Design, synthesis and *in vitro* cytotoxicity evaluation of indolo-pyrazoles grafted with thiazolidinone as tubulin polymerization inhibitors

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1. General Remark

All the starting materials, reagents and solvents were procured from the available commercial sources and used without further purification. All the reactions were carried out using oven-dried glassware and in a round bottom flask. The reactions wherever anhydrous condition was required were carried under positive nitrogen pressure using freshly distilled solvents. The reactions were monitored by thin-layer chromatography, performed on MERCK precoated silica gel 60F-254 (0.5 mm) aluminium plates and visualized under UV radiation at 254 nm. Column chromatography was performed in silica gel 200-400 mesh using 10 - 30% EtOAc in hexane as eluent. ^{1}H and ^{13}C NMR spectrums were recorded on a Bruker Avance spectrometer operating at 500 MHz or 125 MHz, respectively. Chemical shifts are reported in ppm with the solvent resonance as the internal standard for the DMSO-d_6 (δ = 2.50 ppm and 39.52 ppm) and TMS as internal standard for $CDCl_3$ (TMS δ = 0 ppm, $CDCl_3$ δ = 7.26 ppm and 77.16 ppm). The chemical shift (δ) values are given in parts per million (ppm), and coupling constants (J) in hertz. The following abbreviations were used for ^{1}H NMR spectra to indicate the signal multiplicity; s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), hept (heptet) and m (multiplet). HRMS were recorded on 6540 series of Agilent QTOF mass spectrometer. Melting points were determined with an electrothermal digital melting point apparatus IA9100 and are uncorrected. The IUPAC name of all the compounds given in the experimental section were taken from ChemOffice 2020, ChemDraw version 20.1.

2. General procedure for the synthesis of indolo-pyrazole conjugates 6a-ah

To a two neck round bottom flask charged with indole **1** (10 mmol, 1 equiv.) and sodium hydride (12.5 mmol, 1.25 equiv.), were added DMF (20 mL) and alkyl halides (11 mmol, 1.1. equiv.) at 0 °C under inert atmosphere. After the completion (as monitored by TLC), reaction was quenched with cold water and diluted with ethyl acetate (250 mL). The organic phase was washed with cold water (5 x 150 mL), brine solution (30 mL) and dried over Na_2SO_4. The ethyl

acetate was removed under reduced pressure and the crude products **2a-h** were purified by column chromatography in silica (#60-120) using 5% EtOAc in hexane.

Then, to a stirred solution of **2a-h** or **1a-c** (7.5 mmol, 1 equiv.) in acetic anhydride (10 mL, was added cyanoacetic acid (15 mmol, 2 equiv.) at 60 °C. The reaction temperature was increased to 85 °C and reaction was monitored by TLC. After completion (precipitated solid product observed in the reaction mixture), the reaction mixture was cooled to room temperature and added cold methanol (50 mL). The solid precipitate was filtered through vacuum filtration and additionally washed with cold methanol (25 mL) to get pure **3a-j**.

Next, to a stirring suspension of β-ketonitriles **3a-j** (5 mmol, 1 equiv.) and different phenylhydrazine derivatives (5.5 mmol, 1.1 equiv.) in ethanol (20 mL), was added the catalytic amount of methanesulfonic acid (2-3 drops) and reaction was refluxed at 80 °C for 12-15 hours. Alternatively, the reaction was also conducted at 140 °C under microwave heating (using Anton paar monowave 300) for 30 min (to reduce the reaction time). After completion, solvent was directly removed using rotary evaporation and the crude products **4a-ah** were purified through silica column chromatography using 15-30% EtOAc in hexane.

Next, to a solution of pyrazole amines **4a-ah** (2.5 mmol, 1 equiv.) in dry DMF (10 mL), was added 2-chloroacetyl chloride (5.0 mmol, 2 equiv.) under inert condition. The reaction was stirred for 24 hours at room temperature. Then the reaction mixture was poured into ice-cold water (250 mL) with vigorous stirring and the precipitate generated was filtered and washed with n-hexane to obtain crude solid of **5a-ah**. The products **5a-ah** were taken up for the next step without further purification.

Further, a solution of *N*-acylated pyrazole amines **5a-ah** (1 mmol, 1 equiv.) and ammonium isothiocyanate (2.5 mmol, 2.5 equiv.) in absolute ethanol (10 mL), was refluxed for 12 hours. After completion, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic phase was washed with brine solution, dried over Na_2SO_4, filtered, and solvent was removed under reduced pressure using rotary evaporator. The crude solid obtained were subjected to silica (#100-200) column chromatography using 15-20% EtOAc in hexane to obtain pure **6a-ah**.

2-((3-(1*H*-Indol-3-yl)-1-phenyl-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6a): Light yellow solid; Yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.06 (s, 1H), 11.33 (s, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 2.6 Hz, 1H), 7.81 (d, J = 7.5 Hz, 2H), 7.50 (t, J = 8.0 Hz, 2H),

7.44 (d, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.16 (t, *J* = 6.9 Hz, 1H), 7.11 (t, *J* = 6.9 Hz, 1H), 6.52 (s, 1H), 4.14 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.1, 160.5, 148.2, 146.2, 140.0, 137.0, 129.3, 126.5, 125.3, 124.7, 123.3, 122.1, 121.4, 120.1, 112.1, 109.4, 93.4, 35.2 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{20}H_{16}N_5OS^+$ 374.1071, found 374.1094.

2-((1-(2-Ethylphenyl)-3-(1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6b): Light yellow solid; Yield 81%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.92 (s, 1H), 11.29 (s, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 7.83 (d, *J* = 2.6 Hz, 1H), 7.44 – 7.40 (m, 3H), 7.34 (dt, *J* = 8.9, 4.3 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.05 (t, *J* = 7.0 Hz, 1H), 6.50 (s, 1H), 4.12 (s, 2H), 2.48 (q, *J* = 7.6 Hz, 2H), 1.06 (t, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.0, 147.8, 147.2, 141.8, 138.4, 136.9, 129.6, 129.2, 128.9, 126.7, 125.3, 124.2, 121.9, 121.4, 119.8, 112.0, 109.8, 91.7, 35.0, 24.5, 15.0 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{20}N_5OS^+$ 402.1384, found 402.1394.

2-((1-(3-Chlorophenyl)-3-(1H-indol-3-yl)-1H-pyrazol-5-yl)imino)thiazolidin-4-one (6c): Light yellow solid; Yield 79%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.19 (s, 1H), 11.39 (s, 1H), 8.22 (d, *J* = 7.3 Hz, 1H), 7.93 (t, *J* = 2.0 Hz, 1H), 7.92 (d, *J* = 2.6 Hz, 1H), 7.89 (d, *J* = 7.1 Hz, 1H), 7.53 (t, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.20 – 7.11 (m, 2H), 6.57 (s, 1H), 4.16 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.2, 148.8, 146.6, 141.2, 137.0, 133.6, 131.0, 125.9, 125.3, 125.1, 122.2, 122.1, 121.4, 121.3, 120.2, 112.2, 109.1, 93.9, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{20}H_{15}ClN_5OS^+$ 408.0681, found 408.0690.

2-((1-(4-Chlorophenyl)-3-(1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6d): Light yellow solid; Yield 76%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 11.37 (s, 1H), 8.23 (d, *J* = 7.8 Hz, 1H), 7.91 – 7.86 (m, 3H), 7.56 (d, *J* = 8.9 Hz, 2H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.17 (t, *J* = 6.8 Hz, 1H), 7.12 (t, *J* = 6.9 Hz, 1H), 6.55 (s, 1H), 4.16 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 160.8, 148.6, 146.4, 138.9, 137.0, 130.4, 129.2, 125.3, 125.0, 124.5, 122.1, 121.5, 120.1, 112.1, 109.2, 93.7, 56.5, 35.2, 19.0 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{20}H_{15}ClN_5OS^+$ 408.0681, found 408.0688.

4-(3-(1*H*-Indol-3-yl)-5-((4-oxothiazolidin-2-ylidene)amino)-1*H*-pyrazol-1-yl)benzonitrile (6e): Light yellow solid; Yield 72%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.22 (s, 1H), 11.45 (s, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 8.19 (d, *J* = 8.7 Hz, 2H), 7.97 (d, *J* = 8.5 Hz, 3H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.17 (dt, *J* = 15.3, 6.6 Hz, 2H), 6.62 (s, 1H), 4.17 (s, 2H) ppm; ^{13}C NMR (125

MHz, DMSO-d_6): δ 174.0, 161.6, 149.6, 147.2, 143.5, 137.1, 133.6, 130.4, 125.7, 125.2, 122.3, 121.6, 120.3, 119.4, 112.2, 108.9, 107.8, 94.6, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{15}N_6OS^+$ 399.1023, found 399.1029.

2-((1-(2-Ethylphenyl)-3-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6f): Light yellow solid; Yield 81%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.92 (s, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.83 (s, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 3.9 Hz, 2H), 7.34 (dt, J = 8.8, 4.3 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 6.47 (s, 1H), 4.12 (s, 2H), 3.85 (s, 3H), 2.48 (q, J = 7.5 Hz, 2H), 1.05 (t, J = 7.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.0, 147.4, 147.2, 141.8, 138.4, 137.4, 129.7, 129.2, 128.9, 128.4, 126.7, 125.7, 122.0, 121.5, 120.1, 110.3, 108.9, 91.6, 35.0, 33.0, 24.5, 15.0 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{22}N_5OS^+$ 416.1540, found 416.1510.

2-((1-(3-Chlorophenyl)-3-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6g): Light yellow solid; Yield 71%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.19 (s, 1H), 8.22 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 3.0 Hz, 2H), 7.88 (d, J = 8.2 Hz, 1H), 7.56 – 7.48 (m, 2H), 7.38 (d, J = 9.0 Hz, 1H), 7.24 (t, J = 7.2 Hz, 1H), 7.18 (t, J = 7.3 Hz, 1H), 6.53 (s, 1H), 4.16 (s, 2H), 3.86 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.2, 148.4, 146.7, 141.2, 137.5, 133.6, 131.0, 129.2, 126.0, 125.6, 122.2, 121.6, 121.3, 120.4, 110.5, 108.2, 93.8, 35.3, 33.1 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{17}ClN_5OS^+$ 422.0837, found 422.0852.

2-((1-(4-Chlorophenyl)-3-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6h): Light yellow solid; Yield 75%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.90 (s, 1H), 7.88 (d, J = 8.9 Hz, 2H), 7.56 (d, J = 8.9 Hz, 2H), 7.50 (d, J = 8.2 Hz, 1H), 7.24 (t, J = 7.1 Hz, 1H), 7.17 (t, J = 7.4 Hz, 1H), 6.51 (s, 1H), 4.16 (s, 2H), 3.85 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 160.9, 148.2, 146.4, 138.8, 137.5, 130.5, 129.2, 129.1, 125.6, 124.5, 122.2, 121.6, 120.3, 110.5, 108.3, 93.6, 35.3, 33.1 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{17}ClN_5OS^+$ 422.0837, found 422.0822.

4-(3-(1-Methyl-1*H*-indol-3-yl)-5-((4-oxothiazolidin-2-ylidene)amino)-1*H*-pyrazol-1-yl)benzonitrile (6i): Light yellow solid; Yield 71%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.22 (s, 1H), 8.27 (d, J = 7.8 Hz, 1H), 8.19 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 7.7 Hz, 3H), 7.51 (d, J = 8.2 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.4 Hz, 1H), 6.57 (s, 1H), 4.18 (s, 2H), 3.86 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.6, 149.1, 147.2, 143.5, 137.5,

133.6, 129.7, 125.5, 122.4, 121.7, 120.5, 119.3, 110.5, 108.0, 107.8, 94.4, 35.4, 33.2 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{17}N_6OS^+$ 413.1180, found 413.1190.

2-((3-(1-Ethyl-1*H*-indol-3-yl)-1-(2-ethylphenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6j): Light yellow solid; Yield 82%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.93 (s, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.91 (s, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 4.0 Hz, 2H), 7.34 (dt, J = 8.8, 4.3 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 7.18 (t, J = 7.1 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 6.48 (s, 1H), 4.26 (q, J = 7.2 Hz, 2H), 4.13 (s, 2H), 2.48 (q, J = 7.5 Hz, 2H), 1.42 (t, J = 7.2 Hz, 3H), 1.05 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.0, 147.5, 147.2, 141.8, 138.4, 136.4, 129.7, 129.2, 128.9, 126.9, 126.7, 125.8, 122.0, 121.7, 120.0, 110.3, 109.1, 91.6, 40.9, 35.0, 24.5, 15.9, 15.0 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{24}H_{24}N_5OS^+$ 430.1697, found 430.1710.

2-((1-(3-Chlorophenyl)-3-(1-ethyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6k): Light yellow solid; Yield 73%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (s, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.99 (s, 1H), 7.93 (t, J = 1.9 Hz, 1H), 7.89 (d, J = 7.2 Hz, 1H), 7.53 (t, J = 8.2 Hz, 2H), 7.38 (d, J = 9.2 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.17 (t, J = 7.1 Hz, 1H), 6.55 (s, 1H), 4.27 (q, J = 7.2 Hz, 2H), 4.17 (s, 2H), 1.43 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.2, 148.4, 146.6, 141.2, 136.5, 133.6, 131.0, 127.6, 126.0, 125.7, 122.2, 122.2, 121.7, 121.3, 120.4, 110.5, 108.4, 93.8, 40.9, 35.3, 15.9 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{19}ClN_5OS^+$ 436.0994, found 436.1002.

2-((1-(4-Chlorophenyl)-3-(1-ethyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6l): Light yellow solid; Yield 76%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.23 (d, J = 7.9 Hz, 1H), 7.97 (s, 1H), 7.89 (d, J = 8.9 Hz, 2H), 7.58 – 7.51 (m, 3H), 7.22 (t, J = 8.1 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 6.53 (s, 1H), 4.27 (q, J = 7.2 Hz, 2H), 4.16 (s, 2H), 1.43 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 160.8, 148.2, 146.4, 138.8, 136.5, 130.5, 129.2, 127.5, 125.7, 124.5, 122.2, 121.8, 120.3, 110.5, 108.5, 93.6, 40.9, 35.3, 15.9 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{19}ClN_5OS^+$ 436.0994, found 436.1000.

4-(3-(1-Ethyl-1*H*-indol-3-yl)-5-((4-oxothiazolidin-2-ylidene)amino)-1*H*-pyrazol-1-yl)benzonitrile (6m): Light yellow solid; Yield 68%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.22 (s, 1H), 8.28 (d, J = 7.7 Hz, 1H), 8.19 (d, J = 8.9 Hz, 2H), 8.04 (s, 1H), 7.96 (d, J = 8.9 Hz, 2H), 7.55 (d, J = 8.2 Hz, 1H), 7.24 (t, J = 7.0 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 6.60 (s, 1H), 4.27 (q, J = 7.2 Hz, 2H), 4.18 (s, 2H), 1.44 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz,

DMSO-d_6): δ 174.0, 161.5, 149.2, 147.2, 143.5, 136.6, 133.6, 128.1, 125.7, 122.3, 122.3, 121.8, 120.5, 119.3, 110.5, 108.2, 107.8, 94.5, 41.0, 35.3, 15.8 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{19}N_6OS^+$ 427.1336, found 427.1324.

2-((3-(1-Benzyl-1*H*-indol-3-yl)-1-(2-ethylphenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6n): Light yellow solid; Yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.90 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 8.03 (s, 1H), 7.50 (d, J = 8.2 Hz, 1H), 7.43 (d, J = 3.9 Hz, 2H), 7.38 – 7.31 (m, 3H), 7.31 – 7.24 (m, 4H), 7.15 (t, J = 7.1 Hz, 1H), 7.08 (t, J = 7.1 Hz, 1H), 6.50 (s, 1H), 5.48 (s, 2H), 4.12 (s, 2H), 2.48 (q, J = 7.5 Hz, 2H), 1.06 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.1, 147.3, 147.3, 141.8, 138.5, 138.4, 136.8, 129.6, 129.2, 129.0, 128.9, 127.9, 127.8, 127.6, 126.7, 125.9, 122.2, 121.7, 120.3, 110.8, 109.6, 91.7, 49.7, 35.0, 24.5, 15.0 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{29}H_{26}N_5OS^+$ 492.1853, found 492.1854.

2-((3-(1-Benzyl-1*H*-indol-3-yl)-1-(3-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6o): Light yellow solid; Yield 78%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.21 (s, 1H), 8.23 (d, J = 7.0 Hz, 1H), 8.12 (s, 1H), 7.92 (t, J = 2.0 Hz, 1H), 7.88 (d, J = 9.3 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.39 (d, J = 8.0 Hz, 1H), 7.36 – 7.31 (m, 2H), 7.27 (dd, J = 7.8, 2.2 Hz, 3H), 7.22 – 7.14 (m, 2H), 6.56 (s, 1H), 5.49 (s, 2H), 4.16 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.2, 148.2, 146.7, 141.2, 138.4, 136.9, 133.6, 131.0, 129.1, 128.6, 127.9, 127.6, 126.1, 125.9, 122.4, 122.3, 121.7, 121.4, 120.6, 111.0, 108.9, 93.9, 49.7, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{27}H_{21}ClN_5OS^+$ 498.1150, found 498.1145.

2-((3-(1-Benzyl-1*H*-indol-3-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6p): Light yellow solid; Yield 82%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.24 (d, J = 7.2 Hz, 1H), 8.09 (s, 1H), 7.88 (d, J = 8.9 Hz, 2H), 7.56 (d, J = 8.9 Hz, 2H), 7.52 (d, J = 7.6 Hz, 1H), 7.36 – 7.30 (m, 2H), 7.28 (d, J = 7.4 Hz, 3H), 7.21 – 7.13 (m, 2H), 6.54 (s, 1H), 5.49 (s, 2H), 4.16 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 160.9, 148.0, 146.5, 138.8, 138.5, 136.9, 130.6, 129.2, 129.1, 128.5, 127.9, 127.6, 125.9, 124.6, 122.4, 121.8, 120.5, 110.9, 109.1, 93.7, 49.7, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{27}H_{21}ClN_5OS^+$ 498.1150, found 498.1145.

4-(3-(1-Benzyl-1*H*-indol-3-yl)-5-((4-oxothiazolidin-2-ylidene)amino)-1*H*-pyrazol-1-yl)benzonitrile (6q): Light yellow solid; Yield 74%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.18 (s, 1H), 8.31 – 8.25 (m, 1H), 8.19 (d, J = 8.9 Hz, 1H), 8.14 (d, J = 12.7 Hz, 1H), 8.08 (s, 2H), 7.96 (d, J = 8.9 Hz, 1H), 7.54 (d, J = 7.1 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.28 (m, 3H), 7.23 –

7.14 (m, 2H), 6.60 (d, *J* = 9.0 Hz, 1H), 5.49 (s, 2H), 4.17 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 165.7, 161.1, 149.0, 148.6, 1470, 143.5, 138.4, 136.9, 133.6, 130.4, 129.1, 127. 127.6, 127.0, 125.9, 122.4, 122.3, 121.8, 120.6, 111.0, 108.7, 107.9, 94.2, 49.7, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{28}H_{21}N_6OS^+$ 489.1493, found 489.1490.

2-((3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-1-(2-ethylphenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6r): Light yellow solid; Yield 80%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.95 (s, 1H), 8.26 (d, *J* = 1.9 Hz, 1H), 7.94 (s, 1H), 7.49 (d, *J* = 8.7 Hz, 1H), 7.43 (d, *J* = 3.9 Hz, 2H), 7.35 (dt, *J* = 8.5, 4.0 Hz, 1H), 7.32 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 6.47 (s, 1H), 4.13 (s, 2H), 3.85 (s, 3H), 2.48 (q, *J* = 7.5 Hz, 2H), 1.07 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.2, 147.3, 146.9, 141.8, 138.3, 136.1, 130.0, 129.8, 129.3, 128.8, 127.2, 126.8, 124.5, 123.7, 112.8, 112.6, 108.6, 91.6, 35.0, 33.3, 24.5, 15.1 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{21}BrN_5OS^+$ 494.0645, found 494.0613.

2-((3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-1-(3-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6s): Light yellow solid; Yield 73%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (s, 1H), 8.35 (d, *J* = 1.9 Hz, 1H), 8.00 (s, 1H), 7.89 (t, *J* = 2.0 Hz, 1H), 7.84 (d, *J* = 7.1 Hz, 1H), 7.55 (t, *J* = 8.1 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.36 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.53 (s, 1H), 4.17 (s, 2H), 3.85 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.3, 147.8, 146.7, 141.0, 136.2, 133.6, 131.0, 130.7, 127.1, 126.2, 124.7, 123.7, 122.6, 121.5, 113.1, 112.7, 107.9, 93.6, 35.3, 33.4 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{16}BrClN_5OS^+$ 499.9942 and 501.9922, found 501.9914.

2-((3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6t): Light yellow solid; Yield 75%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.35 (d, *J* = 1.9 Hz, 1H), 7.99 (s, 1H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.58 (d, *J* = 8.9 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.35 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.52 (s, 1H), 4.16 (s, 2H), 3.85 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 161.0, 147.7, 146.5, 138.7, 136.2, 130.8, 130.6, 129.3, 127.1, 124.9, 124.7, 123.7, 113.1, 112.7, 108.0, 93.4, 35.3, 33.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{16}BrClN_5OS^+$ 499.9942 and 501.9922, found 501.9914.

4-(3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-5-((4-oxothiazolidin-2-ylidene)amino)-1*H*-pyrazol-1-yl)benzonitrile (6u): Light yellow solid; Yield 70%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (s, 1H), 8.37 (d, *J* = 1.8 Hz, 1H), 8.12 (d, *J* = 8.9 Hz, 2H), 8.04 (s, 1H), 7.98 (d, *J*

= 8.9 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.37 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.58 (s, 1H), 4.18 (s, 2H), 3.86 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 148.6, 147.3, 143.3, 136.3, 133.7, 131.2, 127.1, 124.8, 123.7, 122.7, 119.2, 113.3, 112.8, 108.2, 107.7, 100.0, 94.3, 35.6, 33.4 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{16}BrN_6OS^+$ 491.0285, found 491.0271.

2-((3-(5-Bromo-1-ethyl-1*H*-indol-3-yl)-1-(2-ethylphenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6v): Light yellow solid; Yield 87%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.94 (s, 1H), 8.26 (d, *J* = 2.0 Hz, 1H), 8.02 (s, 1H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.44 (d, *J* = 3.9 Hz, 2H), 7.35 (dt, *J* = 8.9, 4.3 Hz, 1H), 7.30 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 6.49 (s, 1H), 4.26 (q, *J* = 7.2 Hz, 2H), 4.13 (s, 2H), 2.48 (q, *J* = 7.5 Hz, 2H), 1.42 (t, *J* = 7.2 Hz, 3H), 1.06 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.2, 147.3, 147.0, 141.8, 138.3, 135.2, 129.8, 129.3, 128.8, 128.4, 127.3, 126.8, 124.4, 123.8, 112.7, 112.5, 108.8, 91.6, 41.1, 35.0, 24.5, 15.8, 15.1 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{24}H_{23}BrN_5OS^+$ 508.0802 and 510.0781, found 510.0795.

2-((3-(5-Bromo-1-ethyl-1*H*-indol-3-yl)-1-(3-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6w): Light yellow solid; Yield 81%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.16 (s, 1H), 8.35 (s, 1H), 8.06 (s, 1H), 7.89 (s, 1H), 7.84 (d, *J* = 8.1 Hz, 1H), 7.55 (dt, *J* = 8.1, 3.7 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.34 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.55 (s, 1H), 4.27 (q, *J* = 7.2 Hz, 2H), 4.16 (s, 2H), 1.43 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.3, 147.9, 146.7, 141.1, 135.2, 133.6, 131.0, 129.1, 127.3, 126.2, 124.6, 123.8, 122.5, 121.5, 113.0, 112.7, 108.2, 93.7, 41.2, 35.3, 15.8 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{18}BrClN_5OS^+$ 514.0099, found 514.0106.

2-((3-(5-Bromo-1-ethyl-1*H*-indol-3-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6x): Light yellow solid; Yield 86%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.36 (d, *J* = 1.9 Hz, 1H), 8.06 (s, 1H), 7.83 (d, *J* = 8.9 Hz, 2H), 7.58 (d, *J* = 8.9 Hz, 2H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.33 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.54 (s, 1H), 4.26 (q, *J* = 7.2 Hz, 2H), 4.16 (s, 2H), 1.42 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 161.0, 147.8, 146.5, 138.7, 135.2, 130.8, 129.3, 129.0, 127.2, 124.9, 124.6, 123.8, 113.0, 112.7, 108.3, 93.5, 41.2, 35.3, 15.8 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{18}BrClN_5OS^+$ 514.0099, found 514.0105.

4-(3-(5-Bromo-1-ethyl-1*H*-indol-3-yl)-5-((4-oxothiazolidin-2-ylidene)amino)-1*H*-pyrazol-1-yl)benzonitrile (6y): Light yellow solid; Yield 74%; ^{1}H NMR (500 MHz, DMSO-d_6): δ

12.19 (s, 1H), 8.38 (d, *J* = 1.9 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 3H), 7.98 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.7 Hz, 1H), 7.35 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.60 (s, 1H), 4.27 (q, *J* = 7.2 Hz, 2H), 4.18 (s, 2H), 1.43 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.7, 148.7, 147.2, 143.3, 135.3, 133.7, 129.6, 127.2, 124.8, 123.8, 122.7, 119.3, 113.2, 112.8, 108.1, 107.9, 94.4, 41.2, 35.4, 15.8 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{18}BrN_6OS^+$ 505.0441, found 505.0445.

