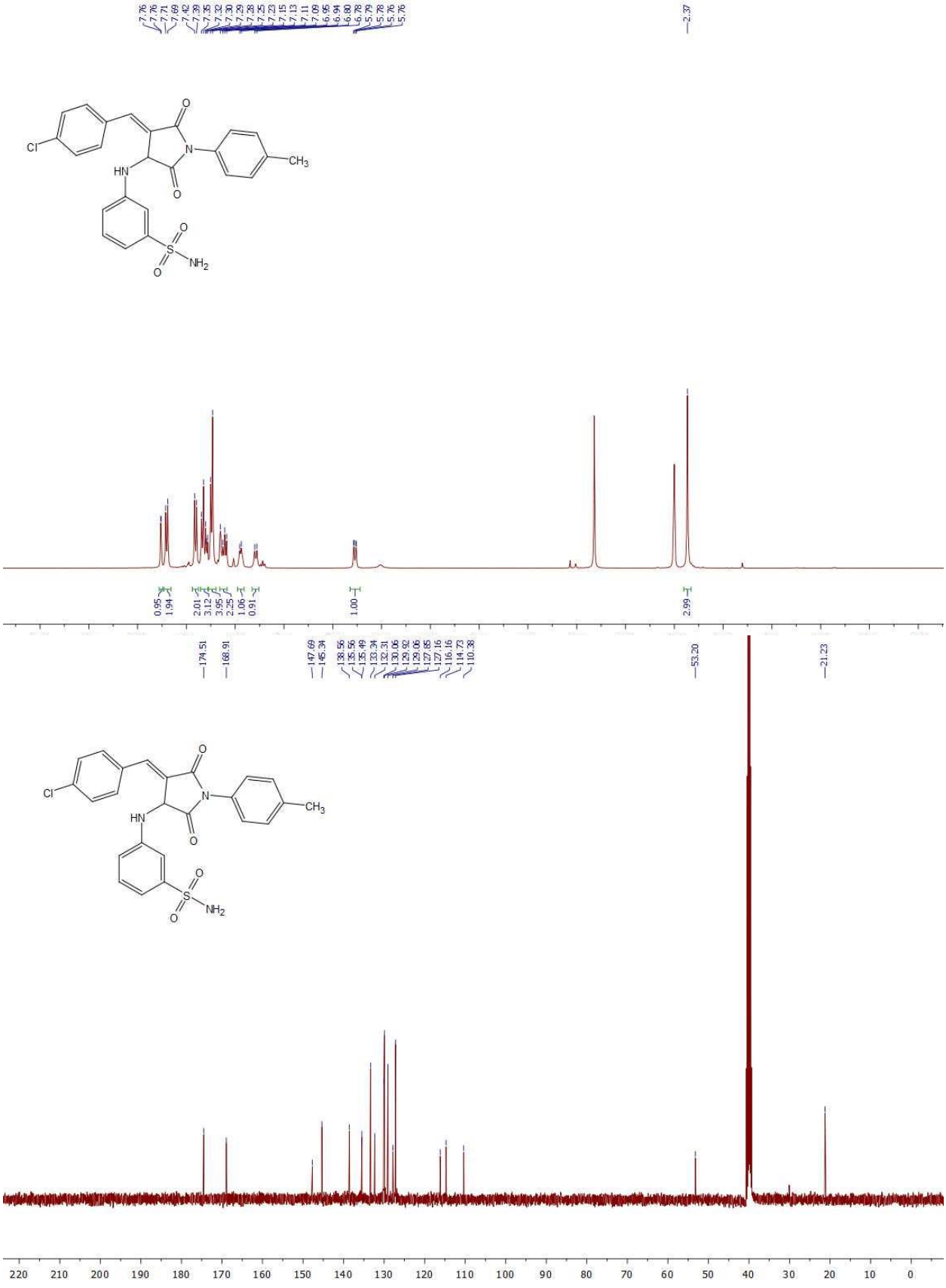




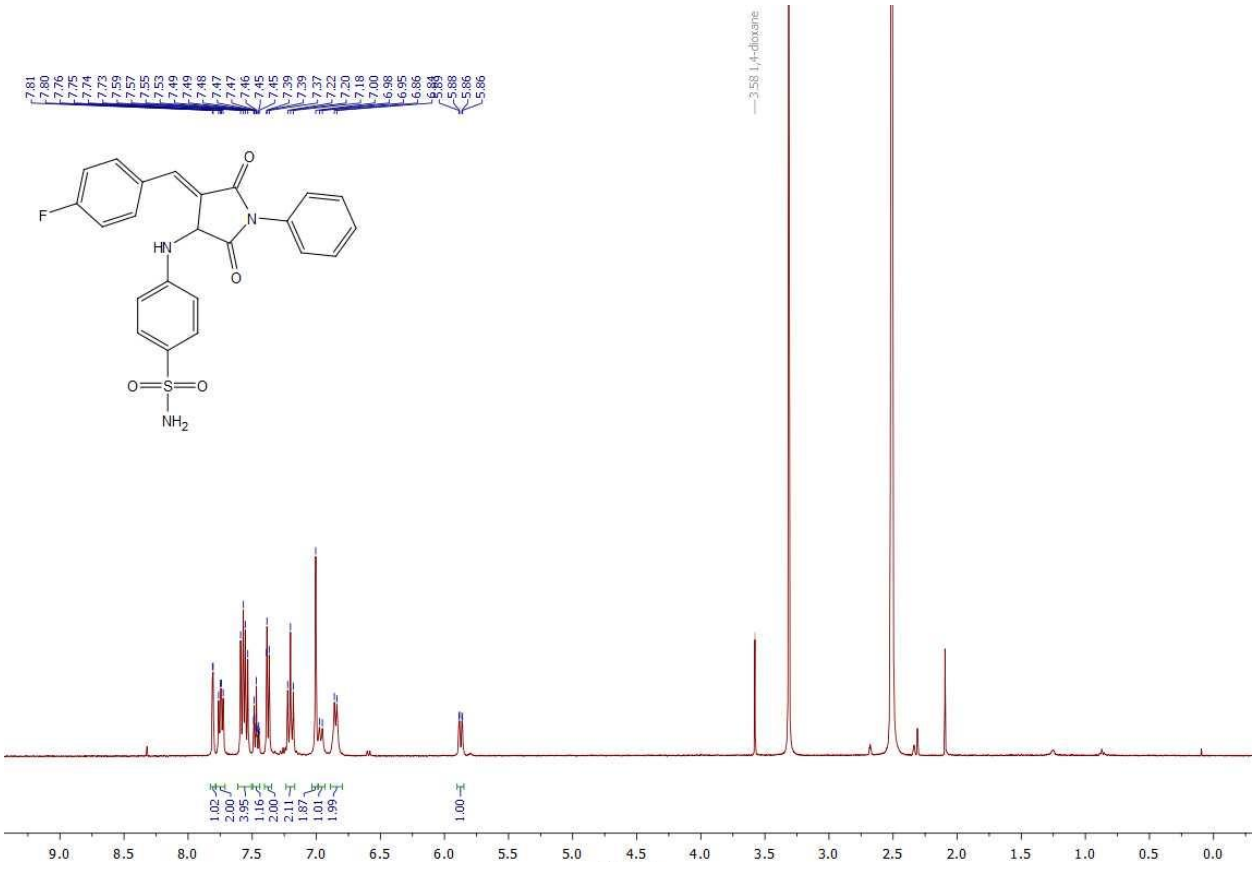
^{1}H and ^{13}C spectra of compound **3m**

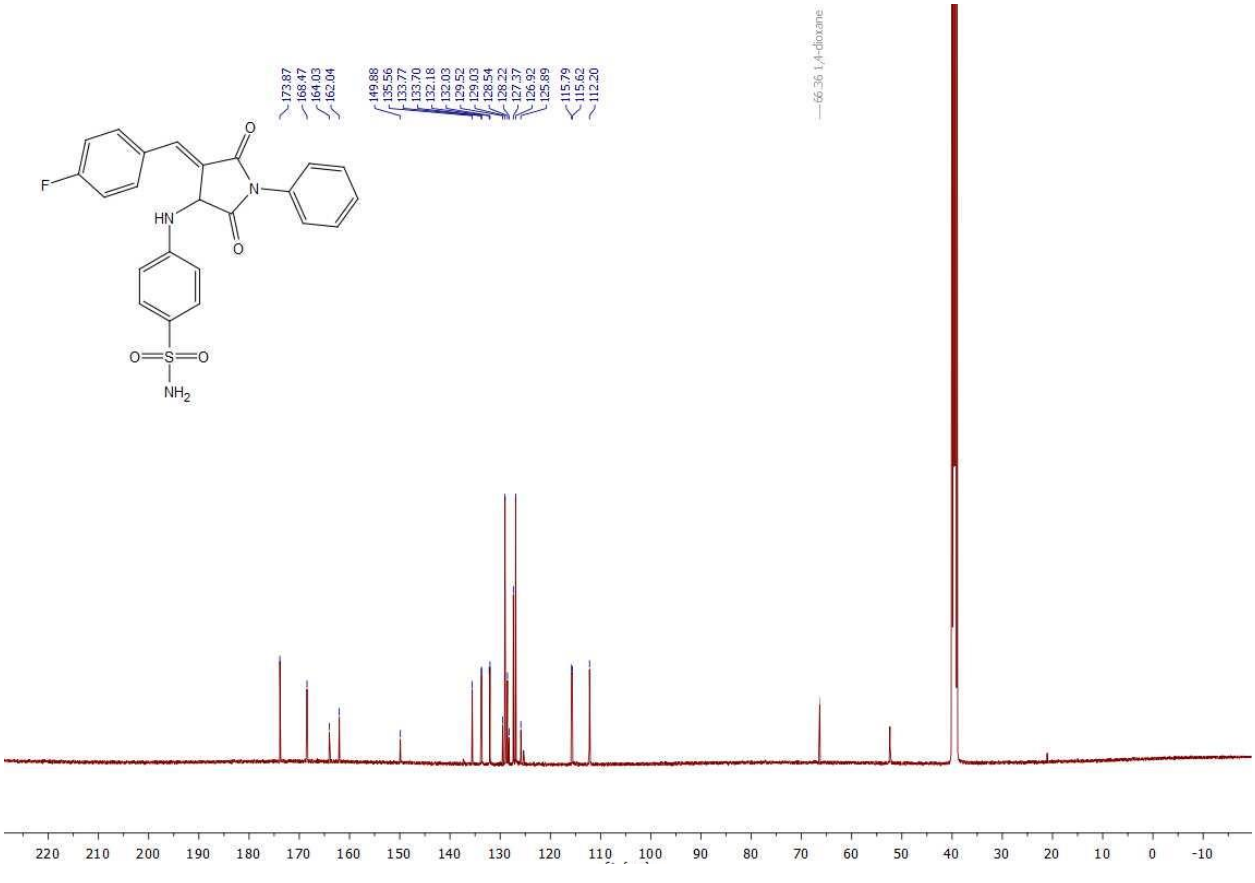


