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Synthesis and X-ray Study of Dispiro 8-Nitroquinolone Analogues and Their Cytotoxic Properties Against Human Cervical Cancer Cells HeLa

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Contents

1. X-Ray crystal structure of compound

Crystallographic data collection and refinement of 6l

The compound **6l** was crystallized from solvent evaporation solution growth technique. Block type single crystals suitable for X-ray diffraction were chosen from the grown sample. The crystallographic data collection, using the X-ray with wavelength of 0.71073 Å, was collected at room temperature with MoK_α radiation using Bruker AXS KAPPA APEX-2 diffractometer equipped with graphite monochromator [1].

Table 1 Crystallographic parameters of **6l**

Empirical formula	$C_{32}H_{25}FN_4O_5$
Formula weight	564.56
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P -1
Unit cell dimensions	a = 7.7342(5) Å α = 97.469(2)°
	b = 11.9996(8) Å β = 92.265(2)°
	c = 14.6465(10) Å γ = 91.035(2)°
Volume	1346.35(16) $Å^3$
Z, Calculated density	2, 1.393 Mg/m^3
Absorption coefficient	0.100 mm^{-1}
F(000)	588
Crystal size	0.432 × 0.216 × 0.199 mm
Theta range for data collection	2.352 to 27.188°
Limiting indices	$-9 \le h \le 9$, $-15 \le k \le 15$, $-18 \le l \le 18$
Reflections collected / unique	41972 / 5937 [R(int) = 0.0229]
Completeness to theta = 25.242	99.90%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5937 / 0 / 381
Goodness-of-fit on F^2	1.046
Final R indices [I>2sigma(I)]	R1 = 0.0483, wR2 = 0.1267
R indices (all data)	R1 = 0.0602, wR2 = 0.1350
Extinction coefficient	0.0159(19)
Largest diff. peak and hole	0.368 and -0.382 $e.A^{-3}$

The structure was solved by direct methods and refined by full-matrix least-squares calculations using SHELXL-2014 [2]. All the H atoms were placed geometrically calculated bond distances, viz., -NH = 0.86 Å, -CH = 0.93 Å (for aromatic), -CH = 0.96 Å (for $-CH_3$), -CH = 0.97 Å (for $-CH_2$) and -CH = 0.98 Å (for aliphatic) constrained to ride on the concerned parent atom with $U_{iso}(H) = 1.2$ or $1.5\ U_{eq}$ (parent atom). The crystallographic data, details of data collection and the structure refinement are presented in Table 1. The ORTEP view of the molecules drawn at 50% probability thermal displacement ellipsoids with the atom numbering scheme is shown in Fig. 1 [3].

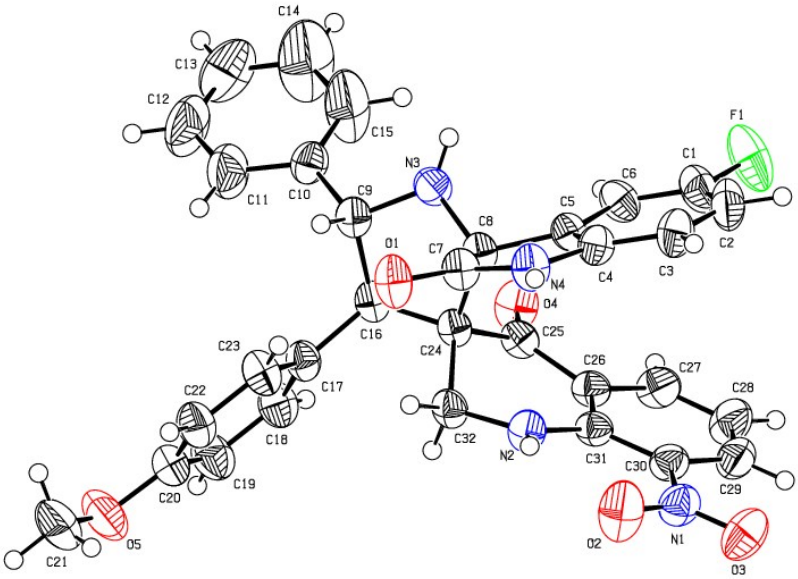


Figure 1. Molecular structure of the compound **6l (CCDC 1843977)** with 50% probability thermal displacement ellipsoids

References

1. Bruker (2001). SAINT and SMART. Bruker AXS Inc., Madison, Wisconsin, USA.
2. G.M. Sheldrick, Acta Cryst. C71 (2015), 3-8.
3. Spek, A. L. (2009). Acta Cryst. D65, 148–155.

Table 2 The selected optimized geometrical values of theoretical (DFT) and single crystal XRD values of the product 6L at M062x/6-31g** level of theory.

Bond length (Å)		
Bonding type	**Experimental**	**Theoretical**
N_9-C_{24}	1.453	1.464
C_{24}-C_{31}	1.512	1.509
C_{24}-C_{18}	1.551	1.550
C_{18}-C_{22}	1.514	1.510
C_{18}- C_{15}	1.560	1.559
C_{15}- C_{14}	1.541	1.542
C_{15}- C_{26}	1.529	1.530
C_{15}- C_{29}	1.598	1.595

C_{29}-N_9	1.449	1.447
C_{29}- C_{20}	1.509	1.513
C_{29}- C_{21}	1.555	1.560
N_9-H_{10}	0.860	1.016
C_{24}-H_{25}	0.980	1.098
C_{18}-H_{19}	0.980	1.093

Bond Angle (θ)		
Bonding type	**Experimental**	**Theoretical**
N_9-C_{24}-C_{31}	112.9	111.8
N_9-C_{29}-C_{15}	103.5	101.4
N_9-C_{29}-C_{20}	112.1	113.8
C_{14}-C_{15}-C_{18}	111.4	109.9
C_{14}-C_{15}-C_{26}	105.5	105.6
C_{14}-C_{15}-C_{29}	107.7	108.8
C_{18}-C_{15}-C_{26}	113.3	115.5
C_{18}-C_{15}-C_{29}	103.6	103.8
C_{15}-C_{18}-H_{19}	106.9	105.4
C_{15}-C_{18}-C_{22}	116.3	116.5
C_{15}-C_{18}-C_{24}	105.7	105.6
C_{26}-C_{15}-C_{29}	115.4	113.3
C_{15}-C_{29}-C_{20}	117.4	117.4
C_{15}-C_{29}-C_{21}	110.1	108.9
H_{19}-C_{18}-C_{22}	106.9	107.8
H_{19}-C_{18}-C_{24}	107.0	106.0
C_{22}-C_{18}-C_{24}	113.6	114.7
C_{18}-C_{24}-H_{25}	109.3	109.4
C_{18}-C_{24}-C_{31}	112.5	112.4

Dihedral angle (θ)		
Bonding type	**Experimental**	**Theoretical**
N_9-C_{29}-C_{15}-C_{14}	98.3	92.3
N_9-C_{29}-C_{15}-C_{26}	-144.2	-150.6
H_{10}-N_9-C_{29}-C_{15}	-142.3	169.1
H_{10}-N_9-C_{29}-C_{21}	98.6	52.0
H_{10}-N_9-C_{24}-C_{18}	140.2	-169.4
C_{31}-C_{24}-C_{18}-C_{22}	-84.6	-86.0
C_{22}-C_{18}-C_{15}-C_{14}	114.5	115.1
C_{22}-C_{18}-C_{24}-N_9	153.2	154.2
C_{18}-C_{15}-C_{29}-C_{20}	-143.9	-149.2
C_{18}-C_{15}-C_{29}-C_{21}	101.4	96.7
C_{31}-C_{24}-C_{18}-C_{22}	-84.6	-86.0

2. Experimental section

2.1 Chemistry

2.1.1 General Methods

All chemicals and reagents were purchased from Sigma Aldrich and were used as such. Commercial grade solvents were distilled according to literature procedure. IR Spectra were recorded on JASCO FT IR 4100 spectrometer using KBr disc and the absorption frequencies quoted in reciprocal centimeters. 1H and ^{13}C NMR spectra were recorded on Bruker advance (400 MHz for ^{1}H and 100 MHz for ^{13}C) DMSO-d_6 solvents. The reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Chemical shifts are reported in δ values (ppm) downfield from tetramethylsilane and coupling constants are reported in Hertz (Hz). The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Elemental analyses were performed on a Perkin Elmer 2400 series II Elemental CHNS analyzer. Mass spectra were obtained on a HR mass spectrometer.

2.1.2 General procedure for synthesis of (*E*)-3-arylidene-2,3-dihydro-8-nitro-4-quinolone (3a-d)

An equimolar mixture of 2,3-dihydro-8-nitro-4-quinolone **1** (1 mmol) and the appropriate aldehydes **2a-d** (1 mmol) was dissolved in 15 ml of ethanol, 5 drops of pyrrolidine base were added and stirred at room temperature for 50 min. The solid formed in the reaction mixture was separated by filtration, dried, and recrystallized from the mixture of chloroform/ethanol (1:1) to obtain the pure product **3a-d** in good yields (89-95%).

2.1.3 General procedure for the synthesis of dispiro 8-nitroquinolone analogues 6a-l

A mixture of Benzylamine **4** (1.2 mmol) and isatin **5** (1 mmol), were refluxed for 10 minutes after that the (*E*)-3-arylidene-2,3-dihydro-8-nitro-4-quinolone (1 mmol) **3a-d** was added slowly to the mixture then the reaction refluxed for 2 - 2.5 hours.. The reaction was monitor by TLC after completion of the reaction the reaction mixture was cooled to room temperature, the solid separated by filtration, dried and recrystallized from the mixture of methanol/ dimethylformamide (3:1) to obtain the pure products **6a-l** in good yields (90–96%). Spectral data for all the compounds were given below.

5-Chloro-4'-(4-chlorophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6a

Yellow solid; Yield (92%); mp: 212-220˚C; IR (KBr): 3404, 3154, 1701, 1615 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52 (s, 1H, Isatin N-H), 8.38 (d, 1H, J = 5.2 Hz, Q N-H), 8.07 (dd, 1H, J = 6.8 Hz, Ar-H), 7.96 (dd, 1H, J = 6.4 Hz, Ar-H), 7.20-7.49 (m, 9H, Ar-H), 6.93 (dd, 1H, J = 6.0 Hz, Ar-H), 6.71 (d, 1H, J = 2.4 Hz, Ar-H), 6.49 (t, 1H, J = 8.0 Hz, Ar-H), 6.50 (d, 1H, J = 7.6 Hz, Ar-H), 5.43 (dd, 1H, J = 5.2 Hz, N-CH), 4.59 (d, 1H, J = 10.0 Hz, -CH), 4.06-3.99 (m, 2H, Q-CH_2), 2.52-2.49 (m, 1H, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.90, 54.02, 58.45, 63.14, 69.79, 110.00, 114.78, 122.40, 124.81, 126.05, 127.35, 128.12, 128.27, 128.38, 130.29, 131.63, 131.68, 131.83, 131.85, 135.64, 135.97, 141.11, 142.02, 144.98, 179.57,

191.06; Anal. Calcd for $C_{31}H_{22}Cl_2N_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97.

5-Chloro-4'-(4-bromophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6b

Yellow solid; Yield (96%); mp: 210-218˚C; IR (KBr): 3401, 3166, 1700, 1614 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.52 (s, 1H, Isatin N-H), 8.37 (d, 1H, *J* = 5.2 Hz, Q N-H), 8.07 (dd, 1H, *J* = 6.8 Hz, Ar-H), 7.96 (dd, 1H, *J* = 6 Hz, Ar-H), 7.21-7.53 (m, 9H, Ar-H), 6.93 (dd, 1H, *J* = 6.0 Hz, Ar-H), 6.71 (d, 1H, *J* = 2.4 Hz, Ar-H), 6.49 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.50 (d, 1H, *J* = 8.0 Hz, Ar-H), 5.43 (dd, 1H, *J* = 5.2 Hz, N-CH), 4.57 (d, 1H, *J* = 10.0 Hz, -CH), 4.06-3.99 (m, 2H, Q-CH_2), 2.52 (d, 1H, *J* = 2.0 Hz, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.90, 54.07, 58.42, 63.05, 69.78, 110.01, 114.79, 120.42, 122.39, 124.81, 126.05, 127.35, 128.13, 128.27, 130.28, 131.30, 131.69, 131.85, 131.99, 135.97, 136.06, 141.11, 142.01, 144.98, 179.56, 191.04; HRMS *m/z* (M+H) 631.0583; Anal. Calcd for $C_{31}H_{22}BrClN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97.

5-Chloro-4'-(4-flurophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6c

Yellow solid; Yield (94%); mp: 205-214˚C; IR (KBr): 3482, 3410, 1702, 1617 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.49 (s, 1H, Isatin N-H), 8.35 (d, 1H, *J* = 4.8 Hz, Q N-H), 8.03 (dd, 1H, *J* = 6.8 Hz, Ar-H), 7.93 (dd, 1H, *J* = 6.0 Hz, Ar-H), 7.46-7.10 (m, 9H, Ar-H), 6.90 (dd, 1H, *J* = 6.0 Hz, Ar-H), 6.86 (d, 1H, *J* = 2.0 Hz, Ar-H), 6.62 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.47 (d, 1H, *J* = 8.4 Hz, Ar-H), 5.40 (dd, 1H, *J* = 5.2 Hz, N-CH), 4.56 (d, 1H, *J* = 9.6 Hz, -CH), 4.02-3.97 (m, 2H, Q-CH_2), 2.49-2.44 (m, 1H, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.93, 53.88, 58.39, 63.30, 69.78, 109.99, 114.76, 115.06, 115.27, 115.69, 115.90, 122.43, 124.79, 126.04, 127.37, 128.09, 128.24, 130.35, 131.67, 131.85, 132.74, 134.62, 135.96, 136.14, 141.11,142.13, 144.98, 160.06, 62.48, 179.62, 191.16; Anal. Calcd for $C_{31}H_{22}FClN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97

5-Chloro-4'-(4-methoxyphenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6d

Yellow solid; Yield (91%); mp: 210-218˚C; IR (KBr): 3469, 3404, 1700, 1614 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H, Isatin N-H), 8.34 (d, 1H, *J* = 4.8 Hz, Q N-H), 8.03 (dd, 1H, *J* = 6.8 Hz, Ar-H), 7.91 (dd, 1H, *J* = 6.0 Hz, Ar-H), 7.47-7.17 (m, 7H, Ar-H), 6.90 - 6.84 (m, 3H, Ar-H), 6.69 (d, 1H, *J* = 2.4 Hz, Ar-H), 6.60 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.46 (d, 1H, *J* = 8.4 Hz, Ar-H), 5.39 (dd, 1H, *J* = 5.2 Hz, N-CH), 4.52 (d, 1H, *J* = 10.0 Hz, -CH), 4.03-3.95 (m, 2H, Q-CH_2), 3.70 (s, 3H, -OCH_3), 2.49-2.44 (m, 1H, Pyrro-N-H) ; ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.96, 53.84, 54.82, 54.85, 54.92, 58.51, 63.06, 69.66, 109.93, 113.79, 114.35, 114.68,

122.45, 124.77, 126.03, 127.21, 127.41, 128.02, 128.18, 128.23, 130.54, 130.72, 131.61, 131.83, 135.94, 141.09, 142.37, 144.97, 158.24, 179.70, 191.30; Anal. Calcd for $C_{32}H_{25}ClN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97

5-Bromo-4'-(4-chlorophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6e

Yellow solid; Yield (90%); mp: 212-214˚C; IR (KBr): 3404, 3178, 1703, 1616 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H, Isatin N-H), 8.35 (d, 1H, J = 5.2 Hz, Q N-H), 8.04 (dd, 1H, J = 6.8 Hz, Ar-H), 7.94 (dd, 1H, J = 6 Hz, Ar-H), 7.18-7.46 (m, 9H, Ar-H), 7.02 (dd, 1H, J = 6.0 Hz, Ar-H), 6.79 (d, 1H, J = 1.6 Hz, Ar-H), 6.63 (t, 1H, J = 8.0 Hz, Ar-H), 6.43 (d, 1H, J = 8.4 Hz, Ar-H), 5.41 (dd, 1H, J = 5.2 Hz, N-CH), 4.55 (d, 1H, J = 10.0 Hz, -CH), 4.04-3.95 (m, 2H, Q-CH_2), 2.49-2.45 (m, 1H, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.92, 54.00, 58.40, 63.15, 69.80, 110.51, 112.48, 114.82, 122.43, 127.34, 128.13, 128.38, 128.81, 130.60, 131.11, 131.67, 131.82, 135.66, 136.01, 141.52, 142.05, 144.97, 179.40, 191.10; Anal. Calcd for $C_{31}H_{22}ClBrN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97.

5-Bromo-4'-(4-bromophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6f

Yellow solid; Yield (93%); mp: 196-206˚C; IR (KBr): 3405, 3334, 1699, 1616 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.53 (s, 1H, Isatin N-H), 8.37 (d, 1H, J = 4.8 Hz, Q N-H), 8.07 (dd, 1H, J = 6.8 Hz, Ar-H), 8.00 (dd, 1H, J = 12 Hz, Ar-H), 7.21-7.53 (m, 9H, Ar-H), 7.06 (dd, 1H, J = 6.0 Hz, Ar-H), 6.82 (d, 1H, J = 7.2 Hz, Ar-H), 6.66 (t, 1H, J = 8.0 Hz, Ar-H), 6.46 (d, 1H, J = 8.0 Hz, Ar-H), 5.44 (dd, 1H, J = 5.2 Hz, N-CH), 4.57 (d, 1H, J = 10.0 Hz, -CH), 4.07-3.97 (m, 2H, Q-CH_2), 2.51 (d, 1H, J = 2.0 Hz, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.92, 54.06, 58.37, 63.07, 69.79, 110.51, 112.48, 114.81, 120.41, 122.42, 127.34, 128.13, 128.80, 130.59, 131.11, 131.31, 131.67, 131.85, 132.01, 136.01, 136.08, 141.51, 142.03, 144.96, 179.39, 191.08; Anal. Calcd for $C_{31}H_{22}Br_2N_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97.