2-((1-(2-Ethylphenyl)-3-(5-methoxy-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6z): Light yellow solid; Yield 80%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.93 (s, 1H), 11.17 (s, 1H), 7.79 (d, *J* = 2.6 Hz, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.36 – 7.30 (m, 2H), 7.26 (d, *J* = 7.4 Hz, 1H), 6.80 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.48 (s, 1H), 4.12 (s, 2H), 3.72 (s, 3H), 2.53 (q, *J* = 7.5 Hz, 2H), 1.10 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.0, 154.2, 147.9, 147.1, 141.8, 138.4, 132.1, 129.8, 129.1, 128.8, 126.7, 125.7, 124.9, 112.7, 111.8, 109.6, 103.3, 91.7, 55.8, 35.0, 24.6, 15.2 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{22}N_5O_2S^+$ 432.1489, found 432.1510.

2-((1-(3-Chlorophenyl)-3-(5-methoxy-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6aa): Light yellow solid; Yield 72%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (s, 1H), 11.26 (d, *J* = 2.2 Hz, 1H), 7.96 (t, *J* = 2.0 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 2.7 Hz, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 7.53 (t, *J* = 8.1 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 6.83 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.54 (s, 1H), 4.16 (s, 2H), 3.82 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.1, 154.3, 148.9, 146.6, 141.3, 133.6, 132.1, 131.0, 125.8, 125.7, 125.7, 122.0, 120.9, 112.8, 111.9, 108.8, 103.4, 93.8, 55.7, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{17}ClN_5O_2S^+$ 438.0786, found 438.0805.

2-((1-(4-Chlorophenyl)-3-(5-methoxy-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6ab): Light yellow solid; Yield 82%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 11.24 (d, *J* = 2.2 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 2H), 7.85 (d, *J* = 2.6 Hz, 1H), 7.73 (d, *J* = 2.5 Hz, 1H), 7.56 (d, *J* = 8.9 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 1H), 6.83 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.52 (s, 1H), 4.16 (s, 2H), 3.81 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 160.8, 154.3, 148.7, 146.3, 138.9, 132.2, 130.4, 129.2, 125.7, 125.6, 124.4, 112.7, 111.9, 108.9, 103.6, 93.6, 55.8, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{21}H_{17}ClN_5O_2S^+$ 438.0786, found 438.0794.

2-((1-(2-Ethylphenyl)-3-(5-methoxy-1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6ac): Light yellow solid; Yield 85%; ^{1}H NMR (500 MHz,

DMSO-d_6): δ 11.93 (s, 1H), 7.79 (s, 1H), 7.60 (d, J = 2.5 Hz, 1H), 7.41 (m, 2H), 7.38 (d, J = 8.9 Hz, 1H), 7.34 (ddd, J = 8.8, 6.1, 2.9 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 6.86 (dd, J = 8.8, 2.5 Hz, 1H), 6.44 (s, 1H), 4.12 (s, 2H), 3.81 (s, 3H), 3.73 (s, 3H), 2.54 (q, J = 7.5 Hz, 2H), 1.09 (t, J = 7.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 160.0, 154.4, 147.5, 147.2, 141.8, 138.3, 132.8, 129.8, 129.1, 129.0, 128.8, 126.7, 126.1, 111.8, 111.1, 108.5, 103.6, 91.6, 55.8, 35.0, 33.2, 24.6, 15.2 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{24}H_{24}N_5O_2S^+$ 446.1646, found 446.1663.

2-((1-(3-Chlorophenyl)-3-(5-methoxy-1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6ad): Light yellow solid; Yield 78%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.21 (s, 1H), 7.95 (t, J = 2.0 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.87 (s, 1H), 7.74 (d, J = 2.5 Hz, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 6.90 (dd, J = 8.8, 2.5 Hz, 1H), 6.50 (s, 1H), 4.16 (s, 2H), 3.83 (s, 3H), 3.82 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.2, 154.6, 148.5, 146.7, 141.3, 133.6, 132.8, 131.0, 129.7, 126.0, 125.8, 122.1, 121.0, 111.9, 111.2, 107.7, 103.6, 93.7, 55.7, 35.3, 33.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{22}H_{19}ClN_5O_2S^+$ 452.0943, found 452.0947.

2-((3-(1-Ethyl-5-methoxy-1*H*-indol-3-yl)-1-(2-ethylphenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6ae): Light yellow solid; Yield 84%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.93 (s, 1H), 7.85 (s, 1H), 7.60 (d, J = 2.5 Hz, 1H), 7.44 – 7.38 (m, 3H), 7.34 (ddd, J = 8.9, 6.0, 2.9 Hz, 1H), 7.26 (d, J = 7.5 Hz, 1H), 6.85 (dd, J = 8.9, 2.5 Hz, 1H), 6.45 (s, 1H), 4.22 (q, J = 7.2 Hz, 2H), 4.12 (s, 2H), 3.73 (s, 3H), 2.54 (q, J = 7.5 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), 1.09 (t, J = 7.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.8, 159.9, 154.3, 147.6, 147.1, 141.8, 138.3, 131.8, 129.8, 129.1, 128.8, 127.4, 126.7, 126.2, 111.7, 111.1, 108.7, 103.8, 91.6, 55.9, 41.0, 35.0, 24.5, 15.9, 15.2 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{25}H_{26}N_5O_2S^+$ 460.1802, found 460.1806.

2-((1-(3-Chlorophenyl)-3-(1-ethyl-5-methoxy-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6af): Light yellow solid; Yield 79%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.21 (s, 1H), 7.95 (t, J = 2.0 Hz, 1H), 7.94 (s, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.74 (d, J = 2.5 Hz, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.45 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 6.88 (dd, J = 8.9, 2.5 Hz, 1H), 6.52 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.17 (s, 2H), 3.83 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 161.1, 154.5, 148.5, 146.6, 141.3, 133.6, 131.8, 131.0, 128.1, 126.2, 125.8, 122.0, 121.0, 111.8, 111.2, 108.0, 103.8, 93.7,

55.7, 41.1, 35.3, 15.9 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{21}ClN_5O_2S^+$ 466.1099, found 466.1102.

2-((1-(4-Chlorophenyl)-3-(1-ethyl-5-methoxy-1*H*-indol-3-yl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6ag): Light yellow solid; Yield 82%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.09 (s, 1H), 7.92 – 7.85 (m, 3H), 7.73 (s, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.9 Hz, 1H), 6.88 (dd, J = 8.9, 2.2 Hz, 1H), 6.50 (s, 1H), 4.22 (q, J = 7.1 Hz, 2H), 4.16 (s, 2H), 3.82 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.0, 160.8, 154.5, 148.4, 146.4, 138.9, 131.8, 130.4, 129.2, 127.9, 126.2, 124.4, 111.8, 111.2, 108.1, 103.9, 93.5, 55.8, 41.1, 35.3, 15.9 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{23}H_{21}ClN_5O_2S^+$ 466.1099, found 466.1101.

2-((3-(1-Benzyl-5-methoxy-1*H*-indol-3-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-5-yl)imino)thiazolidin-4-one (6ah): Light yellow solid; Yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.04 (s, 1H), 7.88 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 2.5 Hz, 1H), 7.56 (d, J = 8.9 Hz, 2H), 7.42 (d, J = 8.9 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.26 (t, J = 6.9 Hz, 3H), 6.84 (dd, J = 8.9, 2.5 Hz, 1H), 6.52 (s, 1H), 5.44 (s, 2H), 4.16 (s, 2H), 3.80 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 160.9, 154.6, 148.2, 146.4, 138.9, 138.5, 132.1, 130.5, 129.2, 129.0, 129.0, 127.9, 127.5, 126.4, 124.5, 112.0, 111.7, 108.6, 104.0, 93.5, 55.8, 49.9, 35.3 ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calculated $C_{28}H_{23}ClN_5O_2S^+$ 528.1256, found 528.1254.

3. Pharmacological evaluations

3.1. Cell culture: All selected cancer cell lines, colon (HCT116), melanoma (SK-MEL-28), lung (A549), mouse melanoma (B-16-F10), and normal human bronchial epithelium cell line (BEAS-2B) were maintained in appropriate media supplemented with 10% fetal bovine serum (FBS) stabilized with 1% antibiotic–antimycotic solution (Sigma Aldrich) and cells were maintained at 37 °C with 5% CO_2 and 98% relative humidity in the incubator. When the cells reach up to 80-90% of confluency, sub-culturing was performed using a 0.25% trypsin/1 mM EDTA solution for further passage. The stock solution of 10 mM was prepared by dissolving test compounds and standards in DMSO. Suitable dilutions further obtain required concentrations with respective media.

3.2. MTT assay: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay is a colorimetric assay that measures the reduction in its quantity. Realistically, MTT forms insoluble formazan by mitochondrial succinate dehydrogenase enzyme in metabolically

active cells, and the activity level indicates the cells' viability. Briefly, cells were seeded in 96-well plates at a density of 3000 to 4000 cells per well in 100 µL of complete medium and allowed to grow overnight. Then after the media was replaced, and cells were treated with various concentrations of the compounds for a period of 48 h. After incubation, the media was aspirated, and 100 µL of MTT (0.5 mg/mL) was added and incubated at 37 °C for 3-4 h. Then MTT reagent was aspirated, and the formazan crystals formed were dissolved by the addition of DMSO. The formazan product quantity was measured using a spectrophotometric microtiter plate reader (Spectra Max, M4 Molecular Devices, USA) at 570 nm wavelength.

3.3. Phase contrast microscopy: SK-MEL-28 cells were plated in 12 well culture plates with a cell density of 1×10^6 cells/mL and allowed to adhere overnight. The cells were incubated with different concentrations of the compound **6c** for 48 h of treatment. Later cells were checked for the morphological changes, such as cell wall deformation, cell shrinkage and number of viable cells, and images were taken using microscope under bright field at 20X magnification (Nikon, Inc. Japan).

3.4. Apoptosis detection assays

***Acridine orange/Ethidium bromide (AO/EB) staining*:** AO/EB staining is used to visualize the characteristic features of cells undergoing apoptosis and to differentiate live and dead cells. SK-MEL-28 cells were plated at a concentration of 1×10^6 cells/mL and treated with various concentrations of compound **6c**, and the plates were incubated for 48 h. Then, fluorescent dye, acridine orange (AO), and ethidium bromide (EB) were added (10 µg/ml). The cells were visualized immediately under a fluorescence microscope (Nikon, Inc. Japan) with excitation (488 nm) and emission (550 nm) at 20X magnification.

***4,6-Diamidino-2-phenylindole (DAPI) staining*:** DAPI staining aids in observing apoptotic changes in the nucleus. After treatment with compound **6c** for 48 h, melanoma SK-MEL-28 cells were washed with PBS, fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X for 15 minutes, followed by staining with 10 µg/ML DAPI. Control and treated cells were observed using fluorescence microscope with excitation at 359 nm and emission at 461 nm applying DAPI filter at 20X magnification.

***2',7'-Dichlorofluorescein diacetate (DCFDA) staining*:** DCFDA is a fluorogenic dye which oxidize to highly fluorescent 2′,7′-dichlorofluorescein (DCF) under the influence of ROS and emits green fluorescence. SK-MEL-28 cells were plated at a concentration of 1 x 10^6 cells/well in a 12 well plate, treated with different concentrations of compound **6c**, and incubated at 37

°C in a CO_2 incubator for 48 h. This was followed by the addition of DCFDA (10 µM) for 30 minutes at 37 °C. The excess dye was washed with PBS, and the images were captured under a fluorescent microscope (Nikon, Inc. Japan) at 20X magnification with excitation and emission wavelength of 498 nm and 530 nm, respectively.

3.5. Flow cytometric analysis

***Annexin V-FITC/PI dual assay*:** Briefly, 1 x 10^6 SK-MEL-28 cells were seeded in a 30 mm petri-plate and subjected to the treatment using various concentrations of compound **6c** for 48 h. After incubation, supernatant was collected, untreated and treated cells were trypsinized using trypsin-EDTA, followed by twice wash with ice-cold PBS. Then Untreated and treated cells were processed with Annexin V-FITC and Propidium Iodide (PI) staining using Apoptosis Detection Kit (Invitrogen) as per the manufacturer's instructions. Initially cells were resuspended in 100 µL of 1x binding buffer and incubated for 15 min at room temperature in the dark after adding 5 µL of FITC-conjugated annexin V and 1 µL propidium iodide (PI). Next 400 µL of 1x binding buffer was added and were kept on ice and analyzed immediately for apoptosis cell population using a flow cytometer (BD FACSVerseTM, USA).

***Cell-cycle analysis*:** The SK-MEL-28 cells were incubated with compound **6c** at various concentrations for 48 h. Untreated and treated cells were harvested, washed and fixed overnight in 70% ethanol in PBS at 2-8 °C. Fixed cells were pelleted and processed using Cell Cycle Analysis Kit (Sigma-Aldrich). As per the manufacturer's protocol pellet was washed in 1x cell cycle buffer and further incubated in 1x cell cycle buffer containing Enzyme A and nuclear dye for 30 min at room temperature in dark and about 10,000 events were acquired and analysed on a flow cytometer (BD FACSVerseTM, US).

3.6. Tubulin inhibition assay

To examine the effect of compound **6c** on tubulin inhibition, a fluorescence-based *in vitro* tubulin polymerization assay was executed based on the manufacturer's protocol (Cytoskeleton, Inc. BK011). The reagents and buffers were reconstituted as per the manuals instructions and were stored under provided storage conditions. Required 10x stock solution of compound was prepared and the reagent mix buffer containing tubulin glycerol buffer, GTP stock and tubulin stock were mixed as per the required quantity for blank, different concentrations of compound **6c**, enhancer and inhibitor reference standards. Before adding the samples pre-warm the plate at 37 °C for 1min and then add reagent mix to the samples followed

by reading the plate immediately for 1hr at provided specification with fluorescence wavelength of 360 nm excitation and 450 nm emission

3.7. Metastasis (cell migration) assay

SK-MEL-28 cells were plated at a cell density of 3.5×10^5 cells/well into 12-well plates in RPMI medium and allowed to form a confluent monolayer, and the scratches were made with 200 µL sterile pipette tip and then treated with different concentration of compound **6c**. The migration of cells was captured using a Zeiss microscope at 0 h and 48 h. Quantification of the wound area was done by imageJ via the plugin wound healing tool and the % wound area was calculated by using the formula

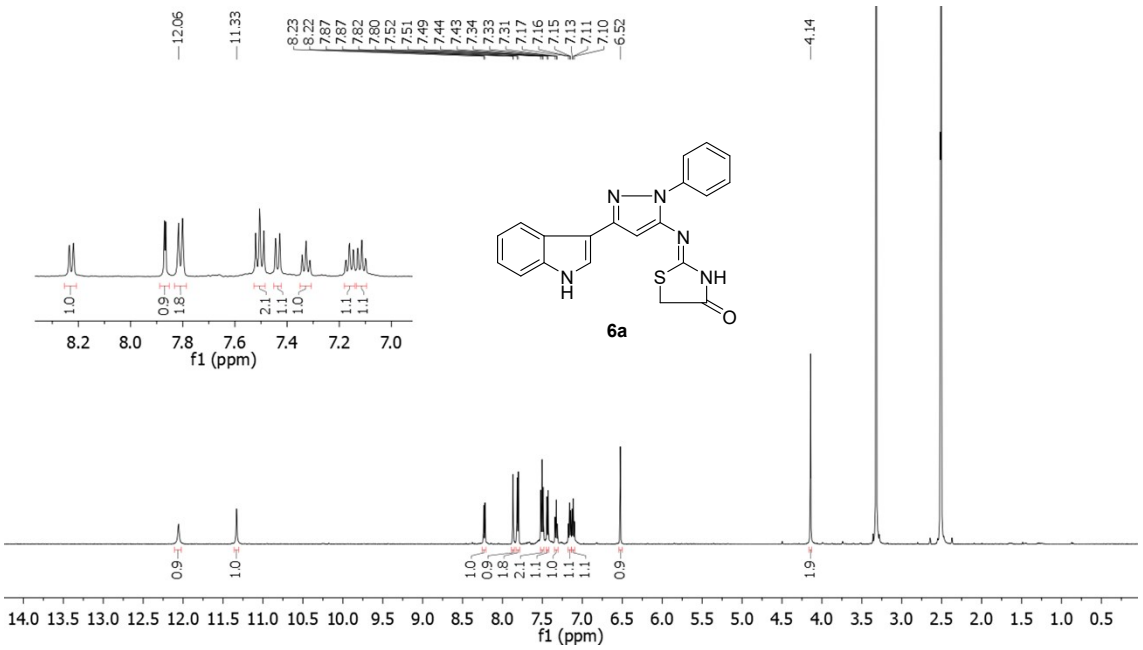
$$\%\, Wound\ Area\ = \frac{Area\ T0\ -\ Area\ T48}{Area\ T0}\, x100$$

where T = time.

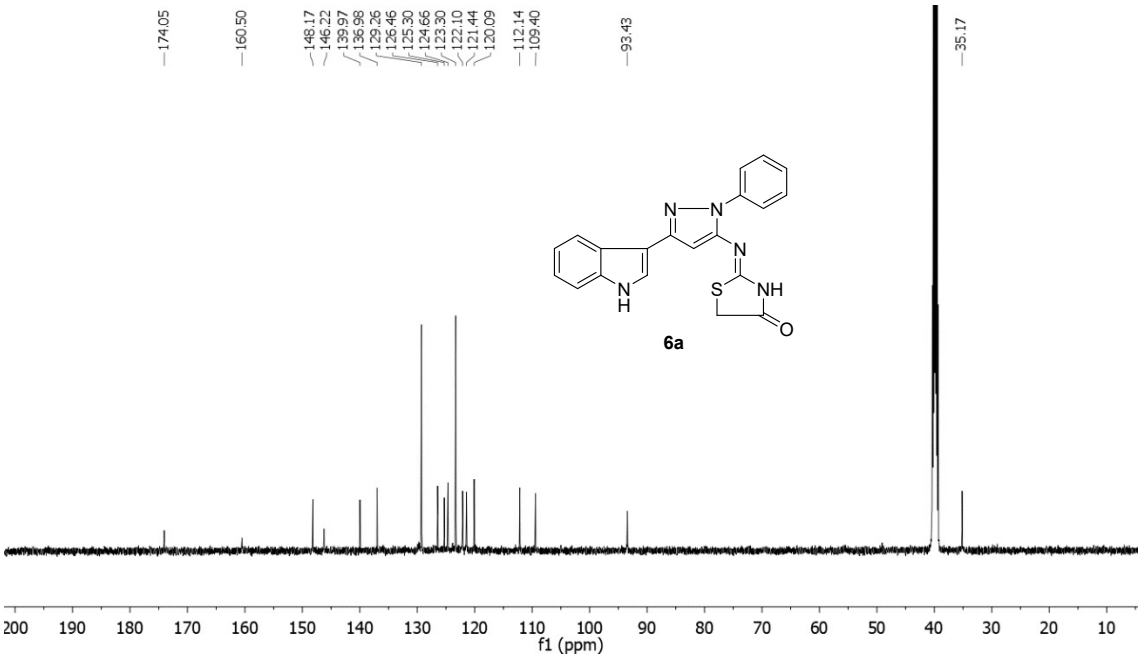
3.8. *In-silico* molecular docking analysis, MD simulation, Drug likeness, and ADMET Profiling

The crystal structure of the human tubulin in complex with colchicine was retrieved from Protein Data Bank (PDB ID: 4O2B). The protein preparation tool in Schrodinger suite 20.3 was used to prepare protein structure. The missing atoms were added, peripheral water molecules were removed at a distance of less than 5 Å from the binding pocket, and the structure was energetically minimized through the protein preparation wizard. The grid was generated by picking the active site where the co-crystal locates with a grid box with an external diameter of 15Å and an internal diameter of 10Å. The potent hybrid **6c** was drawn using 2D sketcher and subjected to energy minimization, followed by ligand preparation for the generation of different conformers using the LigPrep module of the Schrodinger 20.3. The different conformers thus obtained were subjected to molecular docking with Glide in standard precision (SP) mode. The poses generated were evaluated, and the best-ranked pose was described. Furthermore, the lowest energy pose for the compound **6c** was selected, and the docked complex was further optimized using molecular dynamics simulations using Desmond with OPLS-AA force field in an explicit solvent with the TIP3P water model. Before MD simulations, the systems were minimized and pre-equilibrated using the default relaxation routine implemented in Desmond. The complexes present in the trajectory file after the production phase of MD simulations were clustered according to the RMSD of the backbone. The RMSD plot was obtained from the simulation event analysis of Desmond. Additionally,

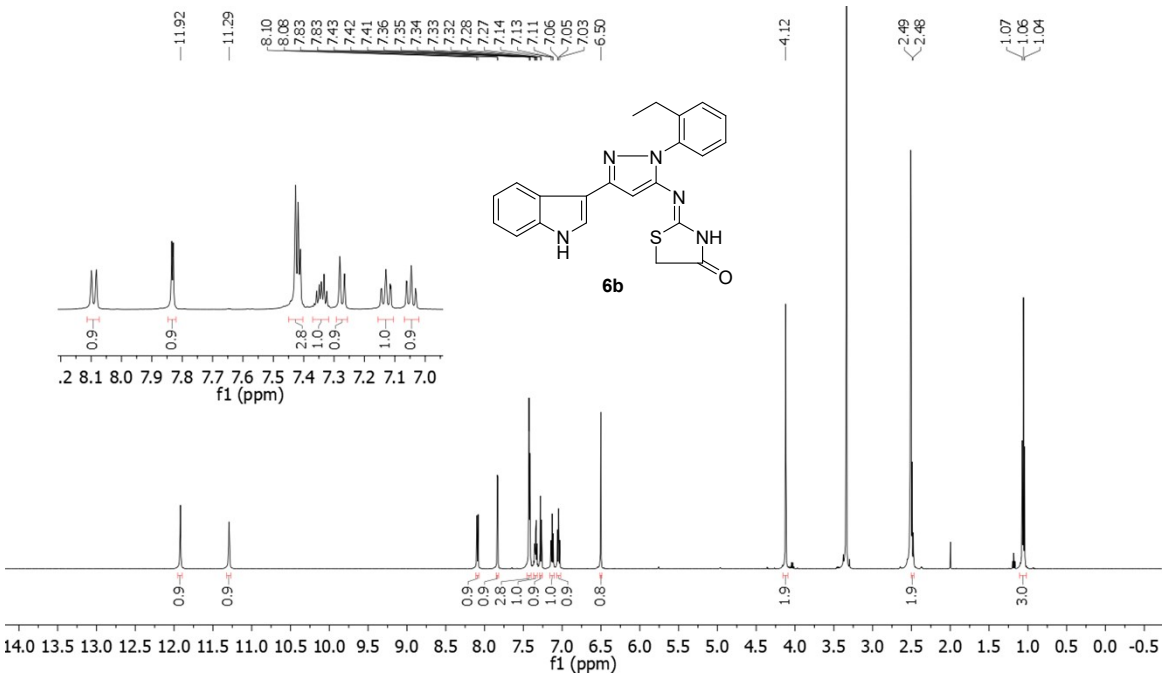
the different conformers of ligand **6c** were envisaged for their physicochemical parameters using QikProp module of the Schrodinger suite 20.3. The different descriptors were thus reported.



