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Supporting Information

Synthesis and *in vitro* cytotoxicity evaluation of β-carboline-linked 2,4-thiazolidinedione hybrids: Potential DNA intercalation and apoptosis inducing studies

Ramya Tokala,[a] Sowjanya Thatikonda,[b] Sravani Sana,[a] Chandraiah Godugu,[b*] Nagula Shankaraiah[a*]

[a]*Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad 500 037, India*

[b]*Department of Regulatory Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad 500 037, India*

*Corresponding authors:

Dr. Nagula Shankaraiah, E-mail.: shankar@niperhyd.ac.in;

Dr. Chandraiah Godugu, E-mail.: chandra.niperhyd@gov.in

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Materials and methods

1. Chemistry

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Analytical thin layer chromatography (TLC) was performed on MERCK precoated silica gel 60-F_{254} (0.5 mm) aluminum plates. Visualization of the spots on TLC plates was achieved by UV light. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz by making a solution of samples in the $CDCl_3$ solvent (or) DMSO using tetramethyl silane (TMS) as the internal standard. Chemical shifts for ^{1}H and ^{13}C are reported in parts per million (ppm) downfield from tetra methyl silane. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constant (*J*) values are reported in hertz (Hz). HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument. Wherever required, column chromatography was performed using silica gel (60-120). The reactions wherever anhydrous conditions required are carried under nitrogen positive pressure using freshly distilled solvents. All evaporation of solvents was carried out under reduced pressure using rotary evaporator below 45 ^{o}C. Melting points were determined with an electro thermal digital melting point apparatus IA9100 and are uncorrected. The names of all the compounds given in the experimental section were taken from Chem Bio Draw Ultra, Version 12.0.

1.0. General procedure for (E)- 5-((1-aryl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19a-aa**)

To a mixture of thiazolidinedione (**9**, **14**, **16a-d**, **18a-d**, 1 equiv.), 1-aryl-9*H*-pyrido[3,4-*b*]indole-3-carbaldehyde (**6a-c**, 1.1 equiv.), in ethanol, catalytic amount of piperdine was added and refluxed at 80 ^{o}C till complete consumption of the starting materials as determined by TLC. The reaction mixture was cooled, precipitate thus formed was filtered, washed using ethanol to yield pure (*Z*)-3-((1-aryl-9*H*-pyrido [3,4-*b*]indol-3-yl)methylene)thiazolidine-2,4-diones **19a-aa** in moderate to good yields (70-90%). All the products were obtained by simple filtration.

*1.1. (Z)-5-((1-(4-Methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19a**).* Yellow solid; 70% yield; mp: 215–220 ^{o}C; FT-IR (cm^{-1}): 3372, 3221, 1728, 1683, 1609, 1552; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.28 (s, 1H), 11.89 (s, 1H), 8.59 (s, 1H), 8.27 (d, *J* = 7.5 Hz, 1H), 8.13 (d, *J* = 8.3 Hz, 2H), 7.95 (s, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.61 (t, *J* = 7.3 Hz,

1H), 7.34 (t, *J* = 7.0 Hz, 1H), 7.27 (dd, *J* = 1.8, 6.9 Hz, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.2, 168.7, 160.5, 143.0, 141.9, 140.9, 132.8, 130.3, 130.2, 130.1, 129.0, 124.0, 122.1, 121.3, 120.8, 119.2, 114.8, 113.3, 55.8; HRMS (ESI): *m/z* calcd. for $C_{22}H_{15}N_3O_3S$ 402.0912 found 402.0911 $[M + H]^+$.

*1.2. (Z)-5-((1-(4-Chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19b**).* Yellow solid; 75% yield; mp: 225–230 °C; FT-IR (cm^{-1}): 3390, 3218, 3028, 2970, 1730, 1688, 1608; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.28 (s, 1H), 11.95 (s, 1H), 8.63 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.15 (d, *J* = 7.7 Hz, 2H), 7.95 (s, 1H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.2 Hz, 1H), 7.35 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.9, 168.5, 142.0, 141.7, 141.1, 136.5, 134.4, 133.0, 130.9, 130.7, 130.5, 129.9, 129.4, 129.3, 124.2, 122.2, 121.3, 120.9, 119.9, 113.3; HRMS (ESI): *m/z* calcd. for $C_{21}H_{12}ClN_3O_2S$ 406.0417 found 406.0412 $[M + H]^+$.

*1.3. (Z)-5-((1-Phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19c**).* Yellow solid; 75% yield; mp: 230–235 °C; FT-IR (cm^{-1}): 3386, 3136, 3028, 1725, 1689, 1627, 1578; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.94 (s, 1H), 8.63 (s, 1H), 8.27 (d, *J* = 7.9 Hz, 1H), 8.15 (d, *J* = 7.0 Hz, 2H), 7.97 (s, 1H), 7.72-7.69 (m, 3H), 7.61 (dd, *J* = 7.6, 14.0 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.1, 168.8, 143.0, 142.0, 141.0, 137.7, 133.0, 130.3, 130.1, 129.6, 129.4, 129.2, 128.9, 124.1, 122.1, 121.3, 120.8, 119.6, 113.3; HRMS (ESI): *m/z* calcd. for $C_{21}H_{13}N_3O_2S$ 372.0807 found 372.0814 $[M + H]^+$.

*1.4. (Z)-5-((1-(4-Methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-morpholino-2-oxo-ethyl)thiazolidine-2,4-dione (**19d**).* Yellow solid; 75% yield; mp: 235–240 °C; FT-IR (cm^{-1}): 3300, 2970, 1664, 1609, 1449, 1384; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.93 (s, 1H), 8.64 (s, 1H), 8.26 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 2H), 8.10 (s, 1H), 7.72 (d, *J* = 6.1 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.28 (d, *J* = 9.2 Hz, 2H), 4.56 (s, 2H), 3.91 (s, 3H), 3.65 (t, *J* = 4.2 Hz, 2H), 3.59 (t, *J* = 4.4 Hz, 2H), 3.55 (t, *J* = 4.5 Hz, 2H), 3.45 (t, *J* = 4.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 191.6, 173.9, 171.8, 166.2, 164.1, 160.6, 143.2, 141.9, 140.7, 133.0, 131.8, 130.4, 130.0, 129.1, 122.1, 121.4, 120.9, 119.7, 114.8, 113.4, 66.4, 55.8, 45.0, 42.4, 42.2; HRMS (ESI): *m/z* calcd. for $C_{28}H_{24}N_4O_5S$ 529.1546 found 529.1548 $[M + H]^+$.

*1.5. (Z)-5-((1-(4-Chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-morpholino-2-oxoethyl)thiazolidine-2,4-dione (**19e**).* Yellow solid; 80% yield; mp: 220–225 °C; FT-IR (cm^{-1}):

3382, 3012, 2954, 2784, 1698, 1674, 1582, 1540; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.00 (s, 1H), 8.69 (s, 1H), 8.27 (d, *J* = 7.9 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 2H), 8.09 (s, 1H), 7.77 (d, *J* = 8.1 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 7.1 Hz, 1H), 4.55 (s, 2H), 3.65 (s, 2H), 3.58 (s, 2H), 3.54 (s, 2H), 3.44 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.6, 166.1, 164.0, 142.0, 141.9, 140.8, 136.4, 134.4, 133.2, 131.5, 130.7, 130.4, 129.4, 122.2, 121.3, 121.0, 120.3, 113.3, 66.4, 45.0, 42.4, 42.2, 40.5; HRMS (ESI): *m/z* calcd. for $C_{27}H_{21}ClN_4O_4S$ 533.1045 found 533.1042 $[M + H]^+$.

*1.6. (Z)-3-(2-Morpholino-2-oxoethyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19f**).* Yellow solid; 85% yield; mp: 258–263 ^{o}C; FT-IR (cm^{-1}): 3297, 2865, 1726, 1670, 1611, 1411, 1243; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.99 (s, 1H), 8.70 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.16 (d, *J* = 6.9 Hz, 2H), 8.12 (s, 1H), 7.73-7.70 (m, 3H), 7.63 (dd, *J* = 7.0, 13.2 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 1H), 4.57 (s, 2H), 3.65 (t, *J* = 4.0 Hz, 2H), 3.59 (t, *J* = 4.6 Hz, 2H), 3.55 (t, *J* = 4.7 Hz, 2H), 3.44 (t, *J* = 5.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.8, 166.2, 164.1, 143.2, 142.0, 140.8, 137.6, 133.2, 131.7, 130.3, 129.7, 129.4, 129.3, 129.0, 122.1, 121.3, 121.2, 120.9, 120.1, 113.4, 66.4, 56.4, 45.0, 42.4, 42.2; HRMS (ESI): *m/z* calcd. for $C_{27}H_{22}N_4O_4S$ 499.1440 found 499.1445 $[M + H]^+$.

*1.7. (Z)-3-Benzyl-5-((1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19g**).* Yellow solid; 75% yield; mp: 250–255 ^{o}C; FT-IR (cm^{-1}): 3420, 3166, 2970, 1736, 1711, 1656, 1463; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.92 (s, 1H), 8.64 (s, 1H), 8.26 (d, *J* = 7.7 Hz, 1H), 8.11 (d, *J* = 8.6 Hz, 3H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.38-7.26 (m, 8H), 4.84 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.0, 166.4, 160.6, 143.2, 141.9, 140.7, 136.3, 133.0, 131.9, 130.3, 130.1, 129.1, 129.1, 128.1, 127.9, 122.1, 121.4, 120.9, 119.6, 114.9, 114.9, 113.4, 55.8, 44.3; HRMS (ESI): *m/z* calcd. for $C_{29}H_{21}N_3O_3S$ 492.1382 found 492.1383 $[M + H]^+$.

*1.8. (Z)-5-((1-(4-Methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(4-methylbenzyl)thiazolidine-2,4-dione (**19h**).* Yellow solid; 85% yield; mp: 210–215 ^{o}C; FT-IR (cm^{-1}): 3442, 2932, 1670, 1341, 1280, 1026; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.92 (s, 1H), 8.61 (s, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.10 (d, *J* = 9.4 Hz, 3H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 7.0 Hz, 1H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 2H), 4.77 (s, 2H), 3.91 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.0,

166.3, 160.6, 143.1, 141.9, 140.7, 137.4, 133.3, 133.0, 131.8, 130.3, 129.6, 128.0, 122.1, 121.3, 120.9, 119.6, 114.8, 113.4, 55.8, 44.0, 21.1; HRMS (ESI): *m/z* calcd. for $C_{30}H_{23}N_3O_3S$ 506.1533 found 506.1528 $[M + H]^+$.

*1.9. (Z)-3-(4-Chlorobenzyl)-5-((1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19i**).* Yellow solid;85% yield; mp: 235–240 °C; FT-IR (cm^{-1}): 3448, 3006, 2970, 2839, 1738, 1718, 1670; ^1H NMR (500 MHz, DMSO-d_6): δ 11.91 (s, 1H), 8.63 (s, 1H), 8.27 (d, *J* = 17.6 Hz, 1H), 8.11 (t, *J* = 17.6 Hz, 3H), 7.71 (d, *J* = 8.0 Hz, 1H),7.61 (t, *J* = 6.9 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.36-7.33 (m, 3H), 7.27 (d, *J* = 8.7 Hz, 2H), 4.83 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.0, 166.3, 160.6, 143.2, 142.0, 140.6, 135.3, 133.1, 132.8, 131.9, 130.3, 130.0, 129.9, 129.2, 129.1, 122.1, 121.4, 120.9, 119.7, 114.8, 113.4, 55.8, 43.6; HRMS (ESI): *m/z* calcd. for $C_{29}H_{20}N_4O_5S$ 526.0987 found 526.0999 $[M + H]^+$.

*1.10. (Z)-5-((1-(4-Methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(4-nitrobenzyl)thiazolidine-2,4-dione (**19j**).* Yellow solid; 85% yield; mp: 240–245 °C; FT-IR (cm^{-1}): 3420, 2970, 1672, 1724, 1378, 1247, 1148; ^1H NMR (500 MHz, DMSO-d_6): δ 11.92 (s, 1H), 8.63 (s, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 8.3 Hz, 2H), 8.11 (d, *J* = 2.9 Hz, 2H), 8.09 (s, 1H), 7.72 (d, *J* = 8.3 Hz, 1H),7.62 (d, *J* = 7.1 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.27 (d, *J* = 8.3 Hz, 2H), 4.97 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.1, 166.3, 160.6, 147.4, 143.8, 143.2, 141.9, 140.6, 133.0, 132.1, 130.3, 130.0, 129.2, 129.1, 129.0, 124.2, 124.1, 122.1, 121.3, 120.9, 120.8, 119.7, 114.8, 113.4, 55.8, 43.7; HRMS (ESI): *m/z* calcd. for $C_{29}H_{20}N_4O_5S$ 537.1227 found 537.1231 $[M + H]^+$.