5-Bromo-4'-(4-flurophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6g

Yellow solid; Yield (95%); mp: 204-212˚C; IR (KBr): 3407, 3330, 1704, 1614 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.50 (s, 1H, Isatin N-H), 8.35 (d, 1H, J = 4.8 Hz, Q N-H), 8.04 (dd, 1H, J = 6.8 Hz, Ar-H), 7.94 (dd, 1H, J = 6.0 Hz, Ar-H), 7.46-7.10 (m, 9H, Ar-H), 7.02 (dd, 1H, J = 6.0 Hz, Ar-H), 6.80 (d, 1H, J = 2.4 Hz, Ar-H), 6.63 (t, 1H, J = 8.0 Hz, Ar-H), 6.43 (d, 1H, J = 8.0Hz, Ar-H), 5.40 (dd, 1H, J = 5.2 Hz, N-CH), 4.56 (d, 1H, J = 10.0 Hz, -CH), 4.02-3.95 (m, 2H, Q - CH_2), 2.49-2.44 (m, 1H, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.95, 53.86, 58.34, 63.32, 69.79, 110.49, 112.47, 114.78, 115.06, 115.27, 122.46, 127.35, 128.10, 128.80, 130.67, 131.08, 131.65, 131.85, 132.75, 136.00, 141.52, 142.15, 144.96, 160.06, 162.48, 179.45,

191.19; Anal. Calcd for $C_{31}H_{22}FBrN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97

5-Bromo-4'-(4-methoxyphenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6h

Yellow solid; Yield (90%); mp: 196-204˚C; IR (KBr): 3405, 3199, 1694, 1614 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.49 (s, 1H, Isatin N-H), 8.37 (d, 1H, *J* = 5.2 Hz, Q N-H), 8.06 (dd, 1H, *J* = 6.8 Hz, Ar-H), 7.94 (dd, 1H, *J* = 6.8 Hz, Ar-H), 7.48-7.19 (m, 7H, Ar-H), 7.05 (dd, 1H, *J* = 6.4 Hz, Ar-H), 6.89 - 6.78 (m, 3H, Ar-H), 6.64 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.45 (d, 1H, *J* = 8.4 Hz, Ar-H), 5.42 (dd, 1H, *J* = 5.2 Hz, N-CH), 4.55 (d, 1H, *J* = 10.0 Hz, -CH), 4.10-3.98 (m, 2H, Q-CH_2), 3.71 (s, 3H, -OCH_3), 2.51-2.46 (m, 1H, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.98, 53.82, 54.91, 54.93, 58.46, 63.08, 69.67, 110.43, 112.45, 113.78, 114.70, 122.48, 127.20, 127.40, 128.02, 128.24, 128.80, 130.73, 130.86, 131.01, 131.59, 131.82, 135.97, 141.50, 142.39, 144.95, 158.24, 179.52, 191.33; HRMS *m/z* (M+H) 627.1083; Anal. Calcd for $C_{32}H_{25}BrN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97

5-Fluro-4'-(4-chlorophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6i

Yellow solid; Yield (92%); mp: 202-210˚C; IR (KBr): 3405, 3174, 1704, 1616 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 1H, Isatin N-H), 8.38 (d, 1H, *J* = 4.8 Hz, Q N-H), 8.07 (dd, 1H, *J* = 6.4 Hz, Ar-H), 7.95 (dd, 1H, *J* = 6 Hz, Ar-H), 7.20-7.50 (m, 9H, Ar-H), 6.74 (m, 1H, Ar-H), 6.62 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.55 (dd, 1H, *J* = 6.0 Hz, Ar-H), 6.48(dd, 1H, *J* = 4.0 Hz, Ar-H), 5.44 (dd, 1H, *J* = 5.2 Hz, N-CH), 4.59 (d, 1H, *J* = 10.0 Hz, -CH), 4.08-4.02 (m, 2H, Q - CH_2), 2.52 (d, 1H, *J* = 2.0 Hz, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.88, 54.06, 58.56, 63.07, 69.88, 109.33, 109.40, 113.41, 113.66, 114.69, 114.76, 114.92, 122.29, 127.38, 128.10, 128.37, 130.13, 130.21, 131.59, 131.71, 131.81, 135.63, 135.99, 138.41, 141.98, 145.06, 156.03, 158.38, 179.943, 191.003; HRMS *m/z* (M+H) 569.14; Anal. Calcd for $C_{31}H_{22}ClFN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97.

5-Fluro-4'-(4-bromophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6j

Yellow solid; Yield (94%); mp: 202-210˚C; IR (KBr): 3400, 3347, 1695, 1616 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 1H, Isatin N-H), 8.38 (d, 1H, *J* = 4.4 Hz, Q N-H), 8.07 (dd, 1H, *J* = 8.0 Hz, Ar-H), 7.95 (dd, 1H, *J* = 8.0 Hz, Ar-H), 7.53-7.22 (m, 9H, Ar-H), 6.73-6.69 (m, 1H, Ar-H), 6.62 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.56 (dd, 1H, *J* = 8.0 Hz, Ar-H), 6.48 (dd, 1H, *J* = 4.0 Hz, Ar-H), 5.44 (dd, 1H, *J* = 4.0 Hz, N-CH), 4.58 (d, 1H, *J* = 10.0 Hz, -CH), 4.08-4.02 (m, 2H, Q-CH_2), 2.53-2.49 (dd, 1H, *J* = 2.0 Hz, Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.88, 54.12, 58.54, 62.99, 69.87, 109.33, 109.41, 113.41, 113.66, 114.70, 114.76, 114.93, 120.40, 122.29, 127.38, 128.11, 130.13, 130.20, 131.30, 131.71, 131.82, 131.95, 136.00, 136.05,

138.41, 141.97, 145.06, 156.03, 158.38, 179.93, 190.98; Anal. Calcd for $C_{31}H_{22}ClFN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97.

5-Fluro-4'-(4-flurophenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6k

Yellow solid; Yield (91%); mp: 208-218˚C; IR (KBr): 3413, 3335, 1704, 1617 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.40 (s, 1H, Isatin N-H), 8.38(d, 1H, *J* = 5.2 Hz, Q N-H), 8.06 (dd, 1H, *J* = 6.8 Hz, Ar-H), 7.95 (dd, 1H, *J* = 6.4 Hz, Ar-H), 7.50 -7.13 (m, 9H, Ar-H), 6.74 - 6.68 (m, 1H, Ar-H), 6.62 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.56 (dd, 1H, *J* = 6.0Hz, Ar-H), 6.47 (dd, 1H, *J* = 4.0 Hz, Ar-H), 5.43 (dd, 1H, *J* = 5.2 Hz, N-CH), 4.60 (d, 1H, *J* = 10.0 Hz, -CH), 4.09-3.99 (m, 2H, Q-CH_2), 2.51-2.48 (d, 1H, *J* = 14.8 Pyrro-N-H) ; ^{13}C NMR(100 MHz, DMSO-d_6): δ 45.86, 53.89, 58.47, 63.20, 69.83, 109.27, 109.34, 113.37, 113.61, 114.69, 114.86, 115.02, 115.23, 122.28, 127.29, 127.36, 128.04, 130.16, 130.24, 131.52, 131.60, 131.65, 131.78, 132.65, 132.68, 135.94, 138.38, 142.05, 145.02, 156.00, 158.35, 160.01, 162.43, 179.95; Anal. Calcd for $C_{31}H_{22}FBrN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97

5-Fluro-4'-(4-methoxyphenyl)-8''-nitro-5'-phenyl-1'',2''-dihydro-4''*H*-dispiro[indoline-3,2'-pyrrolidine-3',3''-quinoline]-2,4''-dione 6l

Yellow solid; Yield (92%); mp: 210-224˚C; IR (KBr): 3403, 3315, 1701, 1613 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H, Isatin N-H), 8.38 (d, 1H, *J* = 4.0 Hz, Q N-H), 8.06 (dd, 1H, *J* = 4.0 Hz, Ar-H), 7.93 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.50-7.19 (m, 7H, Ar-H), 6.86 (d, 2H, *J* = 8.0 Hz, Ar-H), 6.71 - 6.68 (m, 1H, Ar-H), 6.62 -6.55 (m, 2H, Ar-H), 6.46 (dd, 1H, *J* = 4.0 Hz, Ar-H), 5.42 (dd, 1H, *J* = 8.0 Hz, N-CH), 4.56 (d, 1H, *J* = 10.0 Hz, -CH), 4.12-4.06 (m, 2H, Q-CH_2), 3.71 (s, 3H, -OCH_3), 2.51 (d, 1H, Pyrro-N-H) ; ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.93, 53.89, 54.90, 58.63, 63.00, 69.75, 109.24, 113.01, 113.79, 122.35, 127.20, 127.45, 128.01, 128.22, 130.68, 131.64, 131.79, 135.96, 142.33, 145.05, 156.05, 158.24, 180.08, 191.23; HRMS *m/z* (M+H) 564.1899; Anal. Calcd for $C_{32}H_{25}FN_4O_4$; C, 59.11; H, 3.52; N, 8.89; Found C, 59.46; H, 3.48; N, 8.97

2.2 Biology

2.2.1 MTT Cytotoxicity Assay: Cell survival was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as previously described. Briefly, cells were seeded in 100 mL of growth medium at a density of 4000 cells/well in 96-well plates and allowed to establish for 24 h, at which time serially diluted compounds were added in an additional 100 mL of growth medium. Cells were then incubated for 72 h at 37ºC in humidified 5% CO_2, at which time the growth media was drawn and replaced with MTT in IMDM growth media and incubated for 4 h. The MTT solution was then aspirated from the wells, 100 mL of acidified ethanol solution was added to each well, and after following a 15 min lysis step, cell viability was measured spectro photometrically by absorbance at 570 nm and background corrected at 690 nm. All MTT assays were performed three times in triplicate. IC_{50} cytotoxicity

values were determined as the drug concentration that reduced the absorbance to 50% of that in untreated control wells and derived from at least three separate experiments.

2.2.2 Cell Culture and Compounds Treatment: HeLa cells (human cervical carcinoma cells) was procured from National Center for Cell Sciences (NCCS), Pune, India and were cultured in Dulbecco's modified Eagle's medium supplemented with L-glutamine (4 mM), penicillin (50 units mL-1), streptomycin (50 mg mL-1), and 10% (v/v) fetal bovine serum. Cells were maintained in 5% CO_2 humidified incubator at 37ºC. The stock solutions of compounds and were prepared in sterile phosphate buffered saline (PBS). Required volumes of IC_{50} compounds were added to the cultures to obtain appropriate concentrations incubated for 24 h. DMEM was used to dilute the stock to required concentrations. After the treatment with compounds, cells were observed under a phase contrast microscope (Nikon ECLIPSE, TS100, Tokyo) to identify the morphological changes as compared to the non-treated cells.

2.2.3 Hemolysis Assay: Hemolysis assay was performed as per the guidelines of research ethics and Bharathiar University, Department of Chemistry approved these human blood samples analysis. Informed consent was obtained from the human subject for analyzing the human blood samples. Fresh blood sample was obtained and diluted using physiological saline. Red blood cells (RBCs) were then isolated from the serum by centrifugation at 3000 rpm for 10 min. After careful washing three times, a suspension of RBCs was added into the compounds solution at systematically varied IC_{50} concentrations and mixed completely. The mixture was incubated in a 5% CO_2 atmosphere at 37°C. After incubation, all suspensions were centrifuged at 3000 rpm for 5 min, and then photos were taken for each sample. The supernatant of each tube was transferred to a 96-well plate. The OD values of supernatants were measured with a microplate reader at 570 nm. The precipitation of each tube was used to make the cell smear to observe the morphological changes of the erythrocyte. The hemolysis ratio of RBCs was calculated using the following formula: Hemolysis (%) = (OD sample-OD negative control)/(OD positive control-OD negative control) x 100. Physiological saline (PBS) and distilled water were used as the negative and positive controls, respectively.

2.2.4 Determination of Intracellular ROS Generation: The generation of ROS in compounds at IC_{50} treated cells was determined by 2,7-dichlorofluorescein diacetate (DCFH-DA; Sigma-Aldrich, USA) staining. DCFH-DA is non-fluorescent and can diffuse into the cell through the plasma membrane where it is hydrolyzed to DCFH. Non-fluorescent DCFH is finally converted to green fluorescent dichlorofluorescein (DCF) upon intracellular oxidation. For this assay, Cells were seeded in a 6-well plate ($2x10^5$ cells/well) and were grown for 24 h. After 24 h of growth, the cells were treated with IC_{50} concentrations of compounds for 3 h, harvested, and washed twice with PBS. Finally, the cells were resuspended in 1 mL of DMEM with 5 µM DCFH-DA and incubated for 10 min at 37°C. Stock (1 mM) solution of DCFH-DA was prepared in ethanol and stored under liquid nitrogen vapor. Immediately after the incubation, the samples were analyzed for DCF fluorescence in a flow cytometer (FacsCalibur, BD Biosciences, NJ) at an excitation wavelength of 485 nm and emission wavelengths of 530. The fluorescence data were recorded with the Cell Quest program (BD Biosciences) for 20000 cells in each sample. Flow

cytometric data were analyzed using WinMDI software and the ROS generation was expressed in terms of percentage of cells with DCF (green) fluorescence. A parallel batch of treated cells was stained with DCFH-DA visualized under a fluorescence microscope (Nikon ECLIPSE, TS100, Tokyo).

2.2.5 DAPI Staining: The Cells ($1x10^5$ cells/coverslip) were incubated with compounds at their IC_{50} concentration, fixed in methanol:acetic acid (3:1 v/v) and stained with 5 µg/mL of DAPI for 20 min, and analysed for nuclear morphological changes using fluorescence microscope with excitation filter at 510-590 nm.

2.2.6. Flow cytometry and Western blot analysis

The flow cytometric detection of apoptosis in cancer cells, the cells were stained with Annexin V- FITC and propidium iodide (PI). In brief, the cells were pretreated with effective compound concentration. The treated cells were suspended in 200 µL of binding buffer, and the suspension was added with 10 µL of Annexin V-FITC and 5 µL of PI followed by incubation for 15 min in dark at room temperature. Subsequently, 300 µL of binding buffer was added to the cell suspension and the cells were analyzed by a flow cytometer (BD, FACS Calibur, USA). Cells undergoing early apoptosis bind only to Annexin V/FITC, while cells that are either in the late stages of apoptosis or already dead bind to both Annexin V/FITC and PI. All experiments detected at least 10.000 cells, and the data were analyzed with FCS Express V3.

Western blotting of the cells was treated with effective compound for the induction of apoptosis. Western blot analysis for expression of the caspase were incubated for 1h in 5% powdered non-fat milk in PBS (pH 7.4) with antibodies for 30 min in 5% non-fat dry milk with goat anti-mouse serum (1:2000) and visualized. Subsequently, Western blot analysis was performed for cell extracts after determining their protein content by Bradford assay. 50 µg protein samples were resolved by 12% SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose membrane (Pall Corporation, USA). The membranes were immersed in blocking buffer (5% skim milk in PBS) for 1 h at room temperature and incubated overnight with primary antibodies. After normalization with the corresponding expression of β-actin, the protein expression was determined.

2.2.6 Statistical Analysis: Statistical analysis was performed with the Statistical Program for Social Sciences software (SPSS). All data were expressed as means ± standard deviation, and a statistically significant difference was considered to be present at $*p<0.05$ as significant or $**p<0.01$ as highly significant. All assays were carried out in triplicates with three independent experiments.