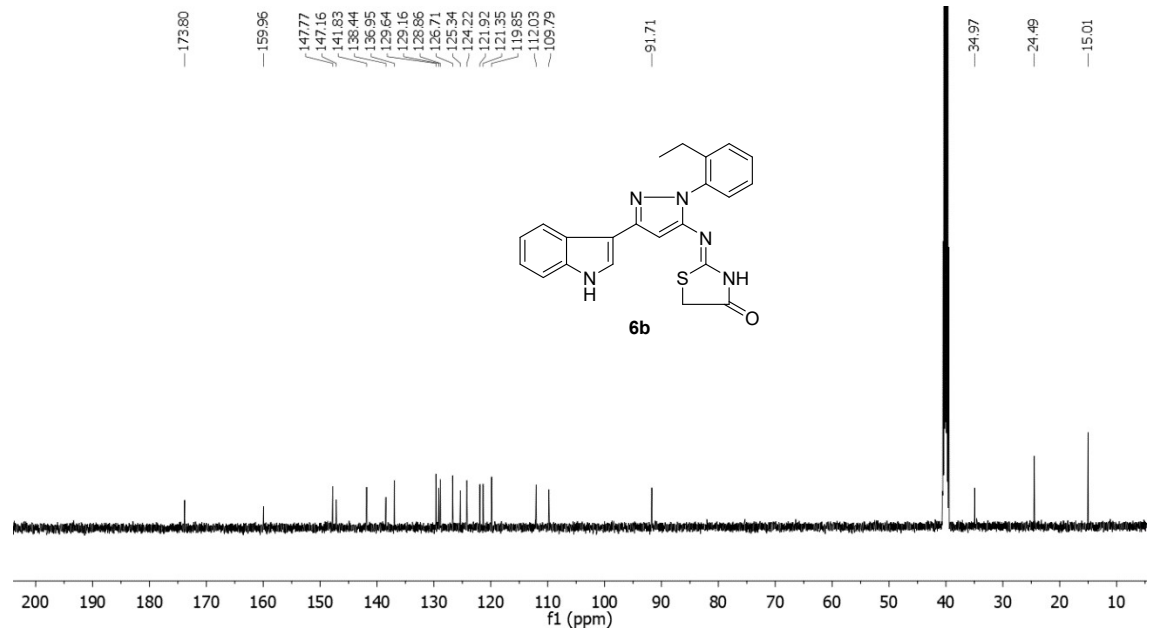
[1]H NMR spectra of 6a (500 MHz, DMSO-d_6)



[13]C NMR spectra of 6a (125 MHz, DMSO-d_6)



^{1}H NMR spectra of 6b (500 MHz, DMSO-d_6)



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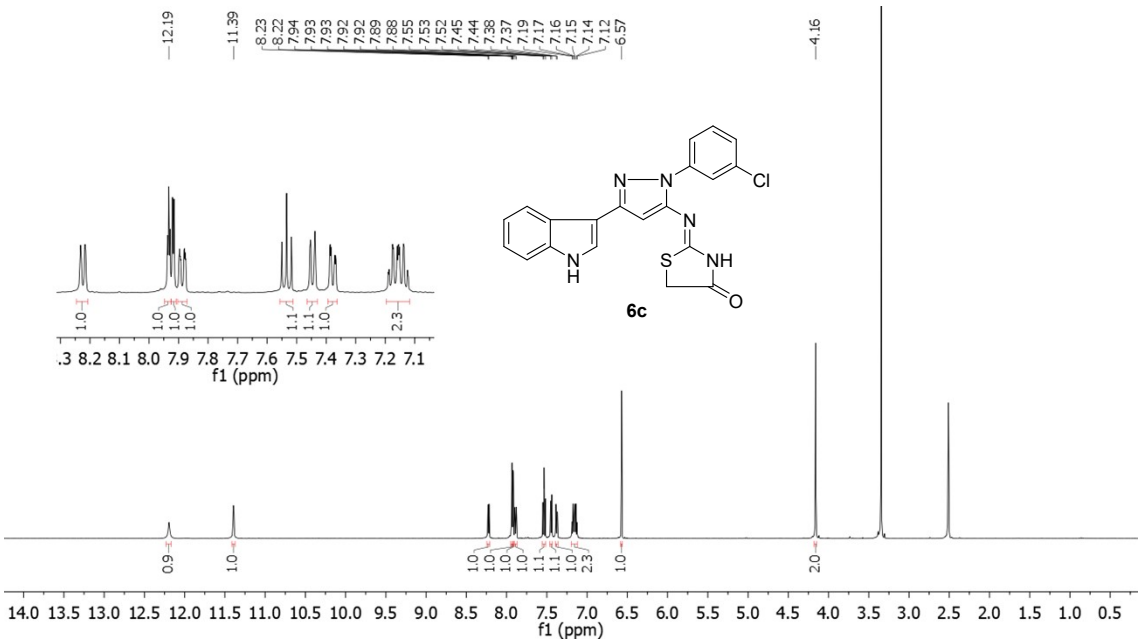
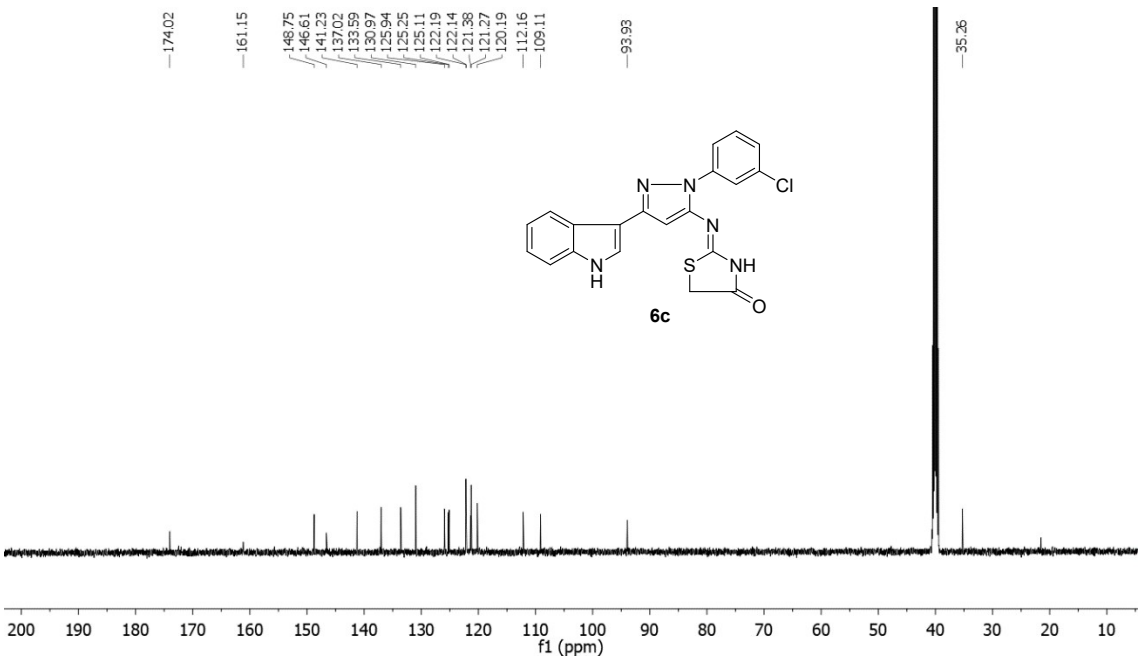
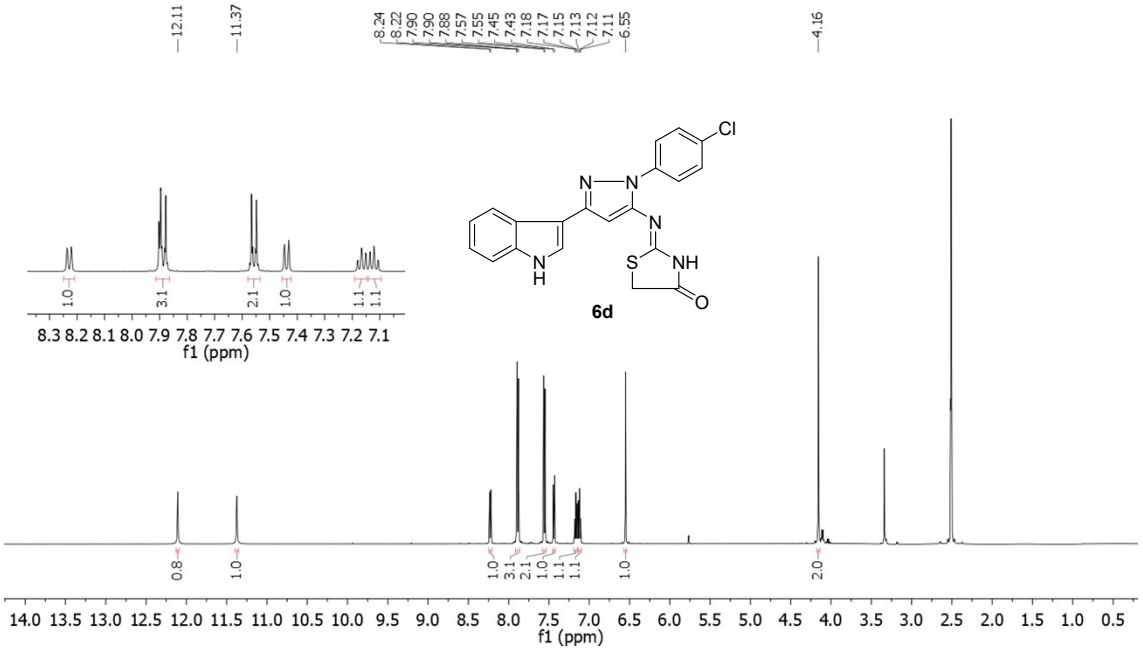


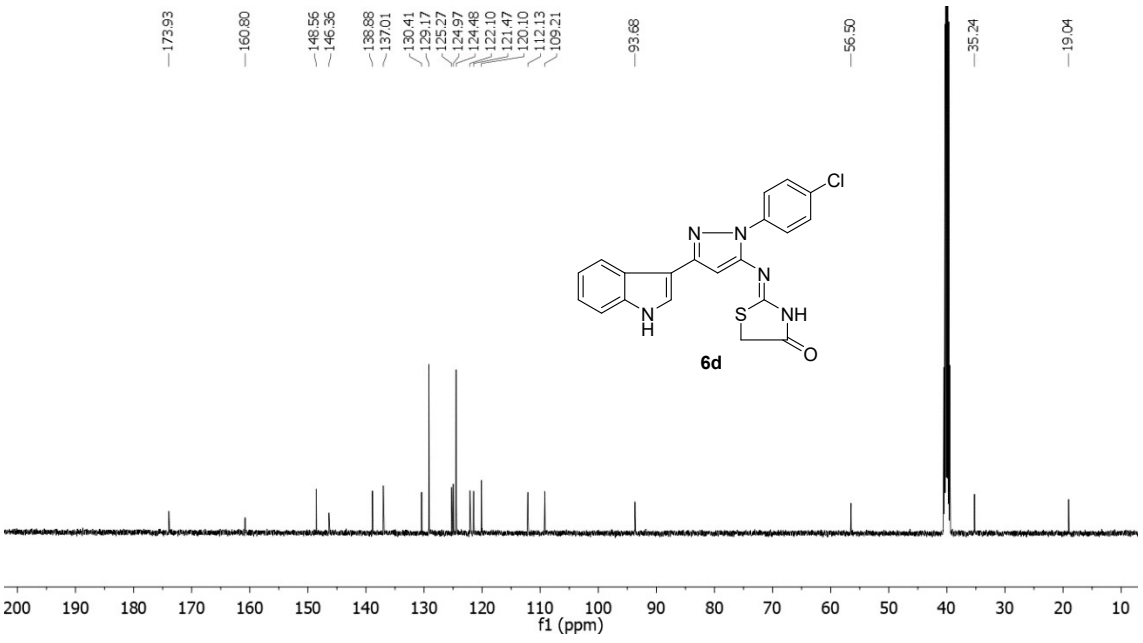
^{1}H NMR spectra of 6c (500 MHz, DMSO-d_6)



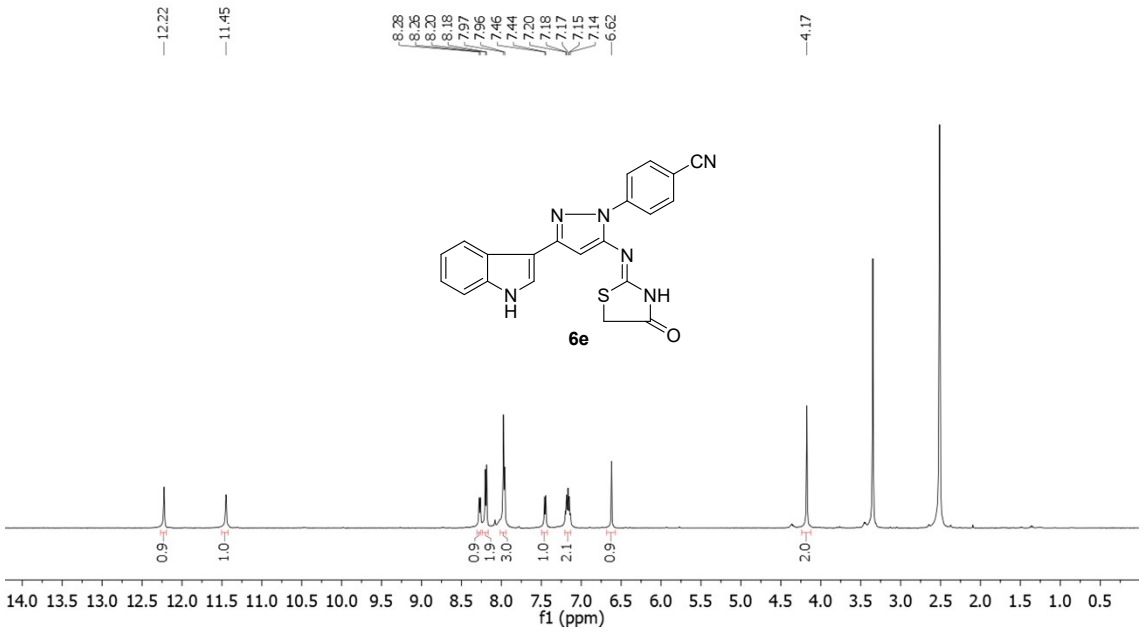
^{13}C NMR spectra of 6c (125 MHz, DMSO-d_6)



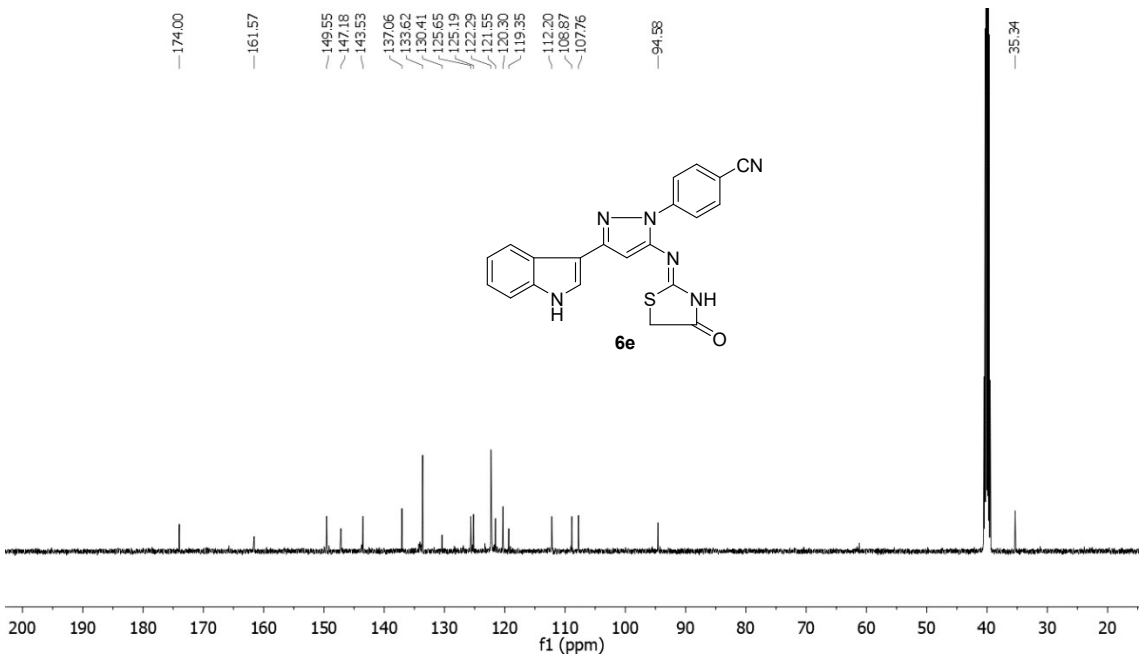
^{1}H NMR spectra of 6d (500 MHz, DMSO-d_6)



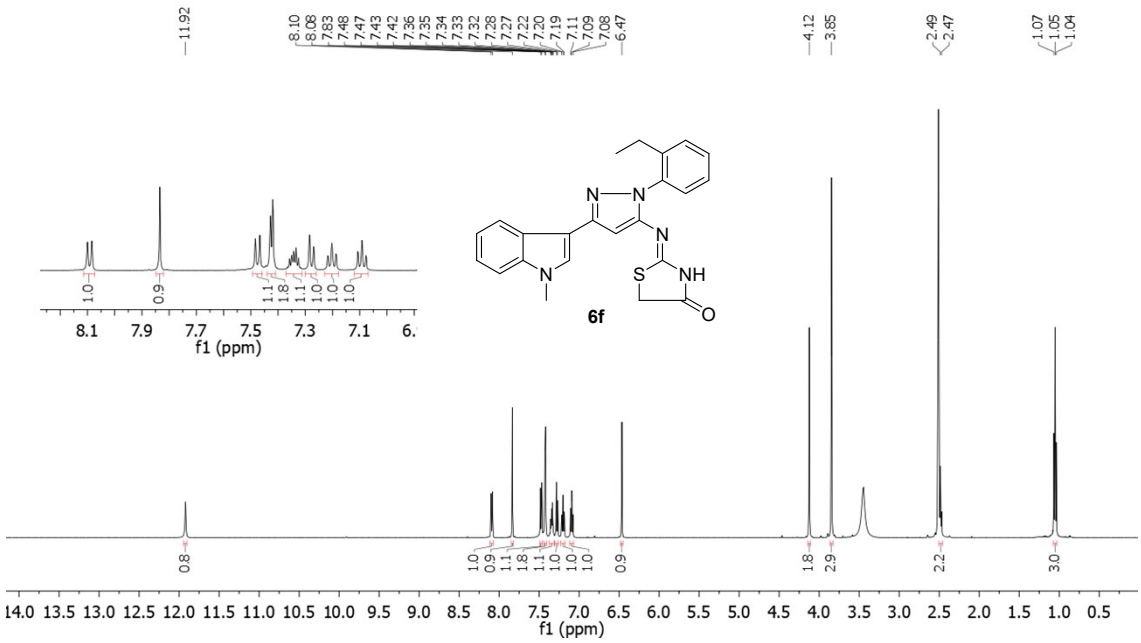
^{13}C NMR spectra of 6d (125 MHz, DMSO-d_6)



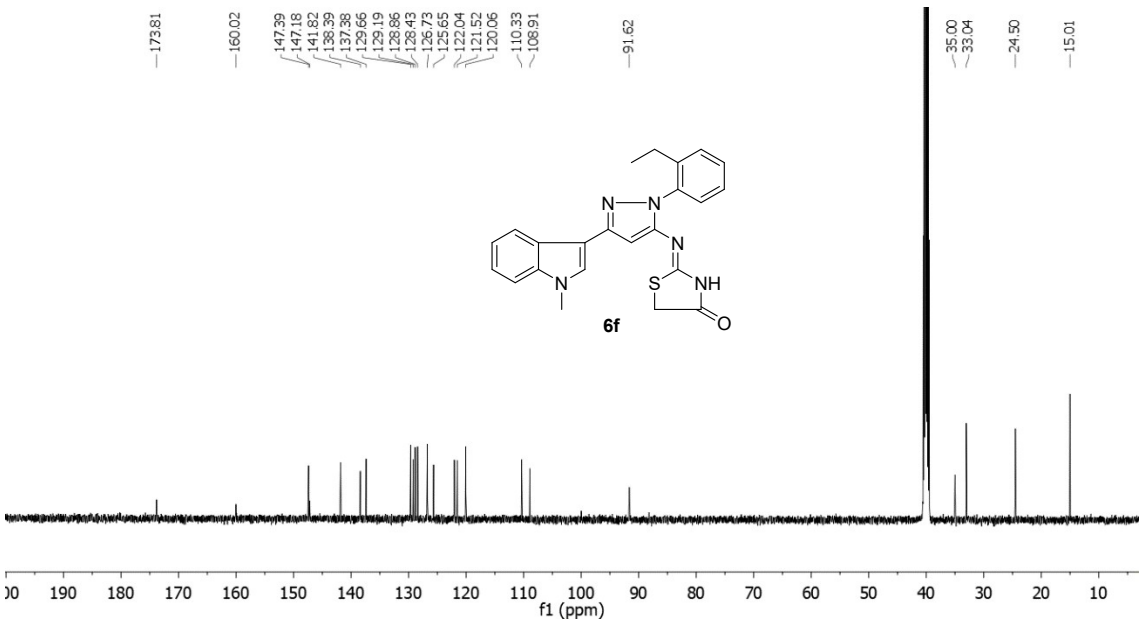
[1]H NMR spectra of 6e (500 MHz, DMSO-d_6)



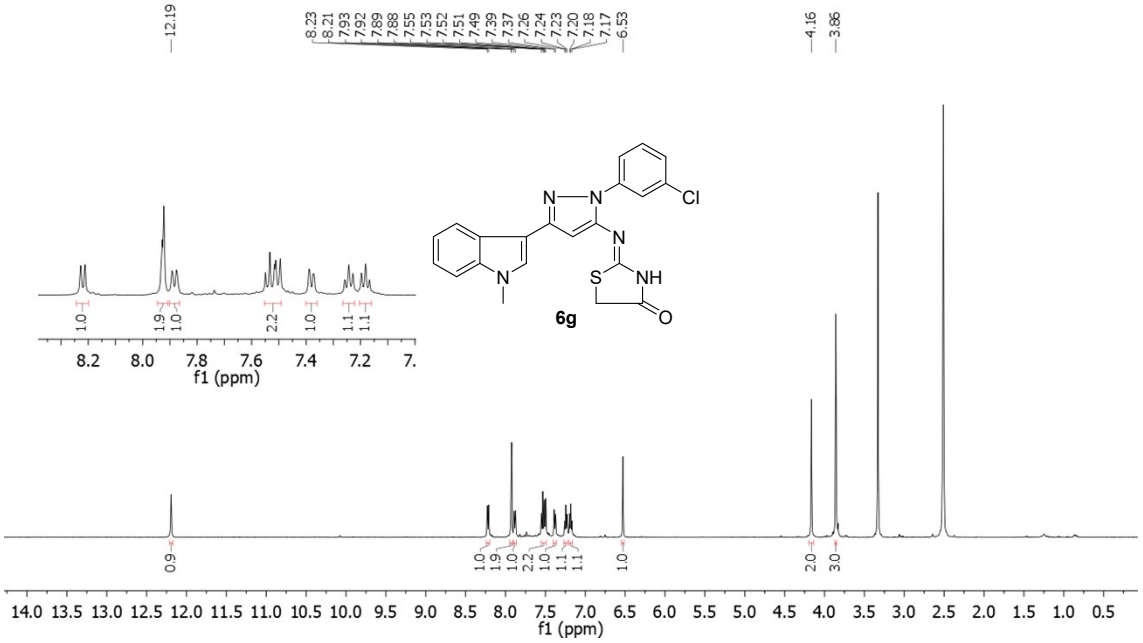
[13]C NMR spectra of 6e (125 MHz, DMSO-d_6)



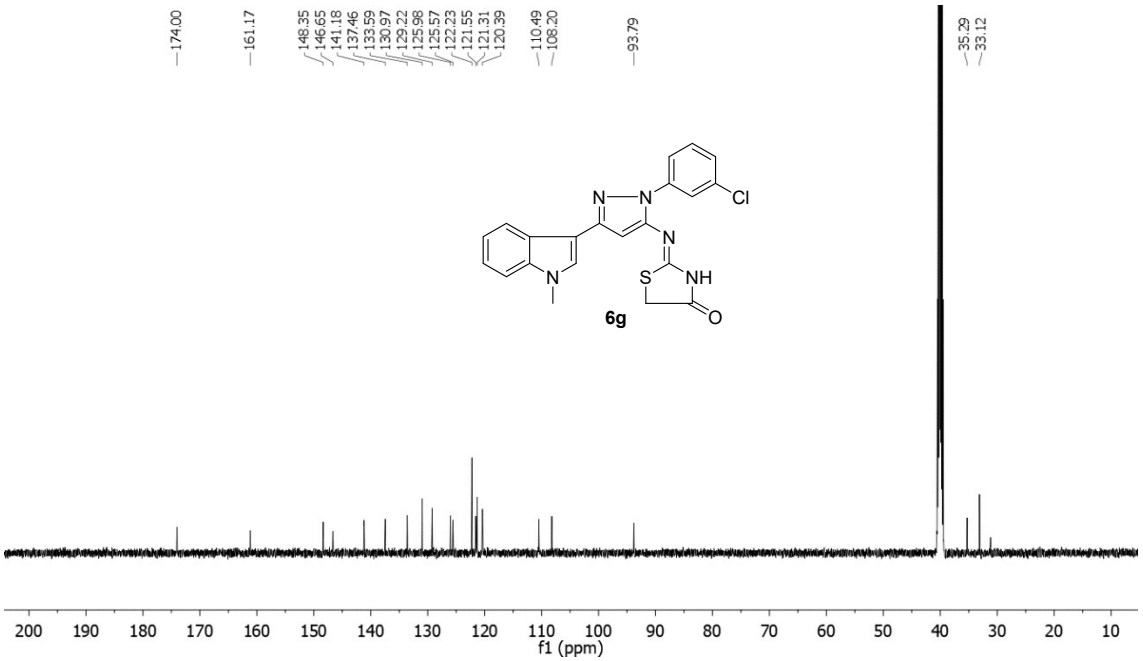
[1]H NMR spectra of 6f (500 MHz, DMSO-d_6)



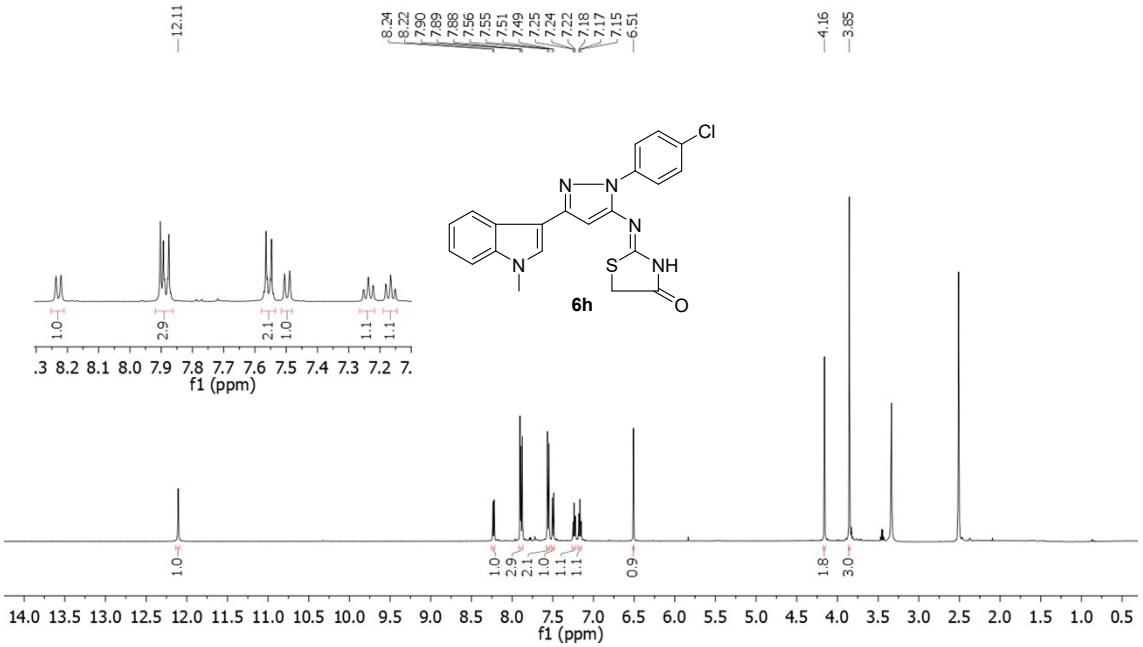
[13]C NMR spectra of 6f (125 MHz, DMSO-d_6)