*1.11. (Z)-5-((1-(4-Chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(4-methylbenzyl)thiazolidine-2,4-dione (**19k**).* Yellow solid; 80% yield; mp: 240–245 °C; FT-IR (cm^{-1}): 3467, 3347, 3029, 2970, 2947, 1719, 1652, 1493; ^1H NMR (500 MHz, DMSO-d_6): δ 11.98 (s, 1H), 8.65 (s, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 2H), 8.10 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.60 (t, *J* = 6.4 Hz, 1H), 7.34 (t, *J* = 6.9 Hz, 1H), 7.21 (d, *J* = 7.5 Hz, 2H), 7.15 (d, *J* = 7.5 Hz, 2H), 4.78 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 166.7, 142.2, 141.9, 140.5, 137.7, 135.9, 135.5, 132.8, 132.6, 130.7, 130.7, 129.6, 129.4, 129.3, 128.7, 123.1, 121.9, 121.7, 121.3, 118.5, 111.9, 44.27, 29.6, 21.1;

*1.12. (Z)-3-Benzyl-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19l**).* Yellow solid; 90% yield; mp: 245–250 °C; FT-IR (cm^{-1}): 3423, 3259, 3060, 1781, 1668,

1658, 1601; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.98 (s, 1H), 8.68 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 8.15 (d, *J* = 7.0 Hz, 2H), 8.12 (s, 1H), 7.70 (t, *J* = 7.6 Hz, 3H), 7.62 (dd, *J* = 7.1, 13.4 Hz, 2H), 7.36 (dd, *J* = 6.4, 12.5 Hz, 3H), 7.32-7.28 (m, 3H), 4.83 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.0, 166.4, 143.2, 142.0, 140.8, 137.6, 136.3, 133.2, 131.7, 130.3, 129.7, 129.4, 129.3, 129.1, 128.9, 128.1, 127.9, 122.1, 121.3, 121.1, 120.9, 120.1, 113.4, 44.2; HRMS (ESI): *m/z* calcd. for $C_{28}H_{19}N_3O_2S$ 462.1276 found 462.1281 $[M + H]^+$.

*1.13. (Z)-3-(4-Methylbenzyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19m**).* Yellow solid; 80% yield; mp: 250–255 °C; FT-IR (cm^{-1}): 3427, 2942, 1740, 1716, 1663, 1606, 1432; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.97 (s, 1H), 8.67 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 8.14 (d, *J* = 7.1 Hz, 2H), 8.10 (s, 1H), 7.70 (t, *J* = 7.4 Hz, 3H), 7.63-7.59 (m, 2H), 7.35 (t, *J* = 7.2 Hz, 1H), 7.19 (dd, *J* = 8.0, 22.0 Hz, 4H), 4.77 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.0, 166.3, 143.1, 142.0, 140.8, 137.6, 137.4, 133.3, 133.2, 131.7, 130.3, 129.7, 129.6, 129.4, 129.2, 128.9, 128.0, 122.1, 121.3, 120.9, 120.1, 113.4, 21.1; HRMS (ESI): *m/z* calcd. for $C_{29}H_{21}N_3O_2S$ 476.1433 found 476.1437 $[M + H]^+$.

*1.14. (Z)-3-(4-Chlorobenzyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19n**).* Yellow solid; 80% yield; mp: 260–265 °C; FT-IR (cm^{-1}): 3300, 1717, 1664, 1610, 1591, 1492, 1376, 1356; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.99 (s, 1H), 8.70 (s, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 3H), 7.73-7.70 (m, 3H), 7.62 (dd, *J* = 7.6, 13.6 Hz, 2H), 7.43 (d, *J* = 8.6 Hz, 2H), 7.35 (t, *J* = 8.6 Hz, 3H), 4.83 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.0, 163.3, 143.2, 142.0, 140.8, 137.6, 135.3, 133.2, 132.7, 131.8, 130.3, 129.9, 129.7, 129.4, 129.3, 129.1, 128.9, 122.1, 121.3, 121.1, 121.0, 120.1, 113.4, 43.6; HRMS (ESI): *m/z* calcd. for $C_{28}H_{18}ClN_3O_2S$ 496.0887 found 496.0890 $[M + H]^+$.

*1.15. (Z)-3-(4-Nitrobenzyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19o**).* Yellow solid; 80% yield; mp: 220–225 °C; FT-IR (cm^{-1}): 3409, 3058, 2934, 1725, 1672, 1614, 1521; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.98 (s, 1H), 8.70 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 1H), 8.23 (d, *J* = 8.7 Hz, 2H), 8.14 (t, *J* = 4.3 Hz, 3H), 7.71 (t, *J* = 4.9 Hz, 3H), 7.64-7.58 (m, 4H), 7.36 (t, *J* = 7.6 Hz, 1H), 4.97 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.1, 166.3, 147.4, 143.8, 143.2, 142.0, 140.8, 137.6, 133.2, 132.0, 130.3, 129.7, 129.4, 129.3, 129.0, 128.9, 124.2, 122.1, 121.3, 121.1, 121.0, 120.2, 113.4, 43.7; HRMS (ESI): *m/z* calcd. for $C_{28}H_{18}N_4O_4S$ 507.1127 found 507.1125 $[M + H]^+$.

*1.16. (Z)-5-((1-(4-Methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-oxo-2-phenylethyl)thiazolidine-2,4-dione (**19p**).* Yellow solid; 80% yield; mp: 225–230 °C; FT-IR (cm^{-1}): 3412, 2894, 2872, 1728, 1614, 1545; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.66 (s, 1H), 8.28 (d, *J* = 8.9 Hz, 1H), 8.14 (d, *J* = 10.2 Hz, 3H), 8.10 (d, *J* = 8.9 Hz, 2H), 7.77-7.72 (m, 2H), 7.64-7.60 (m, 3H), 7.36 (t, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 2H), 5.27 (s, 2H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 192.0, 171.8, 166.1, 160.6, 143.1, 141.9, 140.6, 134.8, 134.3, 133.1, 132.1, 130.4, 130.0, 129.5, 129.1, 128.7, 122.1, 121.4, 120.9, 120.7, 119.8, 114.9, 113.4, 55.8, 47.5; HRMS (ESI): *m/z* calcd. for $C_{30}H_{21}N_3O_4S$ 520.1326 found 520.1320 $[M + H]^+$.

*1.17. (Z)-5-((1-(4-Methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-oxo-2-(p-tolyl)ethyl)thiazolidine-2,4-dione (**19q**).* Yellow solid; 75% yield; mp: 225–230°C; FT-IR (cm^{-1}):3422, 2933, 1728, 1696, 1670, 1511; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.66 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 1H), 8.14 (dd, *J* = 2.9, 6.7 Hz, 3H), 8.00 (d, *J* = 8.1 Hz, 2H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 6.9 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 2H), 7.36 (t, *J* = 6.9 Hz, 1H), 7.28 (dd, *J* = 4.4, 8.8 Hz, 2H), 5.23 (s, 2H), 3.92 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 191.4, 171.8, 166.1, 160.6, 145.5, 143.2, 141.9, 140.6, 133.1, 132.9, 131.9, 130.4, 130.1, 129.2, 128.8, 122.1, 121.4, 120.9, 119.8, 114.9, 113.4, 55.8, 47.4, 21.7; HRMS (ESI): *m/z* calcd. for $C_{31}H_{23}N_3O_4S$ 534.1488 found 534.1493 $[M + H]^+$.

*1.18. (Z)-3-(2-(4-Chlorophenyl)-2-oxoethyl)-5-((1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19r**).* Yellow solid; 85% yield; mp: 265–270 °C; FT-IR (cm^{-1}): 3418, 3064, 2931, 2841, 1811, 1667; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.66 (s, 1H), 8.27 (d, *J* = 7.7 Hz, 1H), 8.14-8.10 (m, 5H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 2H), 5.23 (s, 2H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 191.2, 171.8, 166.1, 160.6, 143.2, 141.9, 140.6, 139.8, 133.1, 133.0, 132.2, 130.6, 130.4, 130.0, 129.6, 129.1, 122.1, 121.4, 120.9, 120.7, 119.8, 114.8, 113.4, 55.8, 47.5; HRMS (ESI): *m/z* calcd. for $C_{30}H_{20}ClN_3O_4S$ 554.0941 found 554.0945 $[M + H]^+$.

*1.19. (Z)-3-(2-(3,4-Dichlorophenyl)-2-oxoethyl)-5-((1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19s**).* Yellow solid; 80% yield; mp: 245–250 °C; FT-IR (cm^{-1}):3406, 2930, 1728, 1708, 1670, 1512; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.97 (s,

1H), 8.67 (s, 1H), 8.35 (d, *J* = 2.7 Hz, 1H), 8.28 (d, *J* = 7.9 Hz, 1H), 8.16 (s,1H), 8.14 (t, *J* = 2.9 Hz, 1H), 8.12 (t, *J* = 2.9 Hz, 1H), 8.05-8.04 (m, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 8.2 Hz, 1H), 7.36 (t, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 2.0 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 5.23 (s, 2H), 3.92 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 190.8, 171.7, 166.0, 160.6, 143.3, 141.9, 140.6, 137.7, 134.4, 133.1, 132.6, 132.3, 131.8, 130.8, 130.4, 130.0, 129.2, 128.7, 122.1, 121.4, 120.9, 120.6, 119.9, 114.9, 113.4, 55.8, 47.4; HRMS (ESI): *m/z* calcd. for $C_{30}H_{19}Cl_2N_3O_4S$ 588.0547 found 588.0546 $[M + H]^+$.

*1.20. (Z)-5-((1-(4-Chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-oxo-2-phenylethyl)thiazolidine-2,4-dione (**19t**).* Yellow solid; 85% yield; mp: 225–230 °C; FT-IR (cm^{-1}): 3407, 3029, 2970, 2933, 1702, 1669, 1494; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.01 (s, 1H), 8.70 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 2H), 8.12 (s, 1H), 8.09 (d, *J* = 7.6 Hz, 2H), 7.77-7.70 (m, 4H), 7.65-7.60 (m, 3H), 7.37 (t, *J* = 8.8 Hz, 1H), 5.24 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 199.9, 171.6, 166.1, 142.1, 142.0, 140.8, 136.4, 134.8, 134.5, 134.4, 133.3, 131.9, 130.8, 130.5, 129.5, 129.4, 128.7, 122.2, 121.3, 121.1, 121.1, 120.4, 113.4, 47.6; HRMS (ESI): *m/z* calcd. for $C_{29}H_{18}ClN_3O_3S$ 524.0836 found 524.0838 $[M + H]^+$.

*1.21. (Z)-5-((1-(4-Chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-oxo-2(p-tolyl)ethyl)thiazolidine-2,4-dione (**19u**).* Yellow solid; 75% yield; mp: 230–235 °C; FT-IR (cm^{-1}): 3422, 3029, 2970, 2938, 1730, 1675, 1670; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.41(s, 1H), 8.16 (s, 1H), 8.09 (t, *J* = 8.6 Hz, 3H), 7.98 (s, 1H), 7.82 (d, *J* = 7.8 Hz, 2H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.50 (t, *J* = 7.3 Hz, 1H), 7.27-7.25 (m, 3H), 5.03 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 190.5, 171.6, 166.0, 145.1, 142.0, 140.7, 136.3, 134.6, 133.2, 131.8, 131.7, 130.5, 130.4, 129.7, 128.5, 121.9, 121.3, 120.7, 119.8, 113.1, 47.0, 21.8; HRMS (ESI): *m/z* calcd. for $C_{30}H_{20}ClN_3O_3S$ 538.0992 found 538.0956 $[M + H]^+$.

*1.22. (Z)-3-(2-(4-Chlorophenyl)-2-oxoethyl)-5-((1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19v**).* Yellow solid; 85% yield; mp: 215–220 °C; FT-IR (cm^{-1}): 3386, 3043, 2970, 2933, 1729, 1702, 1665; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.04 (s, 1H), 8.72 (s, 1H), 8.29 (d, *J* = 7.7 Hz, 1H), 8.17 (d, *J* = 10.1 Hz, 3H), 8.11 (d, *J* = 7.9 Hz, 2H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.70 (t, *J* = 8.3 Hz, 3H), 7.64 (t, *J* = 7.1 Hz, 1H), 7.37 (t, *J* = 6.7 Hz, 1H), 5.28 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 191.2, 171.6, 166.0, 142.0, 140.8, 139.8, 136.4, 134.5, 133.3, 133.0, 131.9, 130.8, 130.6, 130.4, 129.6, 129.5, 122.2, 121.3, 121.1, 121.0,

120.5, 113.4, 47.5; HRMS (ESI): *m/z* calcd. for $C_{29}H_{17}Cl_2N_3O_3S$ 558.0446 found 558.0445 $[M + H]^+$.

*1.23. (Z)-5-((1-(4-Chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methylene)-3-(2-(3,4-dichlorophenyl)-2-oxoethyl)thiazolidine-2,4-dione (**19w**).* Yellow solid; 80% yield; mp: 245–250 °C; FT-IR (cm^{-1}): 3406, 3052, 2930, 1918, 1730, 1707, 1668; ^1H NMR (500 MHz, DMSO-d_6): δ 12.04 (s, 1H), 8.72 (s, 1H), 8.34 (s, 1H), 8.28 (d, *J* = 7.2 Hz, 1H), 8.16 (s, 3H), 8.05 (d, *J* = 12.7 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 6.6 Hz, 1H), 7.37 (s, 1H), 5.34 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 190.5, 171.5, 166.0, 142.0, 140.7, 137.7, 136.3, 134.5, 134.4, 133.3, 132.6, 132.0, 131.8, 130.8, 130.4, 129.5, 128.7, 122.2, 121.3, 121.1, 121.0, 120.5, 113.4, 47.6; HRMS (ESI): *m/z* calcd. for $C_{29}H_{16}Cl_3N_3O_3S$ 592.0056 found 592.0028 $[M + H]^+$.