Spectral data

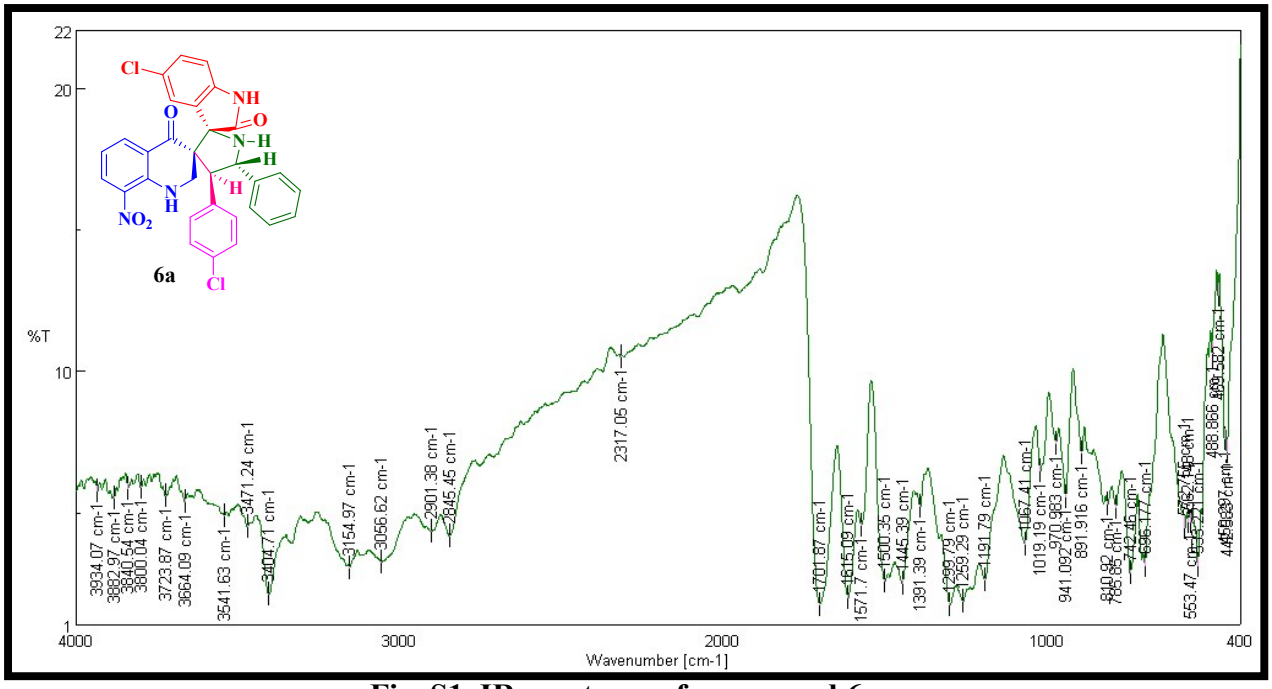


Fig. S1. IR spectrum of compound 6a

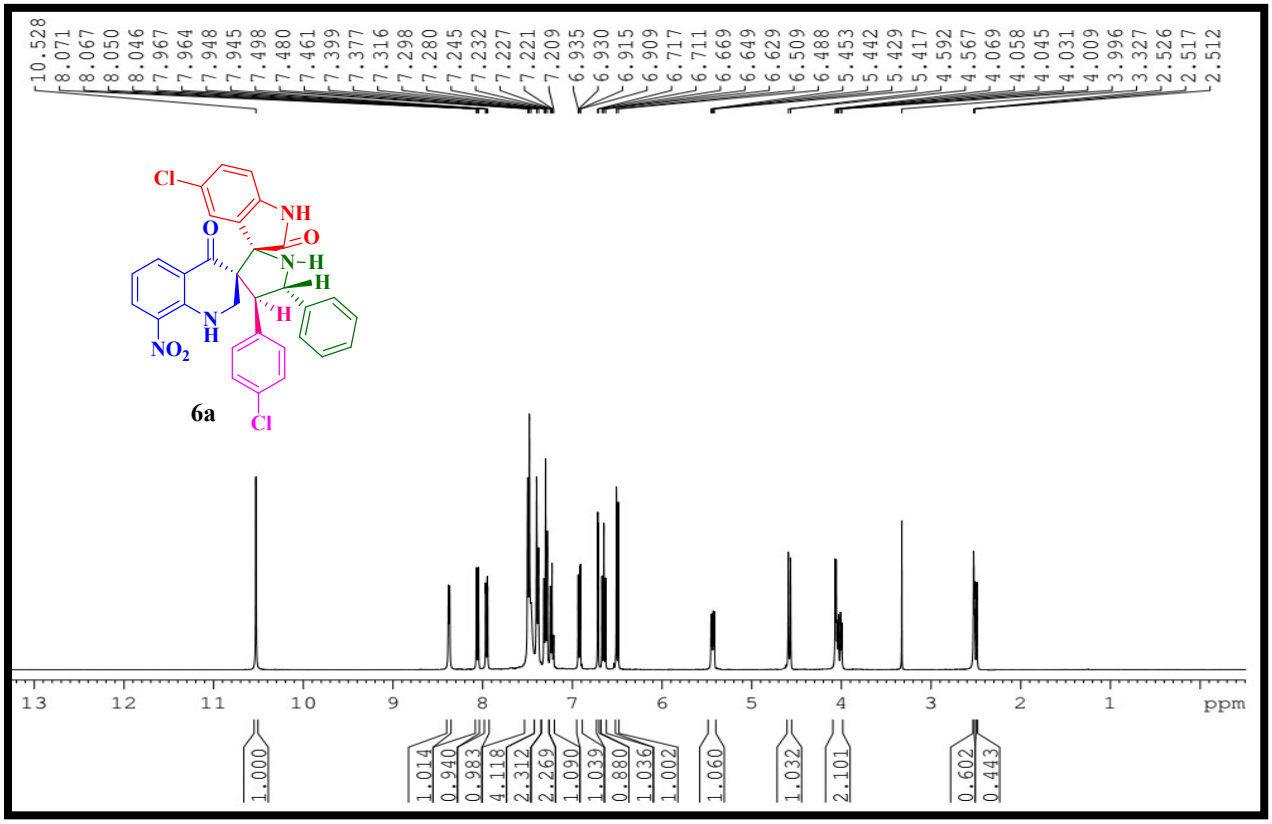


Fig. S2. ^{1}H NMR spectrum of compound 6a

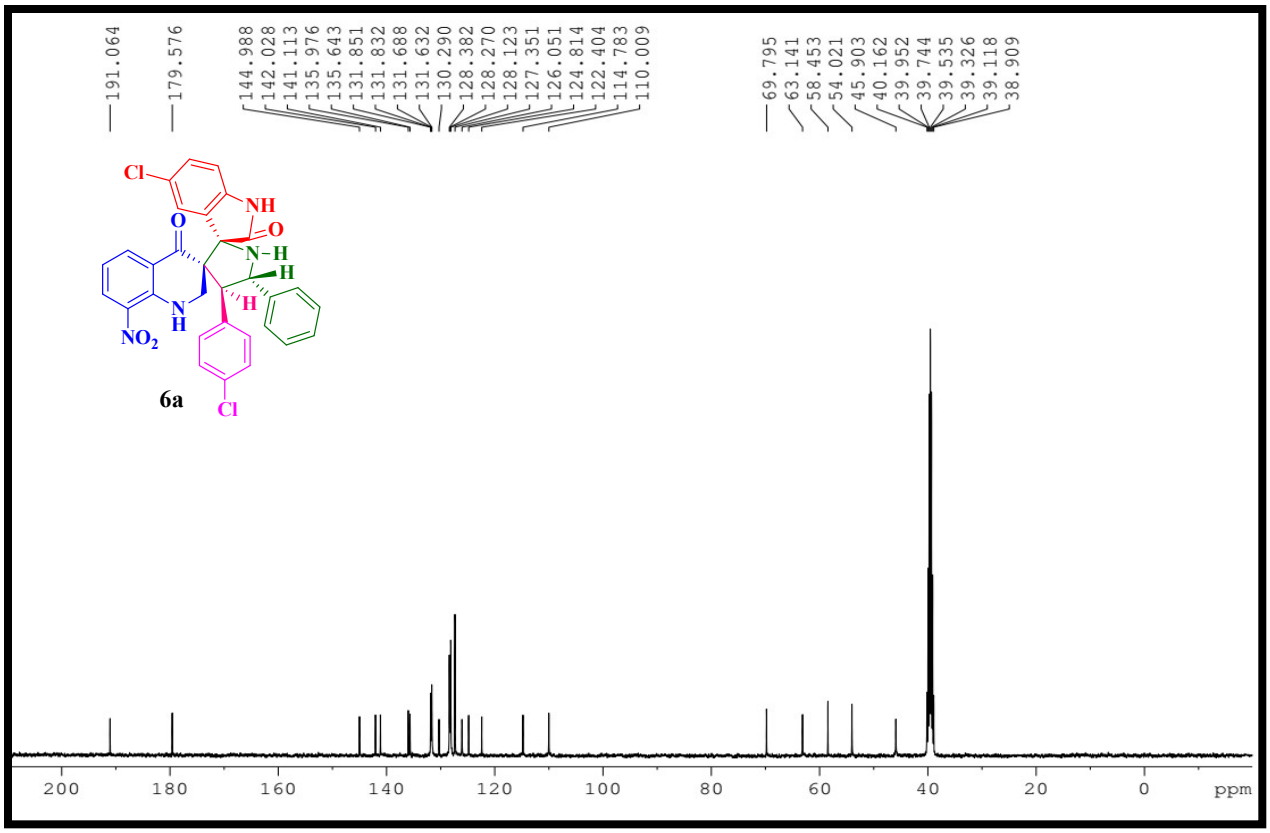


Fig. S3. ^{13}C NMR spectrum of compound 6a

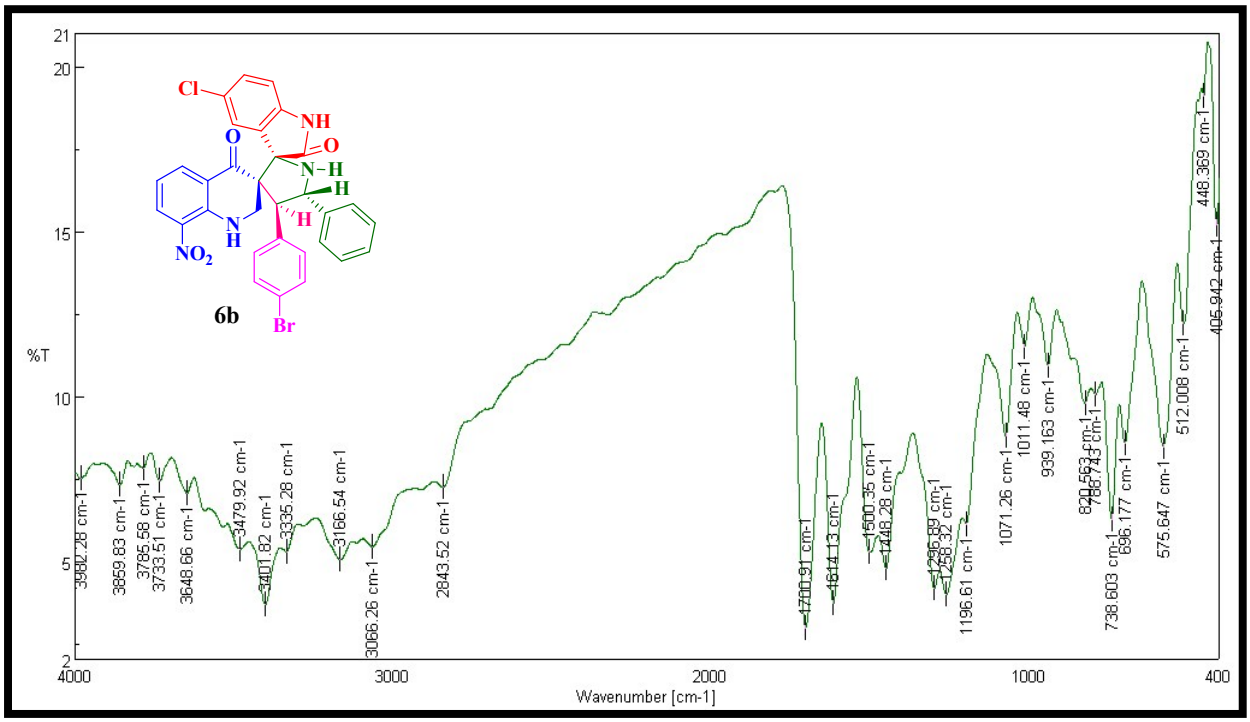


Fig. S4. IR spectrum of compound 6b

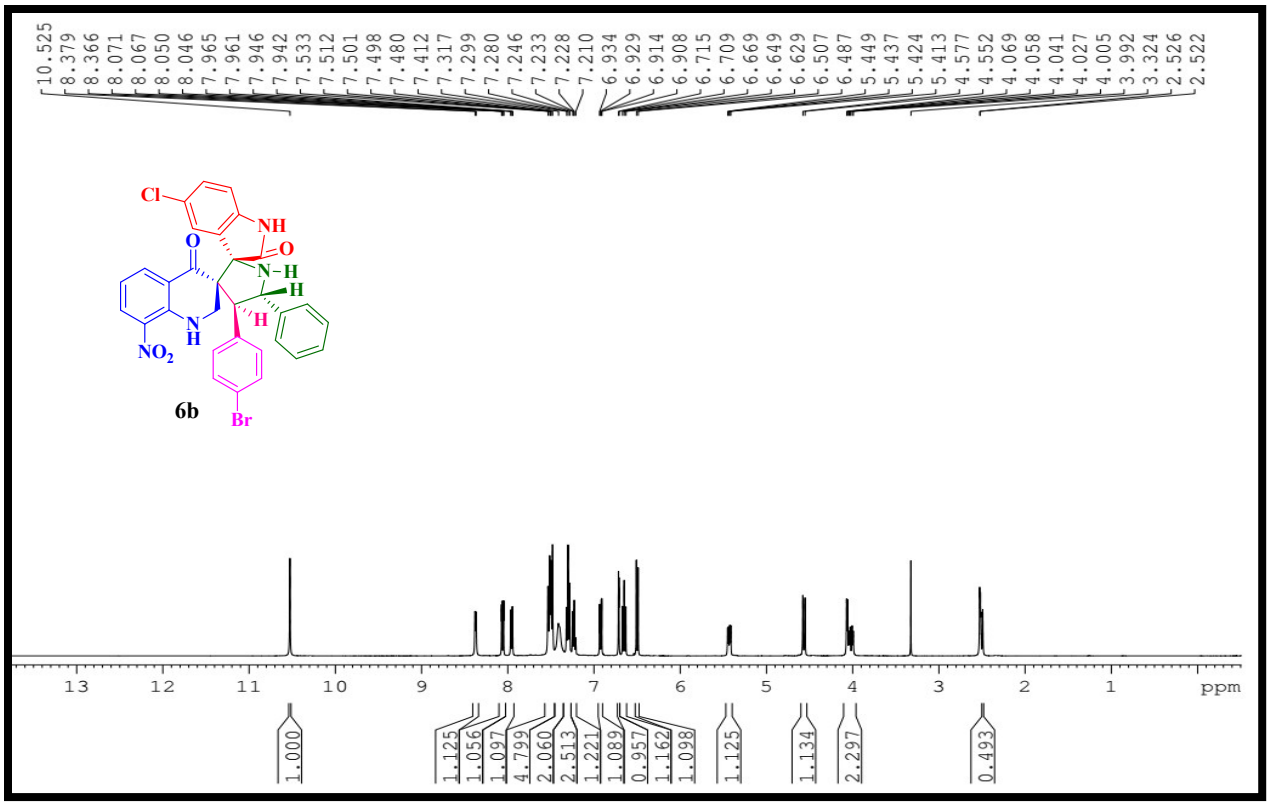


Fig. S5. ^{1}H NMR spectrum of compound 6b

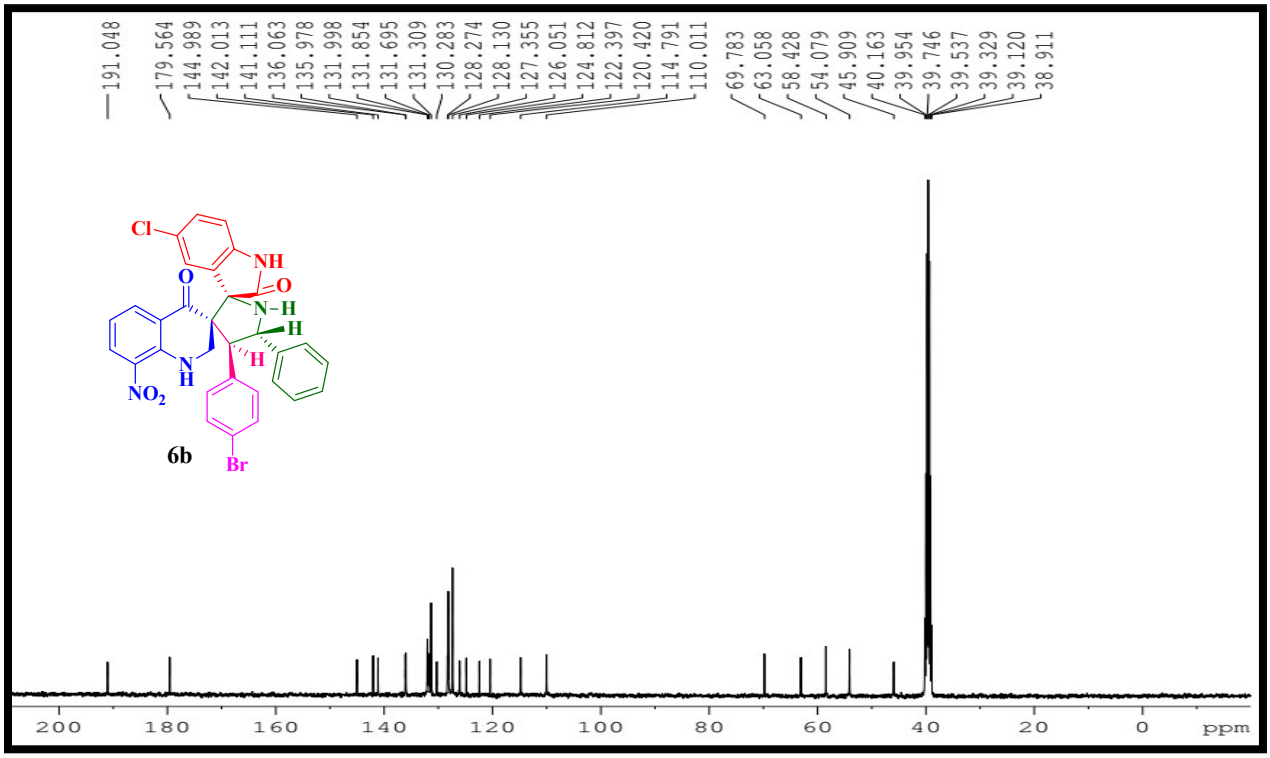