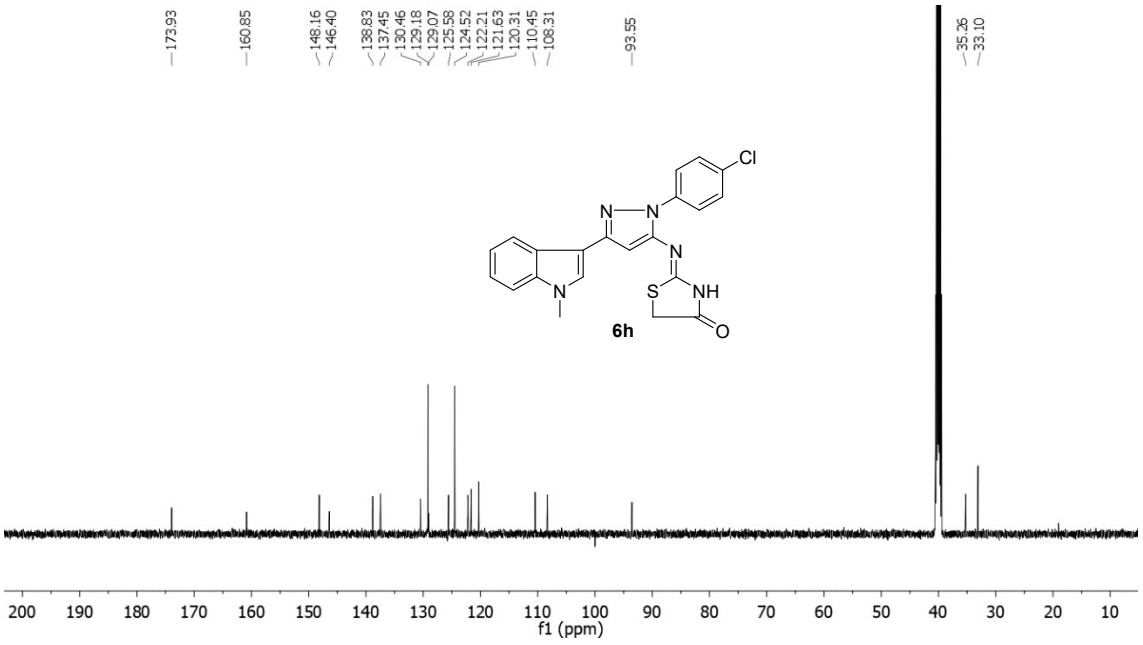
^{1}H NMR spectra of 6g (500 MHz, DMSO-d_6)



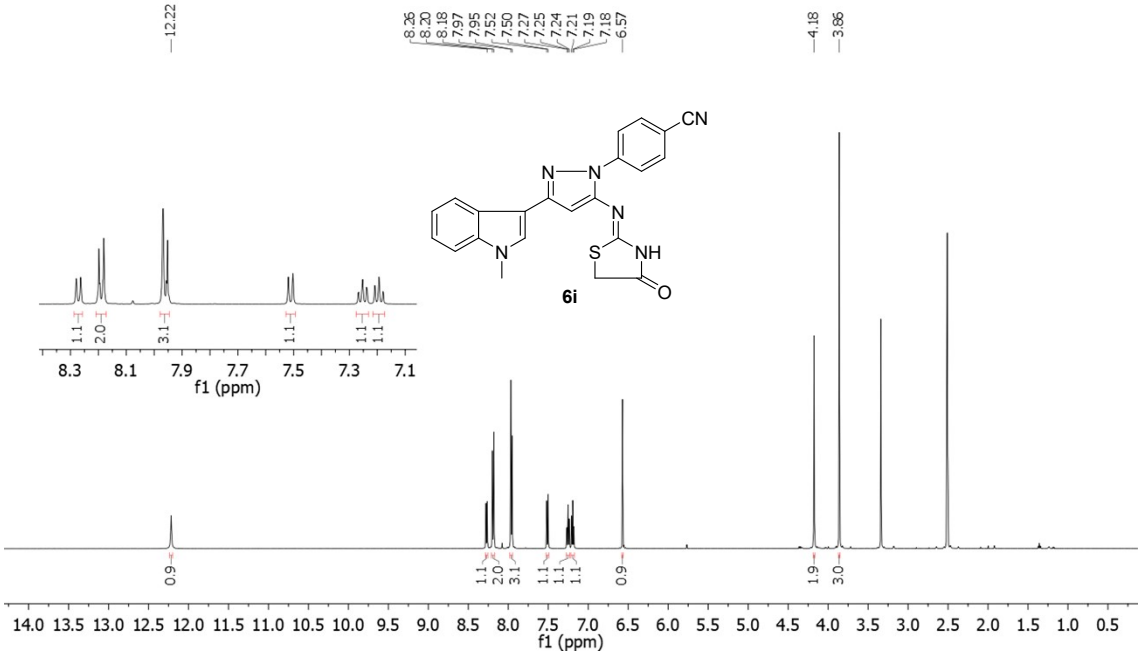
^{13}C NMR spectra of 6g (125 MHz, DMSO-d_6)



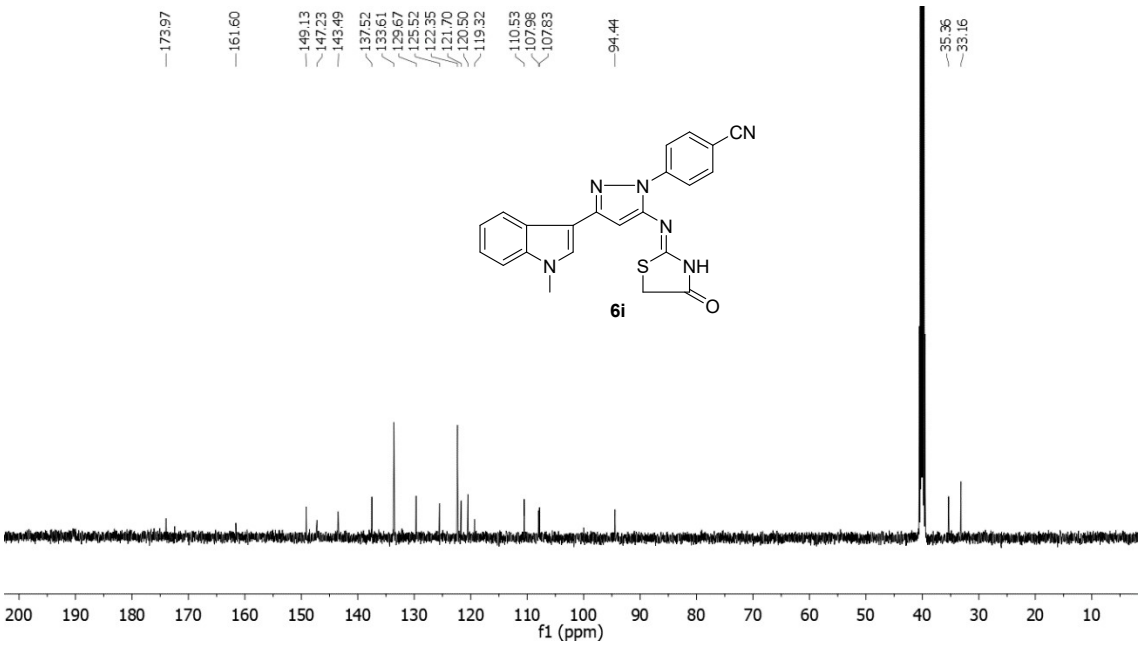
^{1}H NMR spectra of 6h (500 MHz, DMSO-d_6)



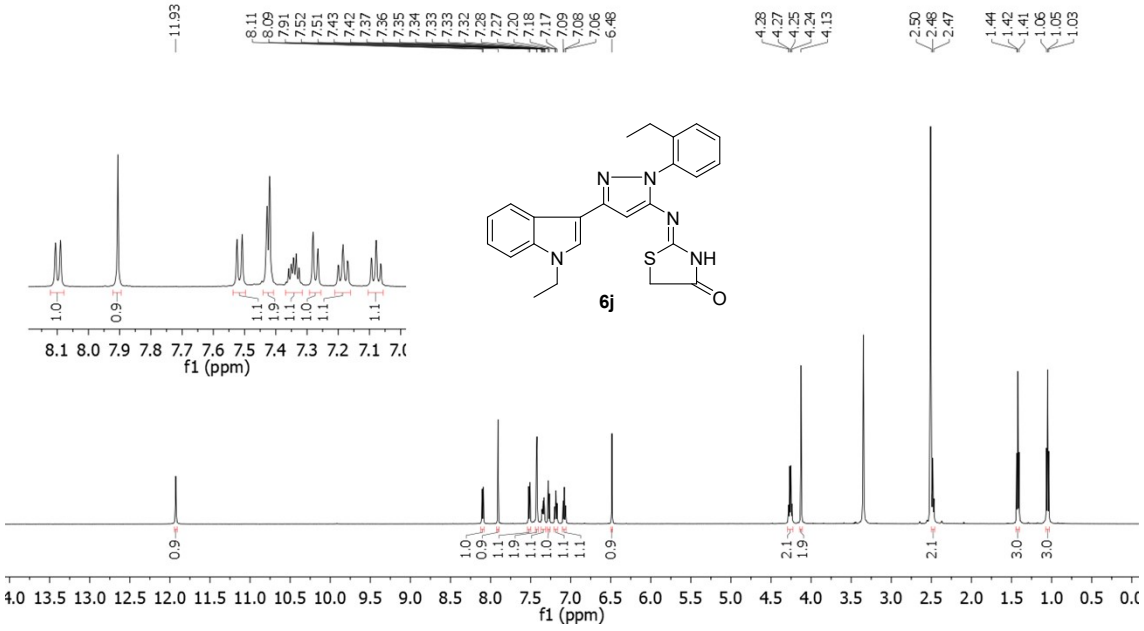
^{13}C NMR spectra of 6h (125 MHz, DMSO-d_6)



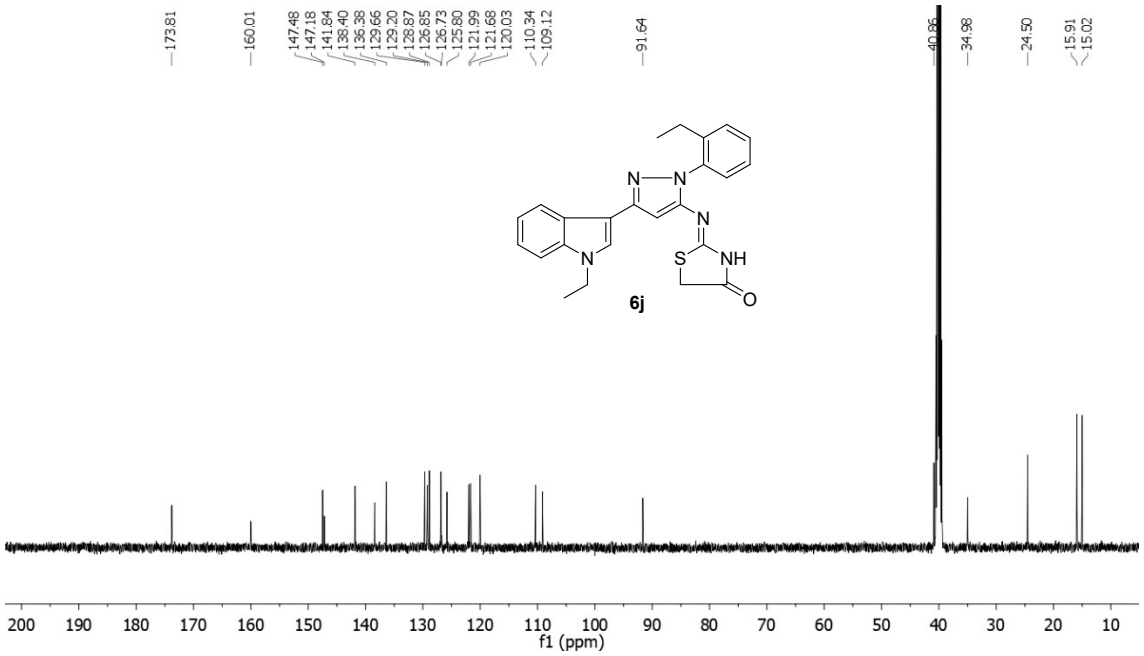
[1]H NMR spectra of 6i (500 MHz, DMSO-d_6)



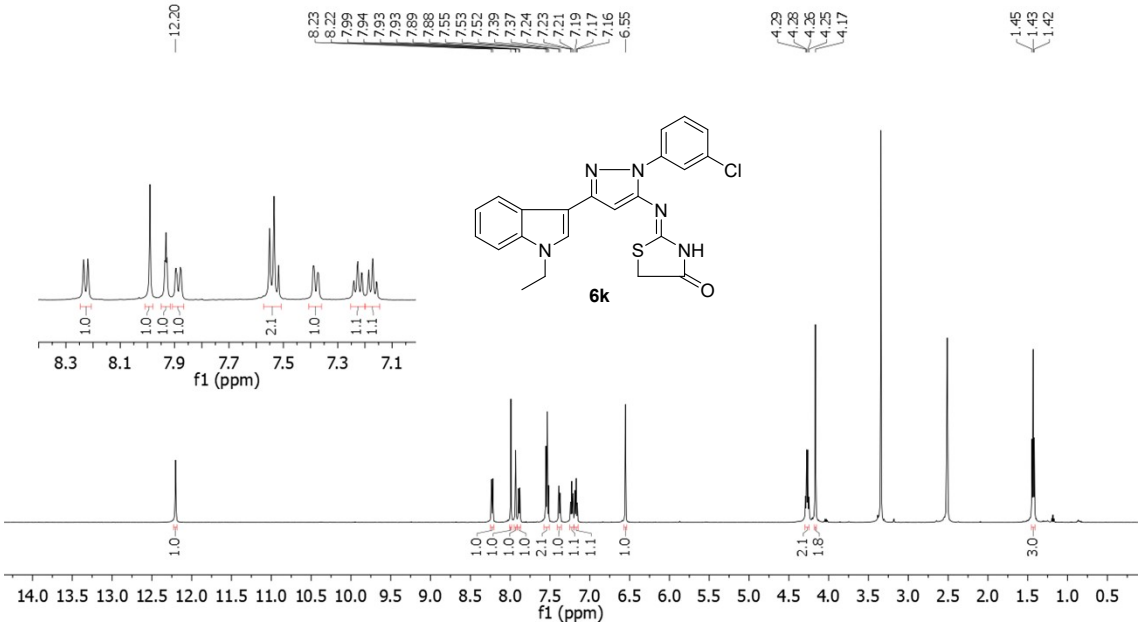
[13]C NMR spectra of 6i (125 MHz, DMSO-d_6)



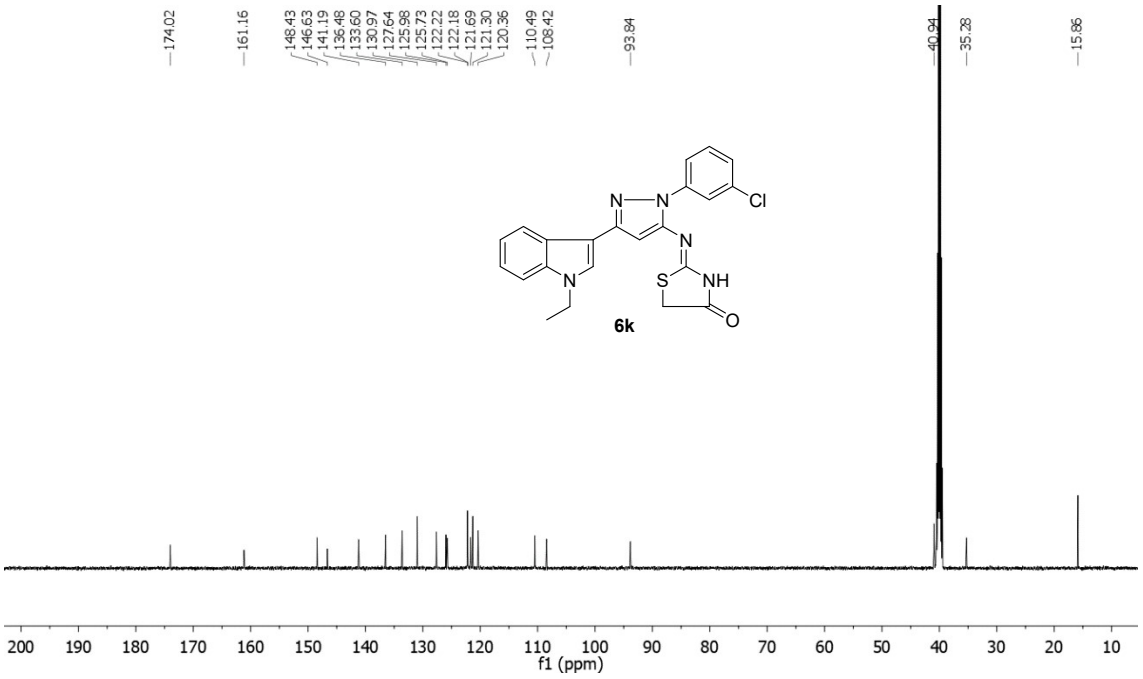
^{1}H NMR spectra of 6j (500 MHz, DMSO-d_6)



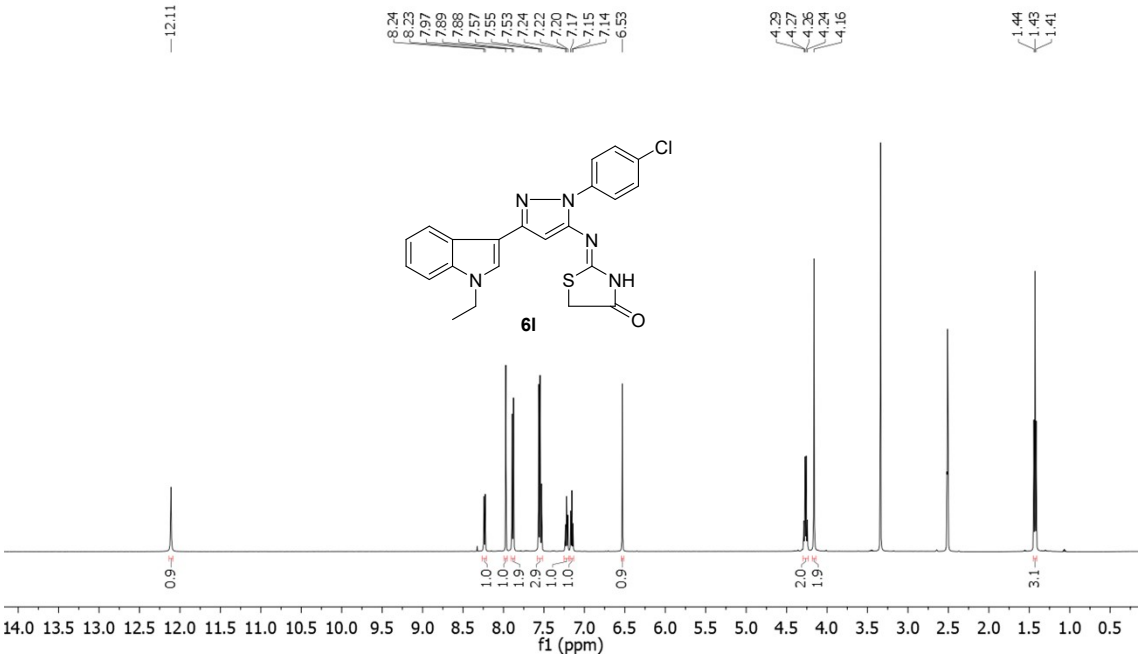
^{13}C NMR spectra of 6j (125 MHz, DMSO-d_6)



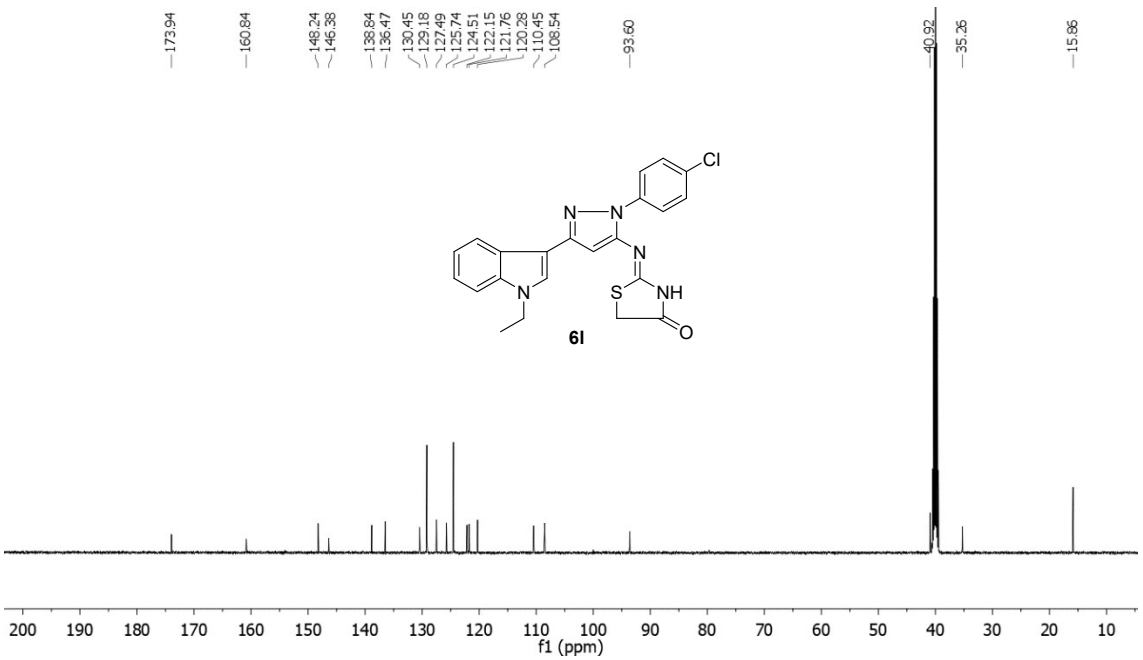
[1]H NMR spectra of 6k (500 MHz, DMSO-d_6)



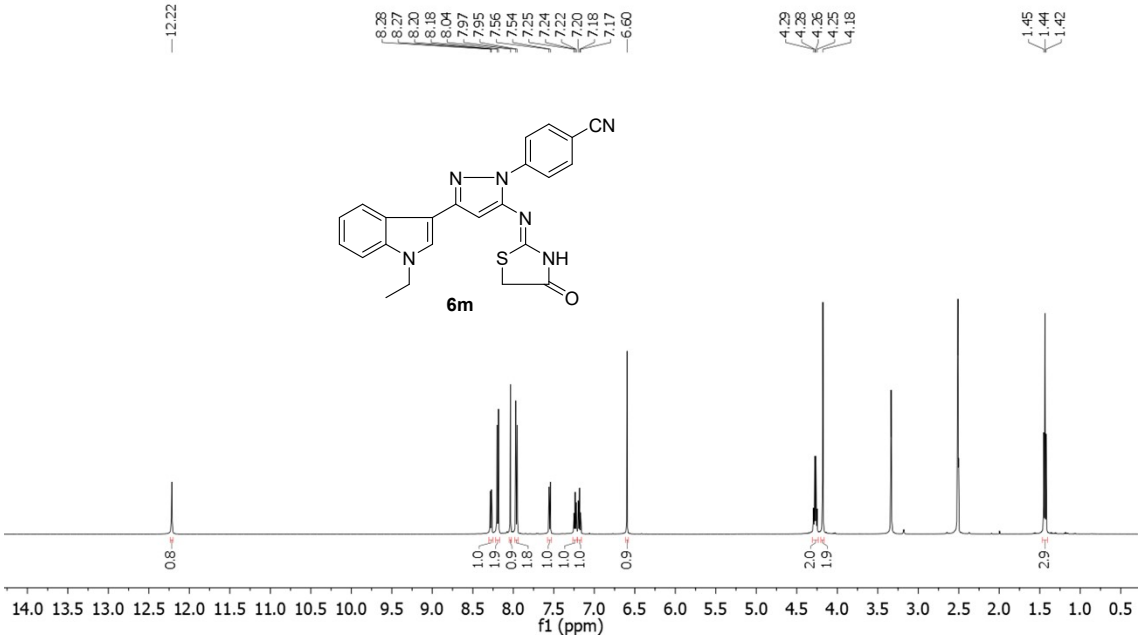
[13]C NMR spectra of 6k (125 MHz, DMSO-d_6)



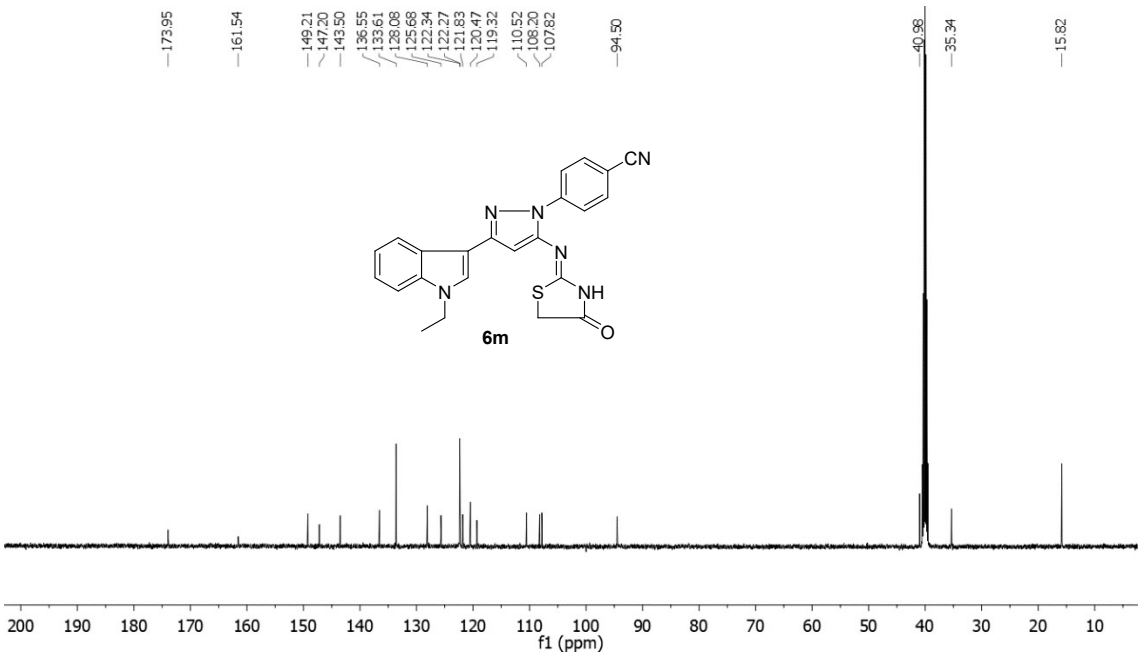
[1]H NMR spectra of 6l (500 MHz, DMSO-d_6)