*1.24. (Z)-3-(2-Oxo-2-phenylethyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19x**).* Yellow solid; 70% yield; mp: 215–220 °C; FT-IR (cm^{-1}): 3427, 2929, 1729, 1709, 1671, 1492, 1442; ^1H NMR (500 MHz, DMSO-d_6): δ 12.02 (s, 1H), 8.73 (s, 1H), 8.29 (d, *J* = 7.7 Hz, 1H), 8.18 (d, *J* = 5.1 Hz, 3H), 8.10 (d, *J* = 7.4 Hz, 2H), 7.77-7.71 (m, 4H), 7.63 (dd, *J* = 8.0, 16.0 Hz, 4H), 7.37 (t, *J* = 7.6 Hz, 1H), 5.29 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 192.0, 171.8, 166.1, 143.3, 142.0, 140.7, 137.6, 134.9, 134.3, 133.3, 132.1, 130.3, 129.7, 129.5, 129.3, 129.0, 128.7, 122.2, 121.3, 121.0, 120.2, 113.4, 47.6; HRMS (ESI): *m/z* calcd. for $C_{29}H_{19}N_3O_3S$ 490.1225 found 490.1228 $[M + H]^+$.

*1.25. (Z)-3-(2-Oxo-2-(p-tolyl)ethyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19y**).* Yellow solid; 80% yield; mp: 235–240 °C; FT-IR (cm^{-1}): 3374, 1722, 1696, 1669, 1605, 1496, 1463; ^1H NMR (500 MHz, DMSO-d_6): δ 8.72 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 8.17 (d, *J* = 8.2 Hz, 3H), 7.98 (d, *J* = 6.9 Hz, 2H), 7.74-7.72 (m, 3H), 7.62 (dd, *J* = 6.7, 14.1 Hz, 2H), 7.41 (d, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 7.2 Hz, 1H), 5.24 (s, 2H), 2.42 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 196.1, 176.5, 170.9, 150.2, 148.0, 146.8, 145.5, 142.4, 138.1, 136.7, 135.1, 134.8, 134.7, 134.3, 134.1, 133.8, 133.7, 133.5, 126.9, 126.1, 125.8, 125.0, 124.9, 52.2, 26.5; HRMS (ESI): *m/z* calcd. for $C_{30}H_{21}N_3O_3S$ 504.1382 found 504.1380 $[M + H]^+$.

*1.26. (Z)-3-(2-(4-Chlorophenyl)-2-oxoethyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19z**).* Yellow solid; 70% yield; mp: 235–240 °C; FT-IR

(cm^{-1}): 3478, 3391, 2931, 1723, 1700, 1662, 1405; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.72 (s, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* = 5.5 Hz, 3H), 8.12 (d, *J* = 8.5 Hz, 2H), 7.74-7.71 (m, 3H), 7.70 (d, *J* = 8.8, 2H), 7.63 (dd, *J* = 7.7, 14.0 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 1H), 5.34 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 191.2, 171.7, 166.1, 143.3, 142.1, 140.7, 139.8, 137.6, 133.3, 133.0, 132.1, 130.6, 130.3, 129.7, 129.6, 129.4, 129.3, 129.0, 122.1, 121.3, 121.0, 120.9, 120.3, 113.4, 47.5; HRMS (ESI): *m/z* calcd. for $C_{29}H_{18}ClN_3O_3S$ 524.0836 found 524.0836 $[M + H]^+$.

*1.27. (Z)-3-(2-(3,4-Dichlorophenyl)-2-oxoethyl)-5-((1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methylene)thiazolidine-2,4-dione (**19aa**).* Yellow solid; 75% yield; mp: 250–255 ^{o}C; FT-IR (cm^{-1}): 3416, 2929, 1809, 1728, 1709, 1618, 1589; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.01 (s, 1H), 8.72 (s, 1H), 8.34 (d, *J* = 2.4 Hz, 1H), 8.27 (d, *J* = 7.9 Hz, 1H), 8.16 (d, *J* = 5.5 Hz, 3H), 8.04 (dd, *J* = 1.9, 8.2 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.74-7.71 (m, 3H), 7.62 (dd, *J* = 6.8, 13.7 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 1H), 5.34 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 190.7, 171.7, 166.0, 143.3, 142.0, 140.7, 137.7, 137.6, 134.4, 133.3, 132.6, 132.1, 131.8, 130.8, 130.3, 129.7, 129.4, 129.4, 129.3, 129.0, 128.7, 122.1, 121.3, 121.0, 120.8, 120.3, 113.4, 47.5; HRMS (ESI): *m/z* calcd. for $C_{29}H_{17}Cl_2N_3O_3S$ 558.0446 found 558.0449 $[M + H]^+$.

2. Pharmacology

2.1. Cell culture

Cancer cell lines such as prostate (PC-3), lung (A549), osteosarcoma (MG-63), colon (HCT-15), breast (MDA-MB-231), skin (A431), pancreatic (PANC-1), normal human pulmonary epithelial cell line (L-132) were maintained in appropriate medium supplemented with 10% fetal bovine serum (FBS) stabilized with 1% antibiotic-antimycotic solution (Sigma Aldrich) cells were maintained in 5% CO_2 and 98% relative humidity at 37 ^oC in incubator. When the cells reached up to 80-90% of confluency, they were sub-cultured using 0.25% trypsin/1 mM EDTA solution for further passage. The compounds were dissolved in DMSO to prepare the stock solution of 10 mM. Further dilutions were made accordingly with respective media to get required concentration.

2.2. MTT assay

MTT assay is a colorimetric assay that measures the reduction of MTT (3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide) to insoluble formazon by mitochondrial succinate dehydrogenase enzyme. Since reduction of MTT can only happen in metabolically active cells, the level of activity is the measure of the viability of the cells. Briefly, cells were seeded in 96-well plates at a density of 1000 to 4000 cells per well in 100 µl of complete medium and allowed to grow overnight for attachment onto the wells. Then the cells were treated with various concentrations of the compounds for a period of 72 h. After 72 h incubation, 100 µl of MTT (0.5 mg/mL) was added and incubated at 37 ^oC for 4 h. Then MTT reagent was aspirated and the formazan crystals formed were dissolved by the addition of 200 µL of DMSO for 20 min. at 37 ^oC. The formazon product quantity was measured by using a spectrophotometric microtiter plate reader (Spectra Max, M4 Molecular devices, USA) at 570 nm wavelength.

2.3. Apoptosis detection studies

2.3.a. Morphological observations

MDA-MB-231 cells were plated in 12 well culture plates with a cell density of $1x10^5$ cells/mL and allowed to adhere for overnight. The cells were incubated with the 0.25, 0.5, 1, 2.5 µM concentrations of the compound **19e**. After 72 h treatment, cells were observed for the

morphological changes and photographs were taken under a phase contrast microscope (Nikon, Inc. Japan).

2.3.b. Acridine Orange Ethidium Bromide (AO/EB) staining

MDA-MB-231 cells were plated at a concentration of $1x10^6$ cells/ml and treated with different concentrations of compound **19e** and the plates were incubated for 72 h. 10 µl of fluorescent dyes containing Acridine Orange (AO) and Ethidium Bromide (EB) were added into each well in equal volumes (10 µg/ml) respectively then the cells were visualized immediately under fluorescence microscope (Nikon, Inc. Japan) with excitation (488 nm) and emission (550 nm) at 200x magnification.

2.3.c. DAPI nucleic acid staining

Morphological changes in nucleus were observed through DAPI staining. After treatment with compound **19e** for 72 h, breast cancer cell line MDA-MB-231 cells were washed with PBS and permeabilized with 0.1% triton X for 10 min followed by staining with 1 µM DAPI. Control and treated cells were observed with fluorescence microscope with excitation at 359 nm and emission at 461 nm using DAPI filter at 200X magnification.

2.4. DCFDA staining

DCFDA staining was performed to determine the reactive oxygen species (ROS) levels as per reported method [27] with slight modifications. For this experiment, MDA-MB-231 cells were plated at cell density of 3.5×10^5 cells/well into 12-well plates in DMEM supplemented with 10% FBS. Then the cells were treated with the compound **19e** at various concentrations for 24 h. Then the DCFDA reagent was added at 10 µM concentration for 15 min and the fluorescent intensity was captured using fluorescent microscope at 200X magnification.

2.5. Clonogenic growth inhibition assay

Breast cancer cells MDA-MB-231 were passaged in DMEM supplemented with 10% fetal bovine serum at 37 ^oC in an atmosphere containing 5% CO_2. Cells at low density 2×10^2 were seeded into 12-well culture plates and kept overnight and treated with the compound **19e**. After 24 h cells were washed with PBS to remove drug. Every 3 days the medium was removed and replaced with fresh medium. After 7 days of incubation, the colonies were fixed and stained with 1% crystal violet in methanol for 3 h. The number of stained colonies was counted under Chemdoc imaging system (Vilber Fusion Fx, France).Colony formation was calculated as a percentage to untreated control cells.

2.6. In vitro cell migration assay/wound healing assay

MDA-MB-231 cells were plated at cell density of 3.5 × 10^5 cells/well into 12-well plates in DMEM medium and allowed to form confluent monolayer, then scratch is made with 200 µL and then treated with 0.5, 1, 2.5 and 5 mM of the compound **19e**. The migration of breast cancer cells was captured by microscopic observations at 0 h and 24 h.

2.7. Flow cytometric analysis:

2.7.a. Measurement of mitochondrial membrane potential

MDA-MB-231 cells (1×10^6 cells/ml) were seeded in 12 well plates and allowed to adhere for overnight. The cells were incubated with compound **19e** at 0.25, 0.5, 1 and 2.5 µM concentrations for 72 h. Cells were collected and washed with PBS and resuspended in solution of JC-1 (1 µM) and incubated for 30 min in incubator at 37 ^oC. The cells were washed twice with PBS and analysed by flow cytometer (BD FACSVerse™, USA).

2.7.b. Cell cycle analysis

Flow cytometric analysis (FACS) was performed to calculate the distribution of the cell population in various cell cycle phases. In general the novel compounds exert their cytotoxic or growth inhibitory effect by arresting the specific checkpoint in cell cycle. Here MDA-MB-231 cancer cells were incubated with compound **19e** at various concentrations from 0.25 to 2.5 µM for 24 h. Untreated and treated cells were harvested, washed and fixed overnight in 70% ethanol in PBS at -20 ^oC. Fixed cells were pelleted and stained with cell cycle analysis reagent propidium iodide (50 µg/ml) with RNase A for 20 min at 37 ^oC in dark according to the manufacturer instructions and about 10,000 events were acquired and analyzed on a flow cytometer BD FACSVerse^{TM} (BD Biosciences, USA).

2.7.c. Annexin V assay

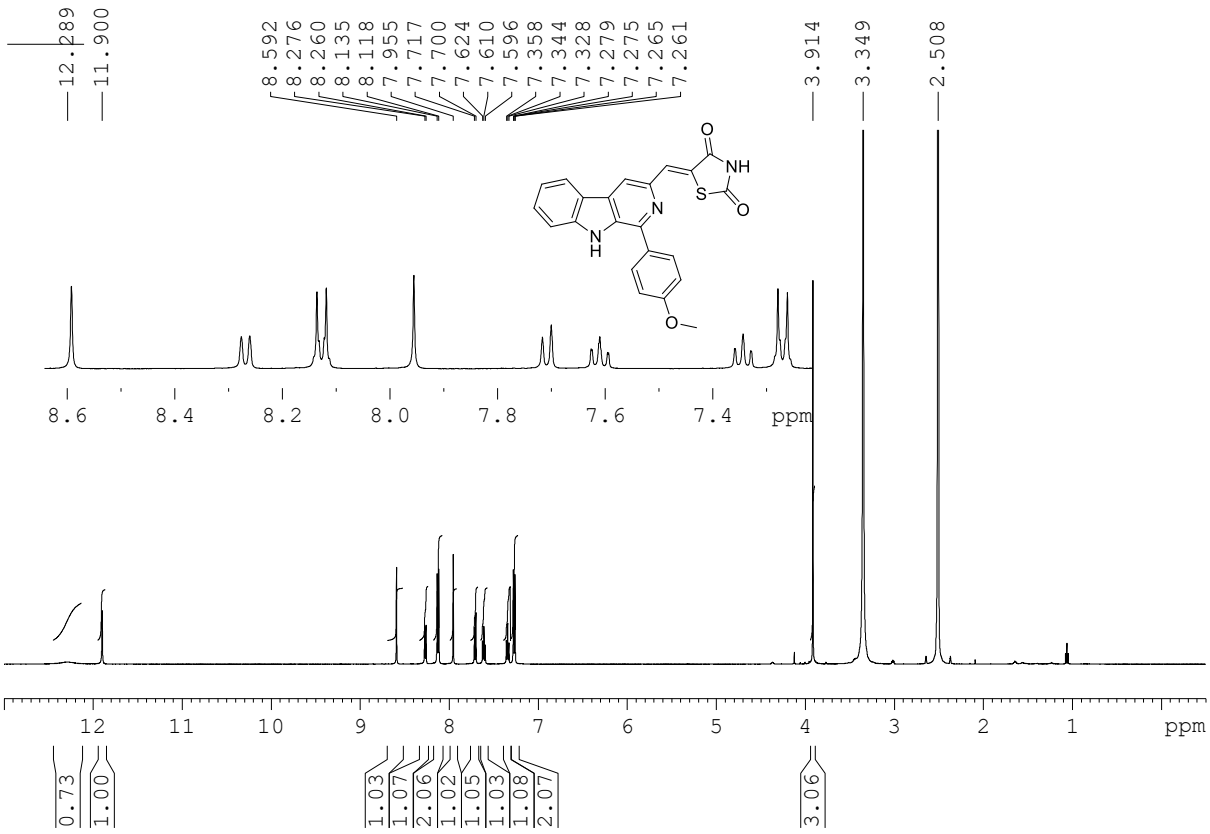
It was performed by the method given by Rieger *et al*. with slight modifications. Briefly, 1 × 10^5 cells were seeded in a 12-well plate and treated with different concentration of compound **19e** for 72 h. The collected cells were washed twice with ice-cold PBS, then incubated with 200 µL of 1 × binding buffer containing 1 µL propidium iodide (PI) for 15 min at room temperature in the dark. After incubation, cells were analyzed for apoptosis using flow-cytometer (BD FACSVerseTM, USA). Apoptosis and necrosis were analyzed with quadrant statistics on propidium iodide-negative cells, fluorescein positive cells and propidium iodide (PI)-positive cells, respectively.