Fig. S6. ^{13}C NMR spectrum of compound 6b

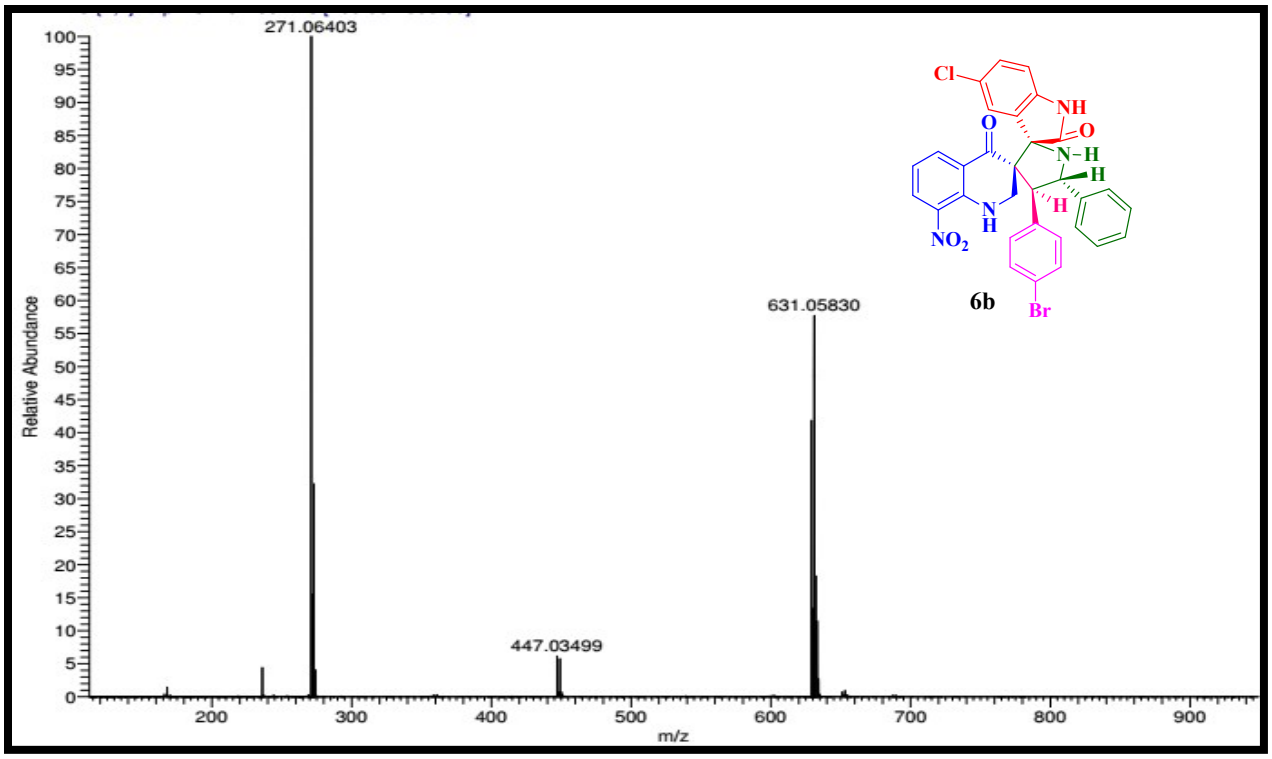


Fig. S7. HRMS spectrum of compound 6b

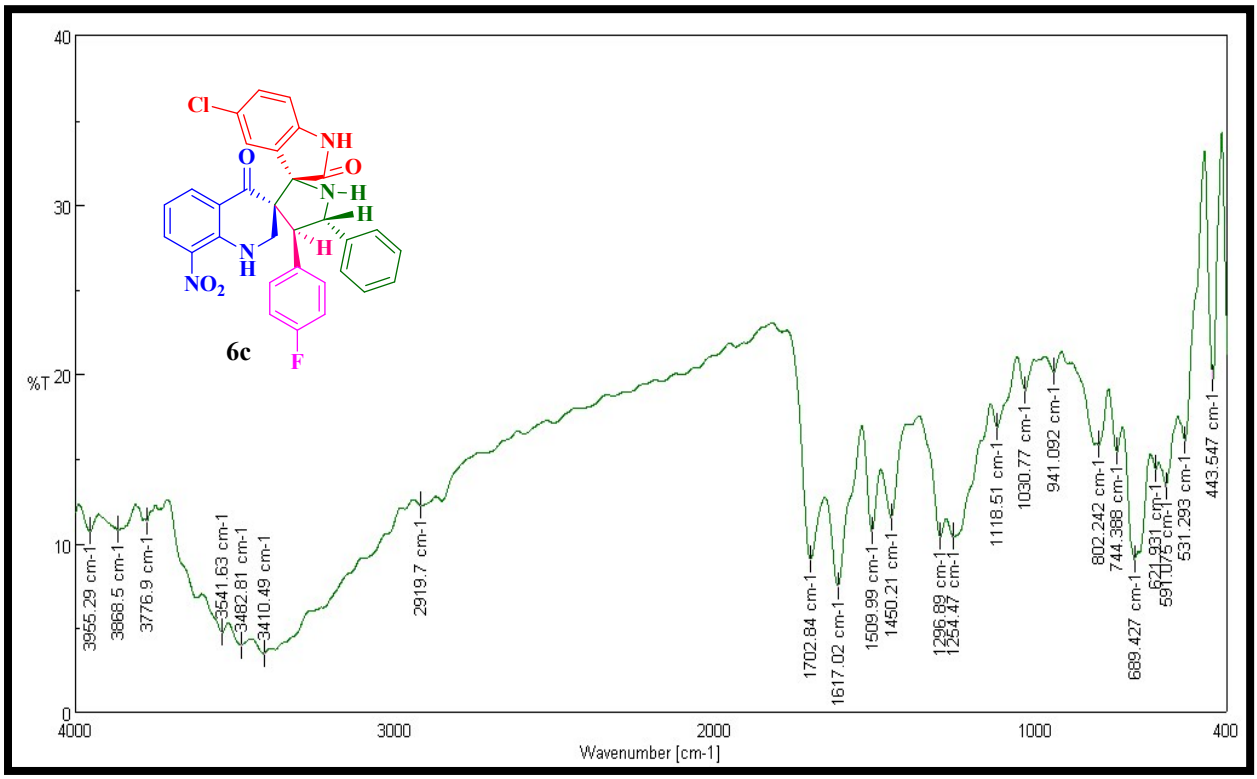


Fig. S8. IR spectrum of compound 6c

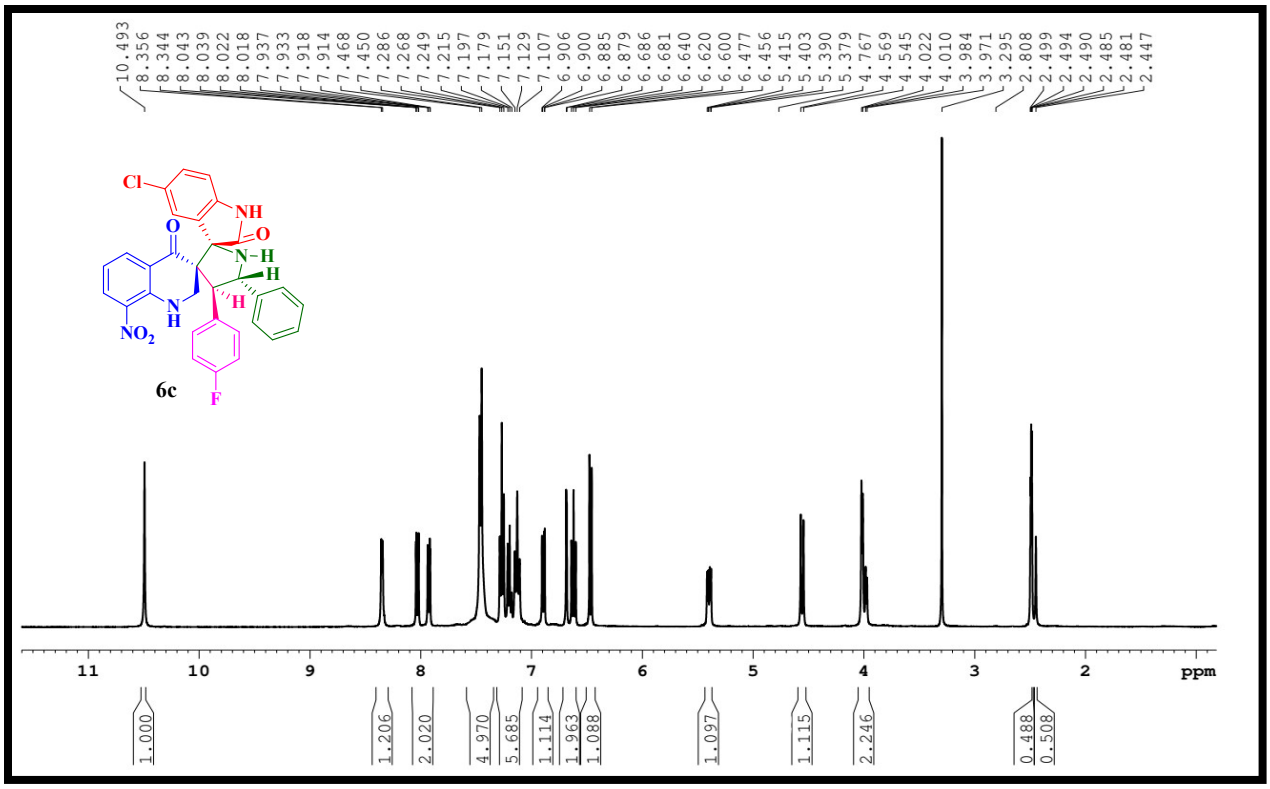


Fig. S9. ^{1}H NMR spectrum of compound 6c

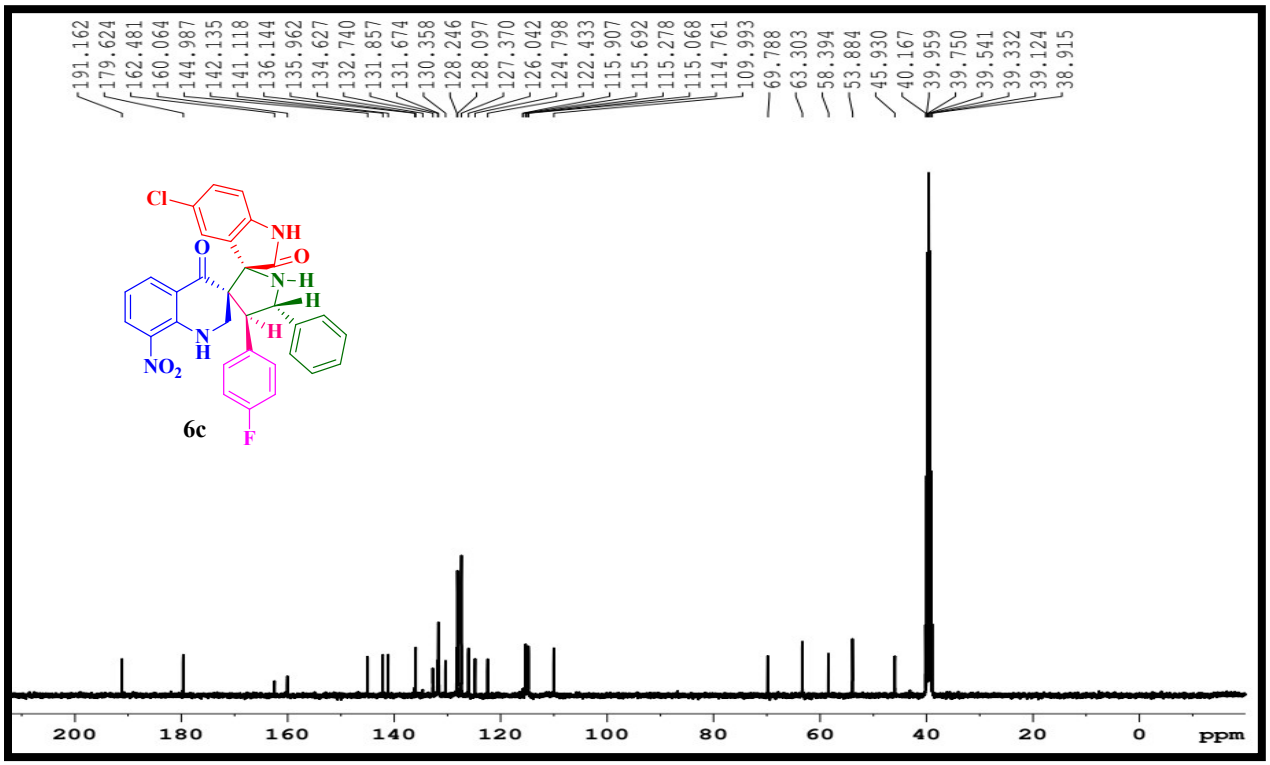


Fig. S10. ^{13}C NMR spectrum of compound 6c

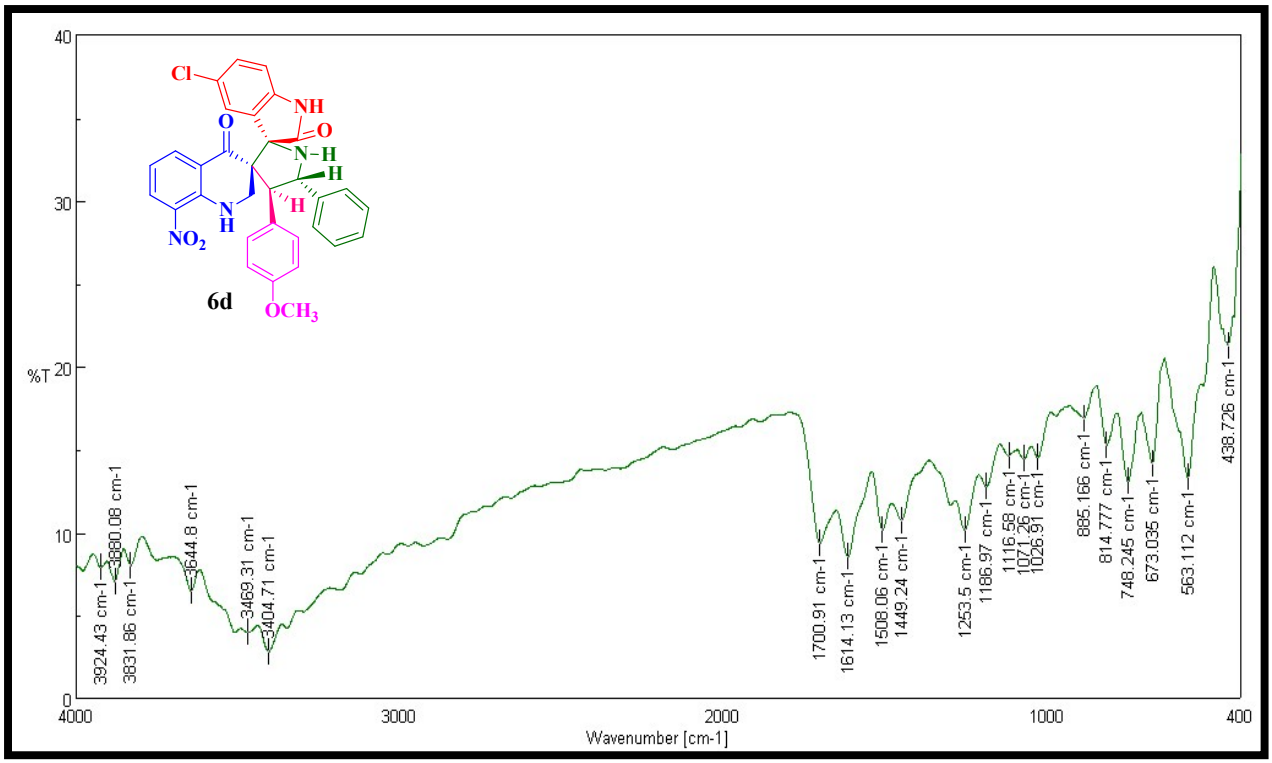


Fig. S11. IR spectrum of compound 6d

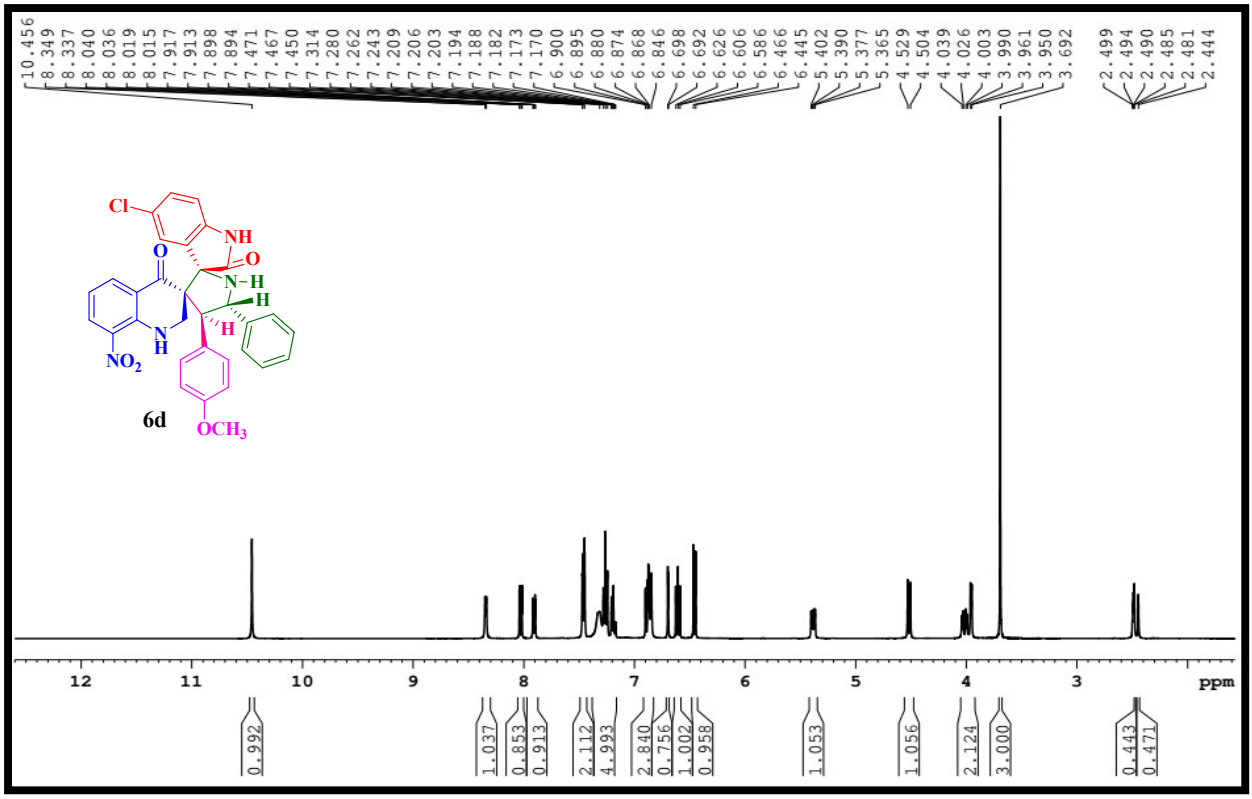