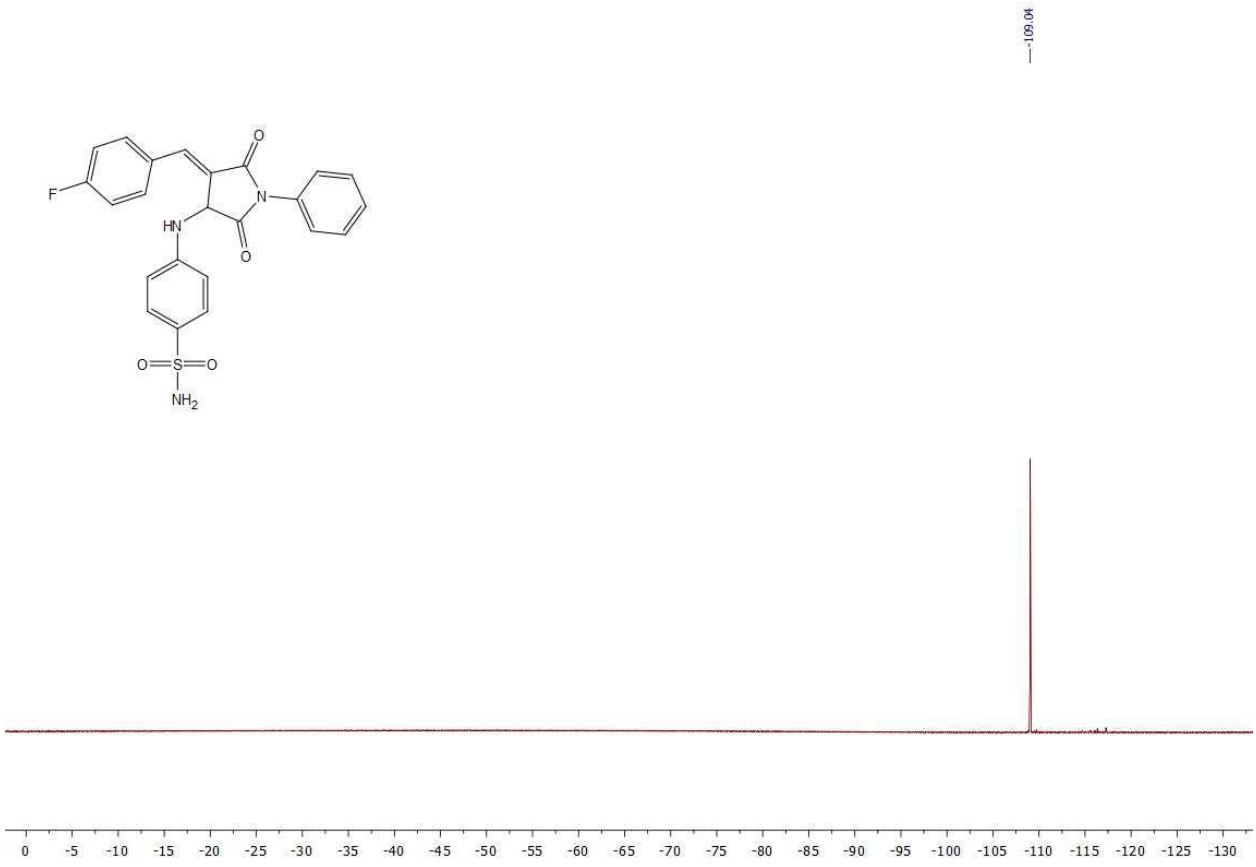
^{1}H and ^{13}C spectra of compound **3n**



^{1}H, ^{13}C and ^{19}F spectra of compound **3o**