3. Relative viscosity studies

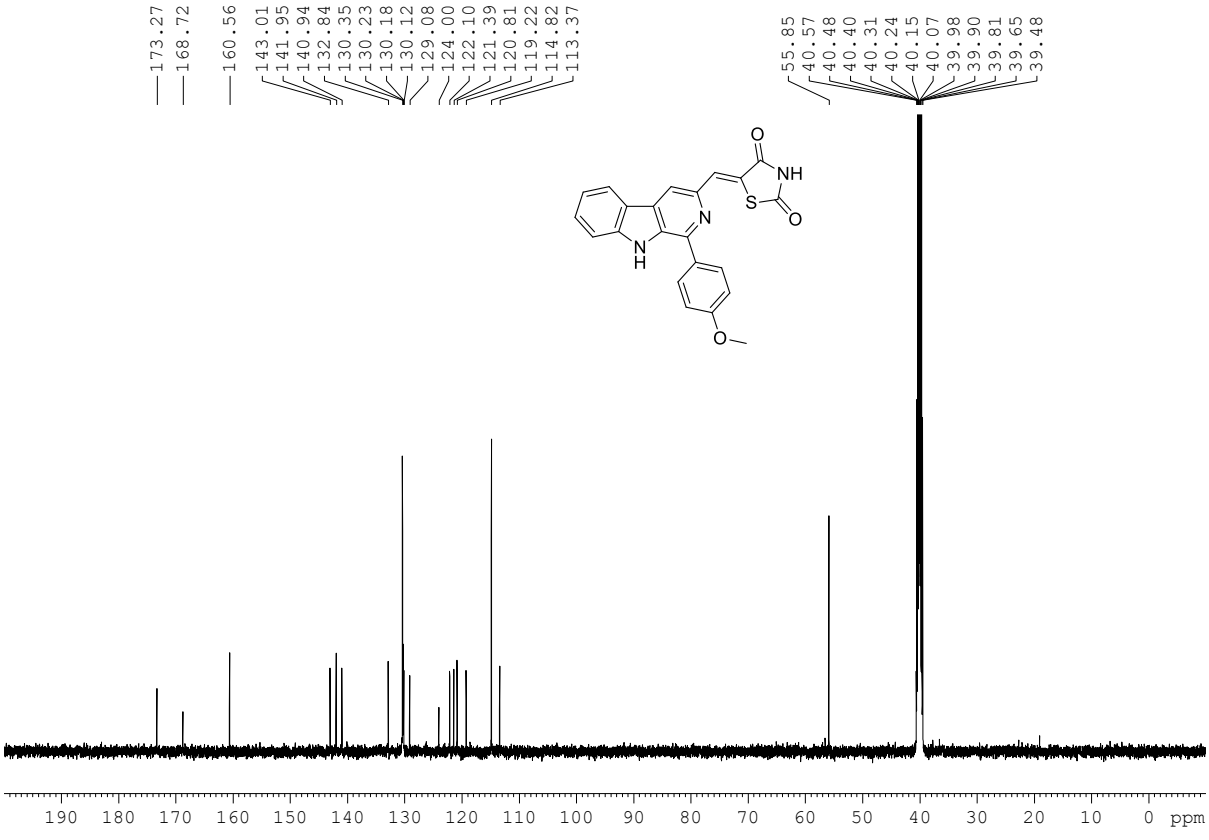
The viscosities of the DNA-ligand complexes were determined by the Lovis 2000 M/ME Rolling-ball viscometer (Anton Paar GmbH, Graz, Austria), based on the falling ball principle. The temperature was controlled at ±0.005 K by means of an internal Peltier thermostat. A calibrated 1.59 mm glass capillary containing a steel ball was filled with the sample for measuring the ball falling time at angles in the range from 20^{o} to 70^{o}. The kinematic as well as dynamic viscosities at 25 ^{o}C were estimated based on the ball falling time and densities DNA solution was prepared in 100 mM Tris-HCl (pH 7.4) and viscosity was measured while each derivative (5µM) was added to CT-DNA solution (50 µM). Doxorubicin, Ethidium bromide, Harmine and Hoechst 33258 at 5µM concentration were used as controls. Data was represented graphically as $(\eta/\eta_0)^{1/3}$ vs. the ratio of the concentration of the hybrid to CT-DNA, where η is the viscosity of CT-DNA in the presence of the derivative and η_0 is the viscosity of CT-DNA solution [30, 31].

4. Molecular docking studies

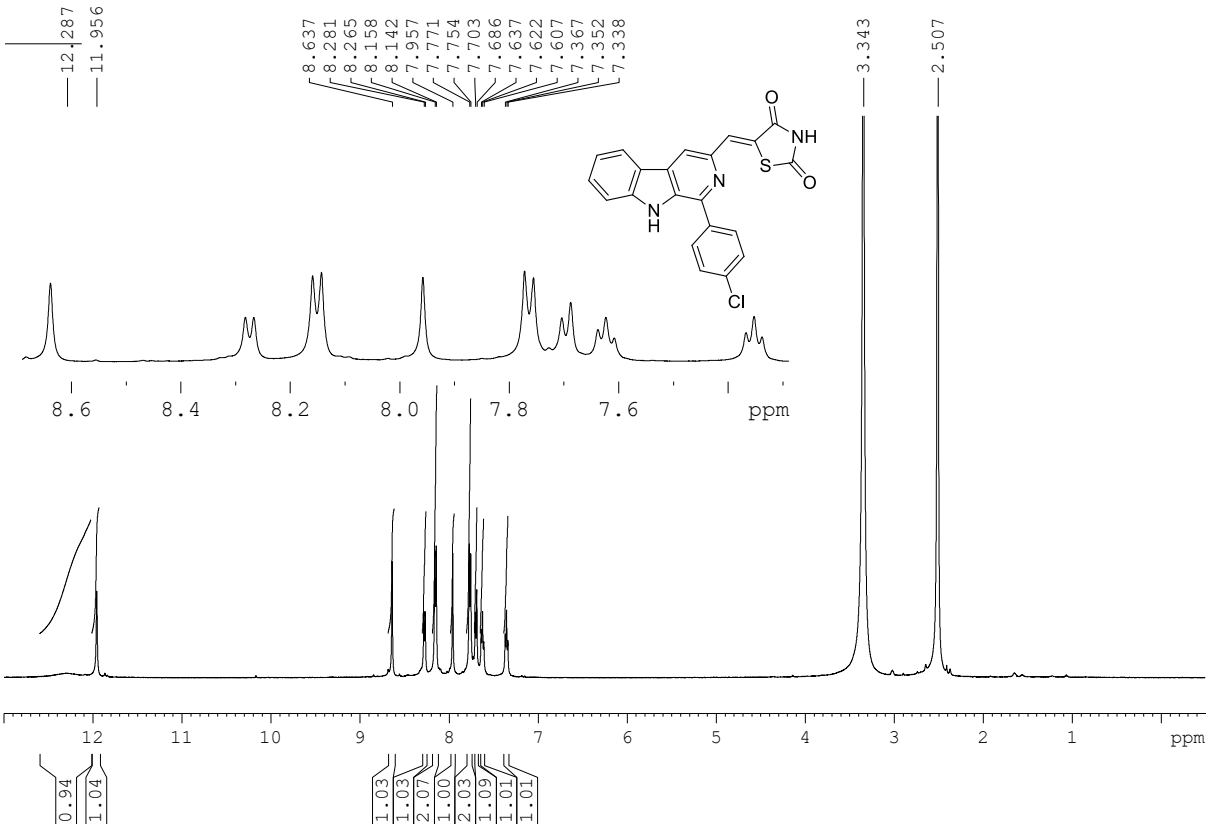
The DNA crystal structure has been retrieved from Protein Data Bank (PDB ID: 209D) [32]. Protein preparation tool was used for the preparation of the DNA hexamer. This adds up the missing atoms and removes peripheral water molecules with a distance of more than 3 Å from the pocket. The grid is generated by picking the active site where the co-crystal is located and grid box of 10 × 10 × 10 Å was generated using Glide 7.4 (Schrödinger 2017-1) [33]. The potent hybrid was sketched (**19e**) by using 2D sketcher and energy minimized, subjected to ligand preparation for generation of different conformers (Schrödinger 2017-1). The different conformers thus generated were subjected to molecular docking with SP Glide 7.4 (Schrödinger 2017-1). The poses obtained were evaluated and best one was reported. The lowest energy pose for the compound was selected and the docked complex were further optimized using molecular dynamics simulations using Desmond 4.4 with OPLS-AA force field in 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 trajectory file after production phase of MD simulations, were clustered according to the RMSD of backbone. The RMSD plot was obtained from simulation event analysis of Desmond.



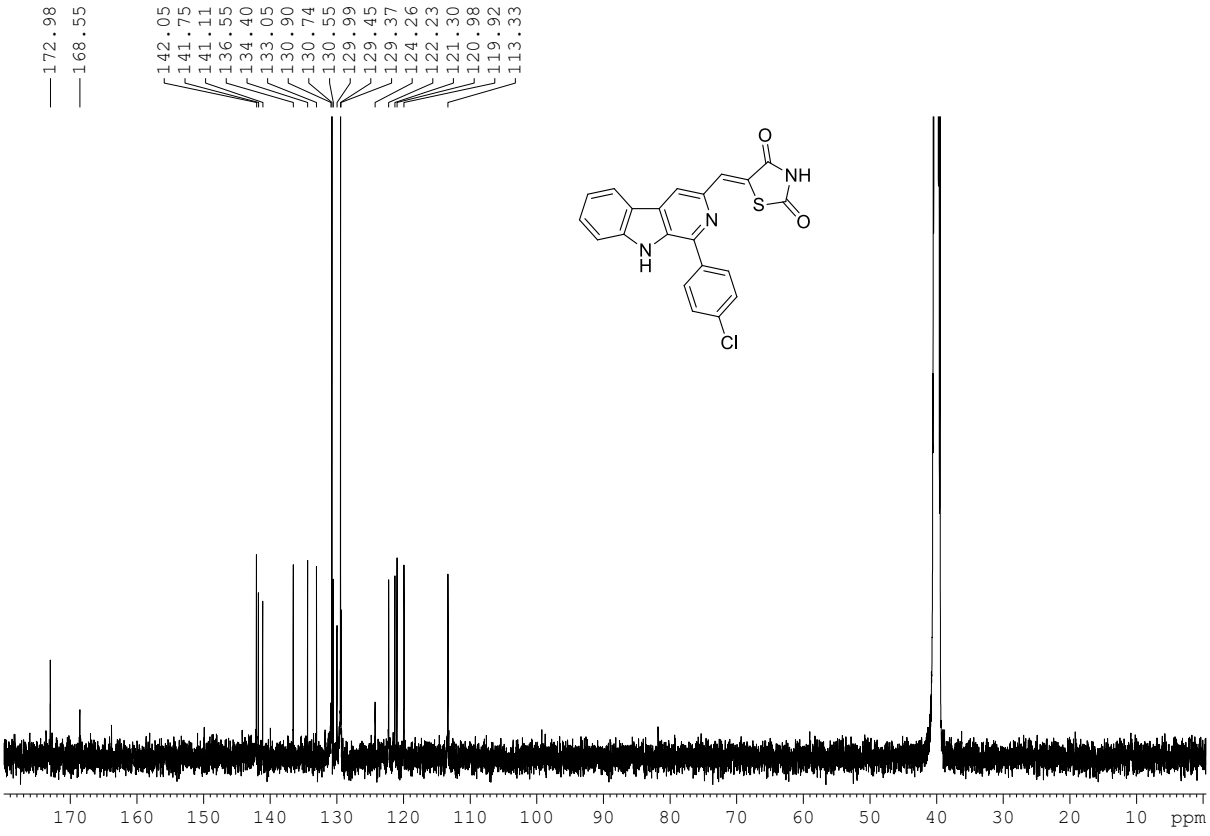
Compound **19a**: ^{1}H NMR (500 MHz, DMSO-d_6)