Fig. S12. ^{1}H NMR spectrum of compound 6d

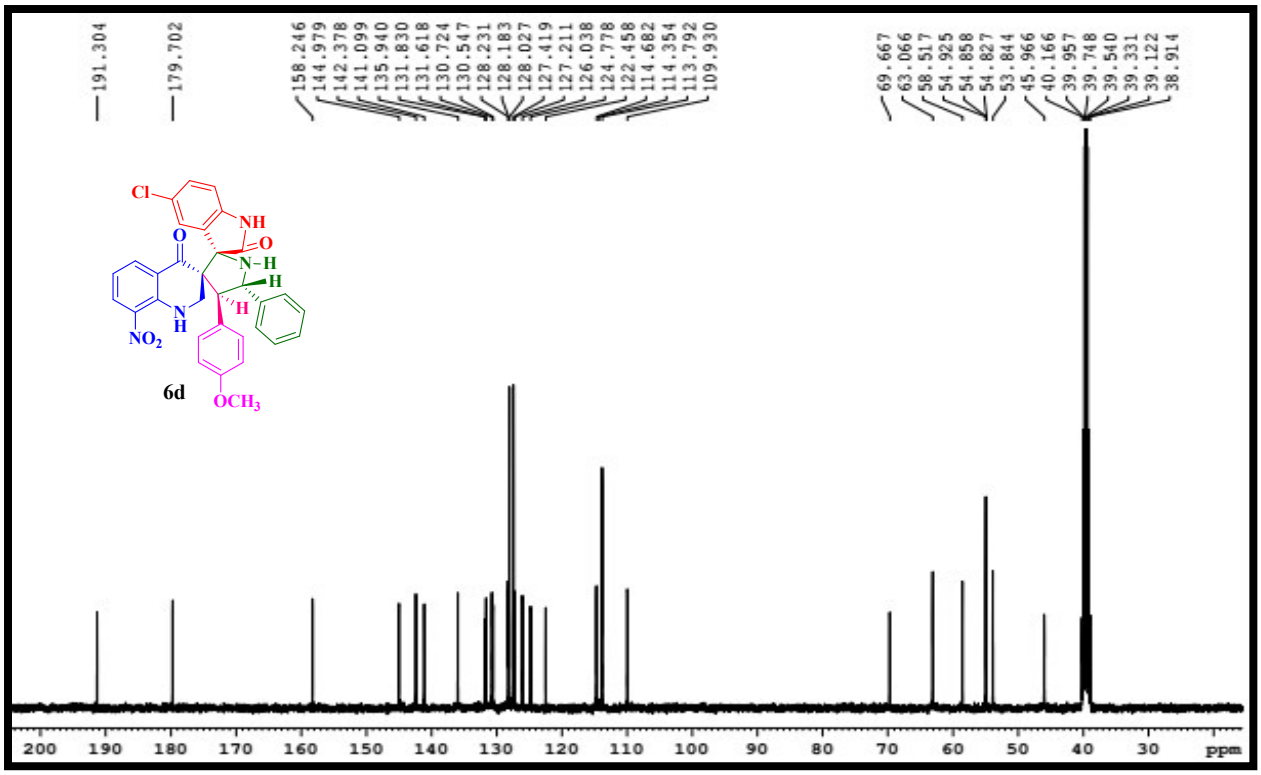


Fig. S13. ^{13}C NMR spectrum of compound 6d

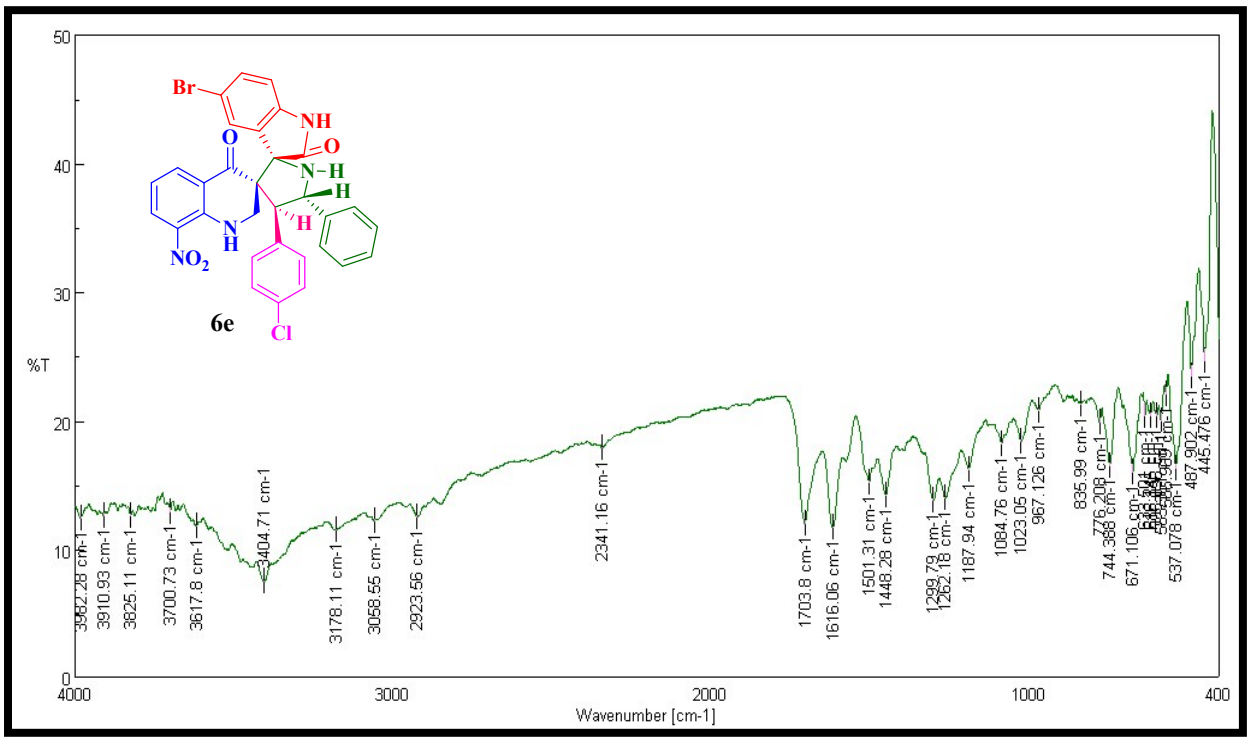
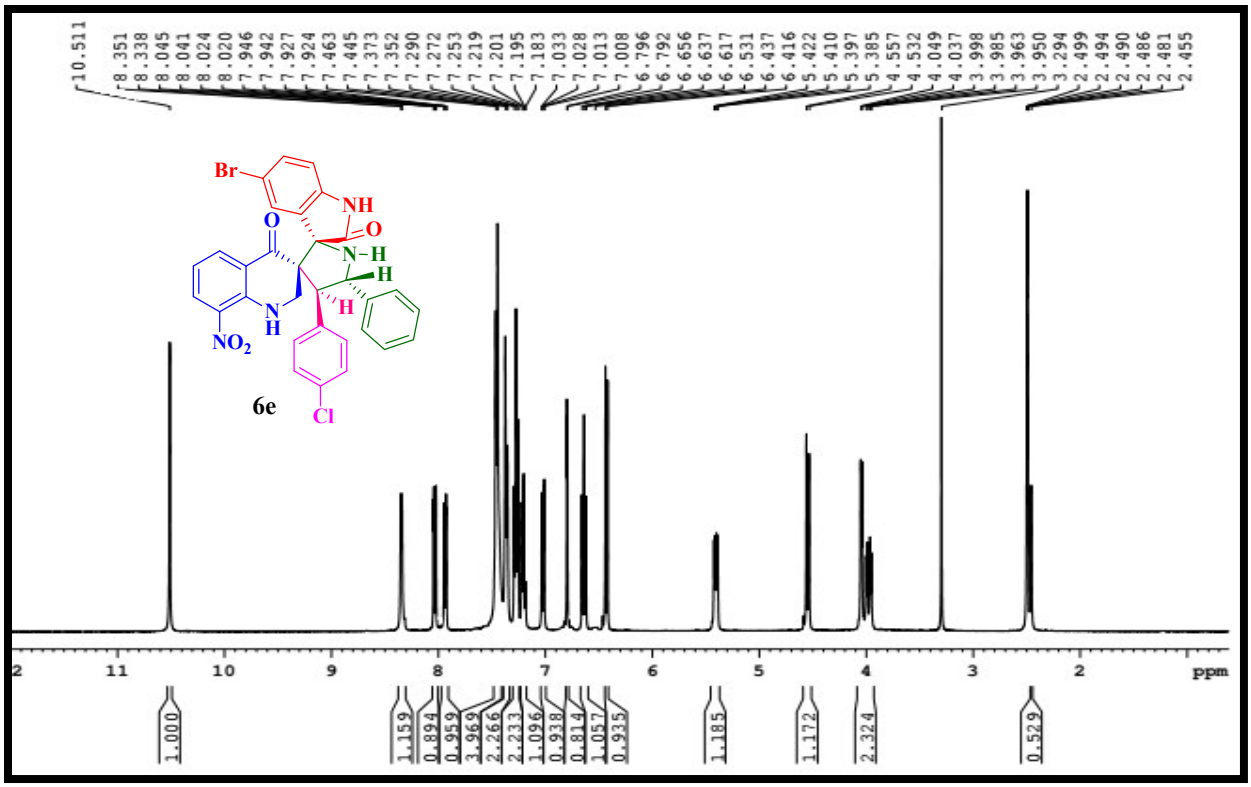
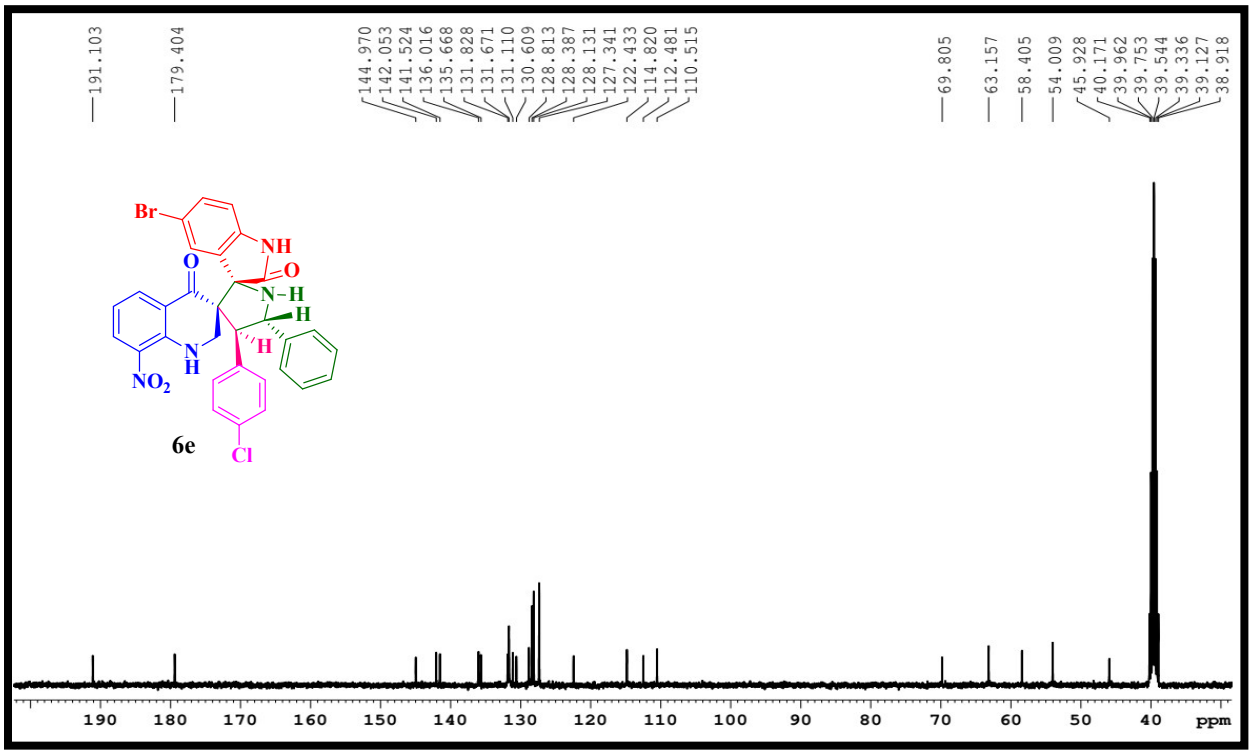


Fig. S14. IR spectrum of compound 6e



S15. ^{1}H NMR spectrum of compound 6e



S16. ^{13}C NMR spectrum of compound 6e

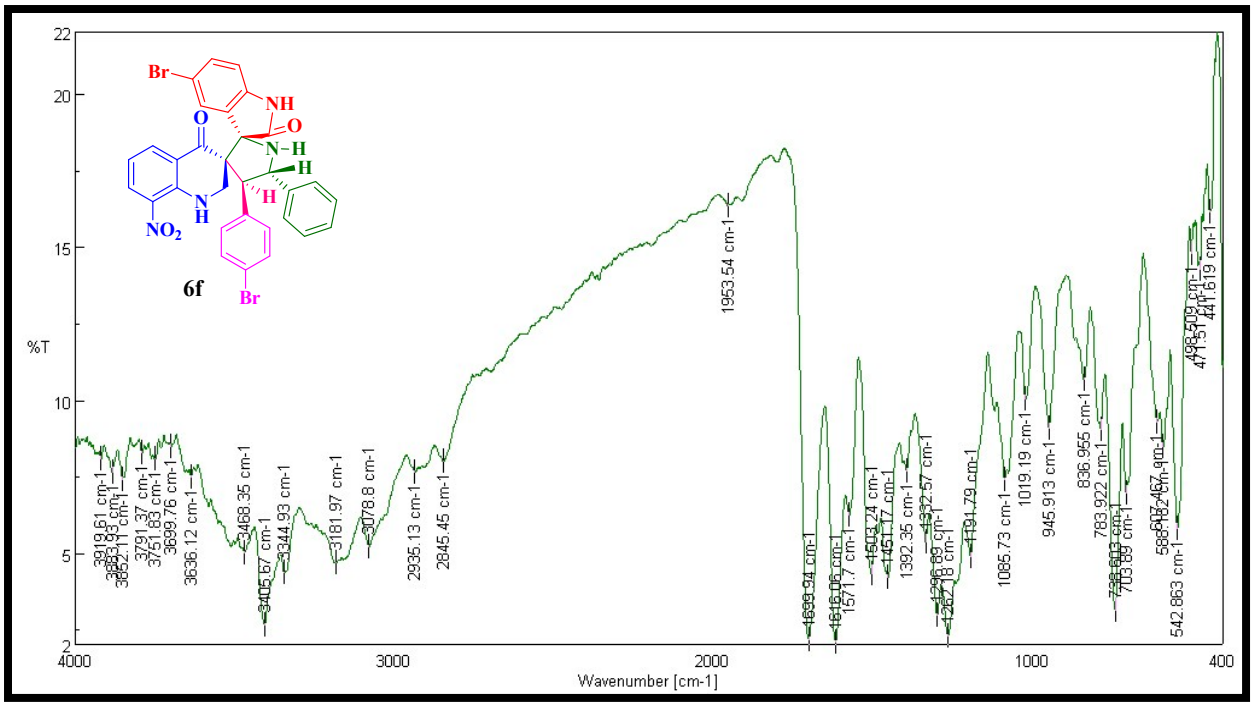
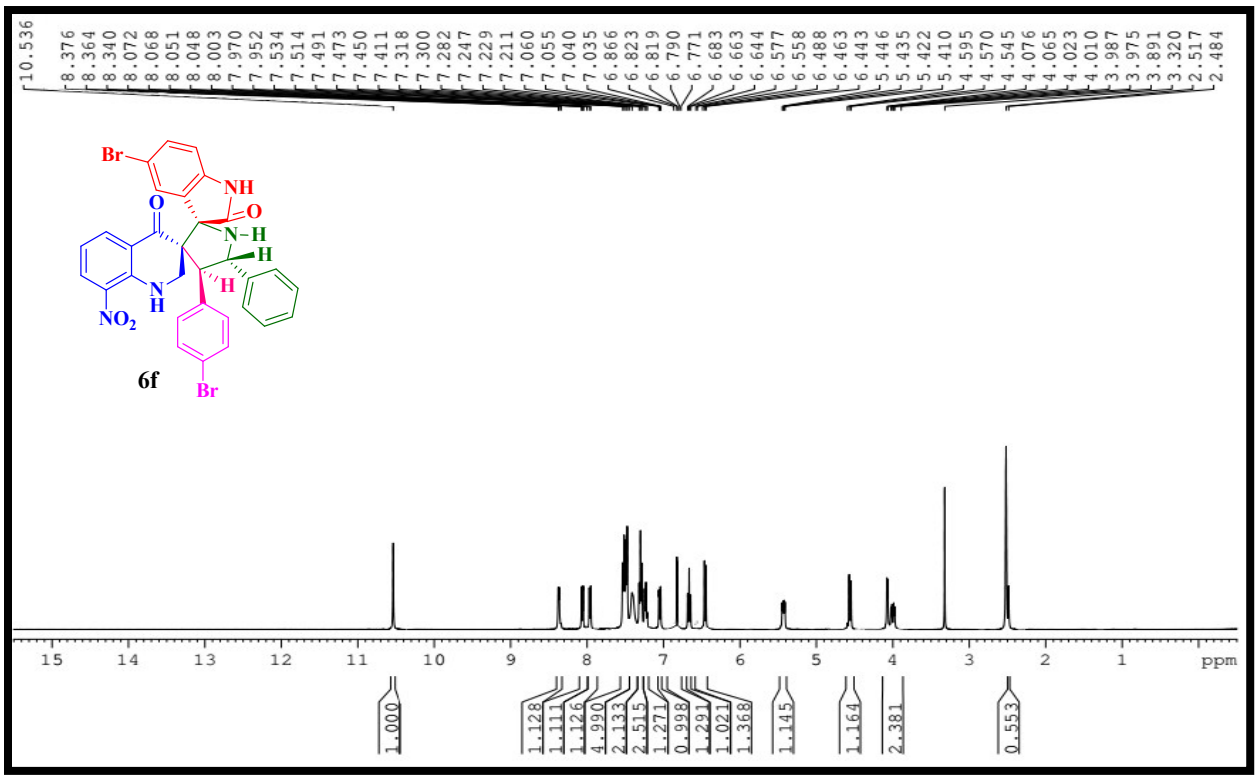
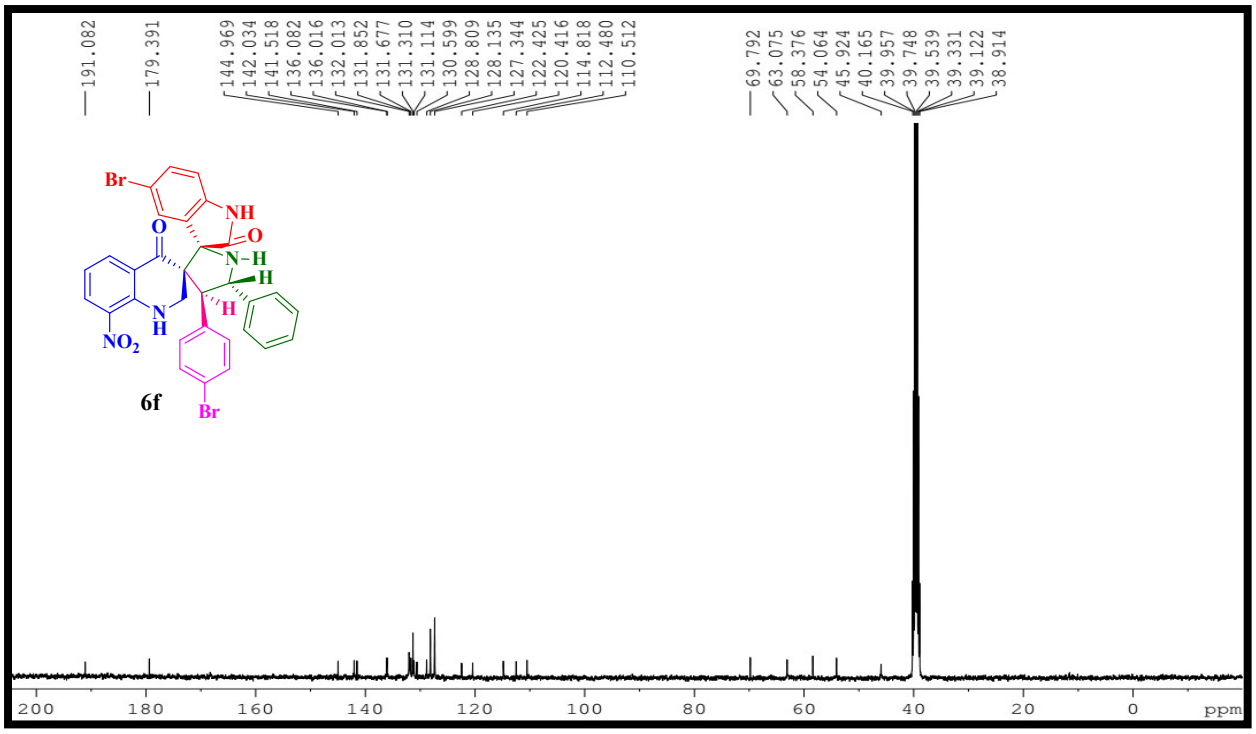