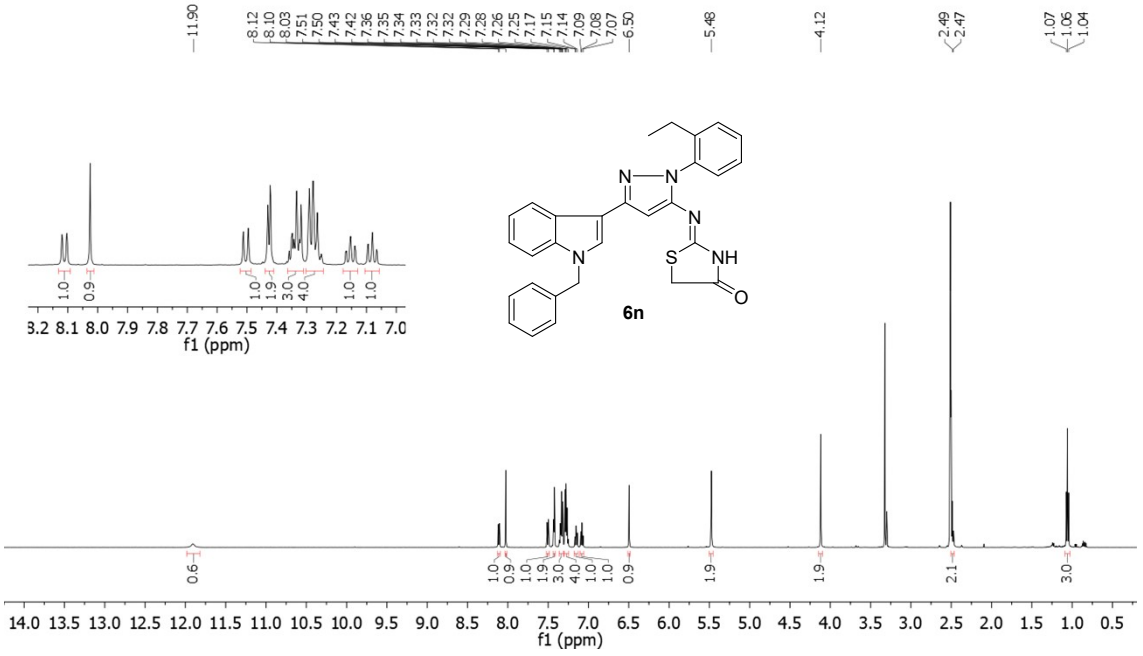
[13]C NMR spectra of 6l (125 MHz, DMSO-d_6)



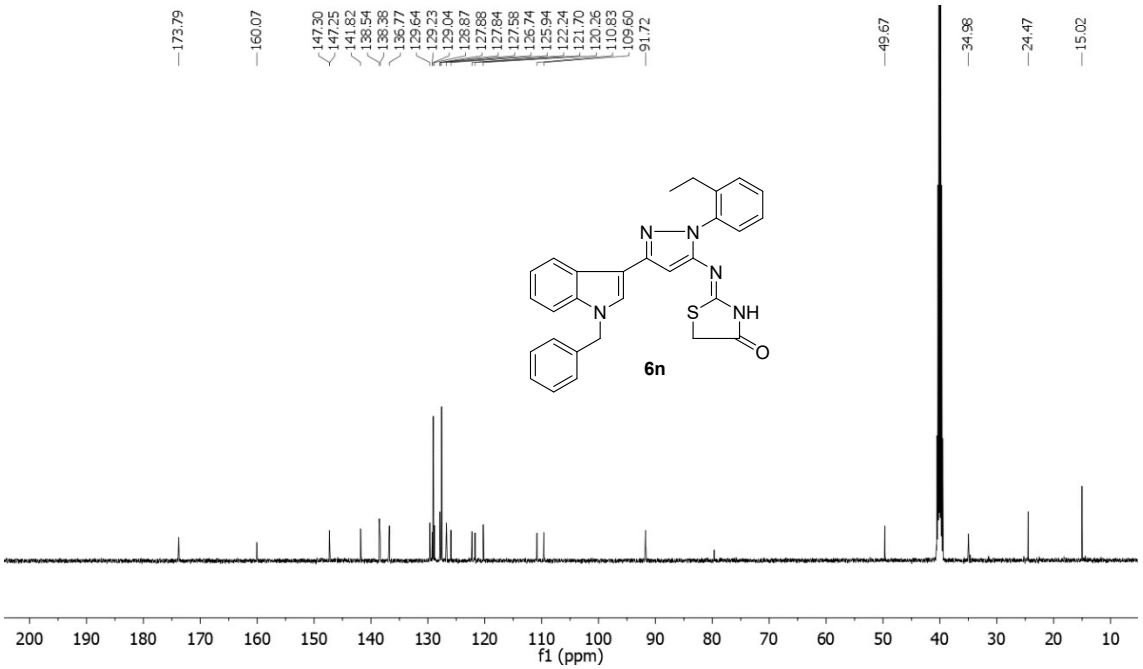
[1]H NMR spectra of 6m (500 MHz, DMSO-d_6)



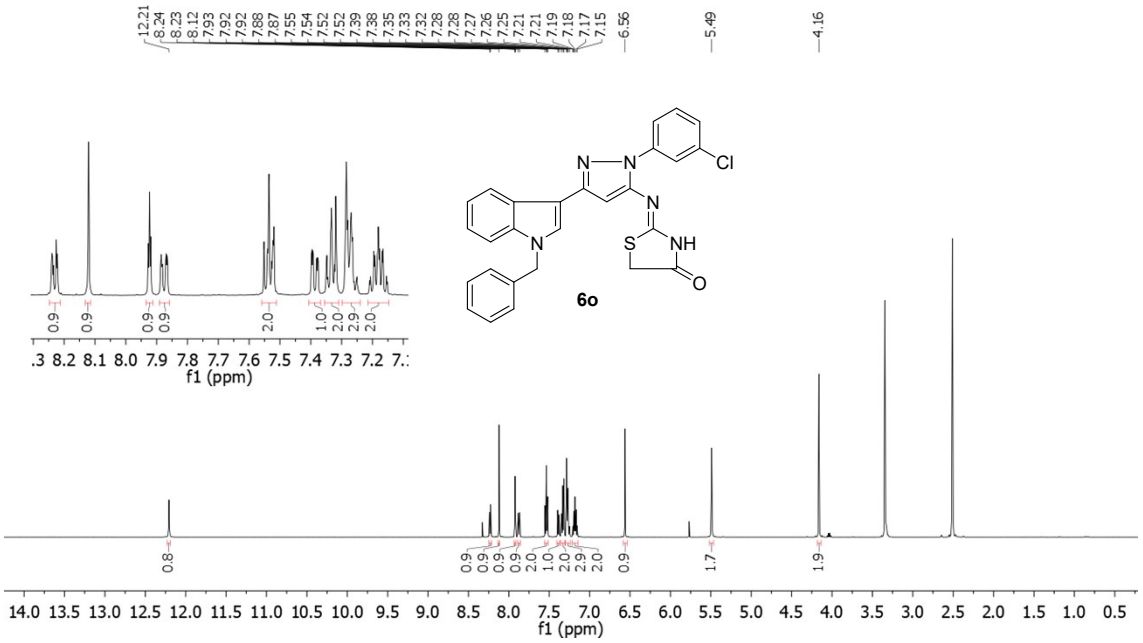
[13]C NMR spectra of 6m (125 MHz, DMSO-d_6)



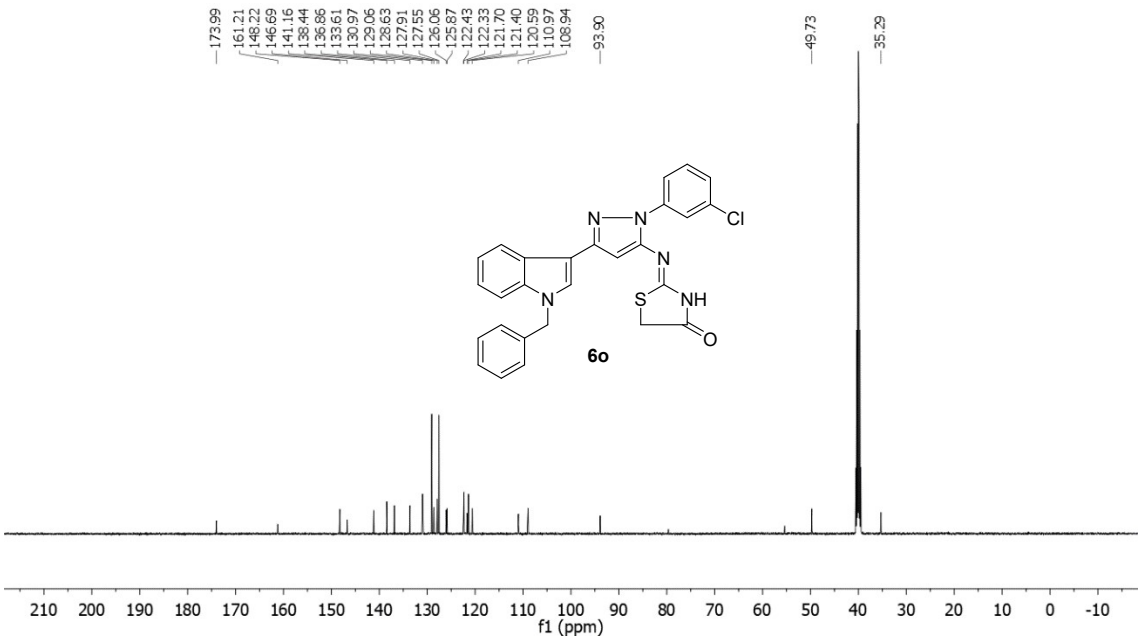
^{1}H NMR spectra of 6n (500 MHz, DMSO-d_6)



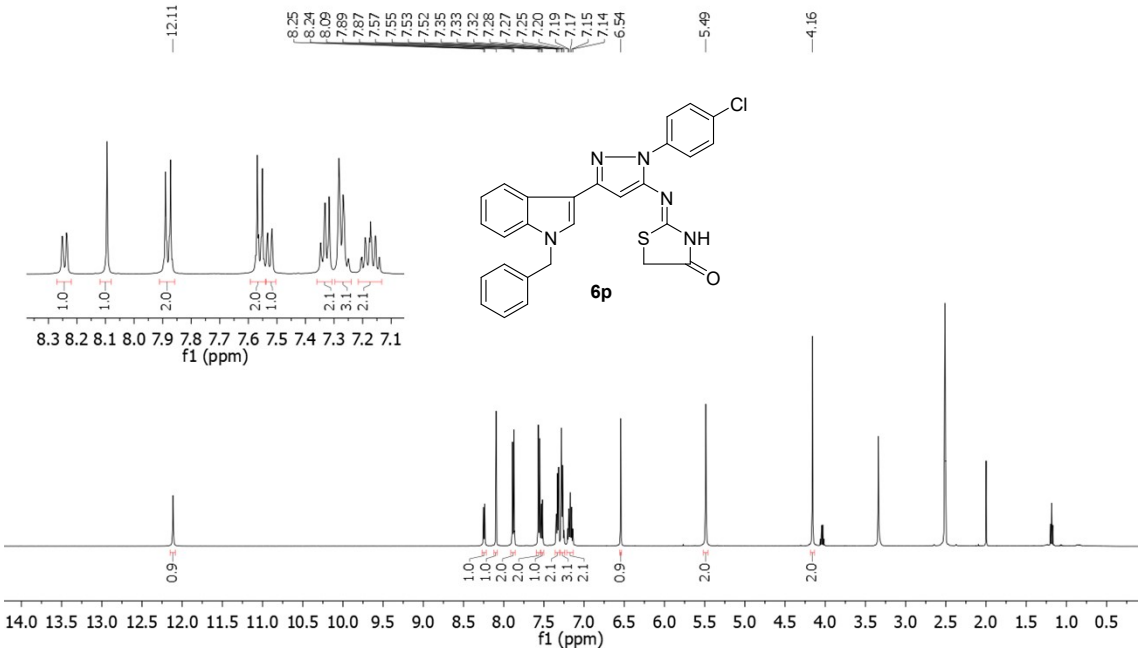
^{13}C NMR spectra of 6n (125 MHz, DMSO-d_6)



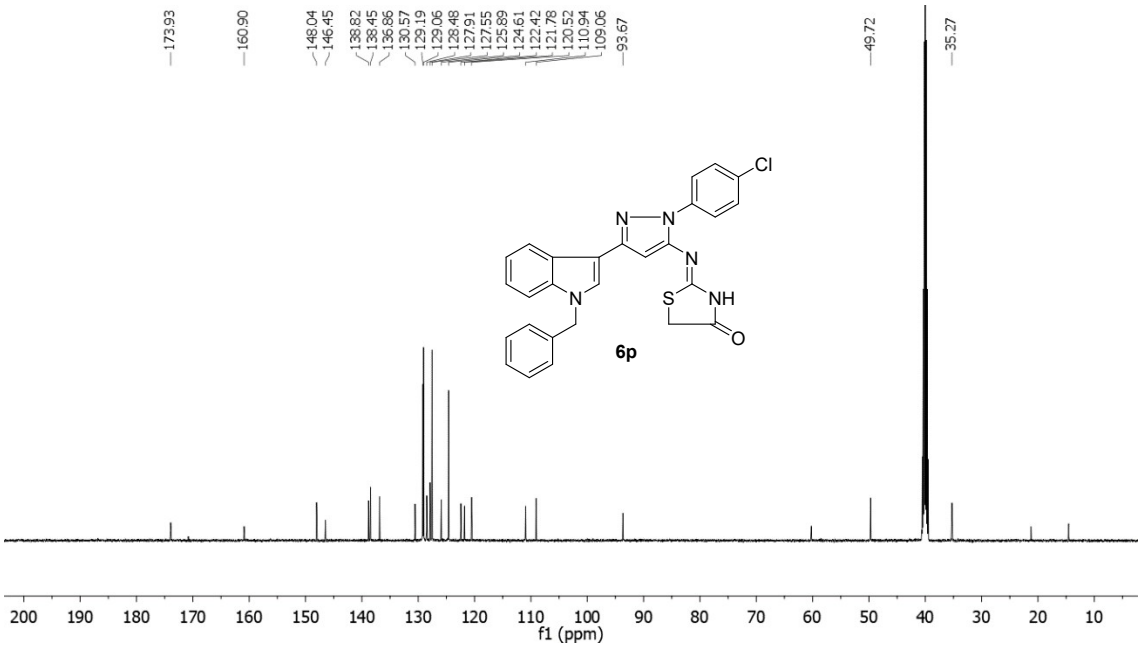
[1]H NMR spectra of 6o (500 MHz, DMSO-d_6)



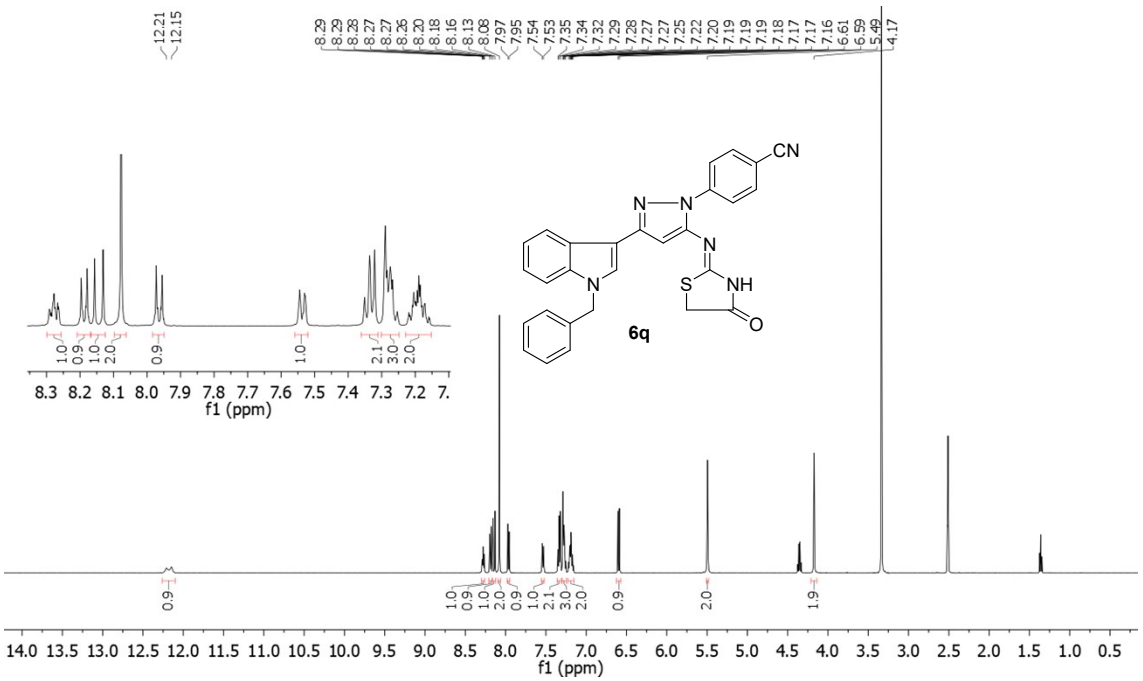
[13]C NMR spectra of 6o (125 MHz, DMSO-d_6)



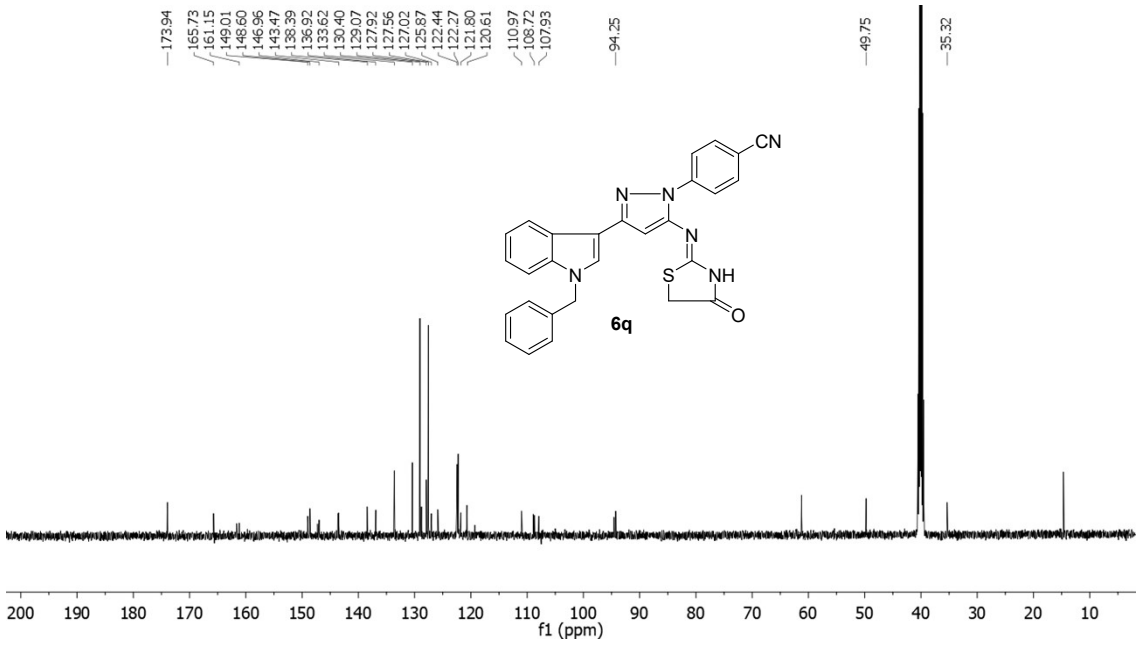
^{1}H NMR spectra of 6p (500 MHz, DMSO-d_6)



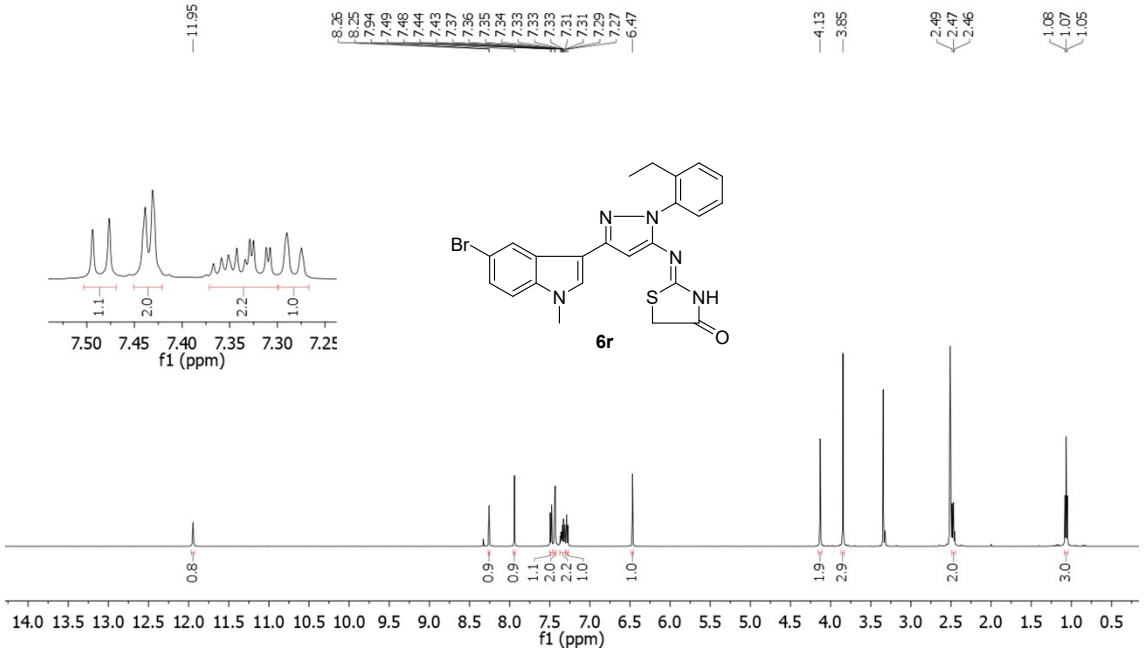
^{13}C NMR spectra of 6p (125 MHz, DMSO-d_6)



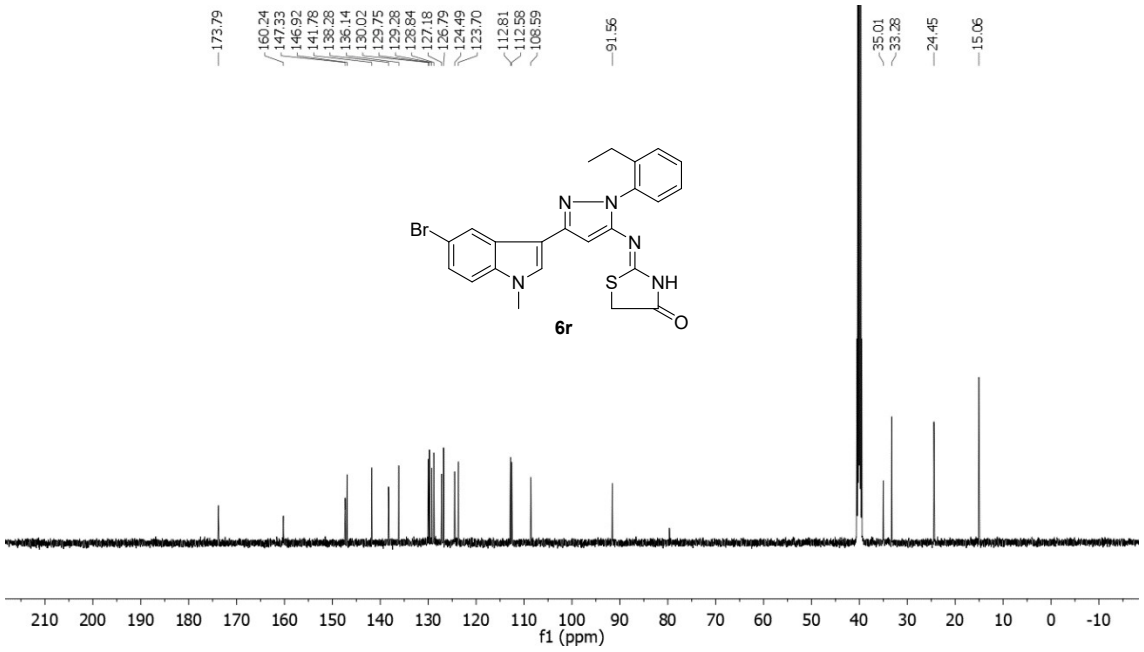
^{1}H NMR spectra of 6q (500 MHz, DMSO-d_6)