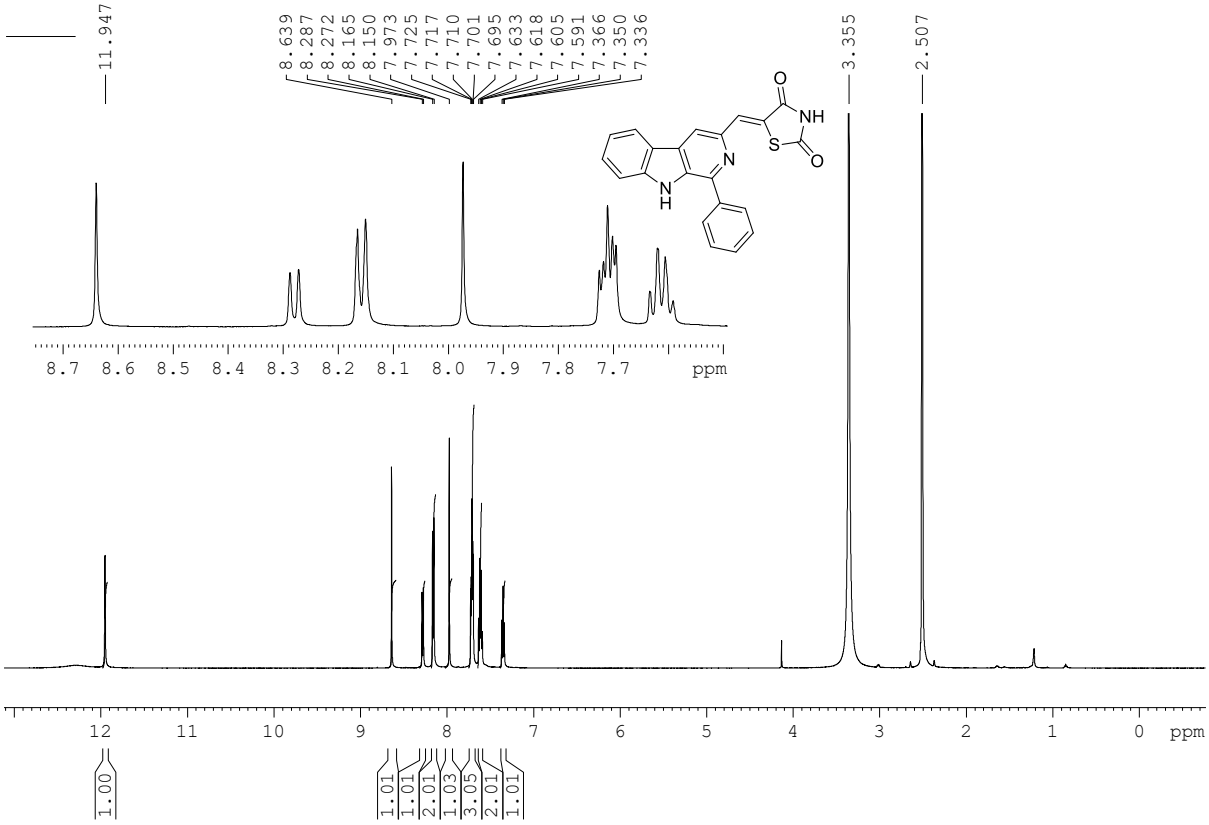
Compound **19a**: ^{13}C NMR (125 MHz, DMSO-d_6)



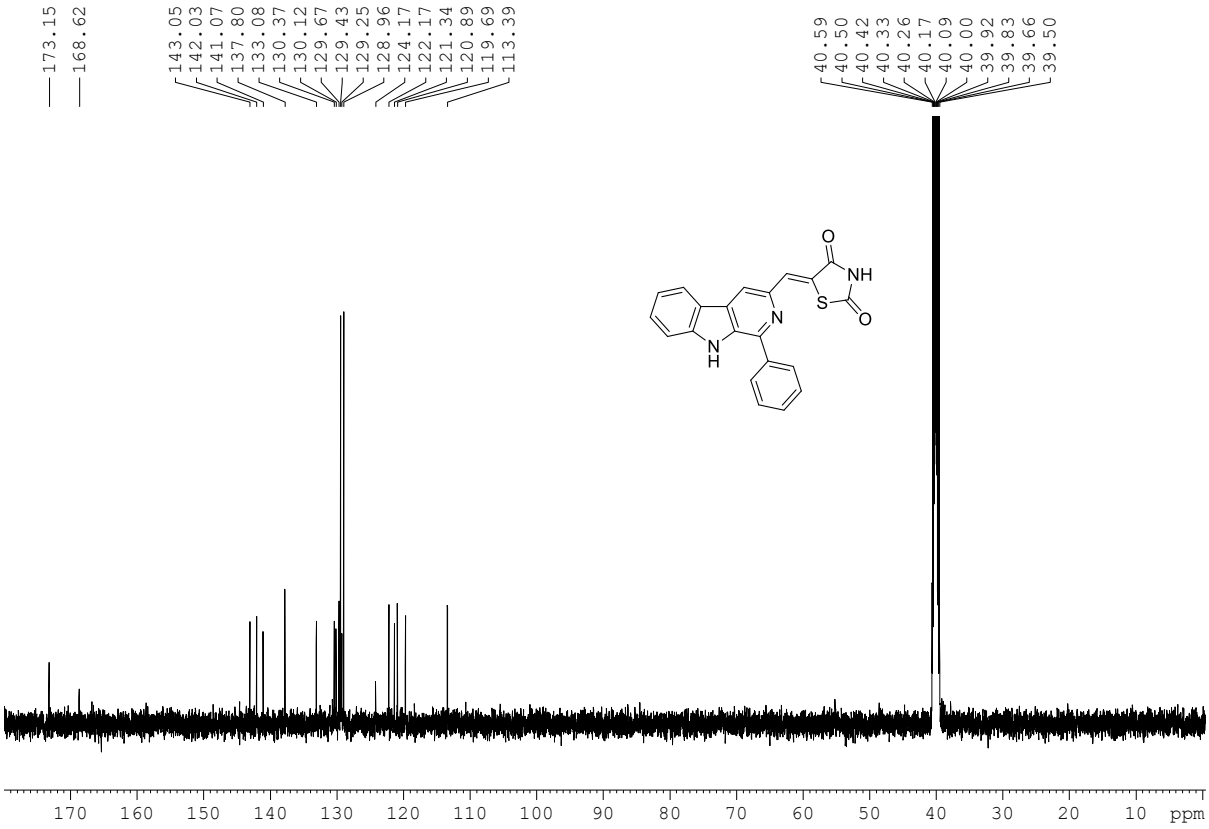
Compound **19b**: ^{1}H NMR (500 MHz, DMSO-d_6)



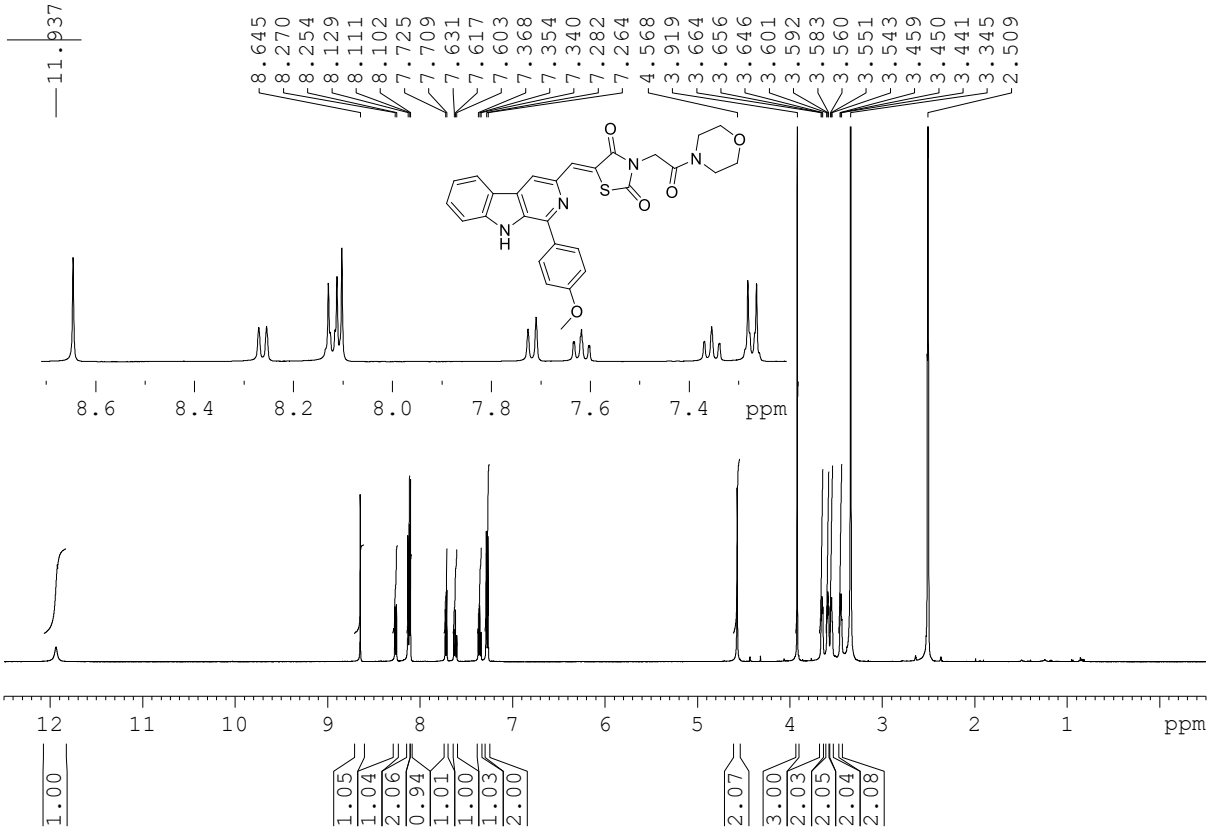
Compound **19b**: ^{13}C NMR (125 MHz, DMSO-d_6)



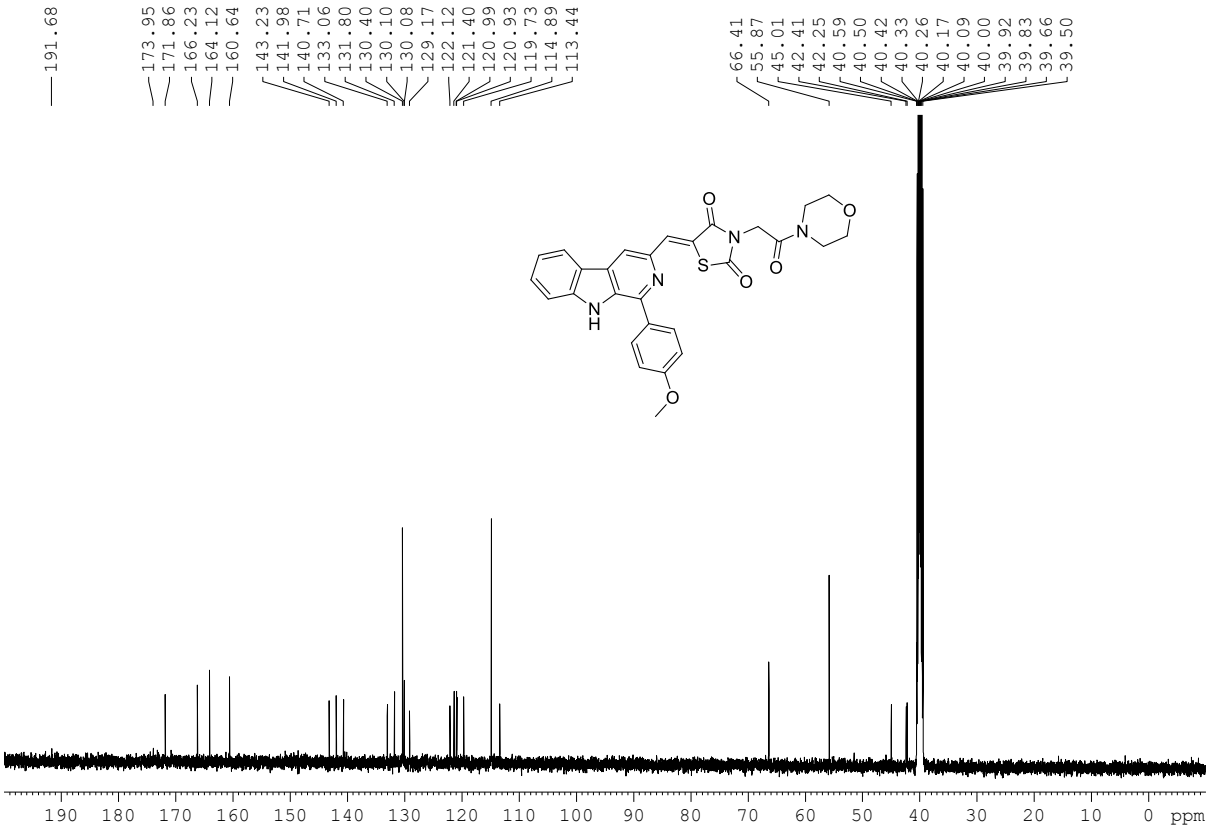
Compound **19c**: ^{1}H NMR (500 MHz, DMSO-d_6)