Fig. S17. IR spectrum of compound 6f



S18. ^{1}H NMR spectrum of compound 6f



S19. ^{13}C NMR spectrum of compound 6f

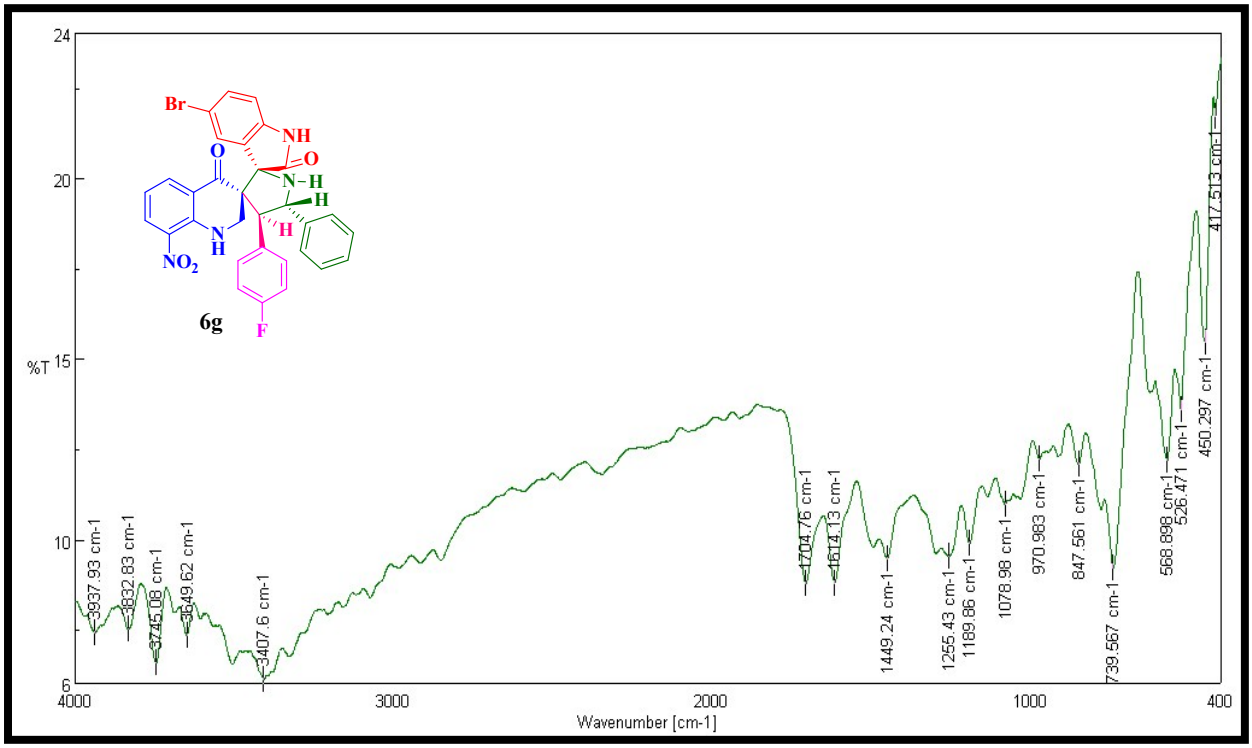


Fig. S20. IR spectrum of compound 6g

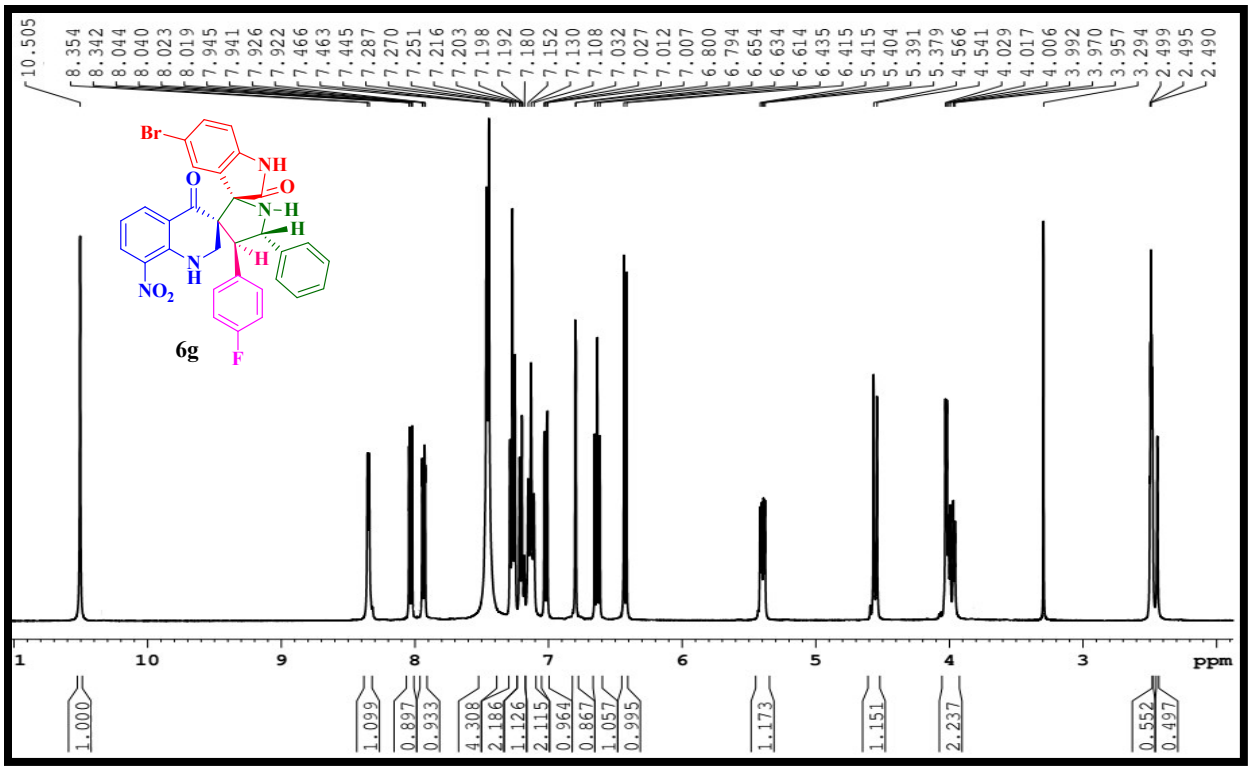


Fig. S21. ^{1}H NMR spectrum of compound 6g

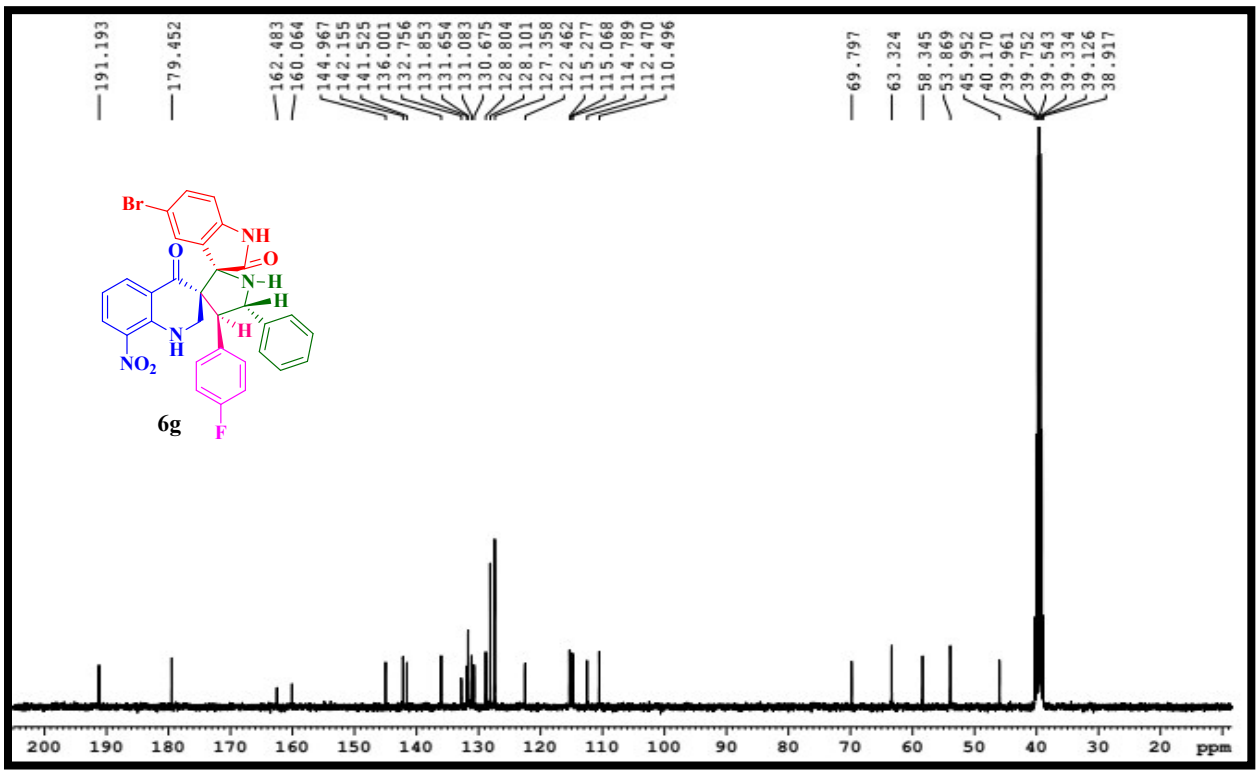


Fig. S22. ^{13}C NMR spectrum of compound 6g

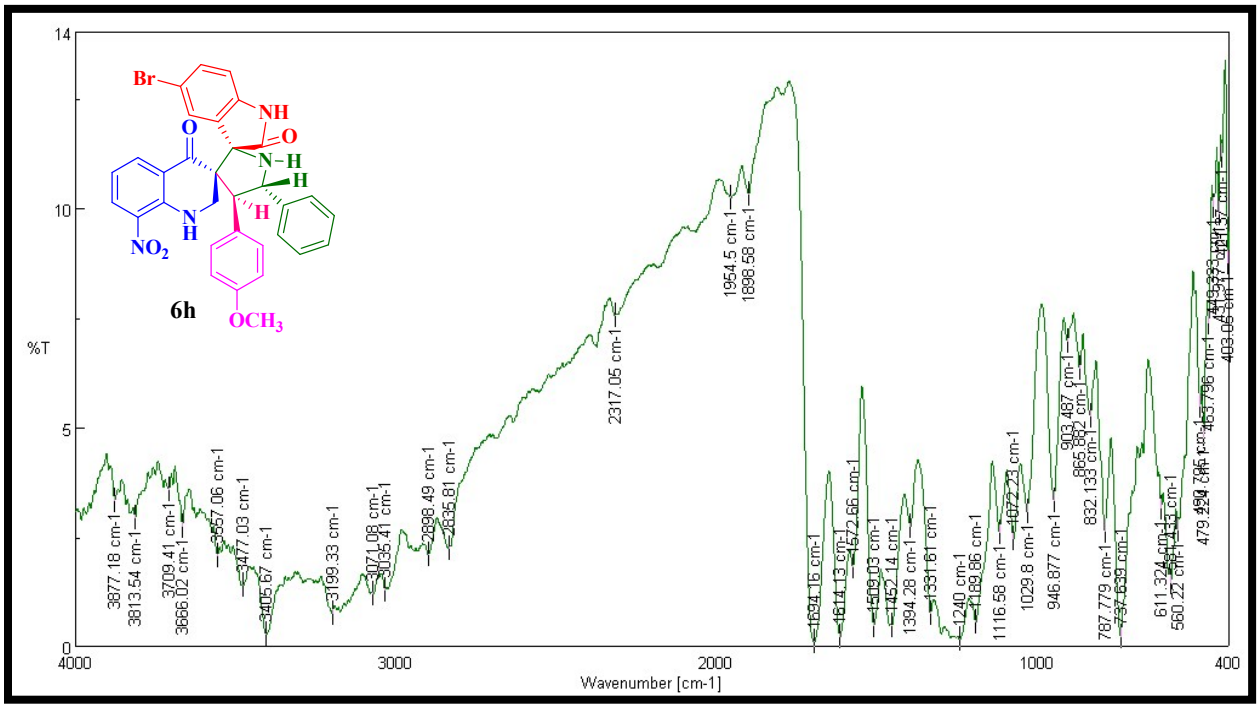


Fig. S23. IR spectrum of compound 6h

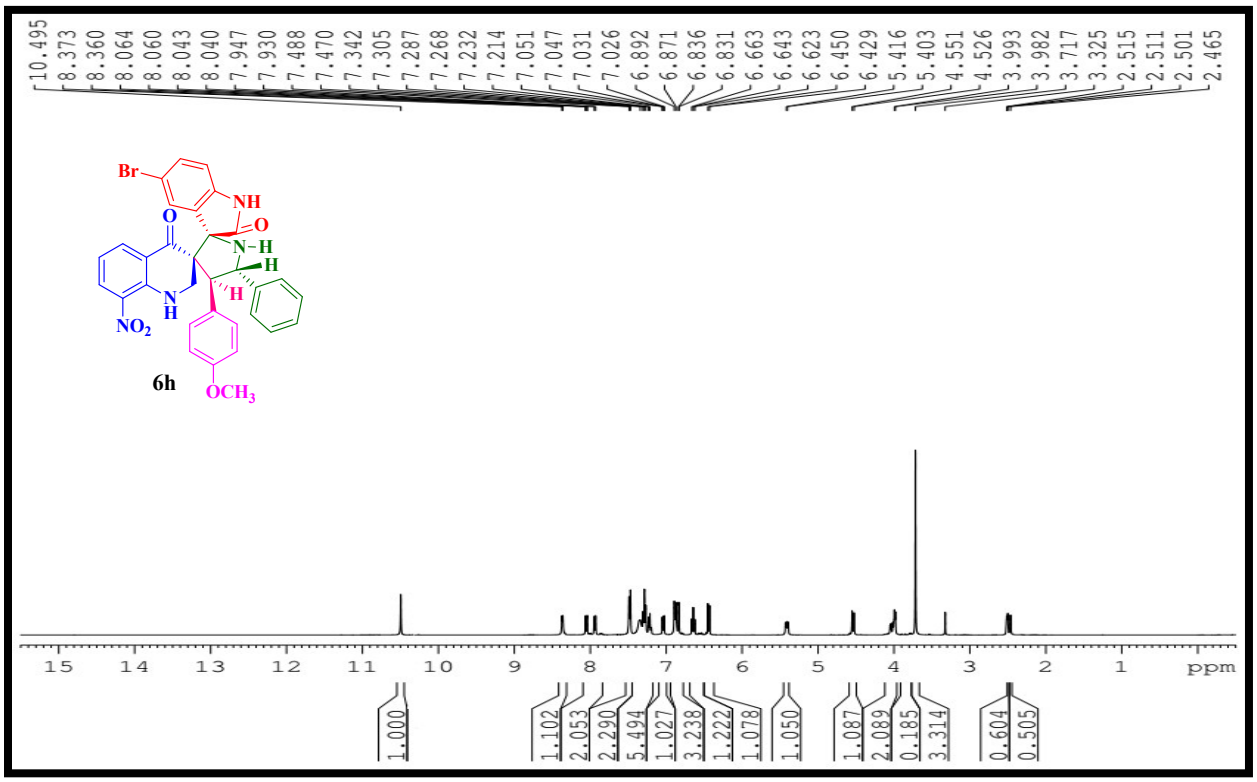


Fig. S24. ^{1}H NMR spectrum of compound 6h

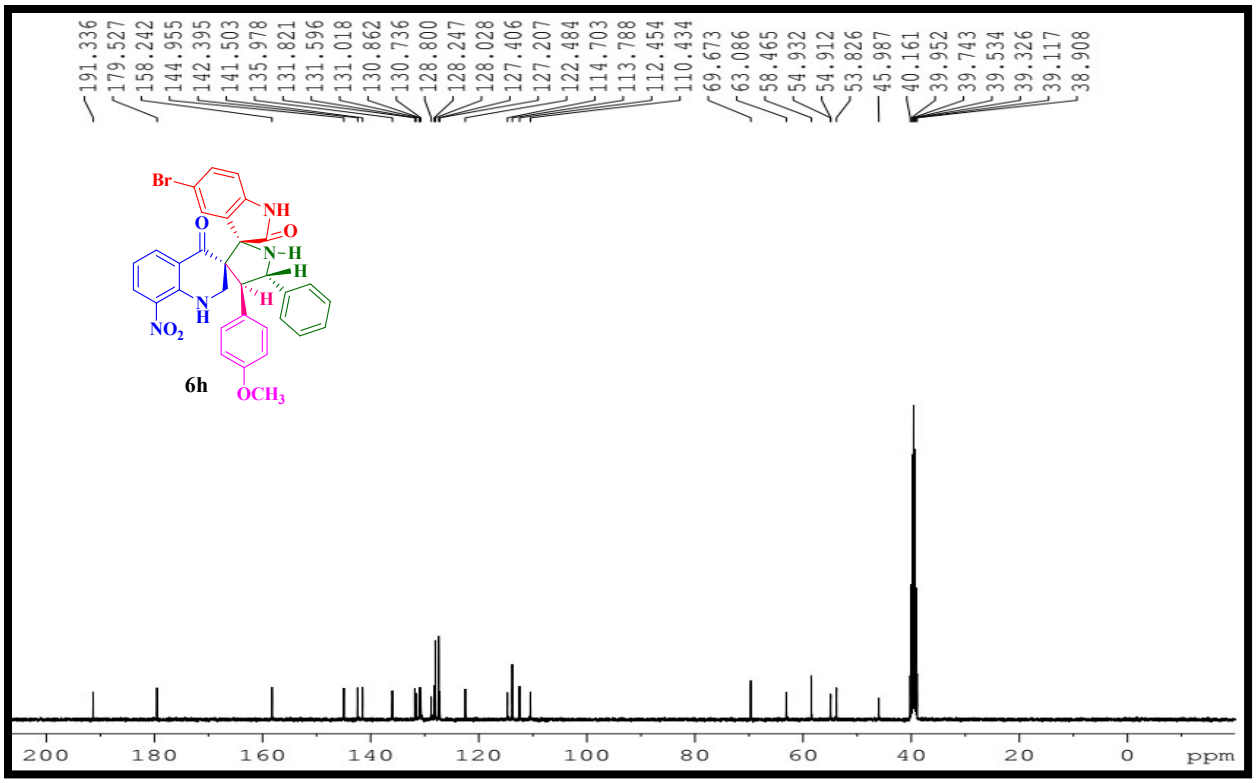


Fig. S25. ^{13}C NMR spectrum of compound 6h