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O
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NH2
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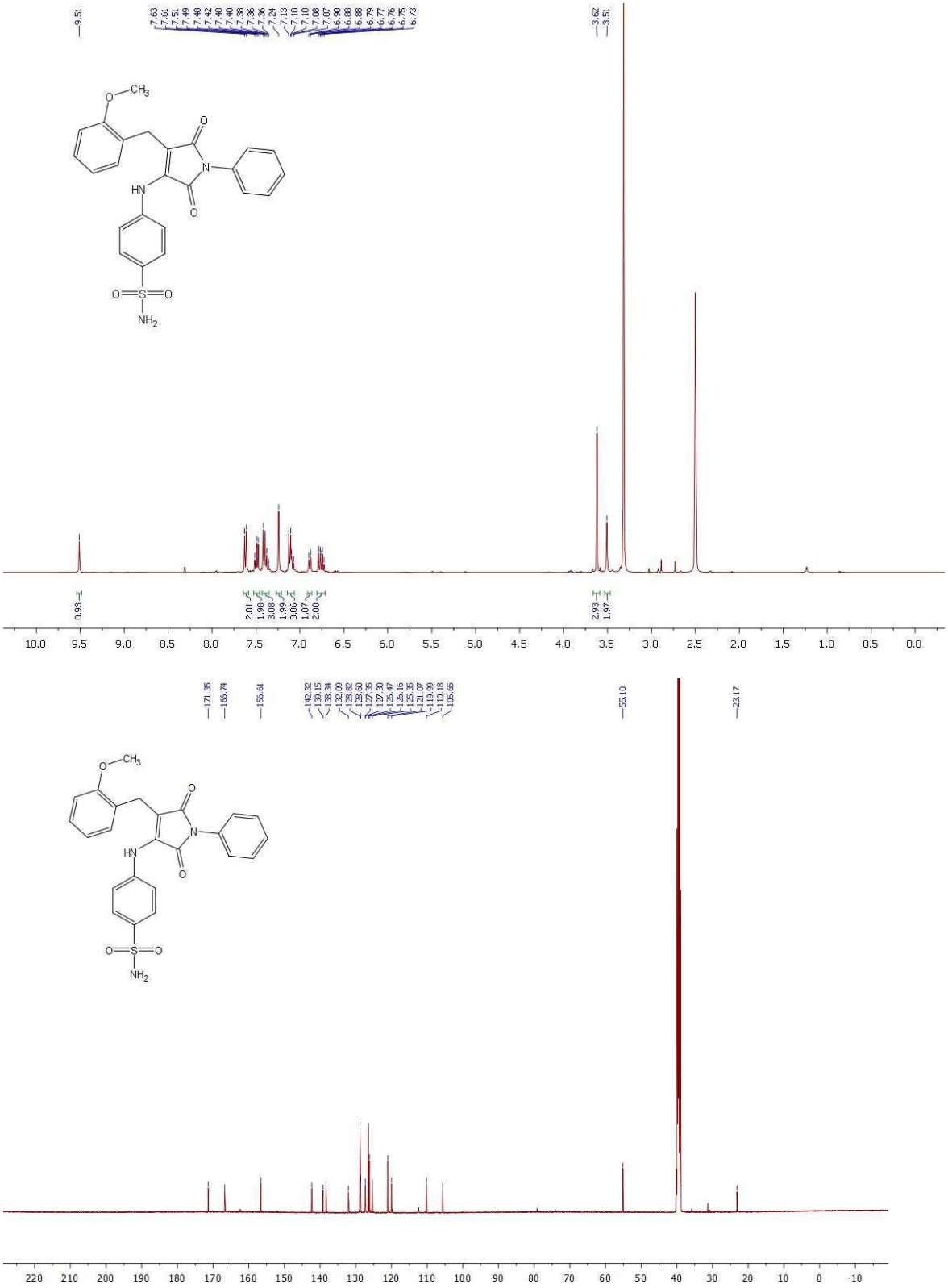
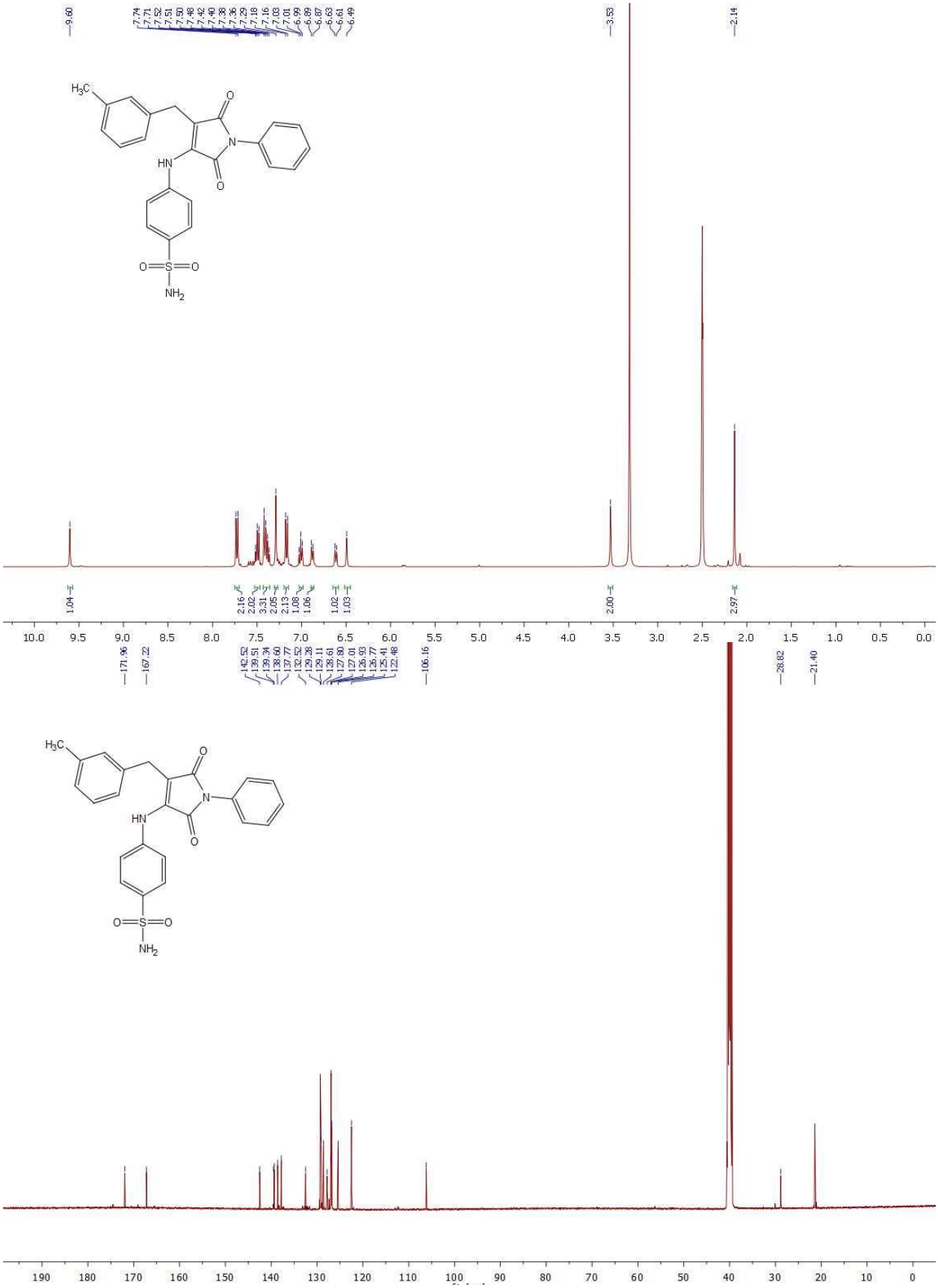
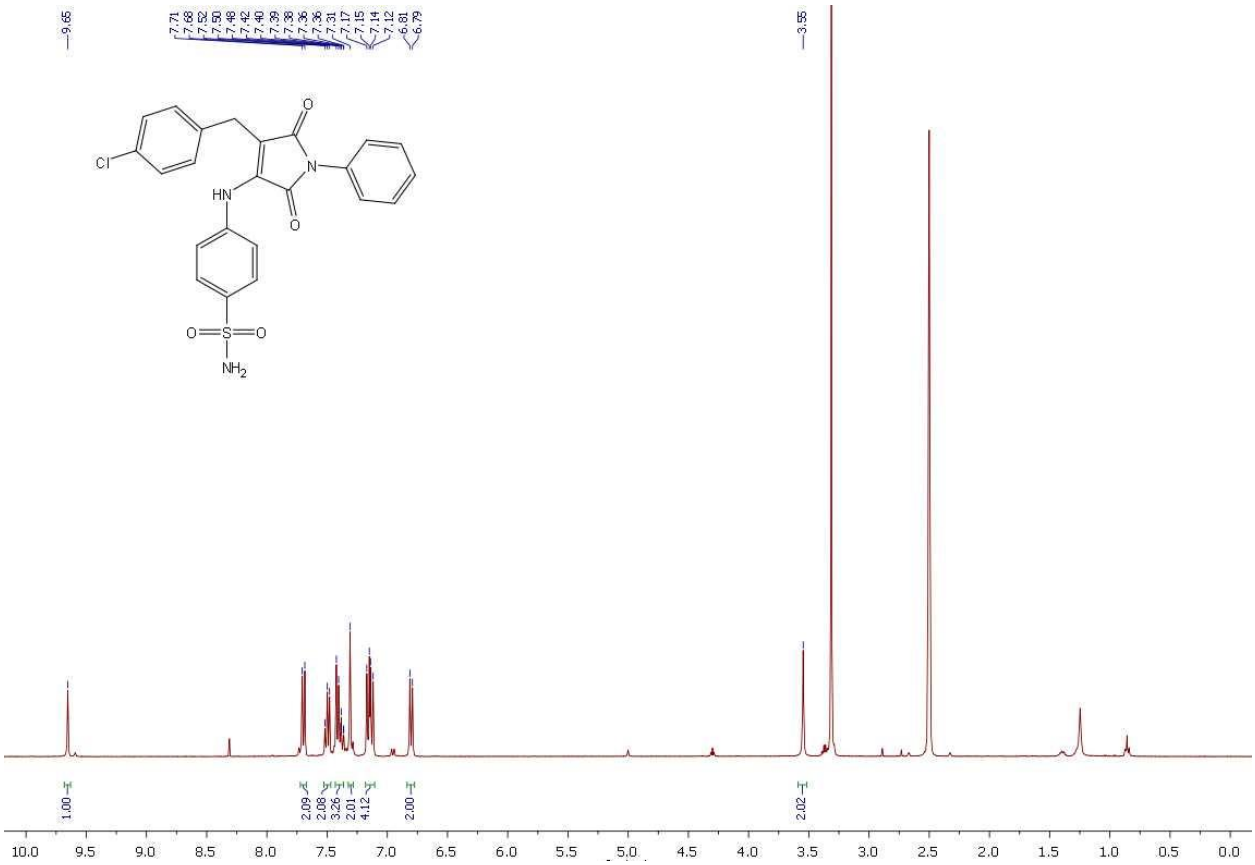