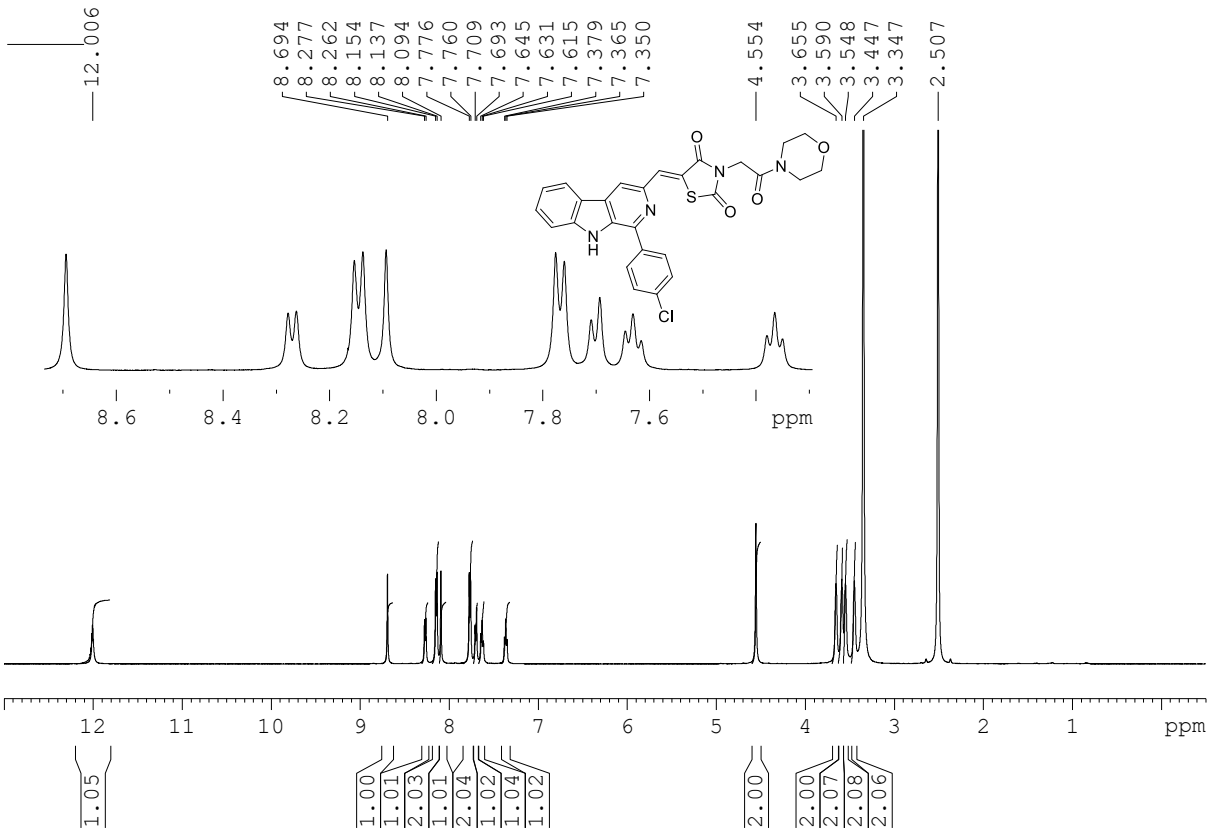
Compound **19c**: ^{13}C NMR (125 MHz, DMSO-d_6)



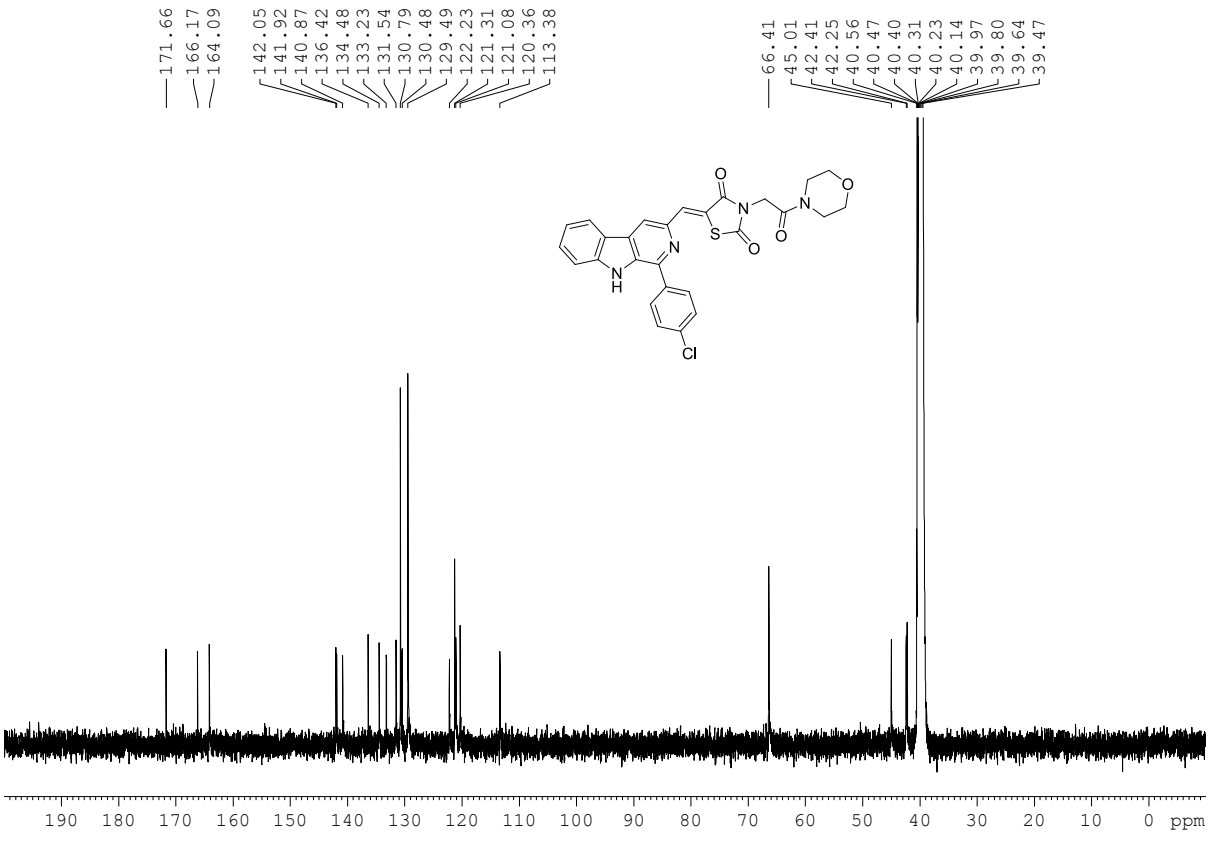
Compound **19d**: ^{1}H NMR (500 MHz, DMSO-d_6)



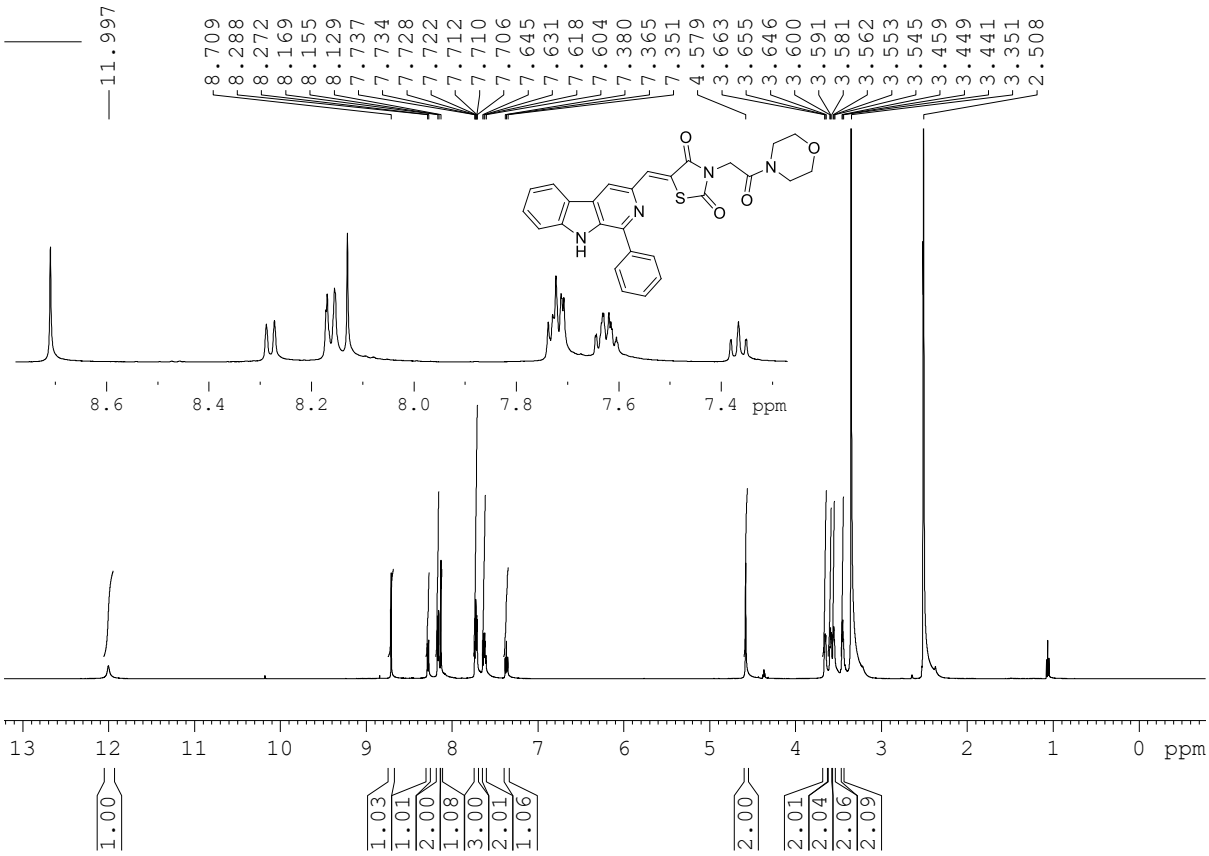
Compound **19d**: ^{13}C NMR (125 MHz, DMSO-d_6)



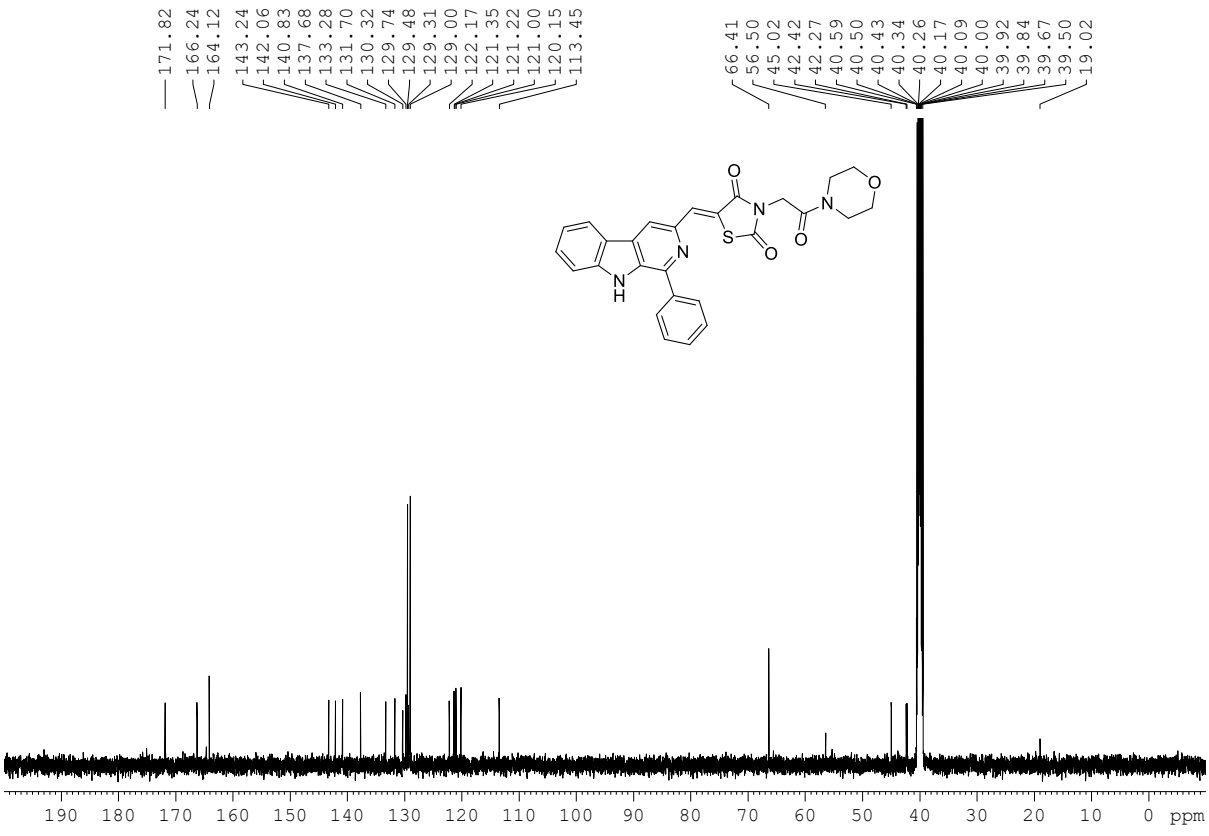
Compound **19e**: ^{1}H NMR (500 MHz, DMSO-d_6)