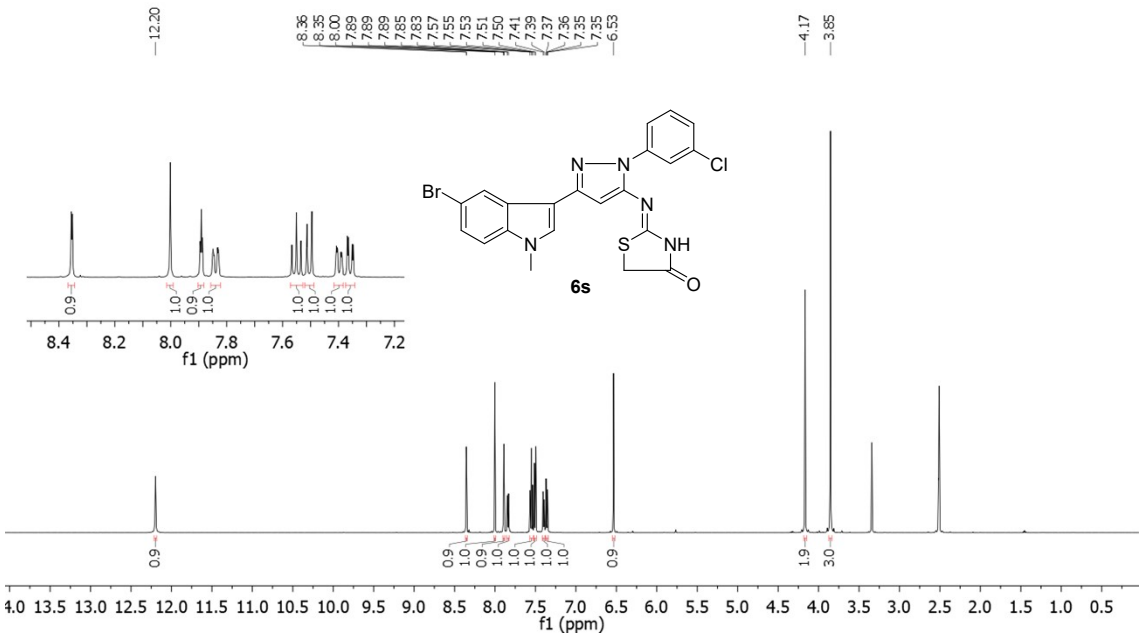
^{13}C NMR spectra of 6q (125 MHz, DMSO-d_6)



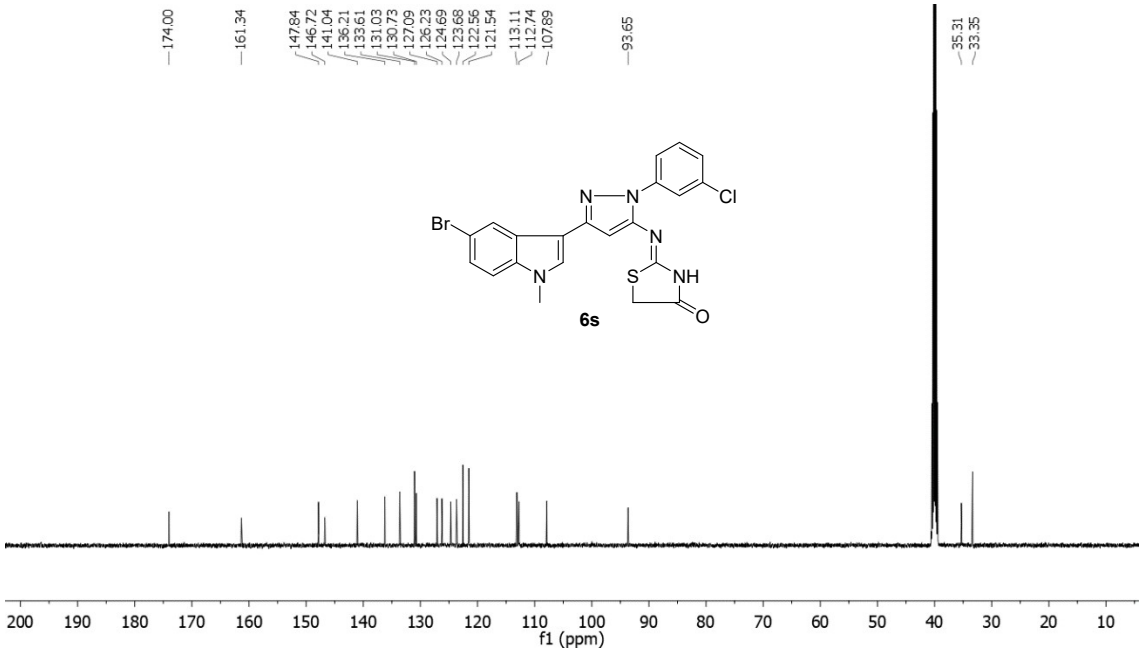
[1]H NMR spectra of 6r (500 MHz, DMSO-d_6)



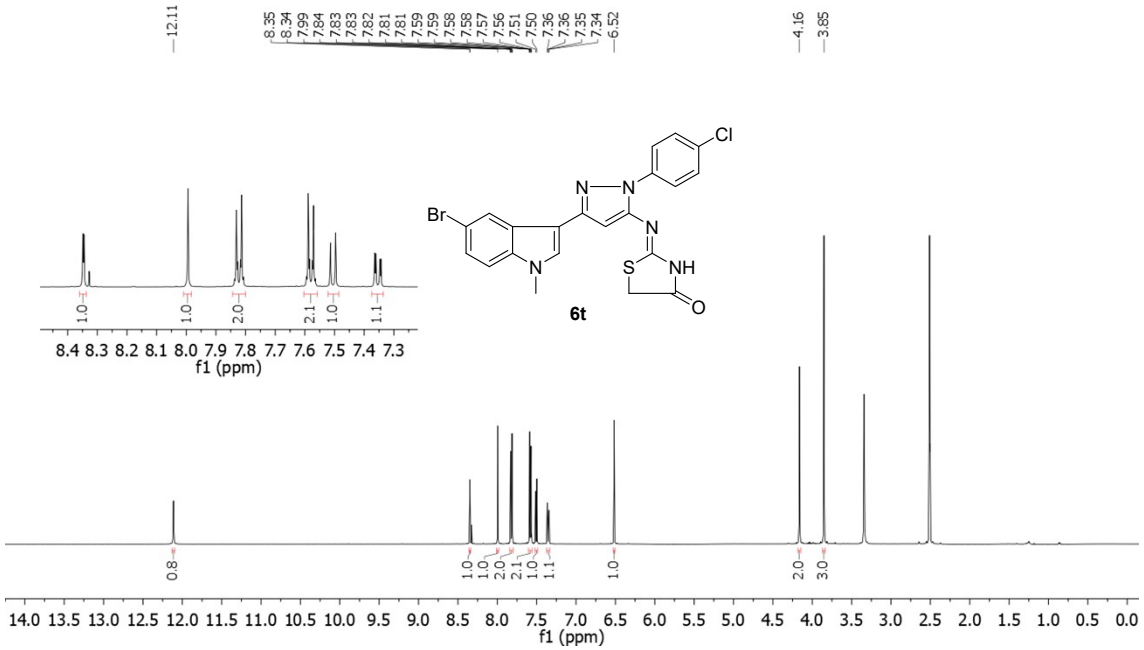
[13]C NMR spectra of 6r (125 MHz, DMSO-d_6)



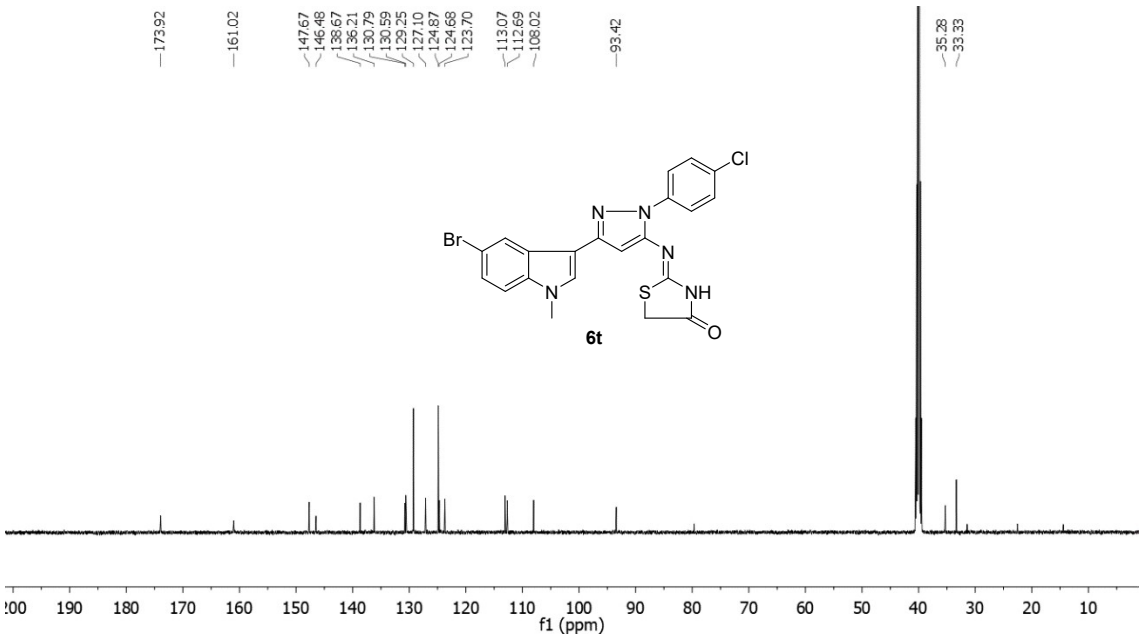
[1]H NMR spectra of 6s (500 MHz, DMSO-d_6)



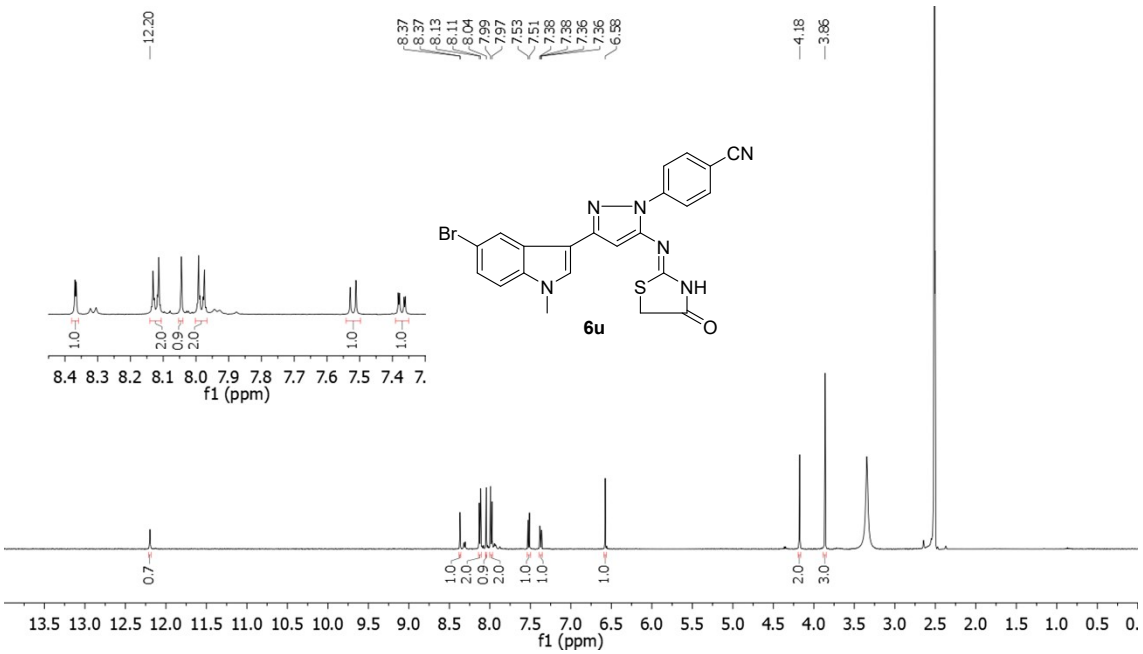
[13]C NMR spectra of 6s (125 MHz, DMSO-d_6)



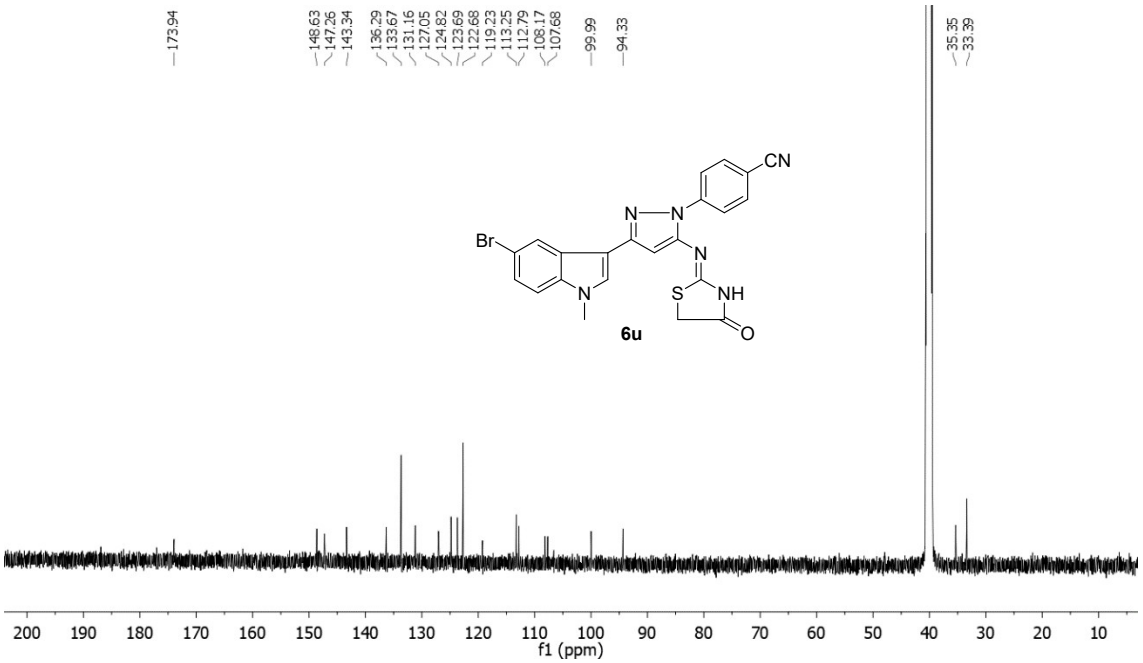
^{1}H NMR spectra of 6t (500 MHz, DMSO-d_6)



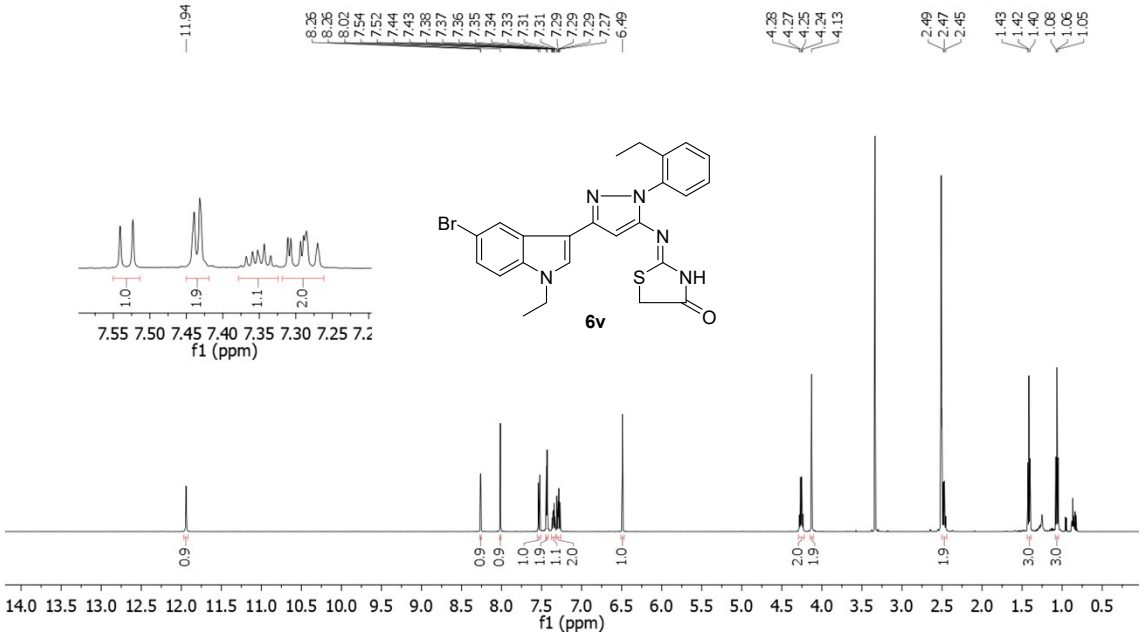
^{13}C NMR spectra of 6t (125 MHz, DMSO-d_6)



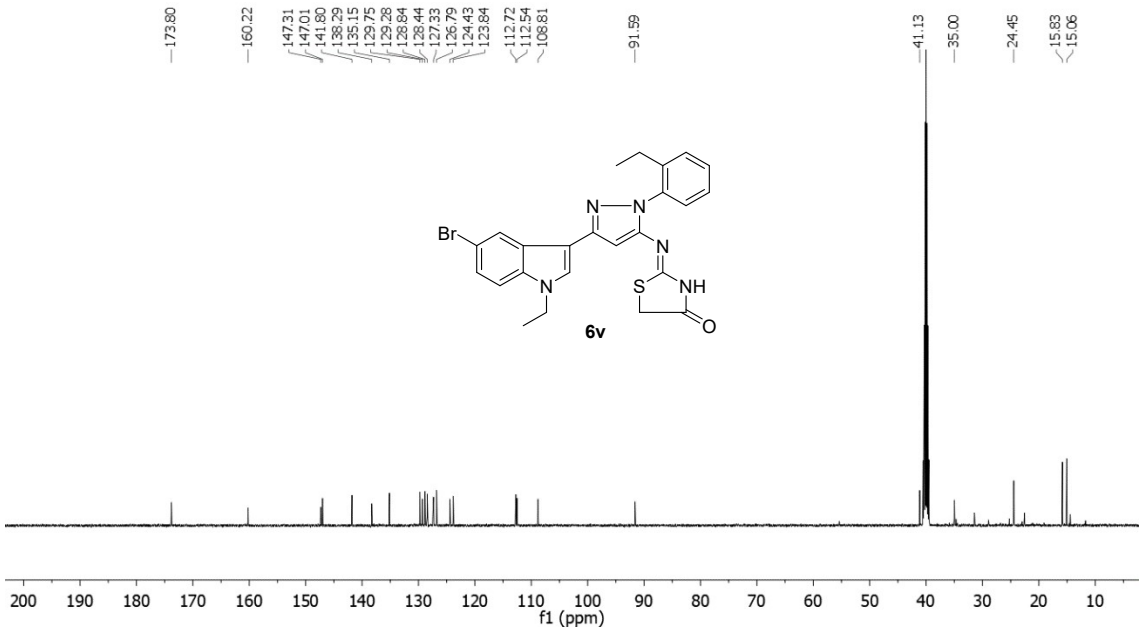
[1]H NMR spectra of 6u (500 MHz, DMSO-d_6)



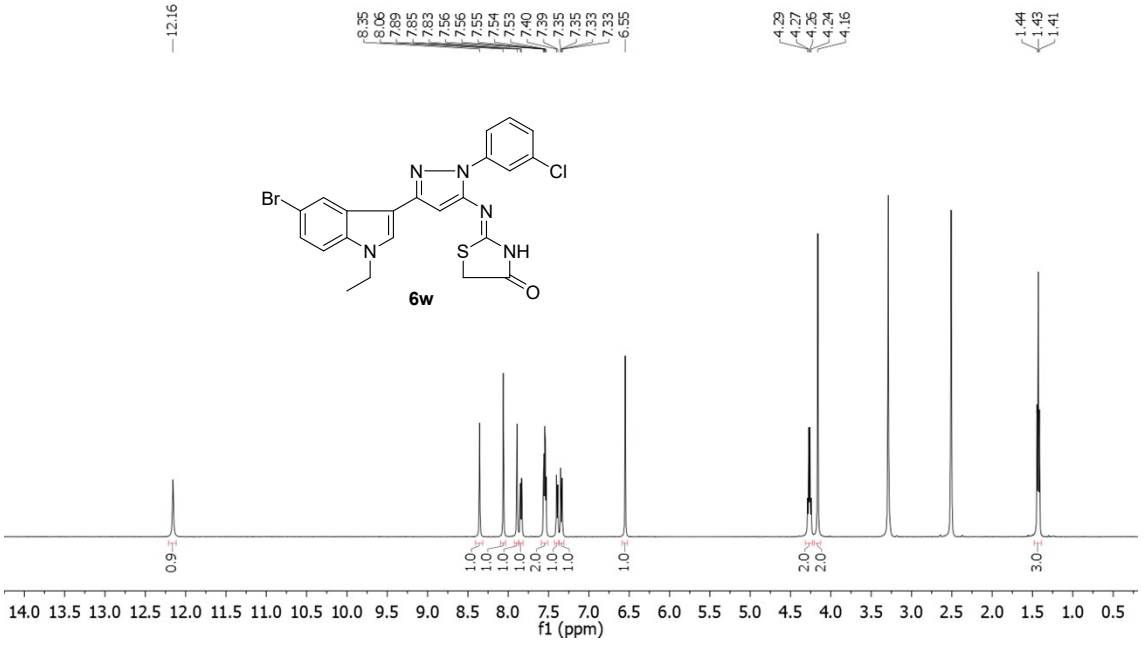
[13]C NMR spectra of 6u (125 MHz, DMSO-d_6)



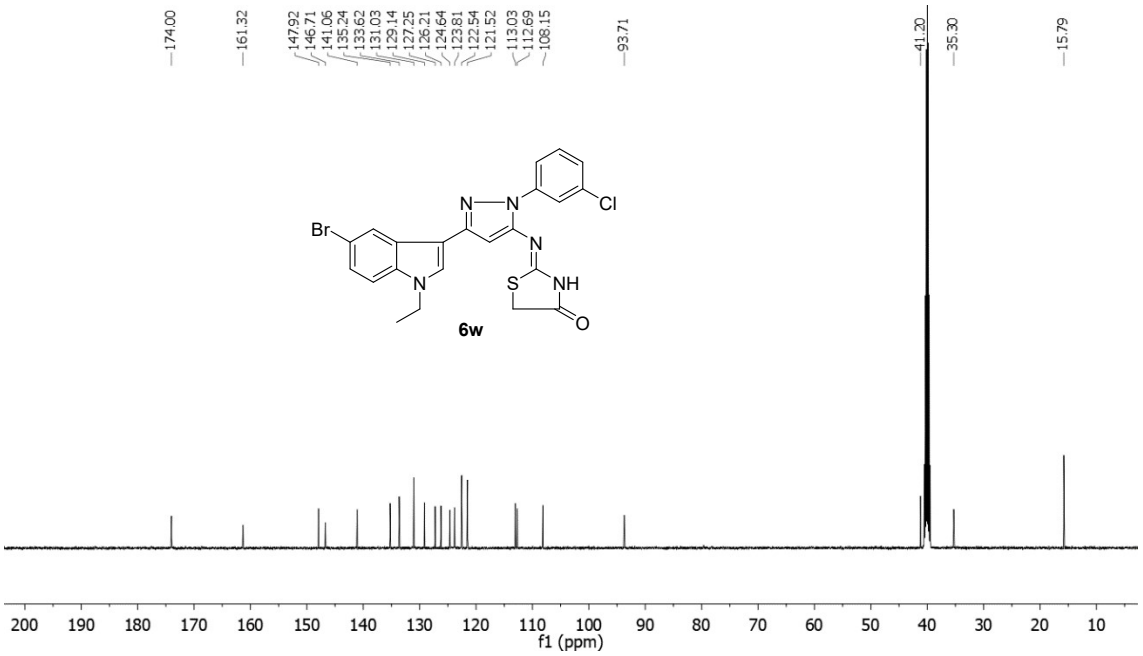
^{1}H NMR spectra of 6v (500 MHz, DMSO-d_6)



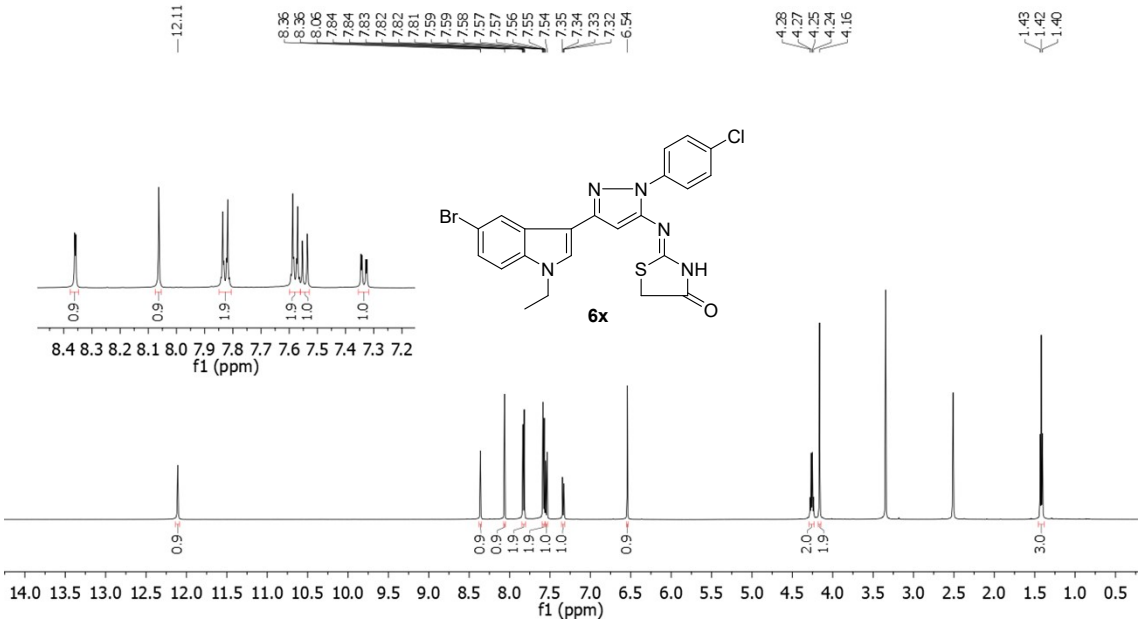
^{13}C NMR spectra of 6v (125 MHz, DMSO-d_6)