^{1}H and ^{13}C spectra of compound **2a**

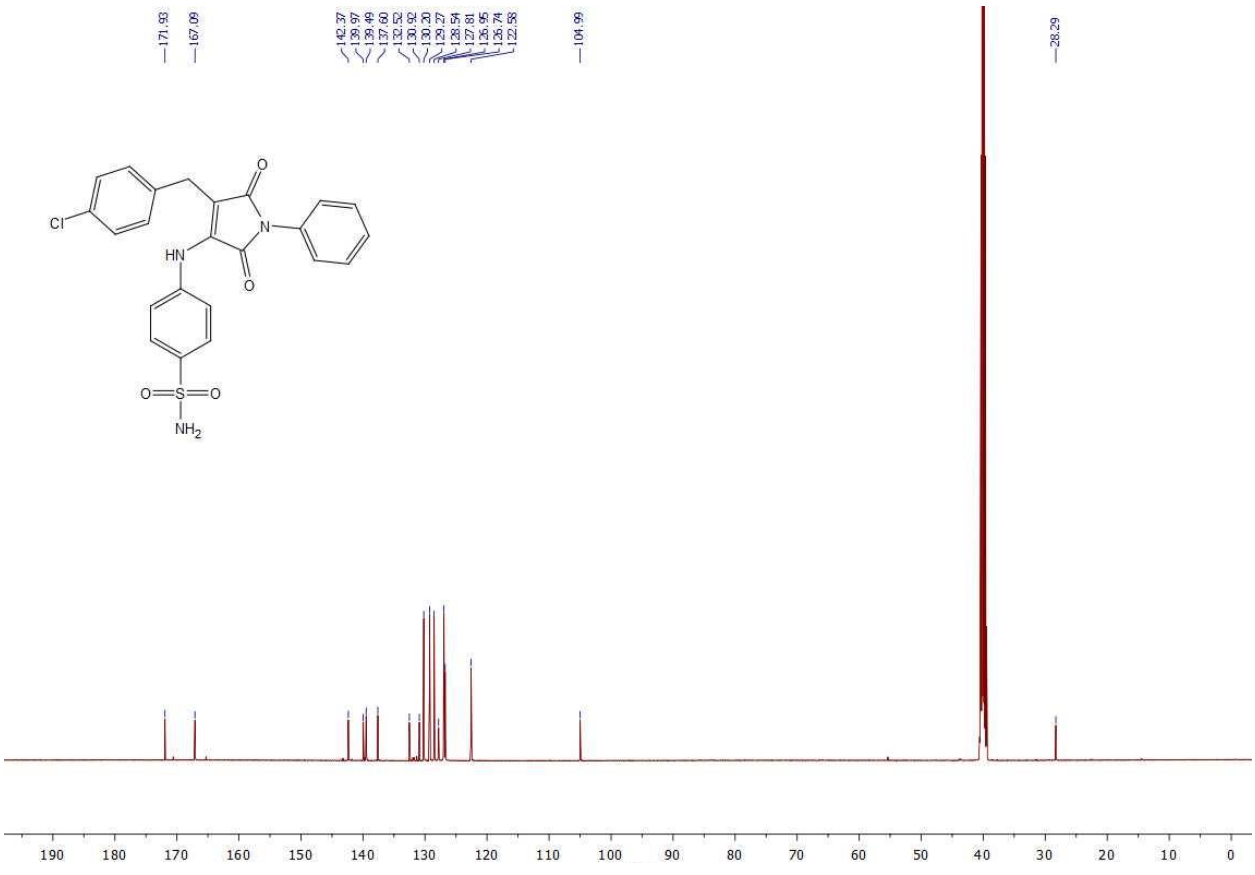


^{1}H and ^{13}C spectra of compound **2b**

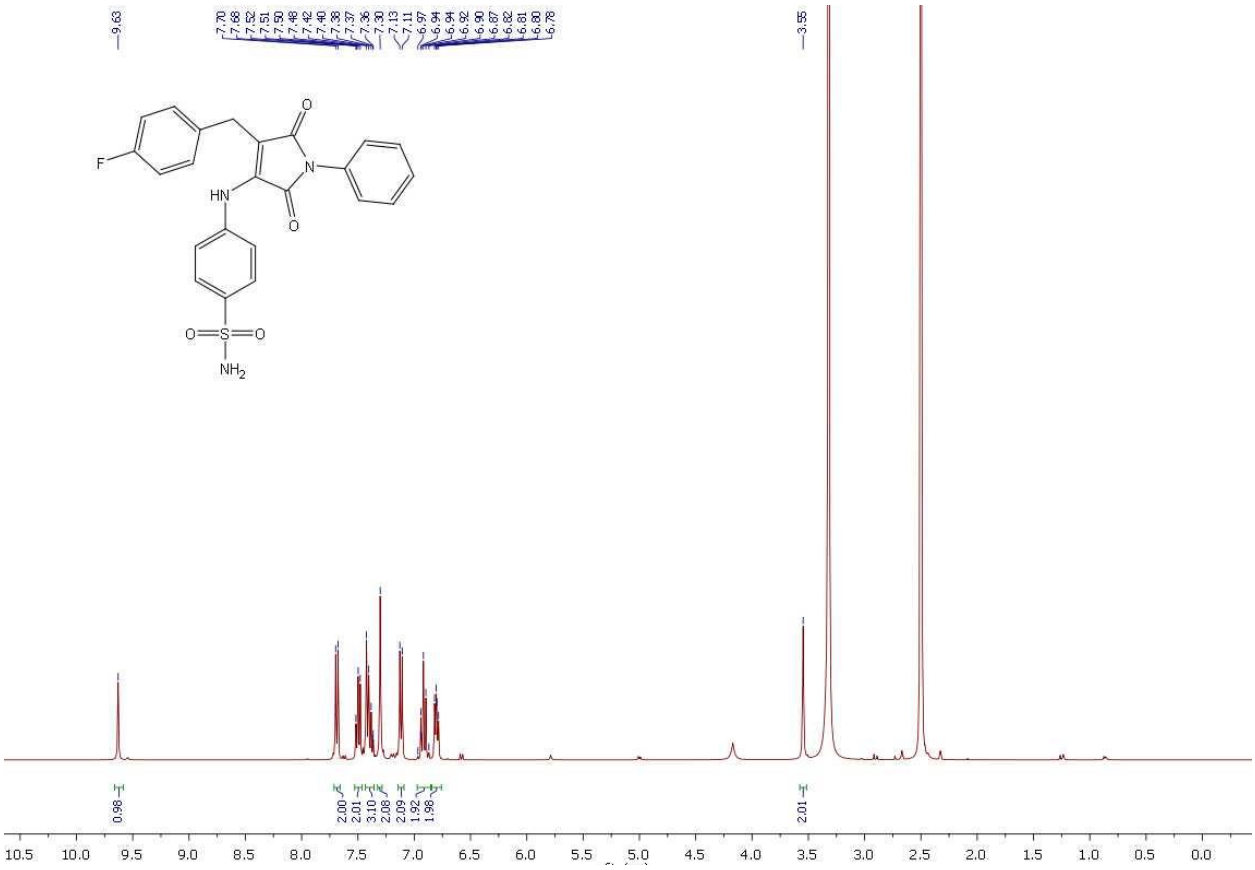