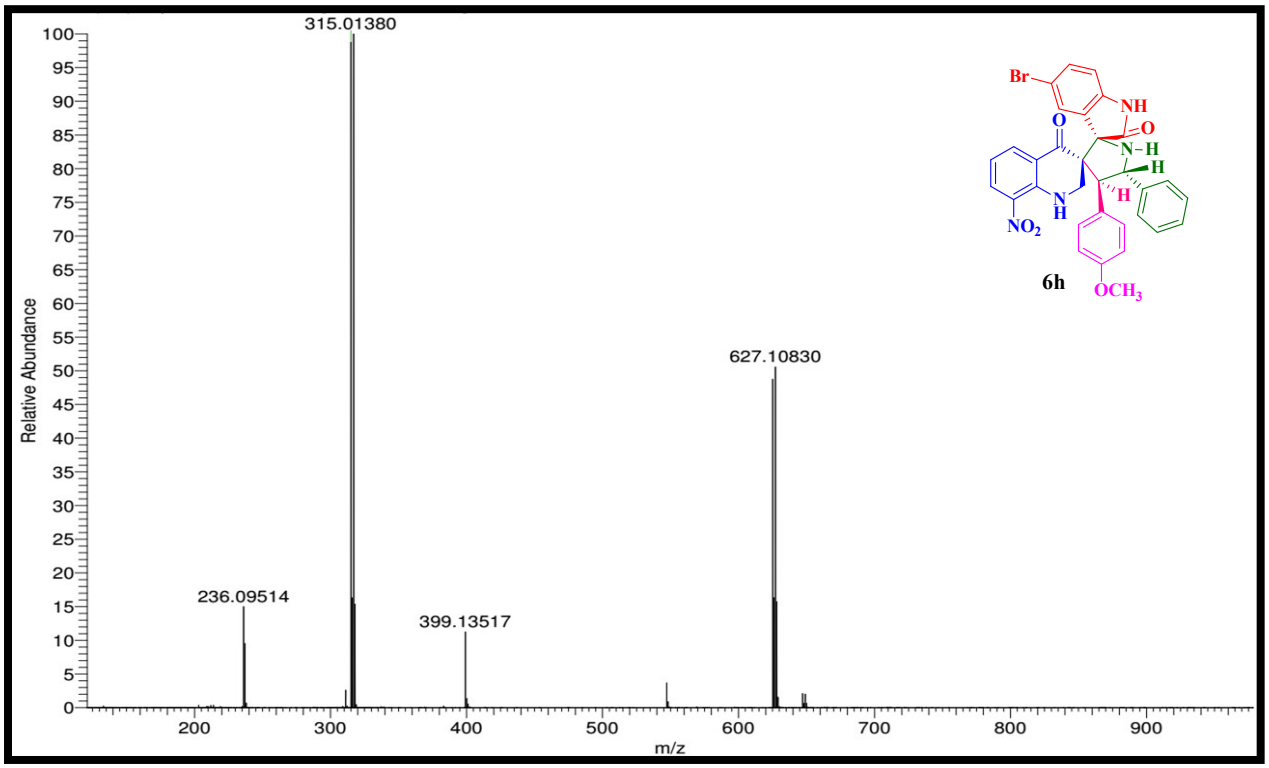


Fig. S26. HRMS NMR spectrum of compound 6h

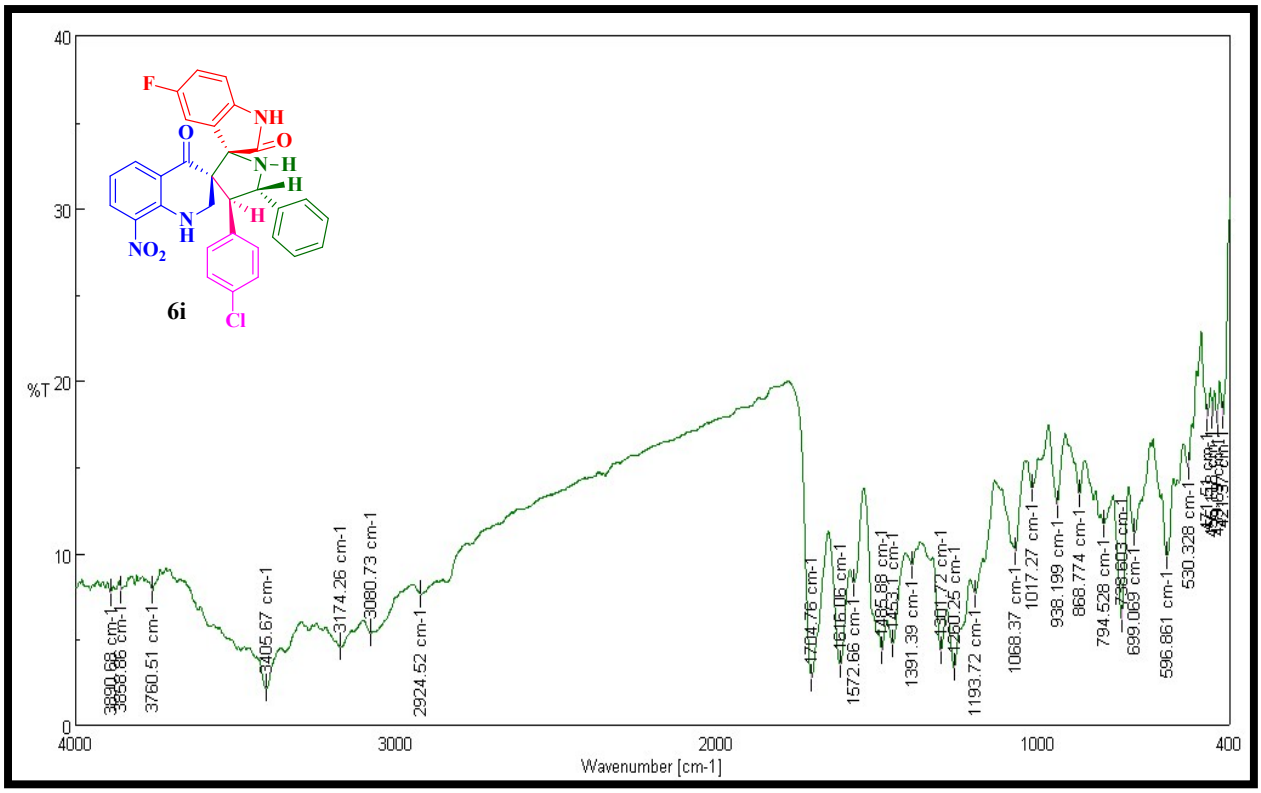


Fig. S27. IR spectrum of compound 6i

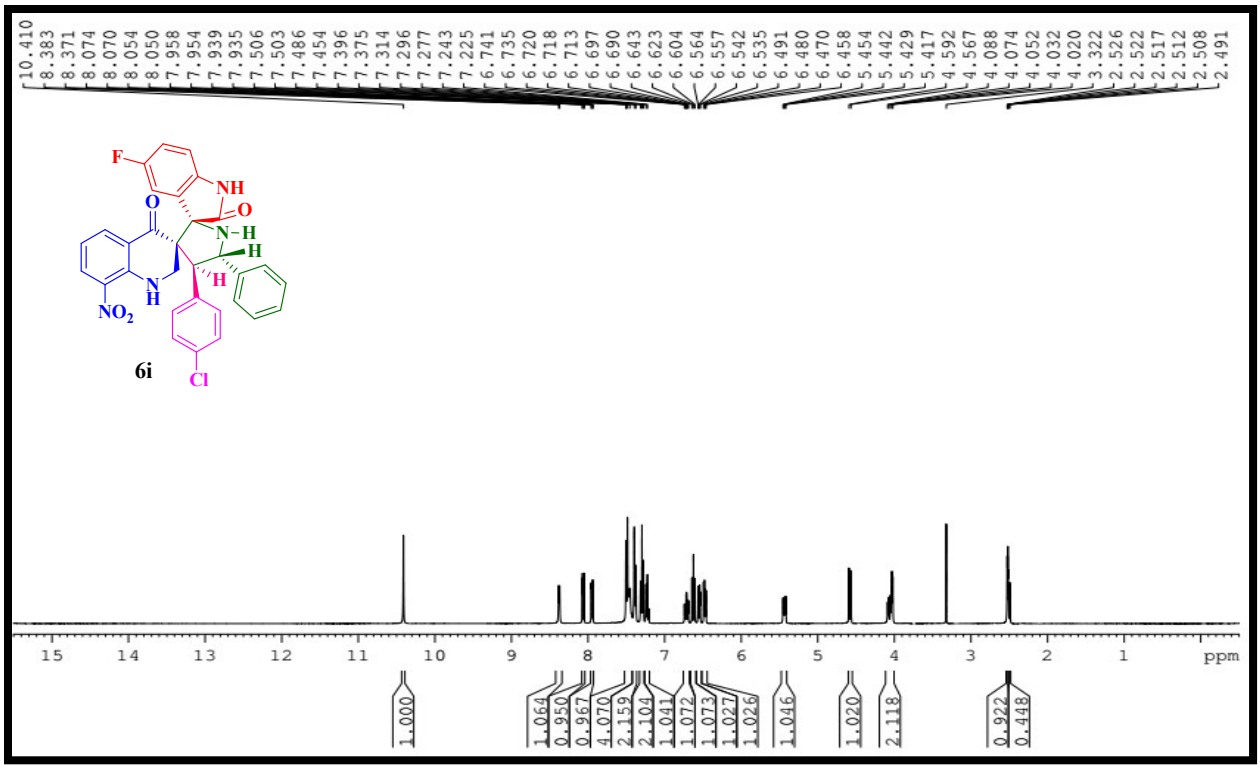


Fig. S28. ^{1}H NMR spectrum of compound 6i

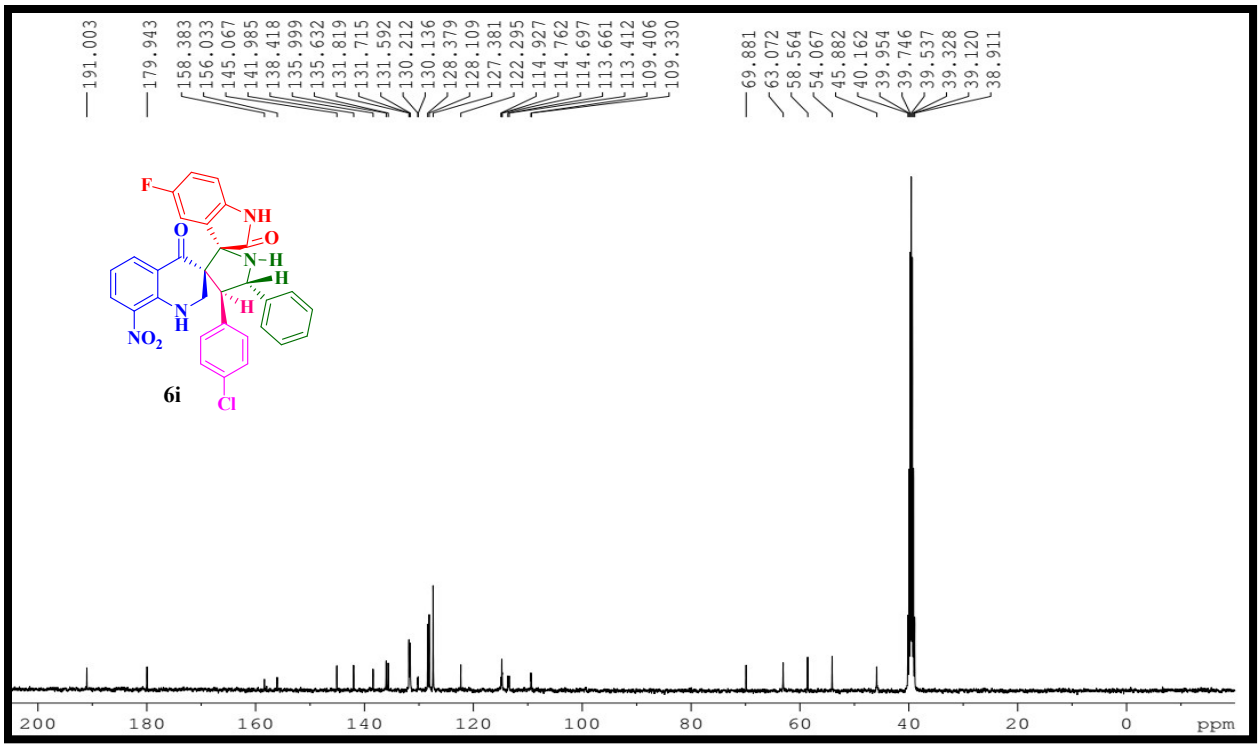


Fig. S29. ^{13}C NMR spectrum of compound 6i

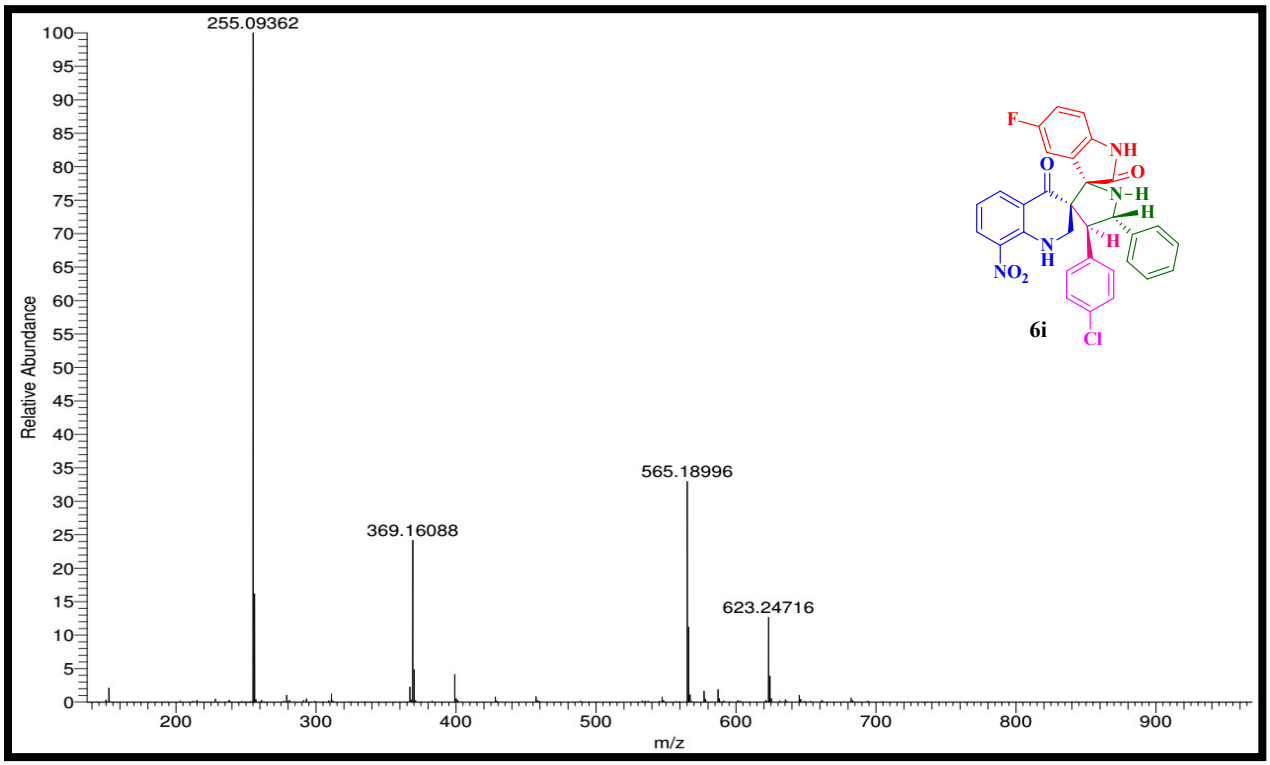


Fig. S30. HRMS spectrum of compound 6i

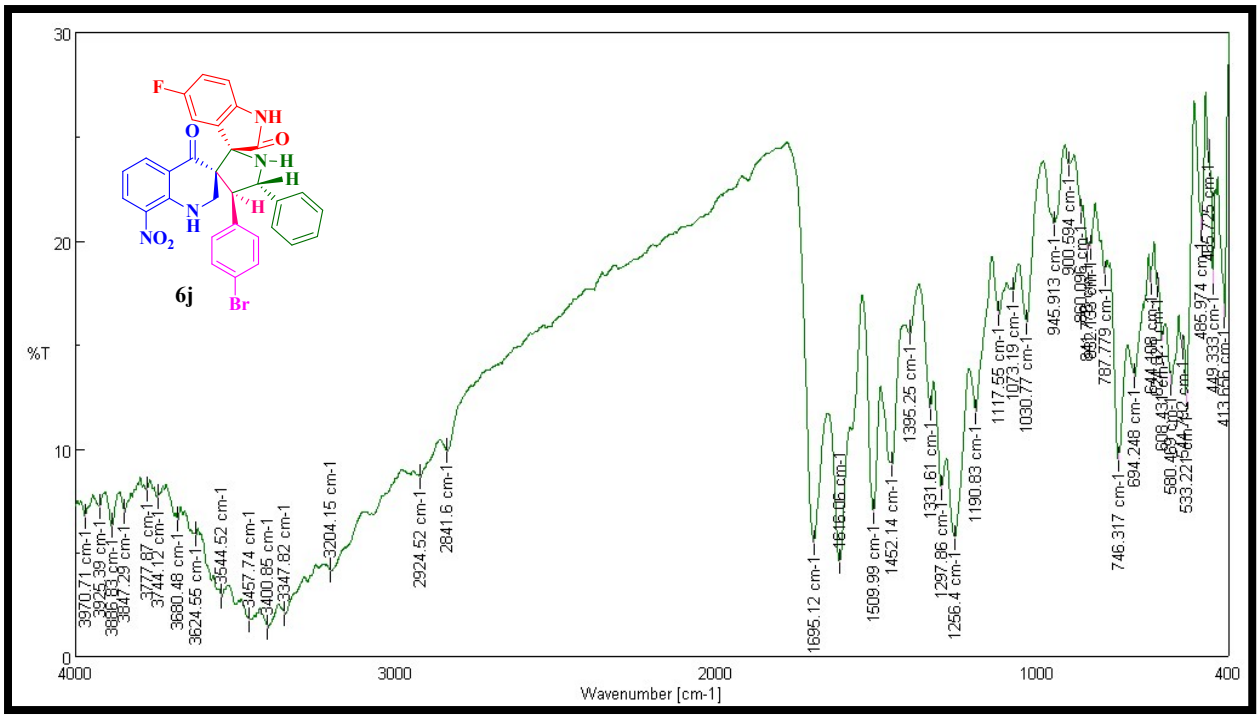


Fig. S31. IR spectrum of compound 6j

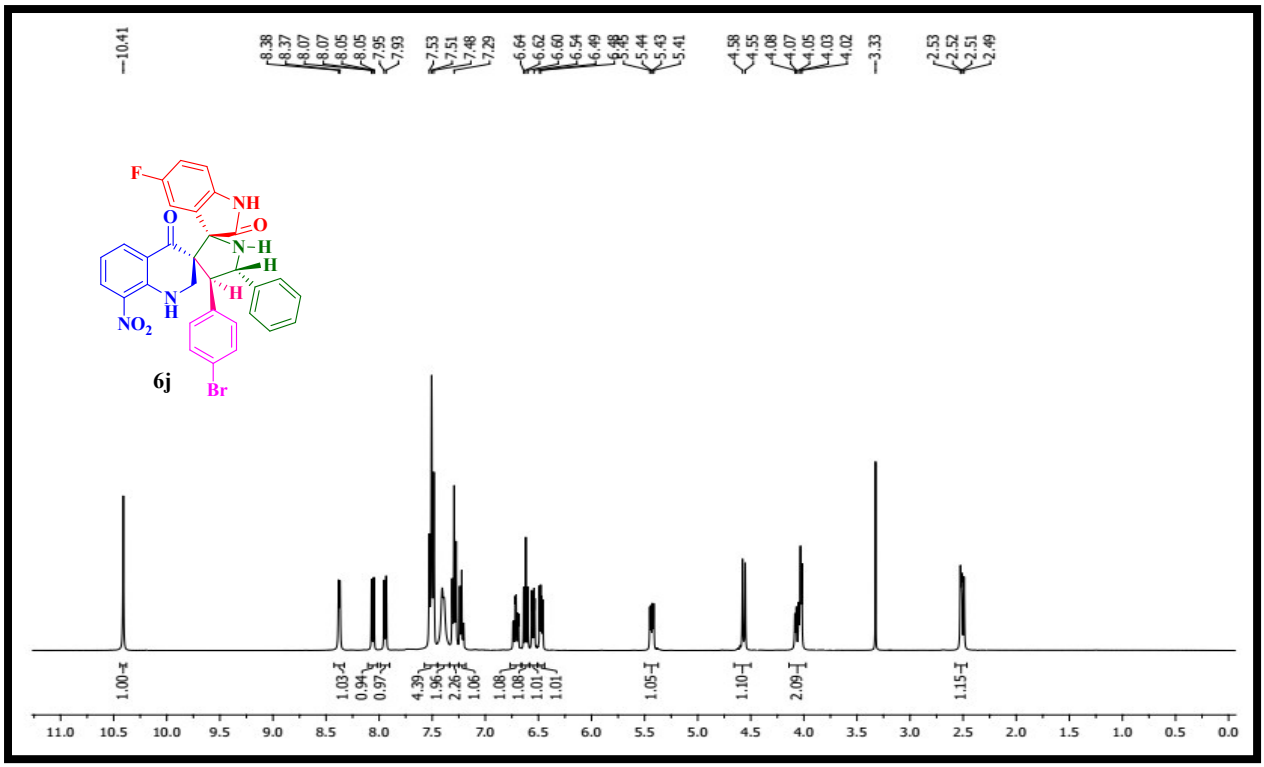


Fig. S32. ^{1}H NMR spectrum of compound 6j