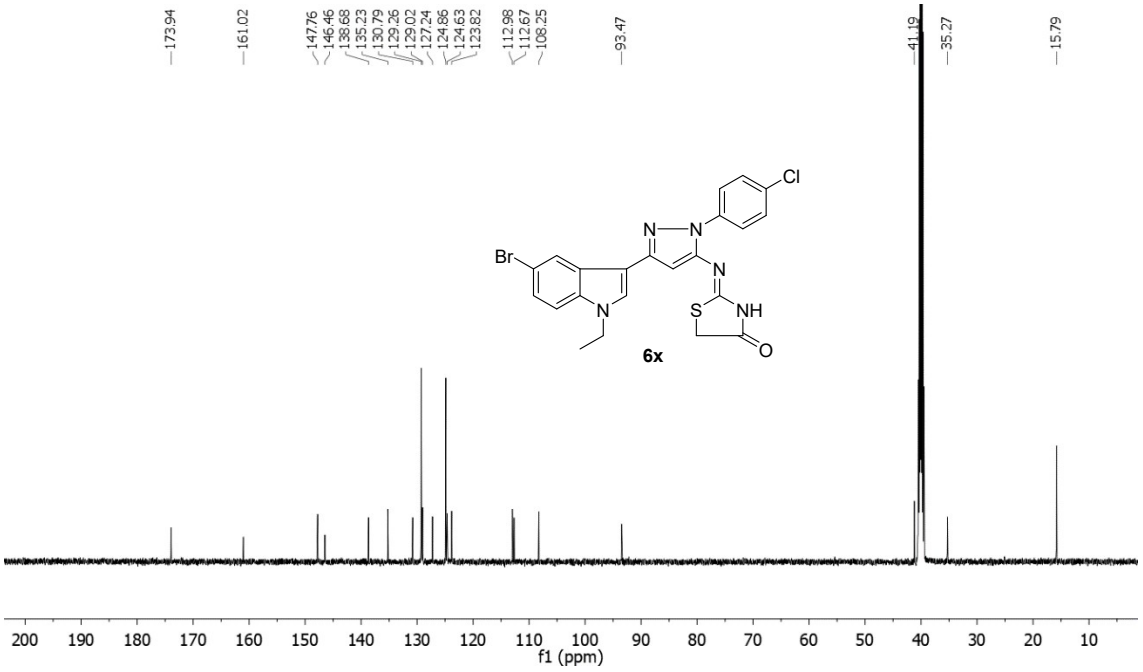
[1]H NMR spectra of 6w (500 MHz, DMSO-d_6)



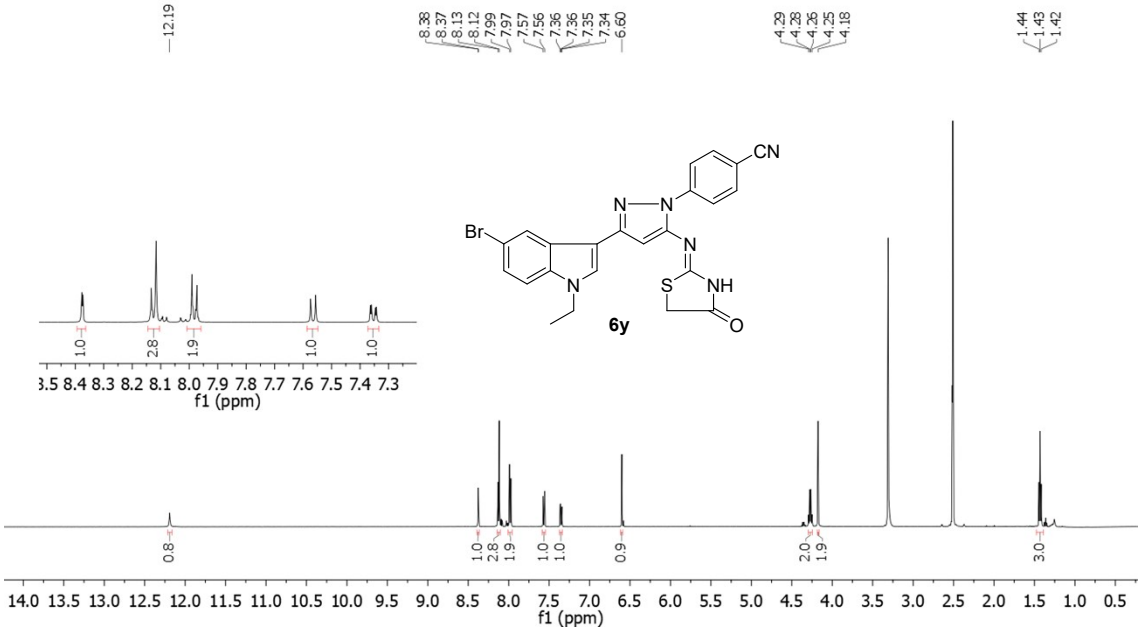
[13]C NMR spectra of 6w (125 MHz, DMSO-d_6)



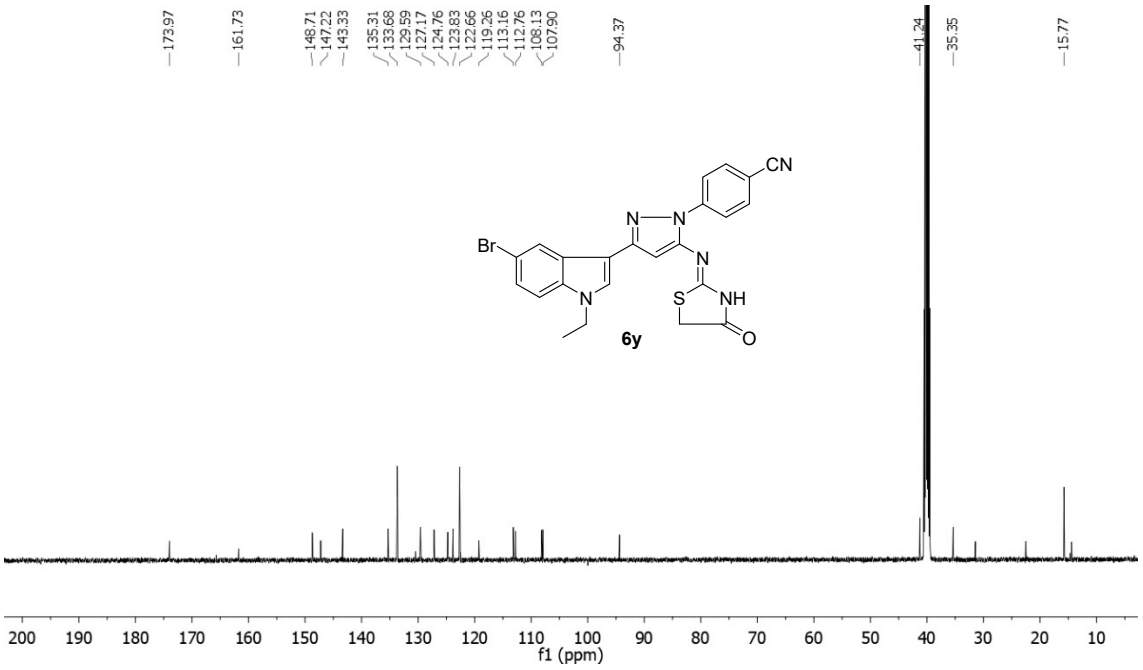
[1]H NMR spectra of 6x (500 MHz, DMSO-d_6)



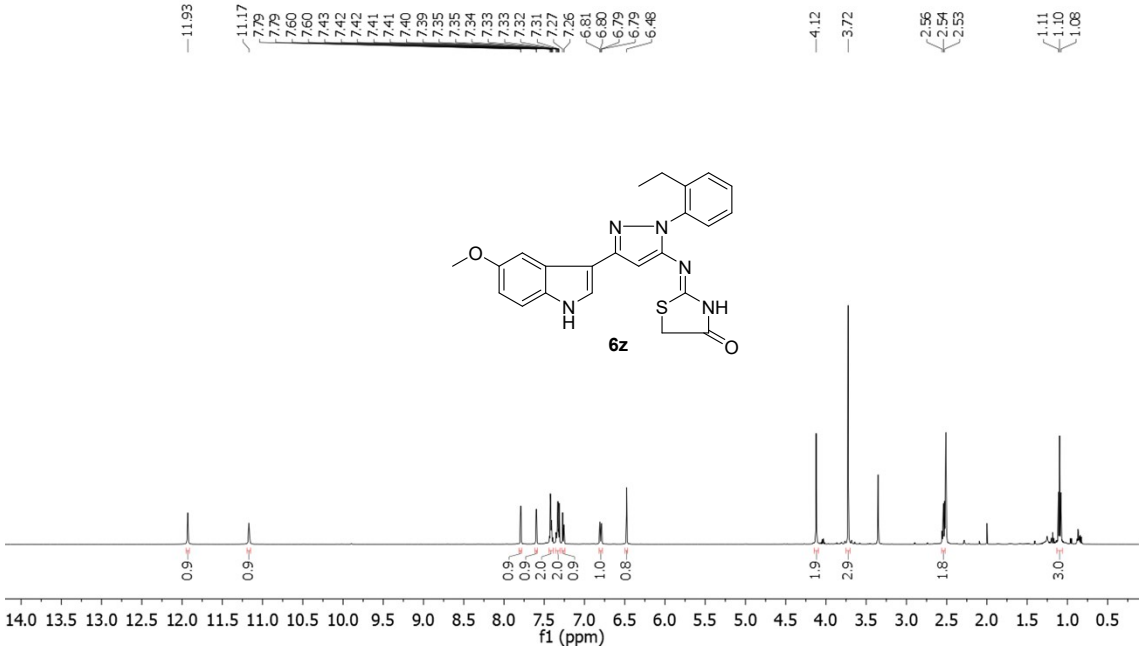
[13]C NMR spectra of 6x (125 MHz, DMSO-d_6)



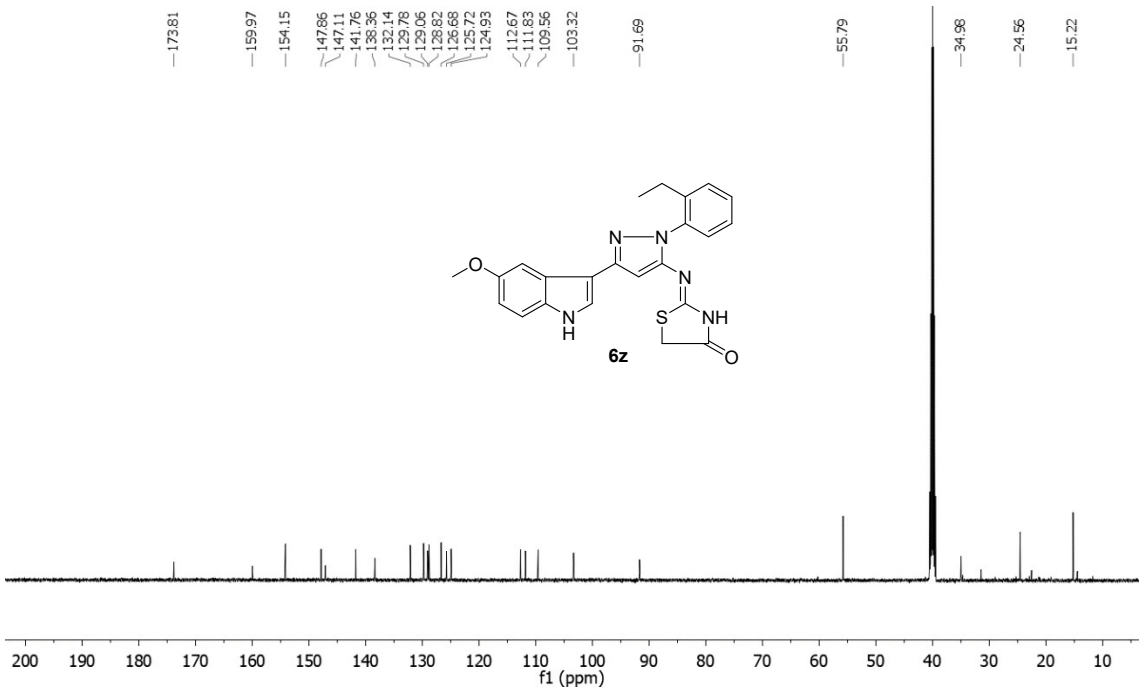
[1]H NMR spectra of 6y (500 MHz, DMSO-d_6)



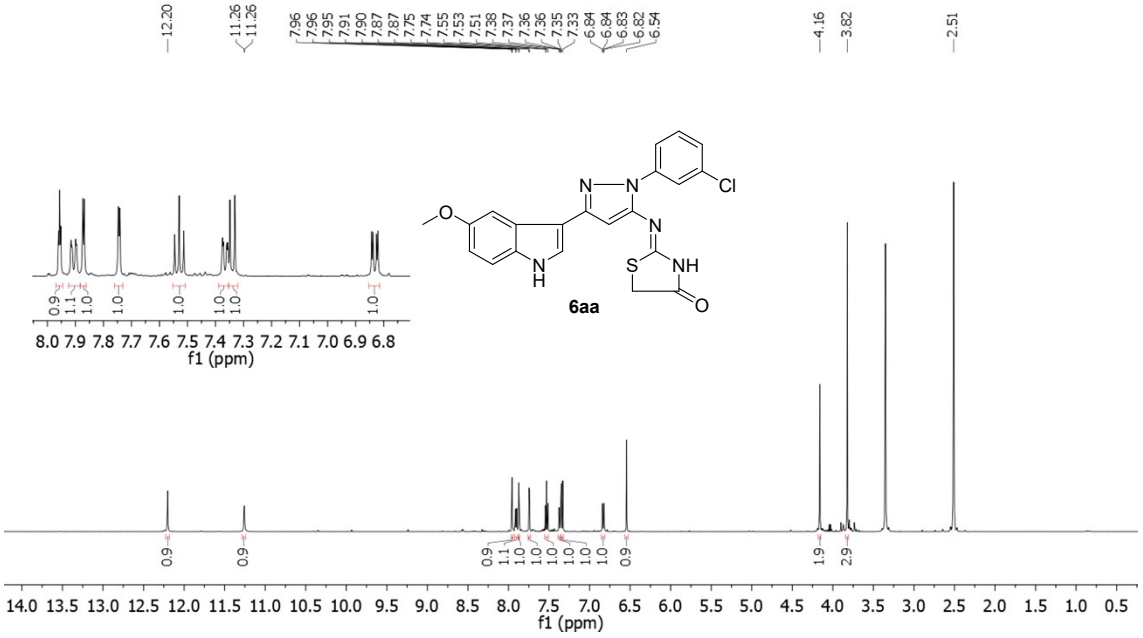
[13]C NMR spectra of 6y (125 MHz, DMSO-d_6)



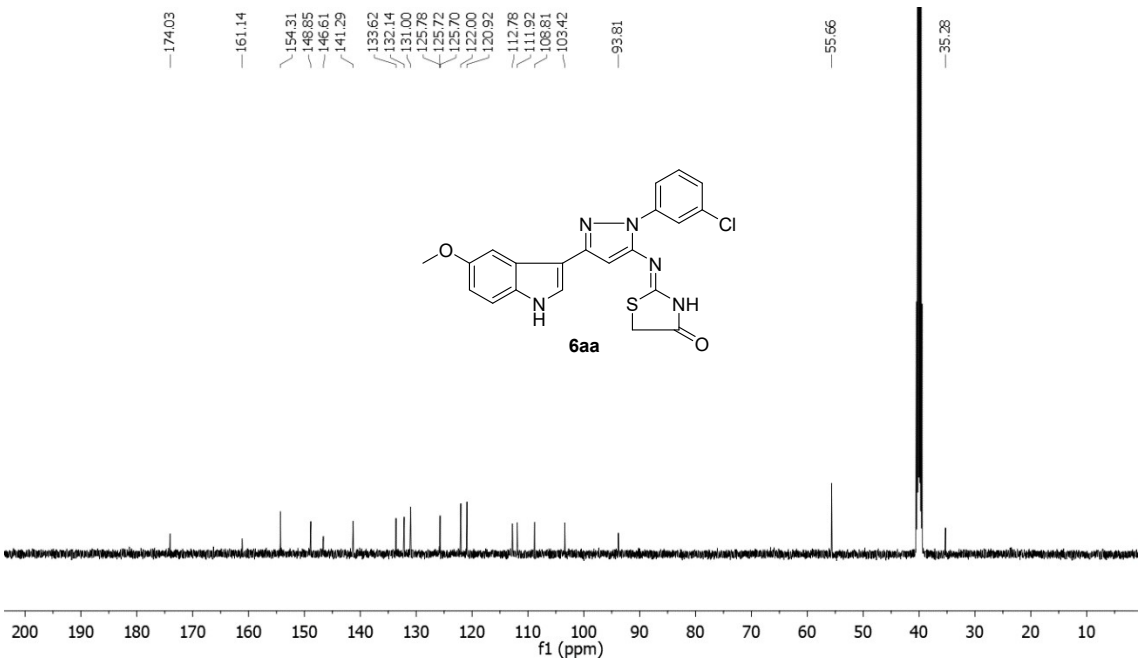
^{1}H NMR spectra of 6z (500 MHz, DMSO-d_6)



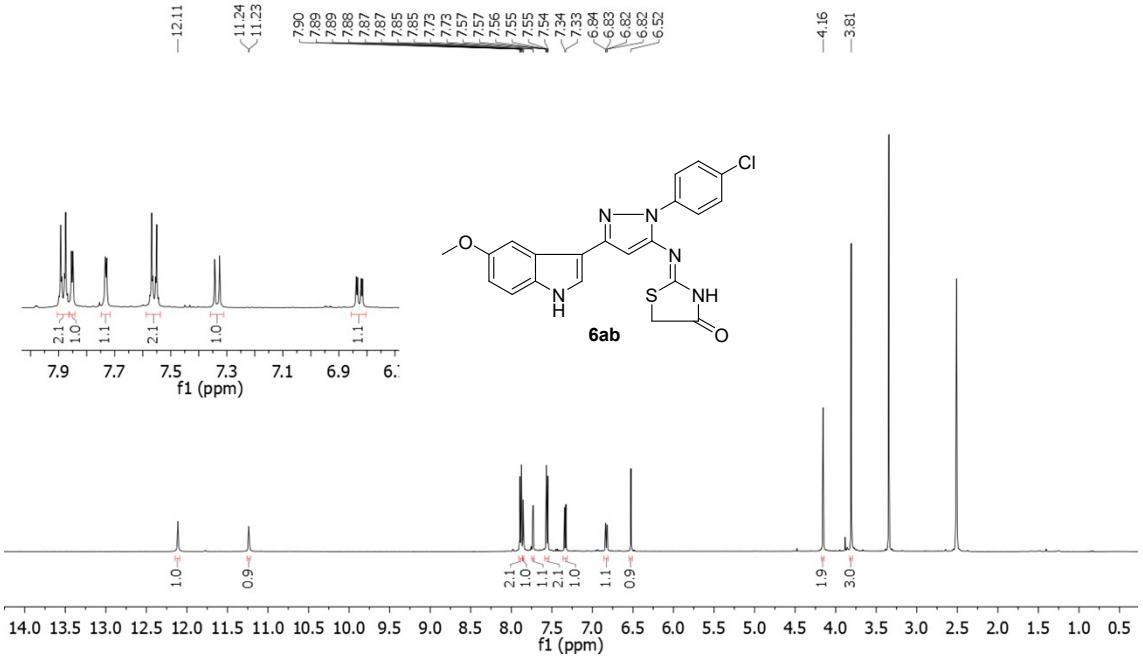
^{13}C NMR spectra of 6z (125 MHz, DMSO-d_6)



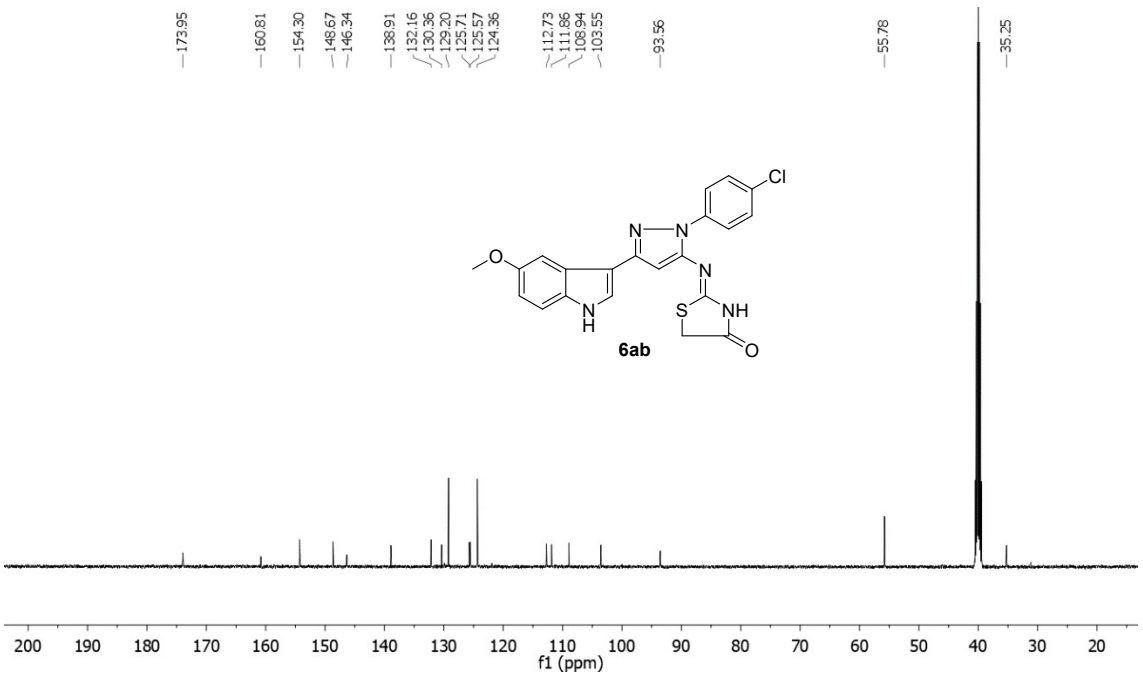
[1]H NMR spectra of 6aa (500 MHz, DMSO-d_6)



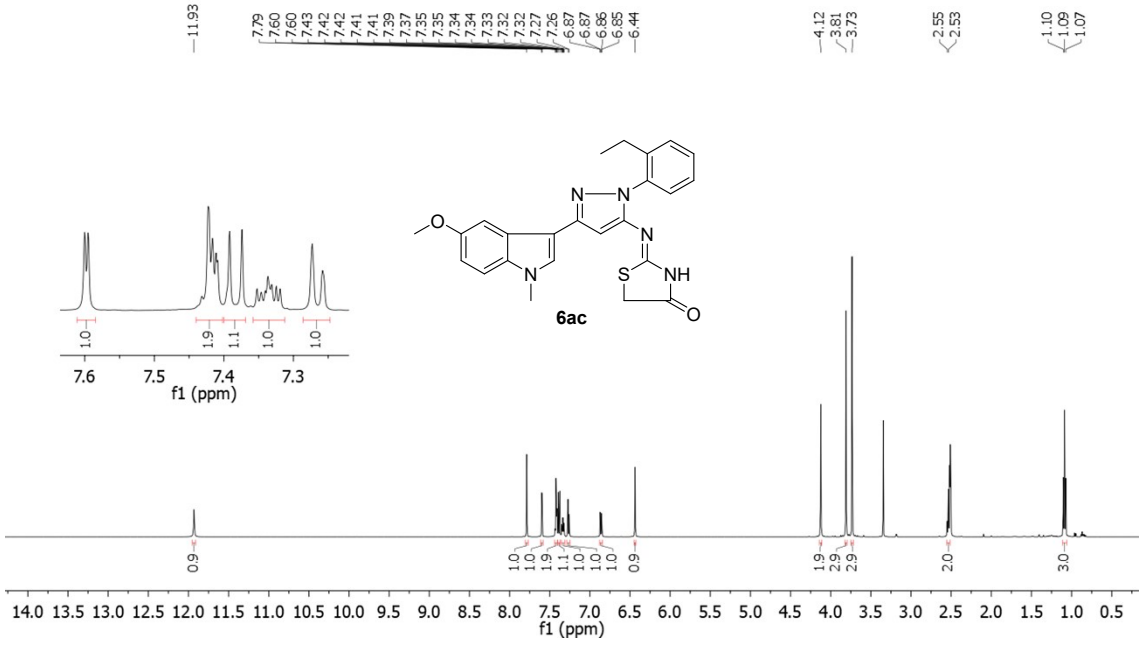
[13]C NMR spectra of 6aa (125 MHz, DMSO-d_6)



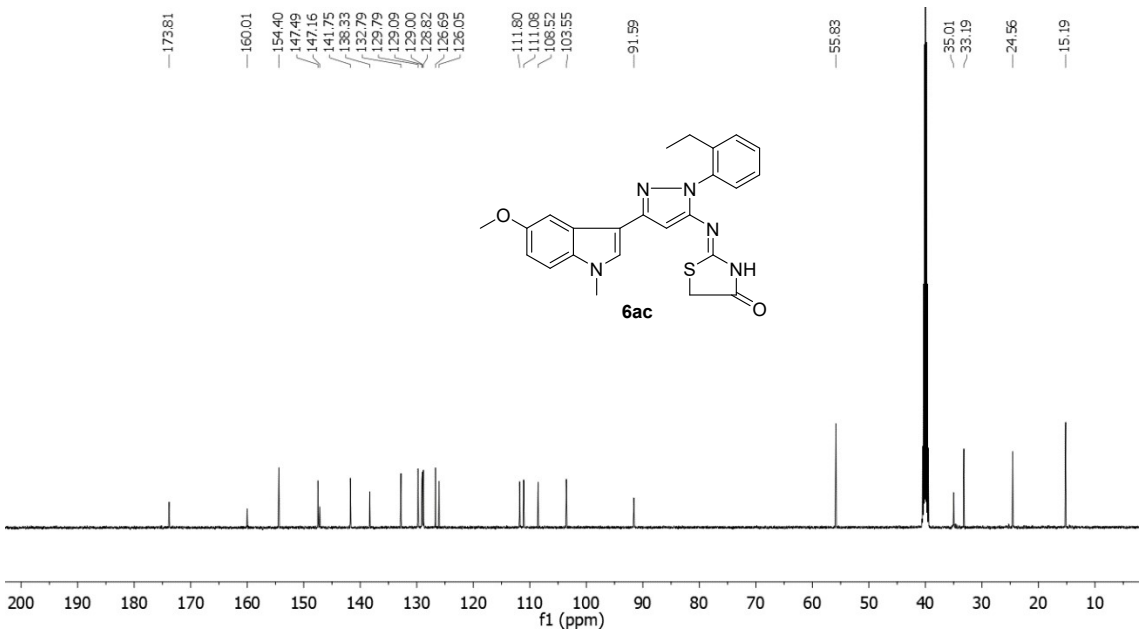
[1]H NMR spectra of 6ab (500 MHz, DMSO-d_6)