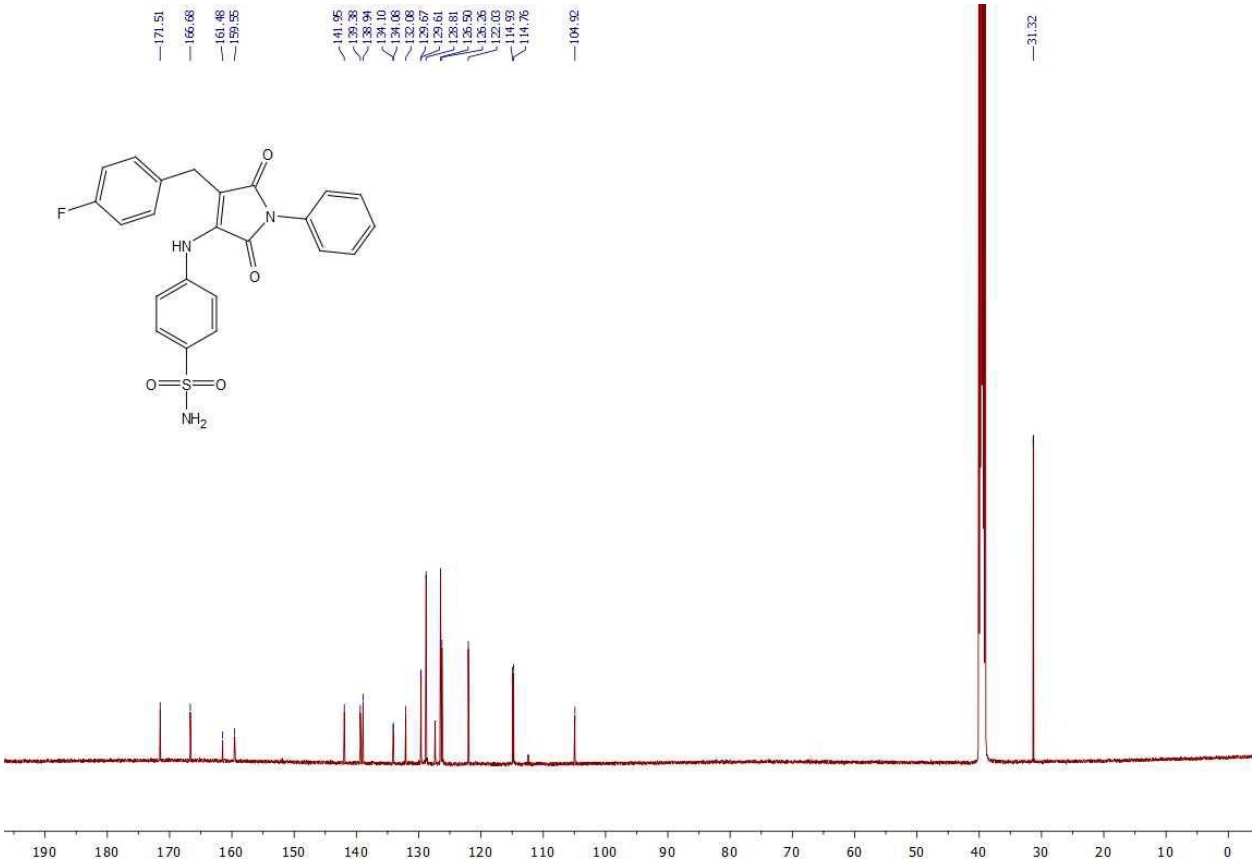
^{1}H and ^{13}C spectra of compound **2c**

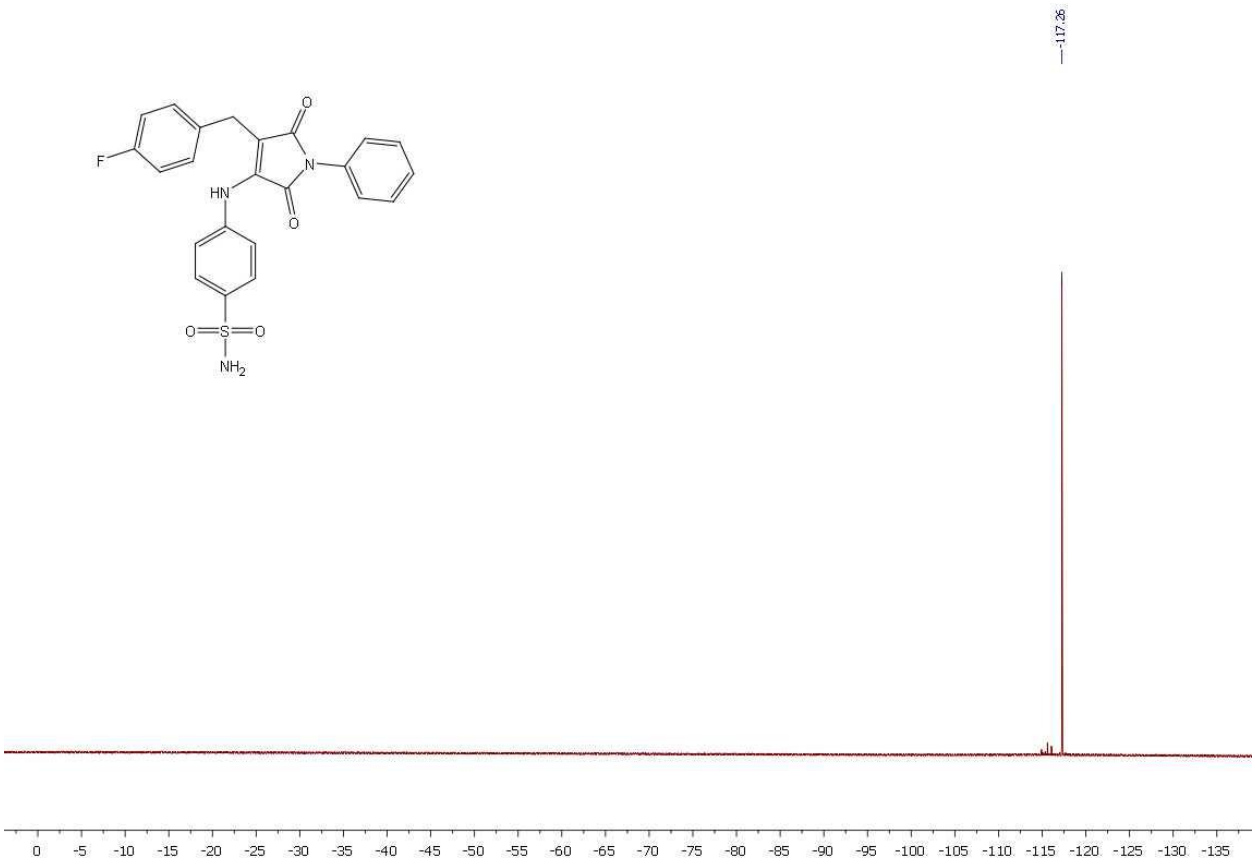




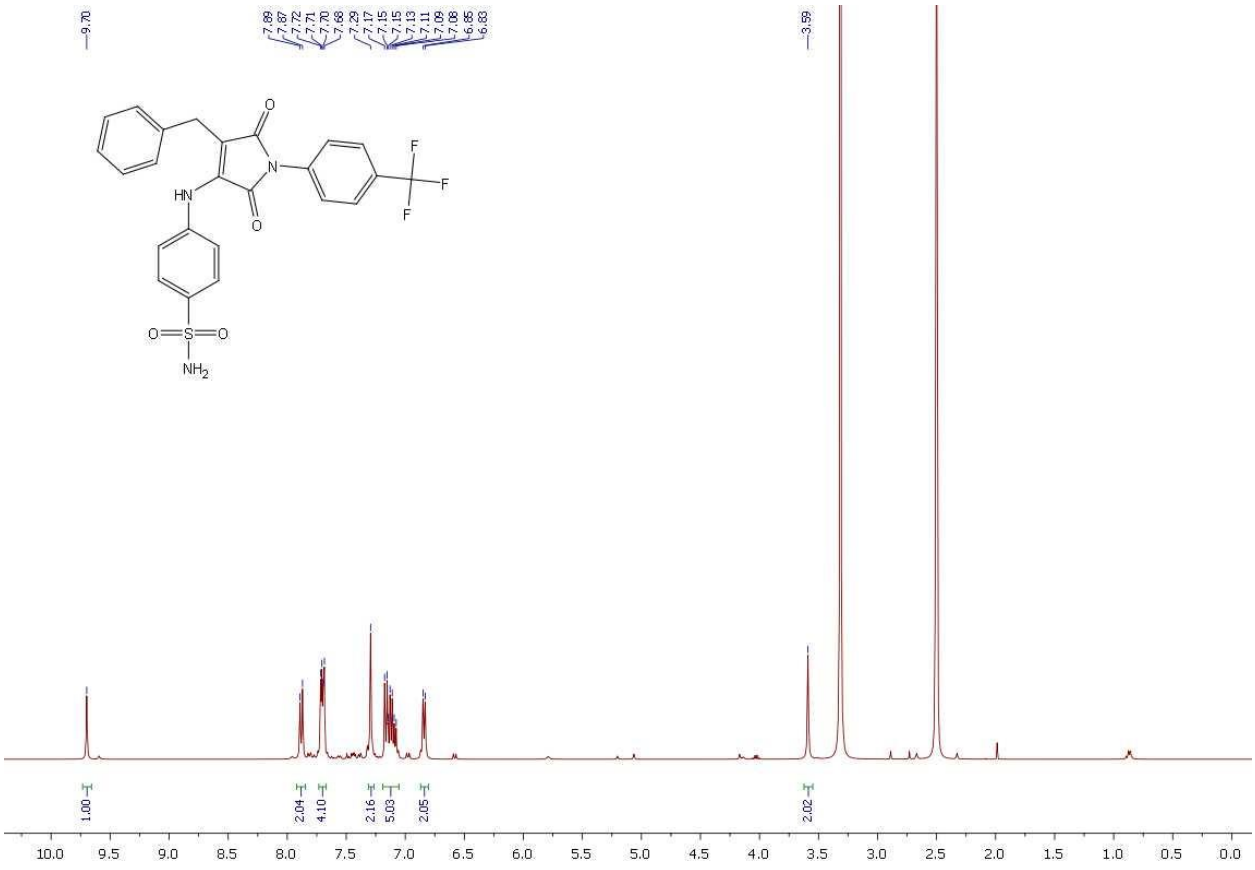