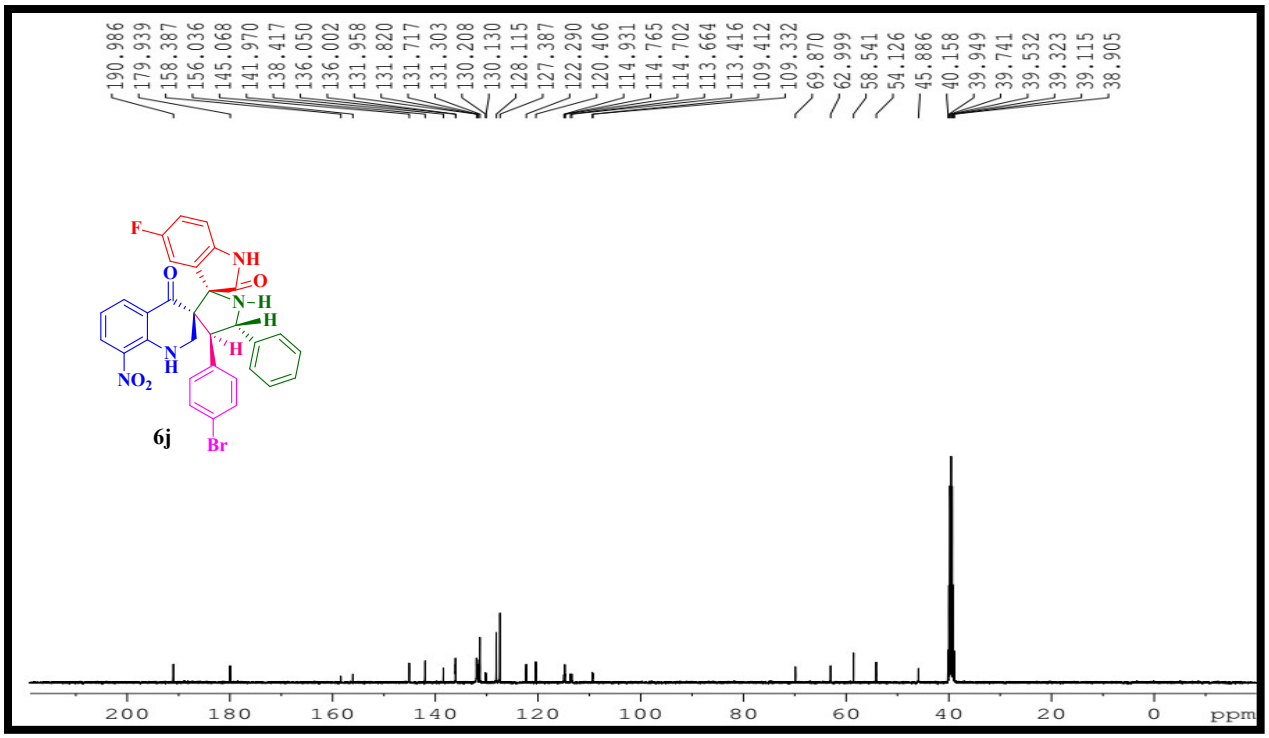


Fig. S33. ^{13}C NMR spectrum of compound 6j

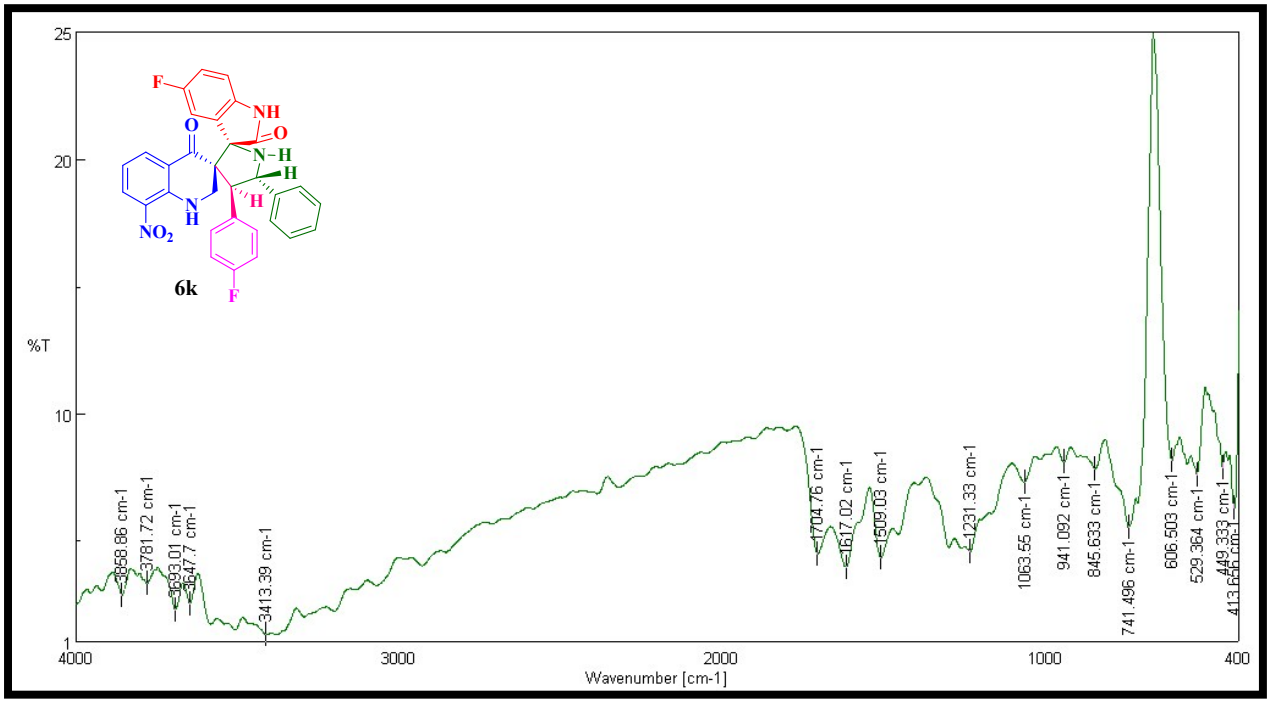


Fig. S34. IR spectrum of compound 6k

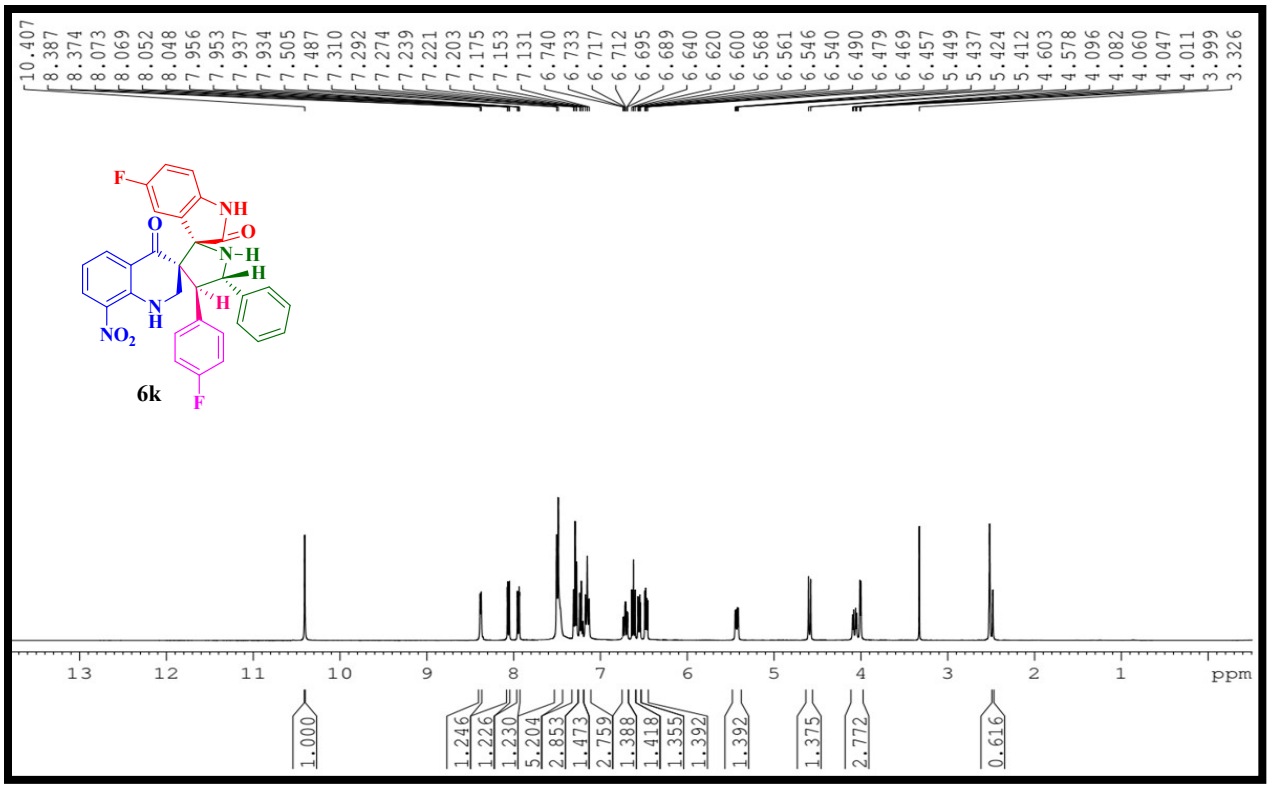


Fig. S35. ^{1}H NMR spectrum of compound 6k

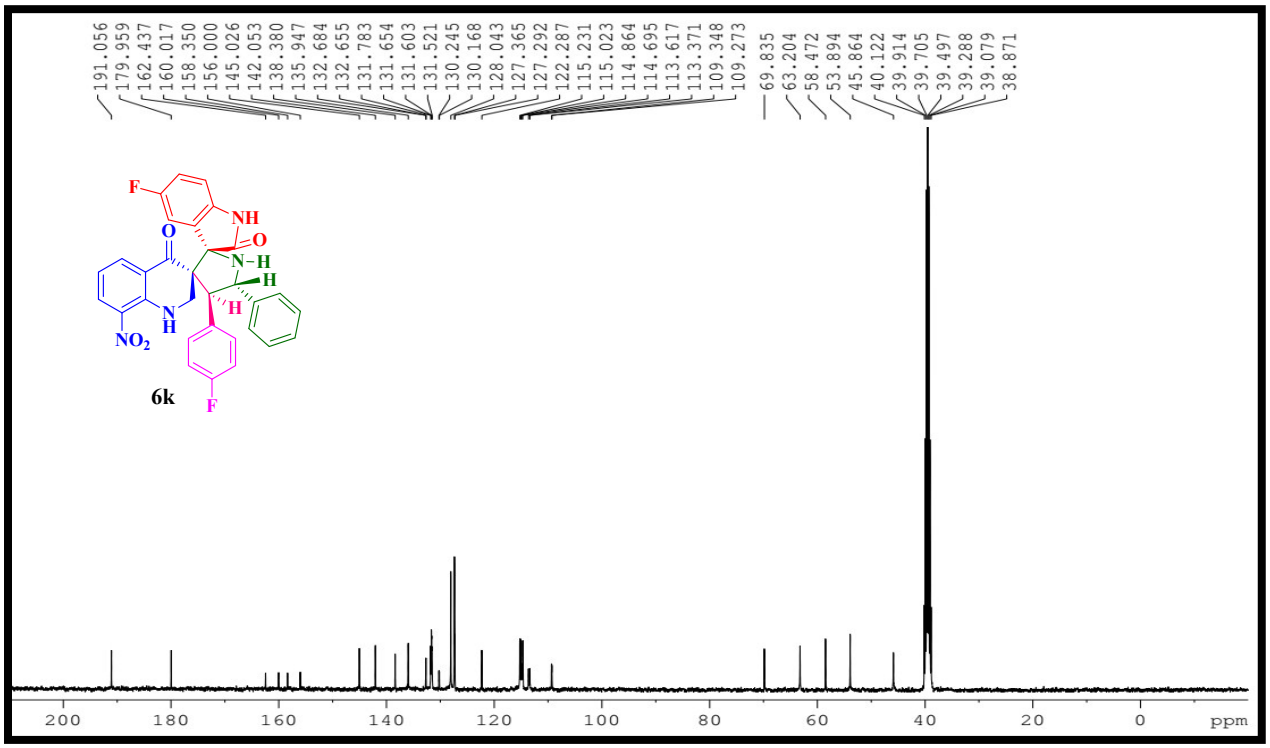


Fig. S36. ^{13}C NMR spectrum of compound 6k

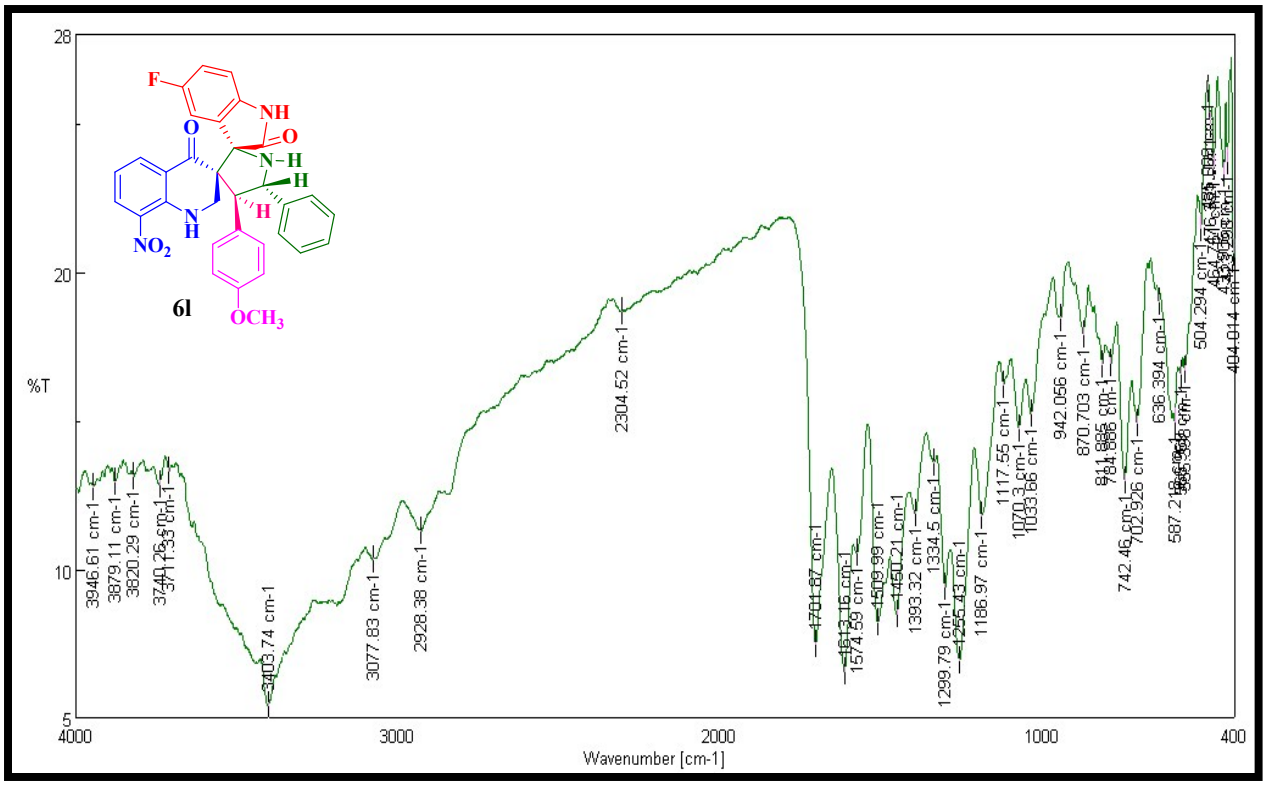


Fig. S37. IR spectrum of compound 6l

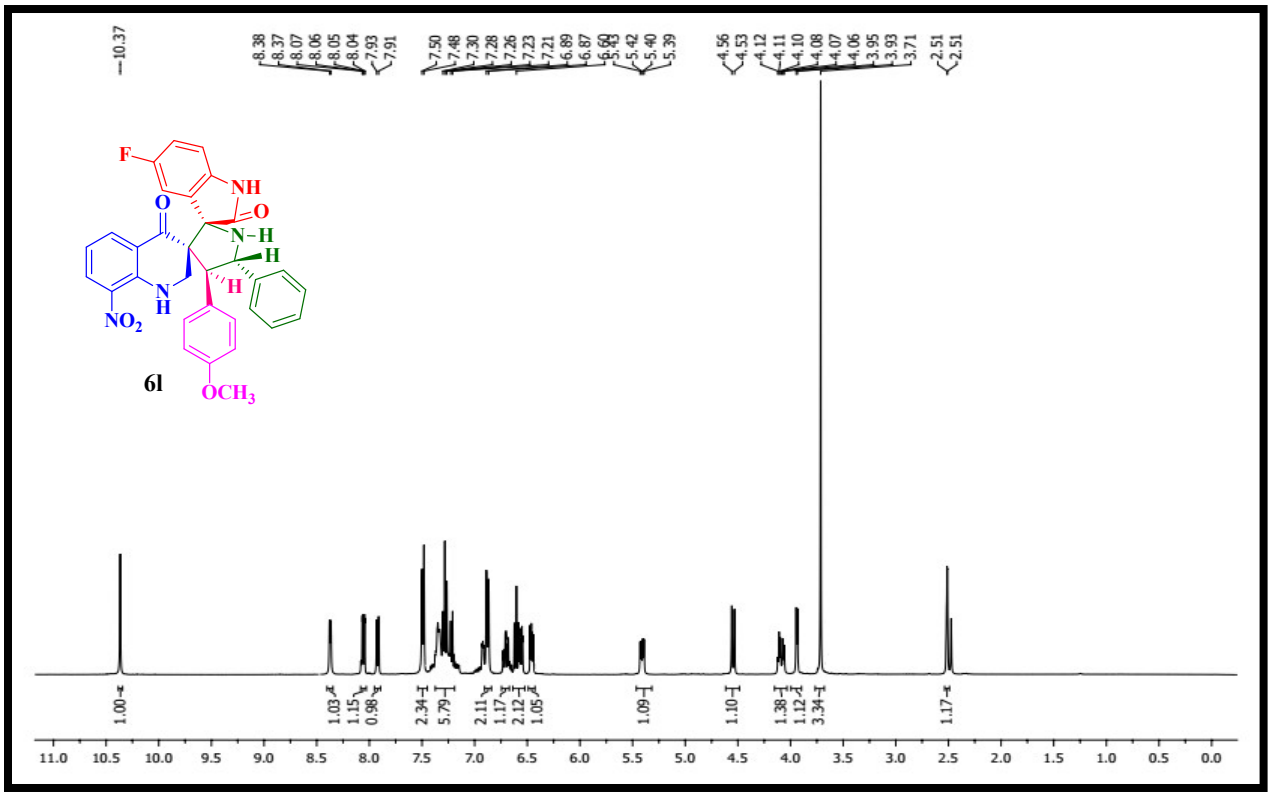


Fig. S38. ^{1}H NMR spectrum of compound 6l

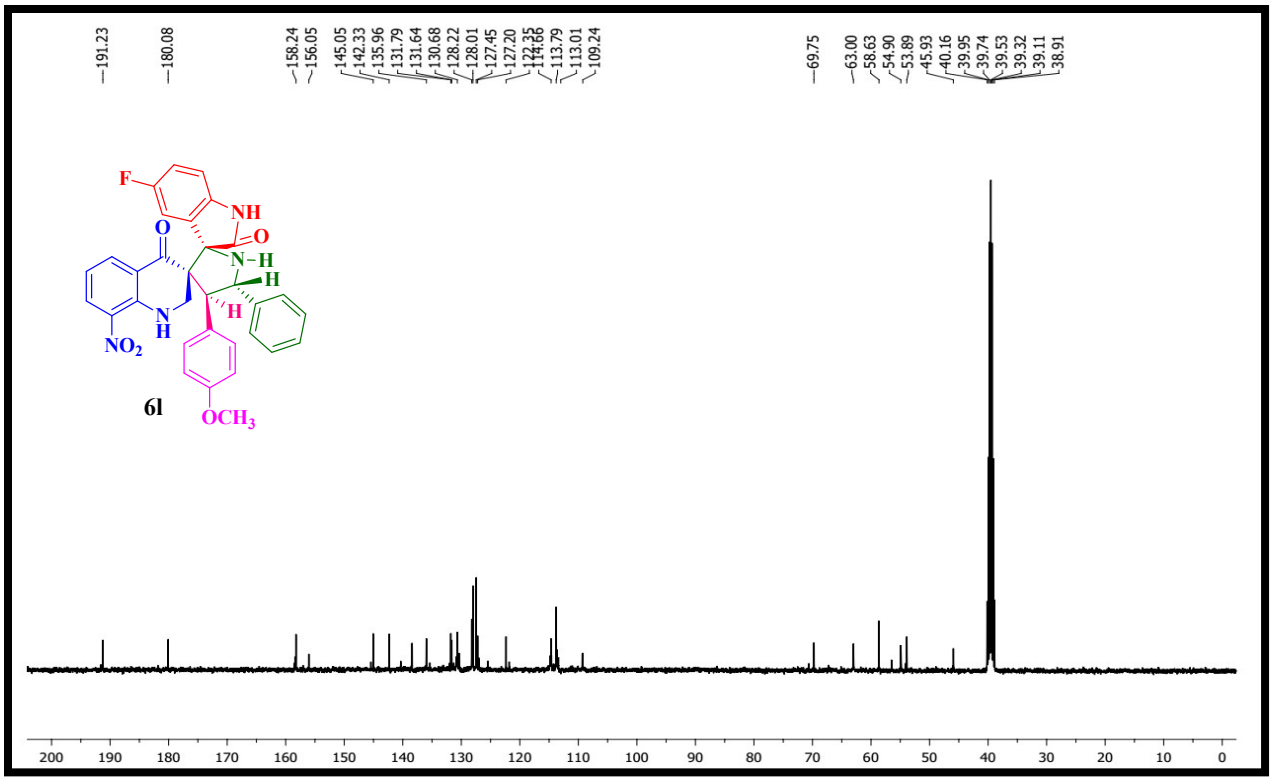


Fig. S39. ^{13}C NMR spectrum of compound 6l

Fig. S40. HRMS NMR spectrum of compound 6l