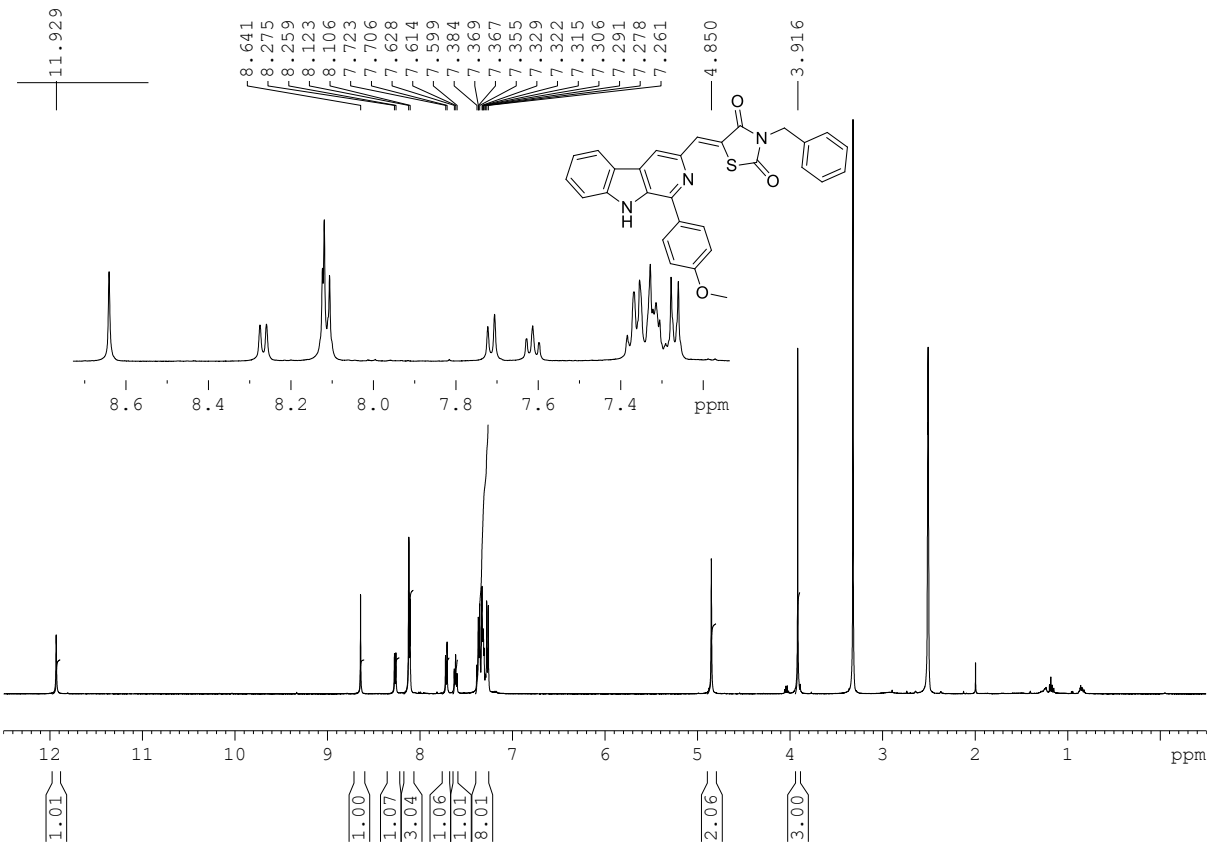
Compound **19e**: ^{13}C NMR (125 MHz, DMSO-d_6)



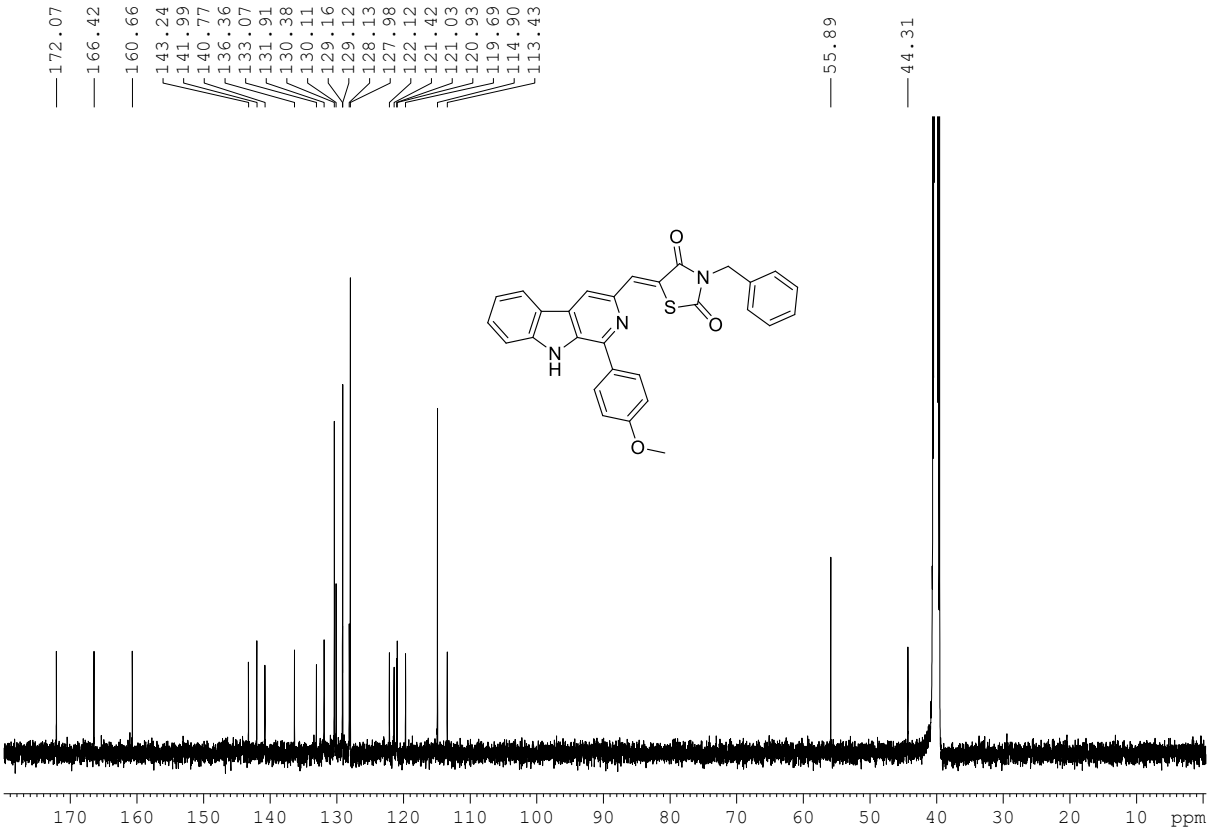
Compound **19f**: ^{1}H NMR (500 MHz, DMSO-d_6)



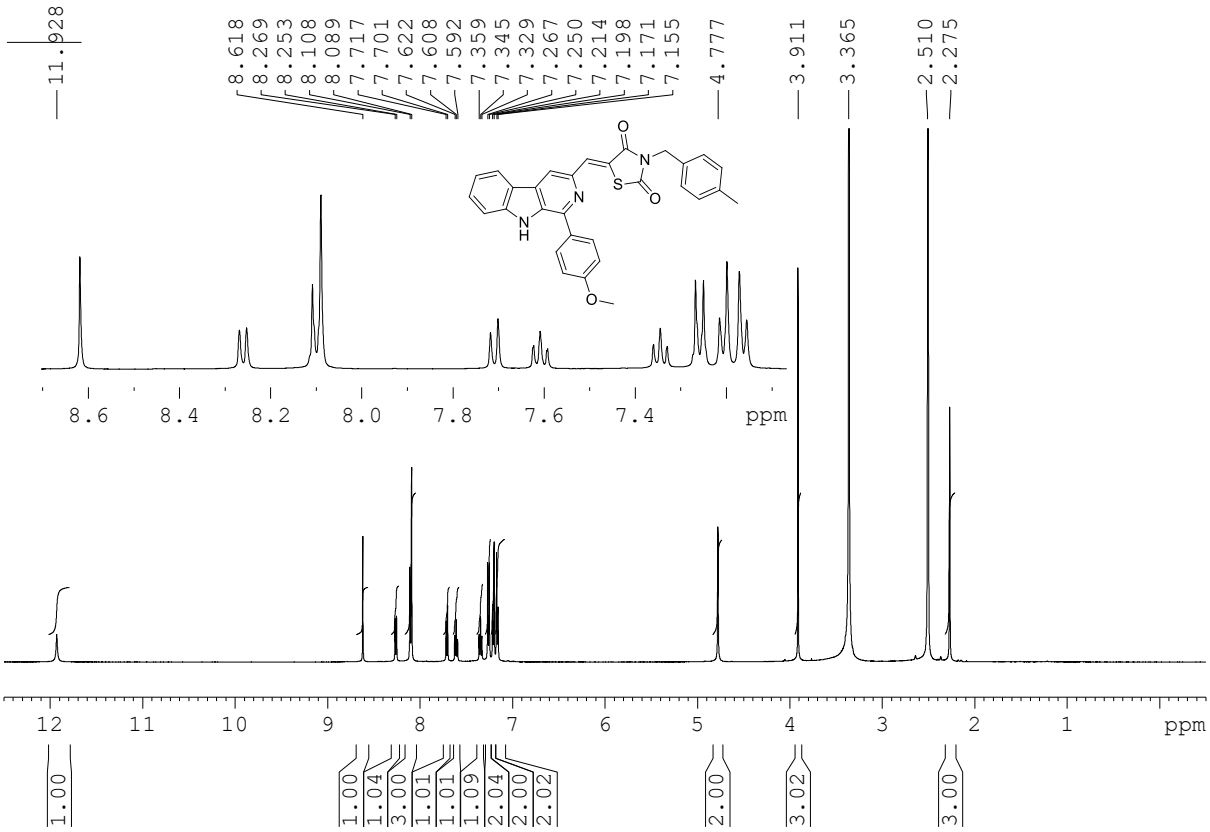
Compound **19f**: ^{13}C NMR (125 MHz, DMSO-d_6)



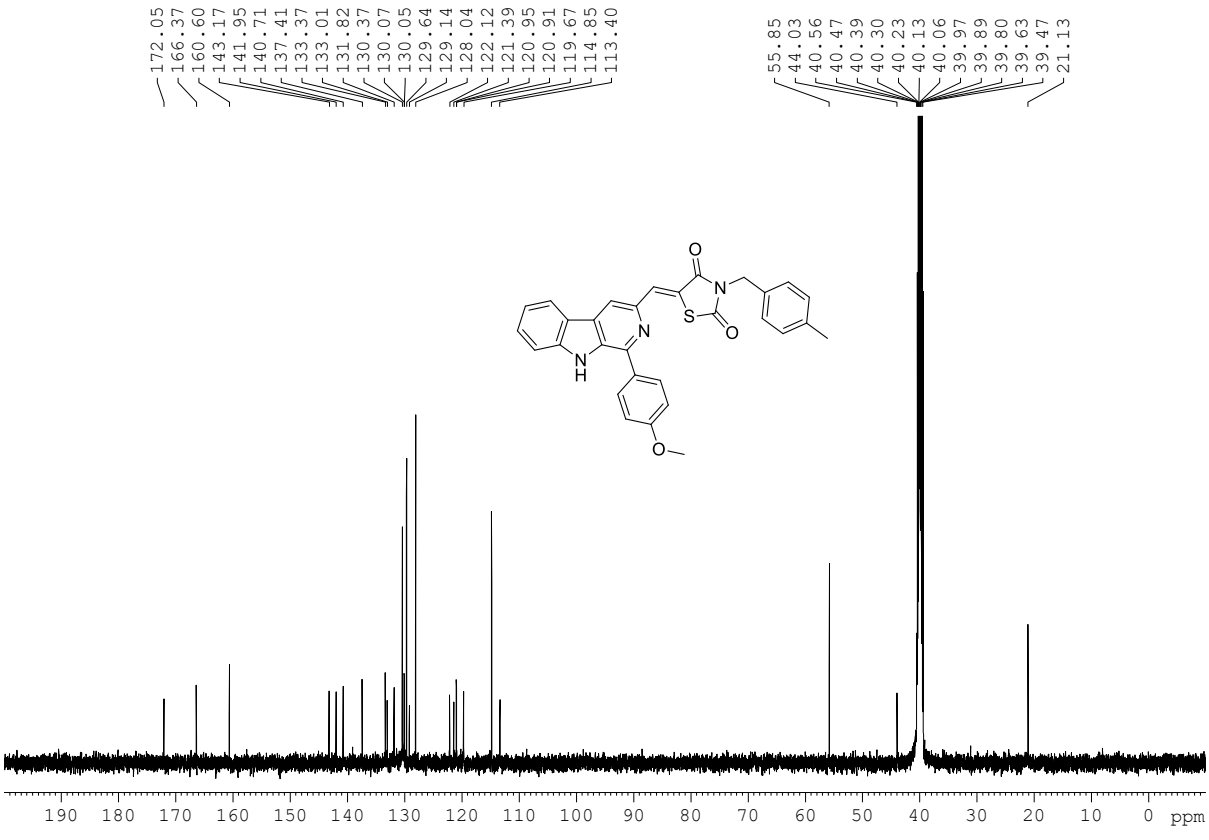
Compound **19g**: ^{1}H NMR (500 MHz, DMSO-d_6)