^{1}H, ^{13}C and ^{19}F spectra of compound **2d**

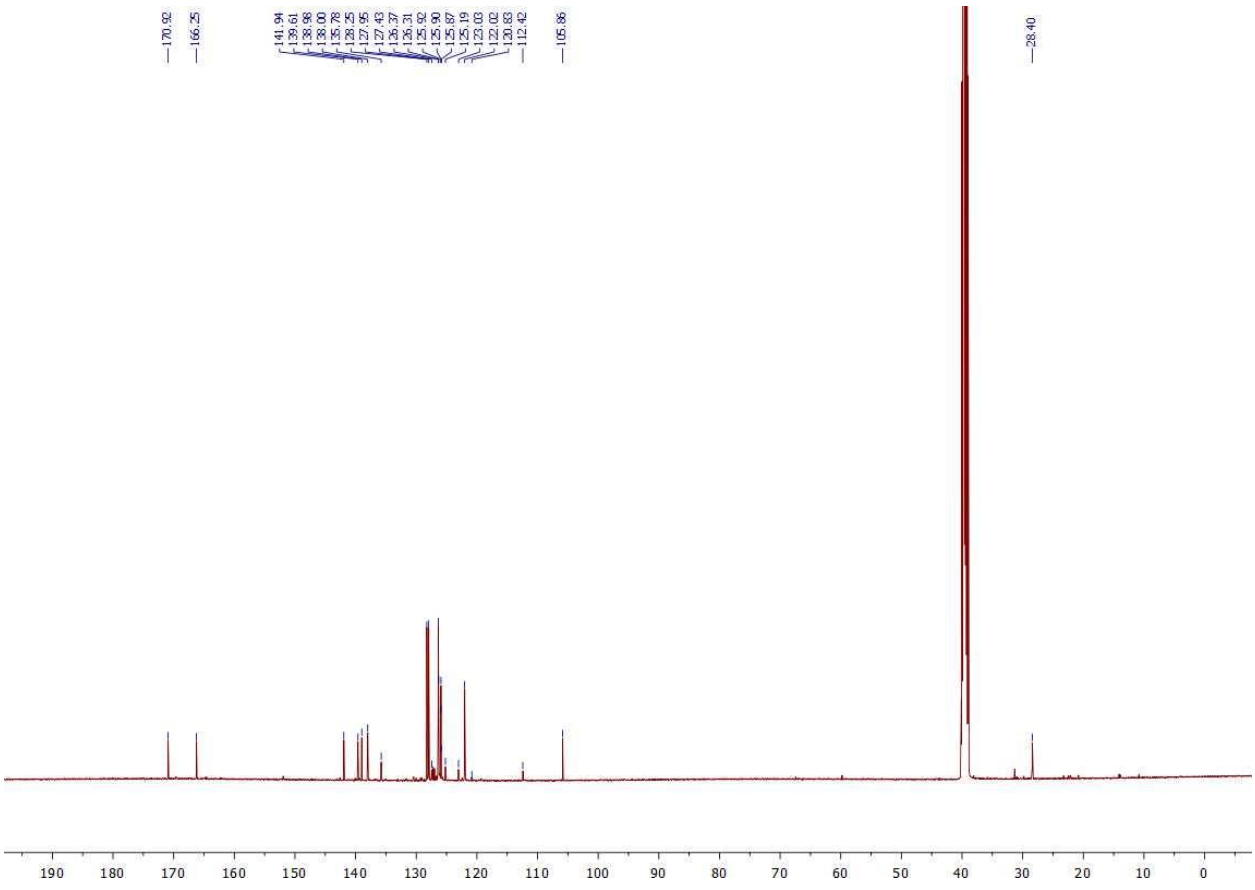






^{1}H, ^{13}C and ^{19}F spectra of compound **2e**