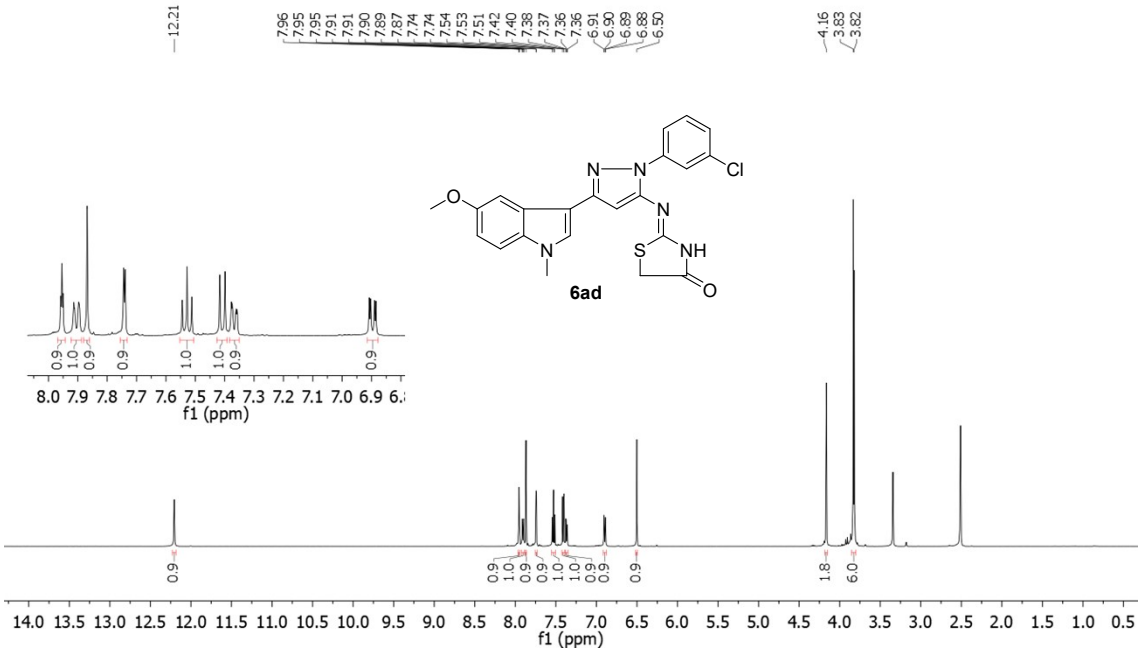
[13]C NMR spectra of 6ab (125 MHz, DMSO-d_6)



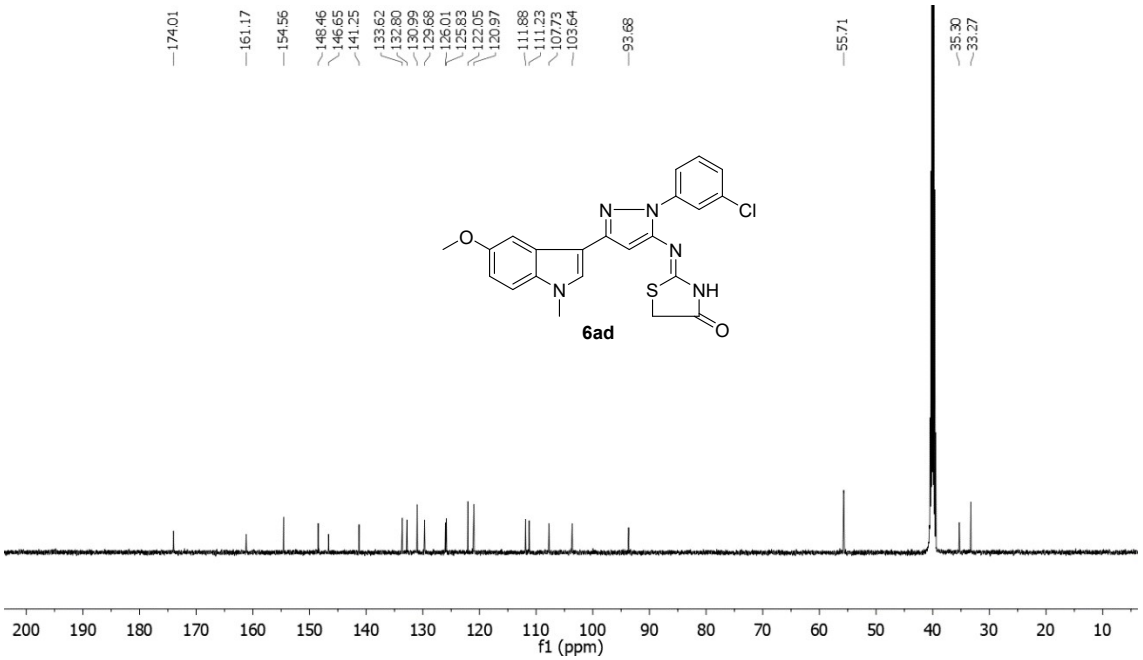
[1]H NMR spectra of 6ac (500 MHz, DMSO-d_6)



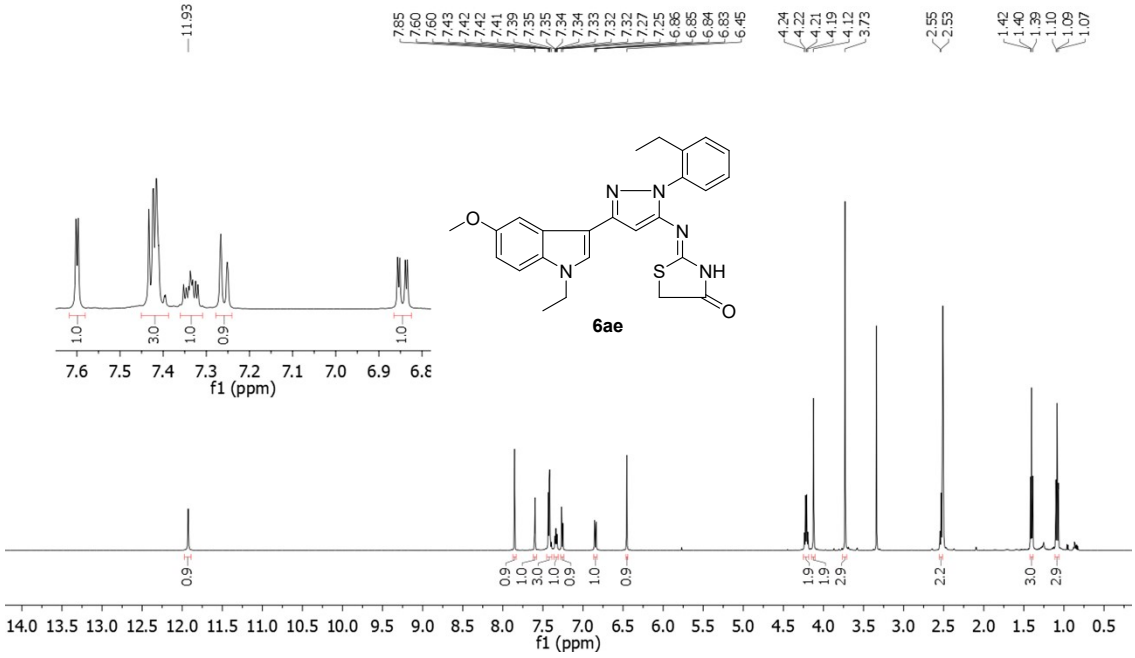
[13]C NMR spectra of 6ac (125 MHz, DMSO-d_6)



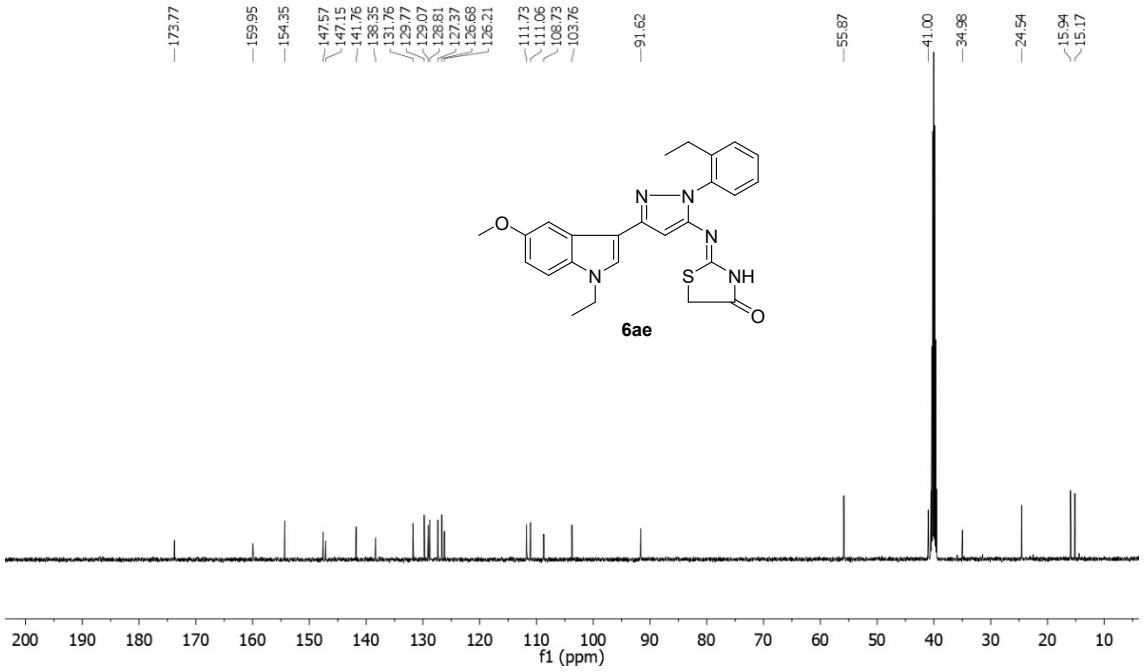
^{1}H NMR spectra of 6ad (500 MHz, DMSO-d_6)



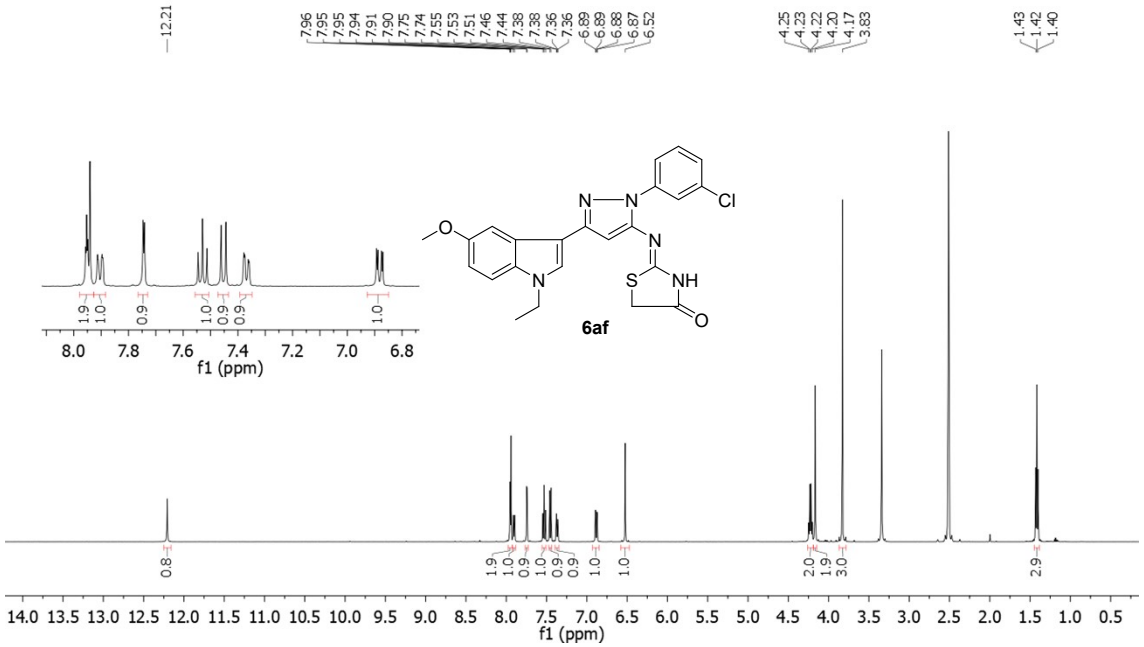
^{13}C NMR spectra of 6ad (125 MHz, DMSO-d_6)



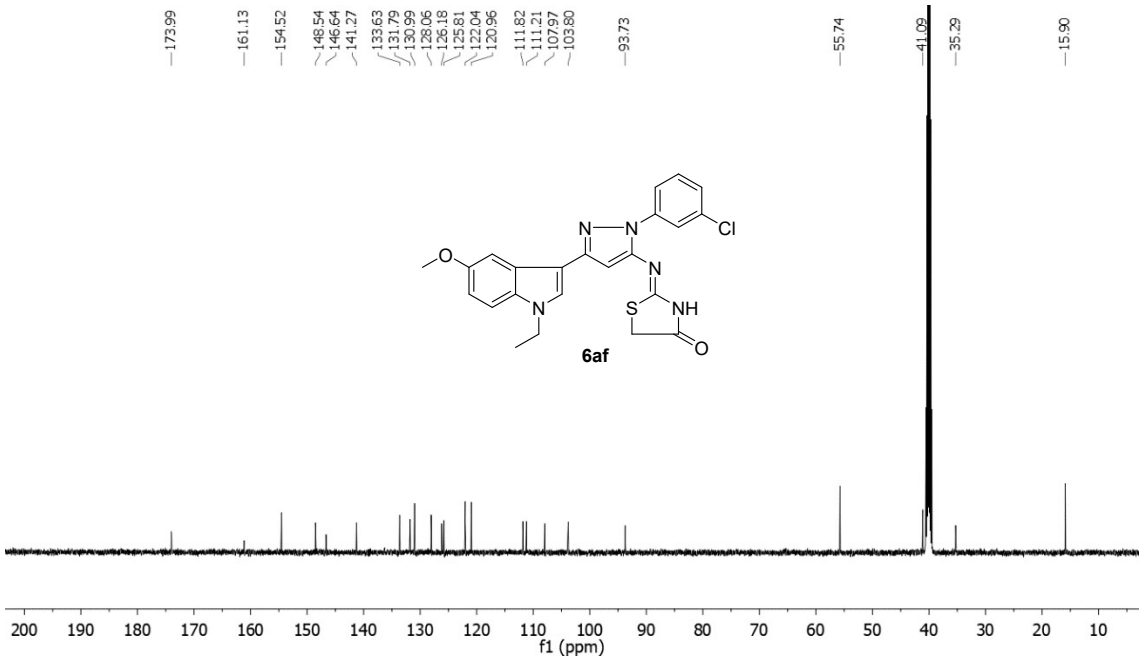
[1]H NMR spectra of 6ae (500 MHz, DMSO-d_6)



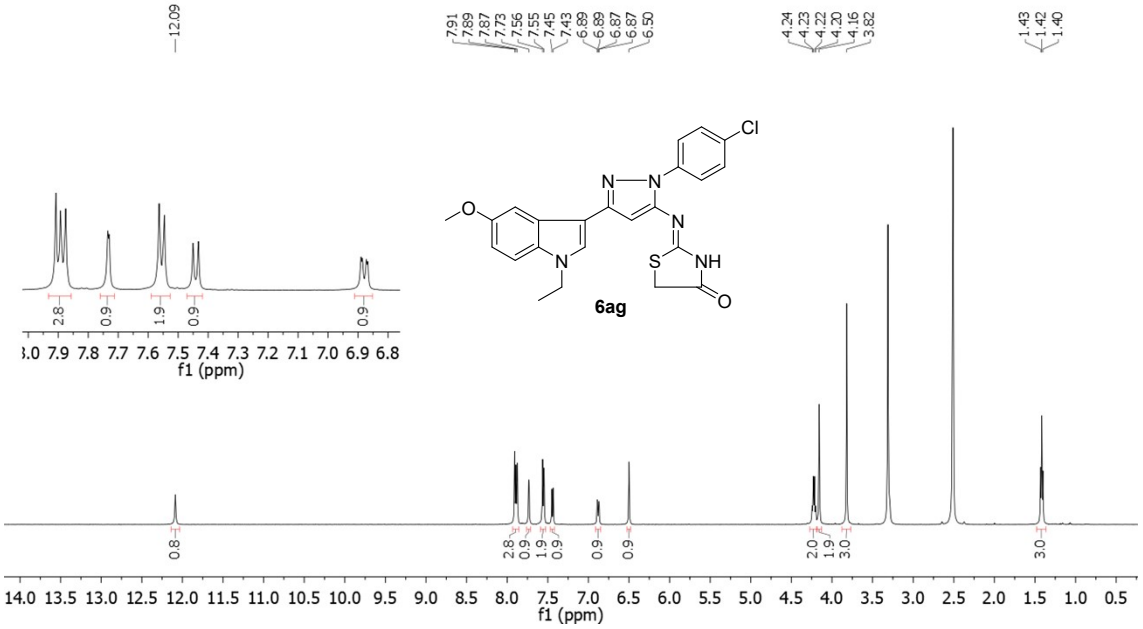
[13]C NMR spectra of 6ae (125 MHz, DMSO-d_6)



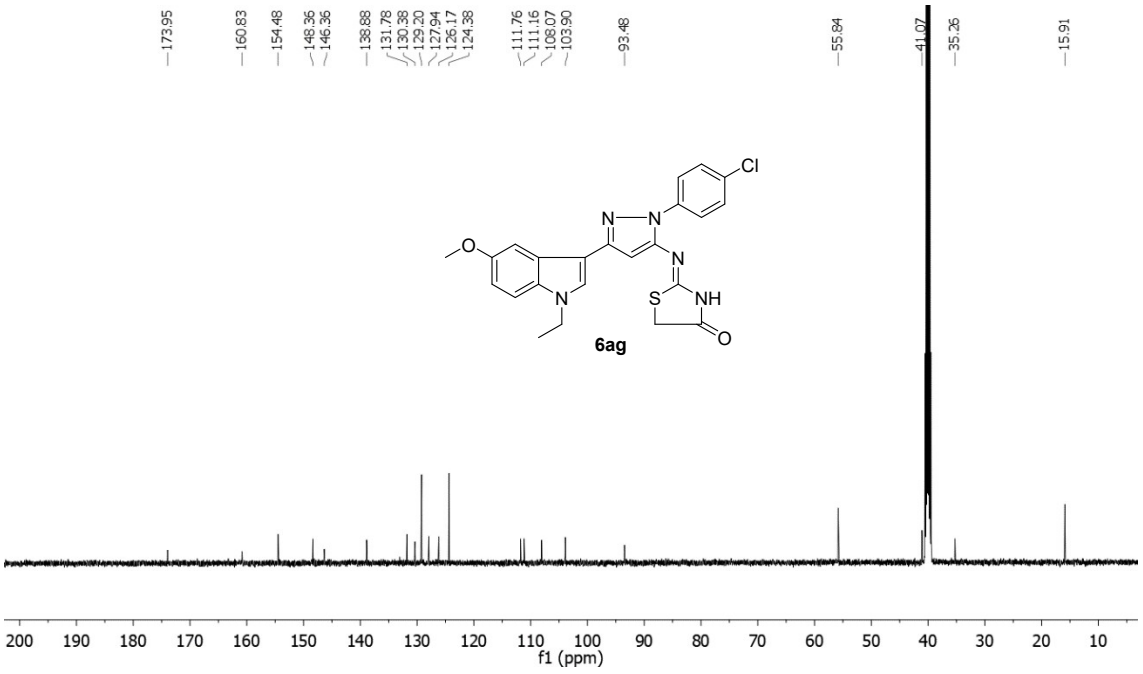
^{1}H NMR spectra of 6af (500 MHz, DMSO-d_6)



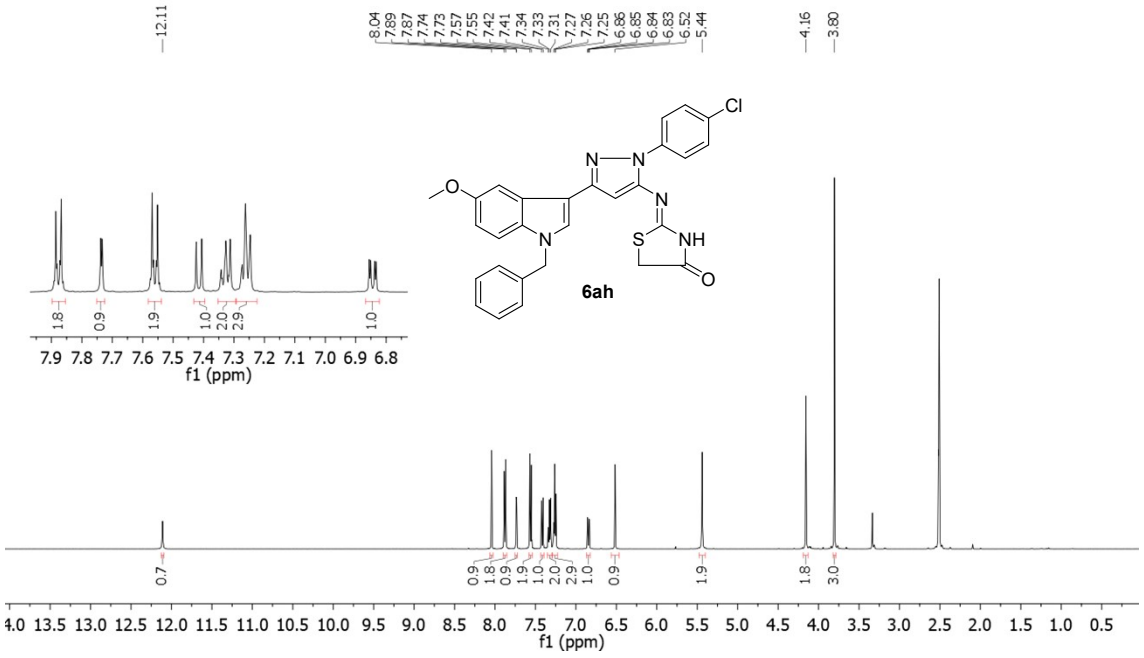
^{13}C NMR spectra of 6af (125 MHz, DMSO-d_6)



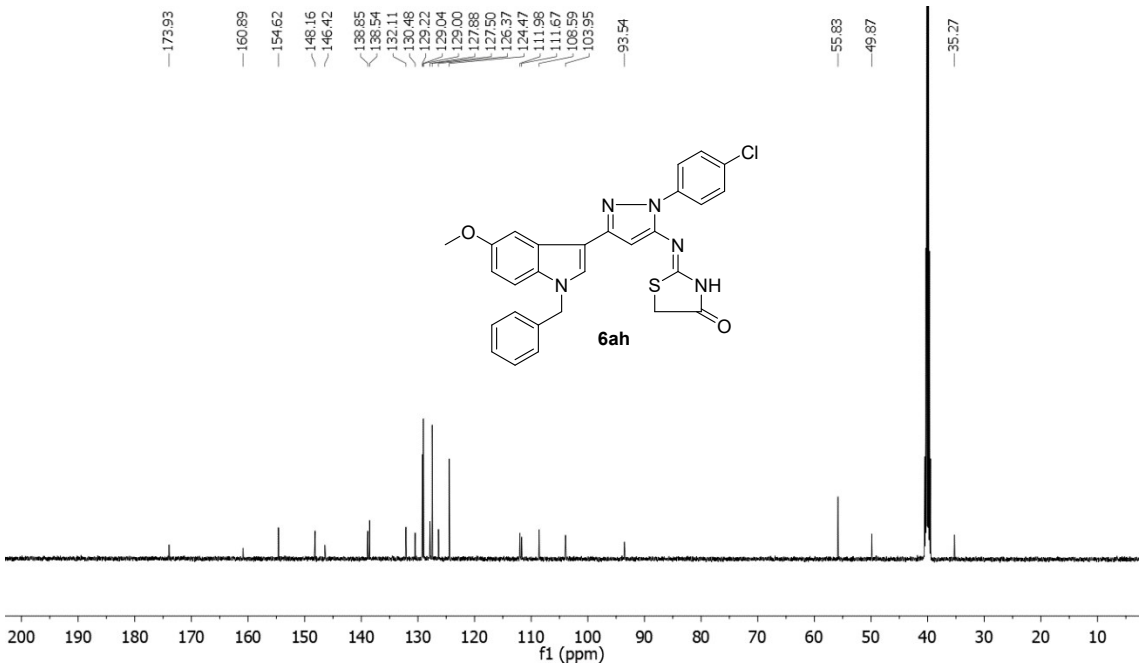
^{1}H NMR spectra of 6ag (500 MHz, DMSO-d_6)



^{13}C NMR spectra of 6ag (125 MHz, DMSO-d_6)



^{1}H NMR spectra of 6ah (500 MHz, DMSO-d_6)



^{13}C NMR spectra of 6ah (125 MHz, DMSO-d_6)