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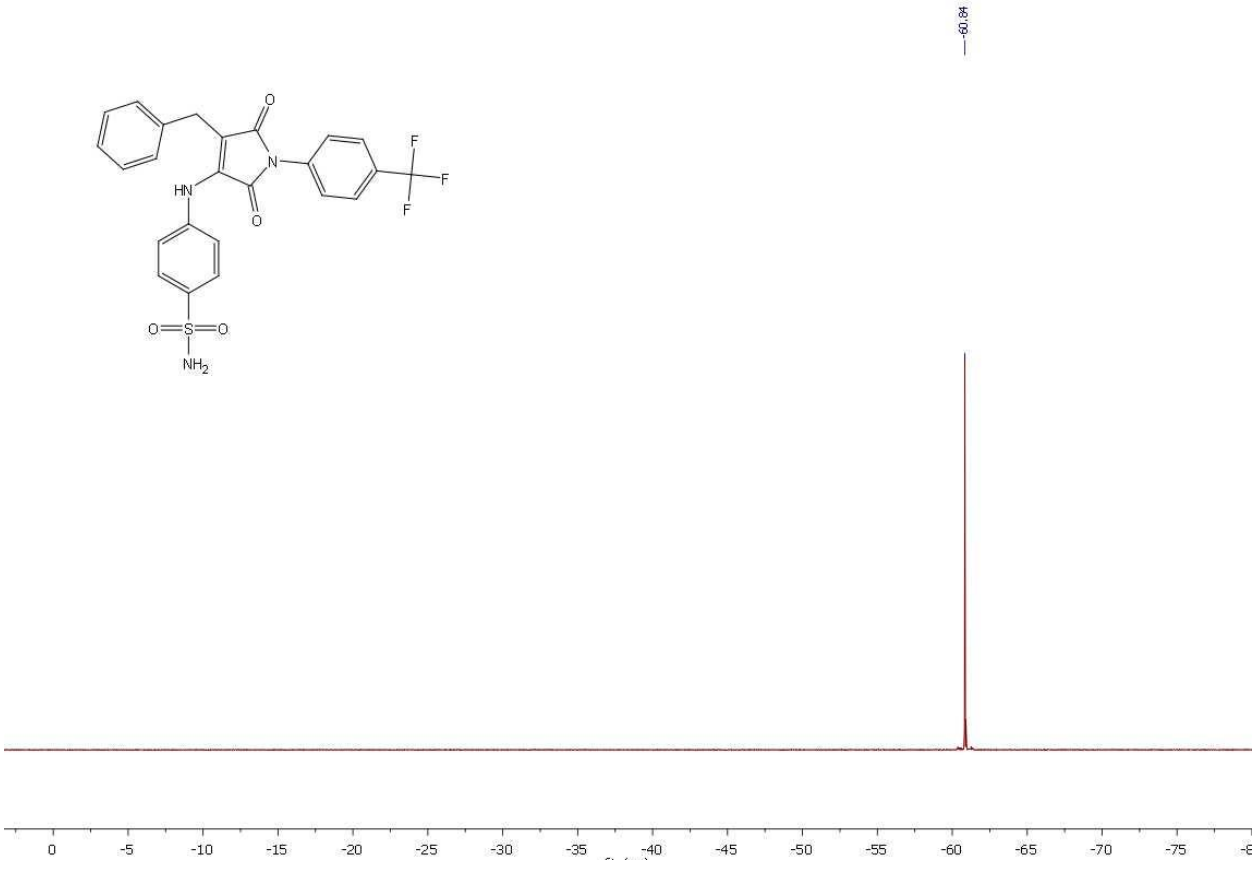
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