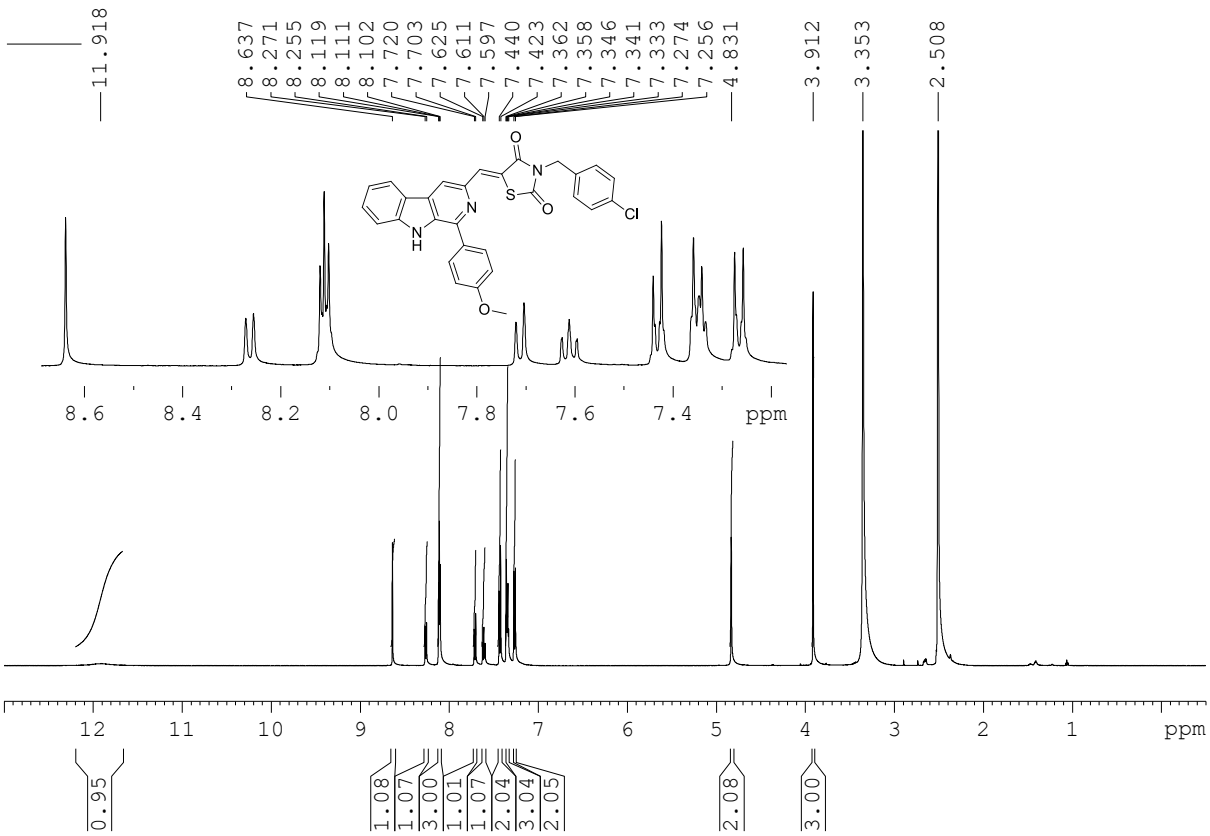
Compound **19g**: ^{13}C NMR (125 MHz, DMSO-d_6)



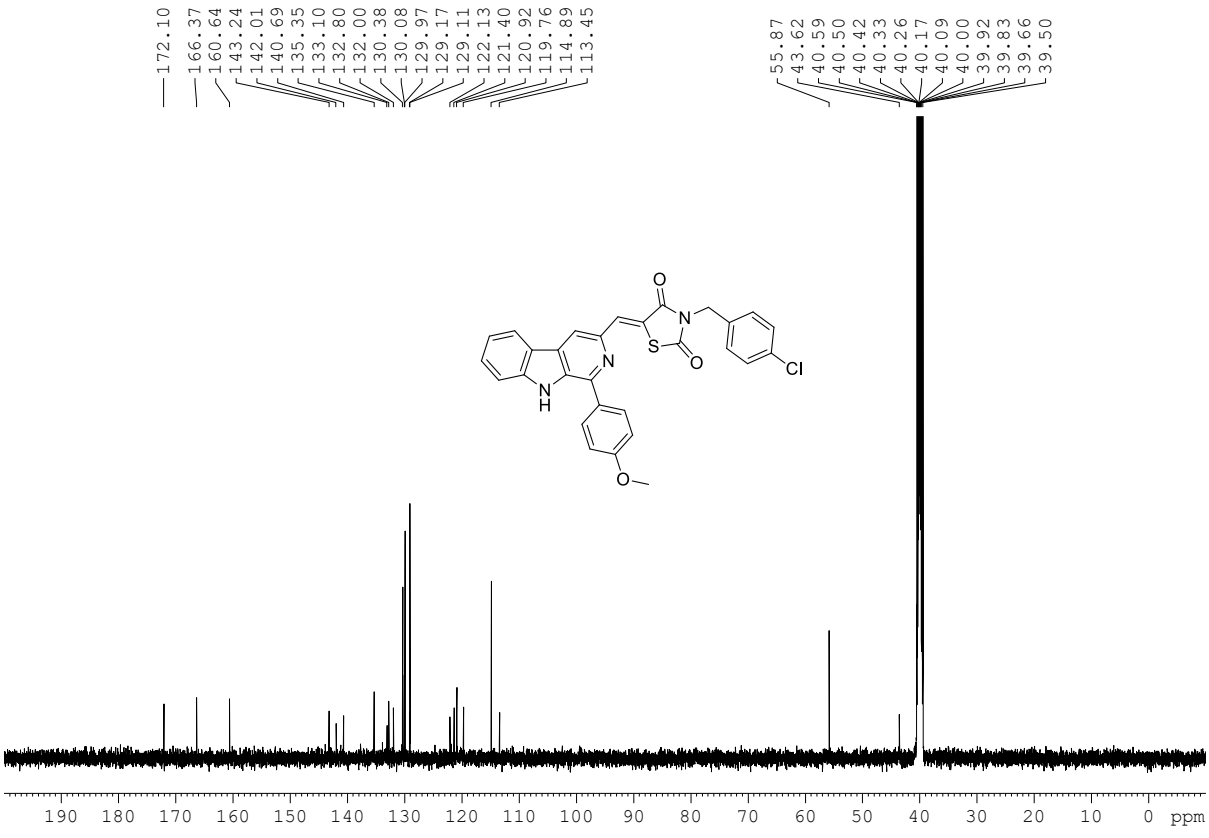
Compound **19h**: ^{1}H NMR (500 MHz, DMSO-d_6)



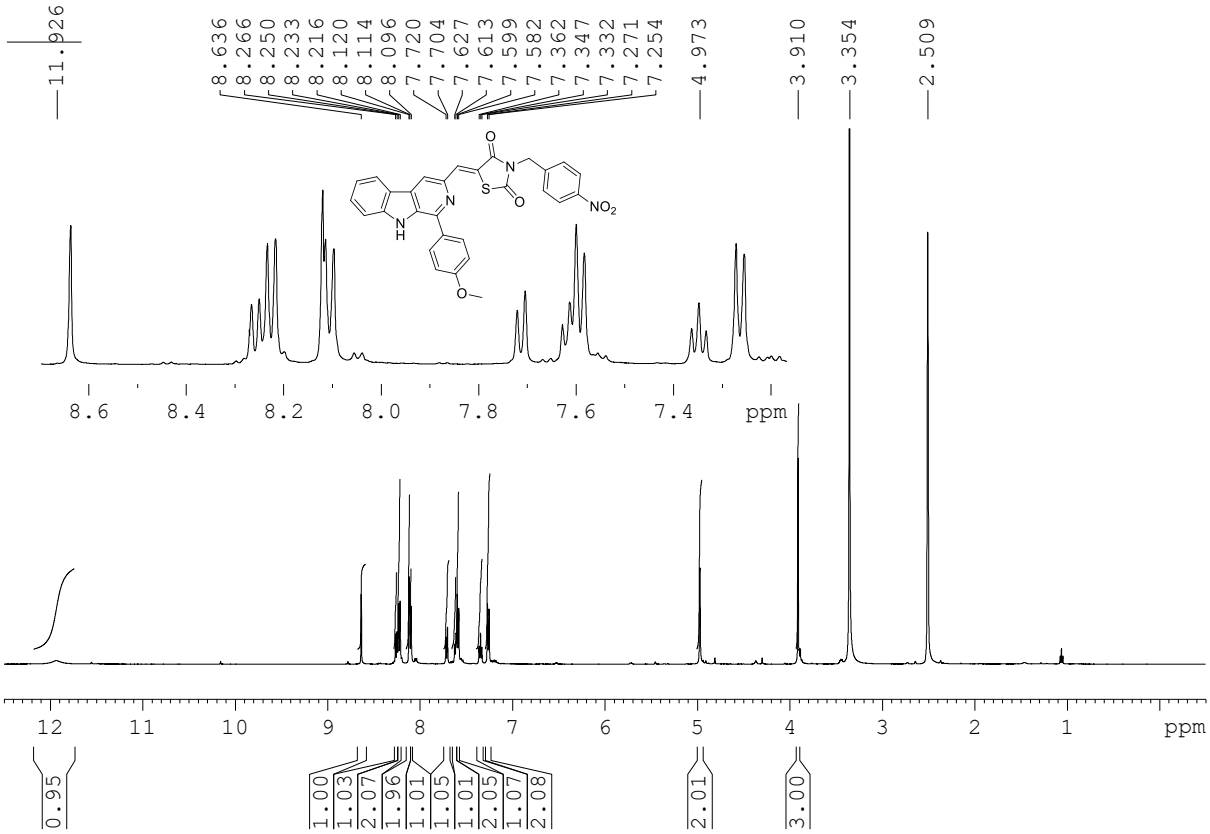
Compound **19h**: ^{13}C NMR (125 MHz, DMSO-d_6)



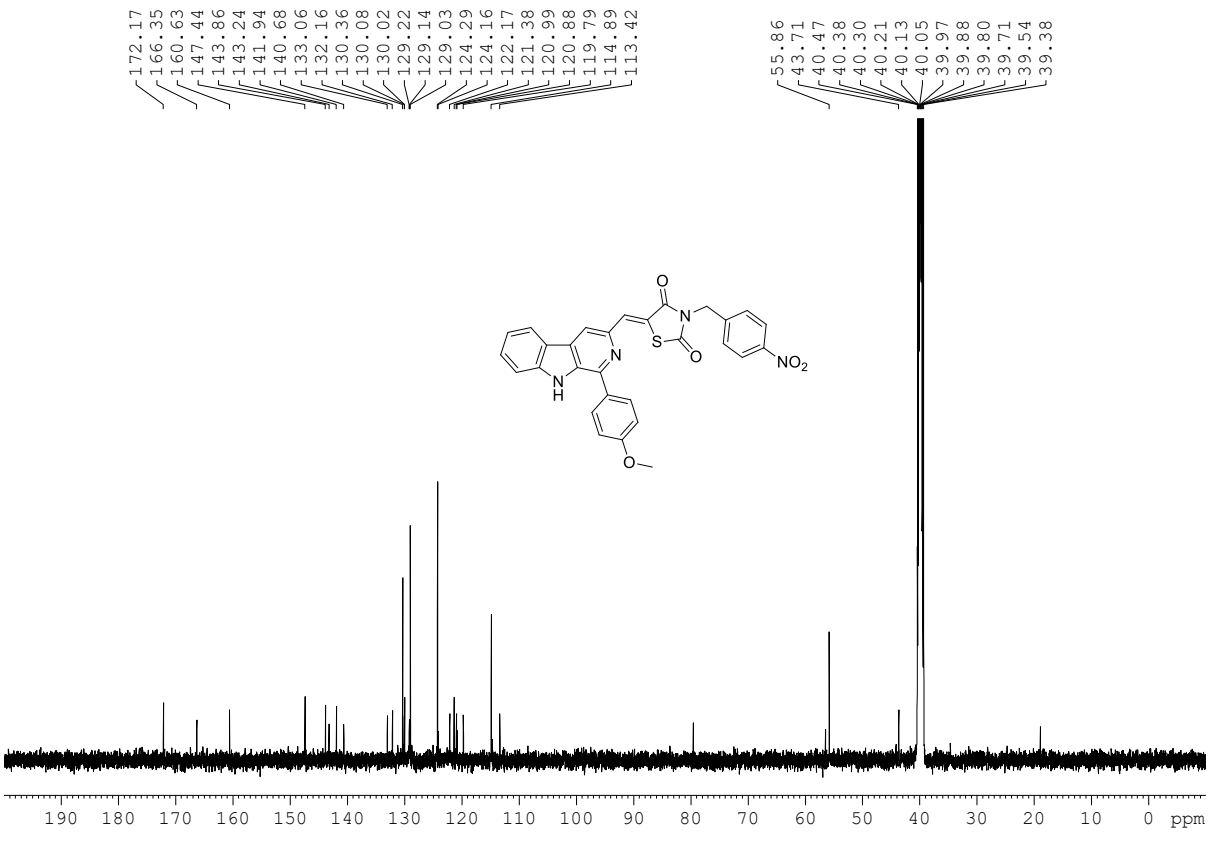
Compound **19i**: ^{1}H NMR (500 MHz, DMSO-d_6)



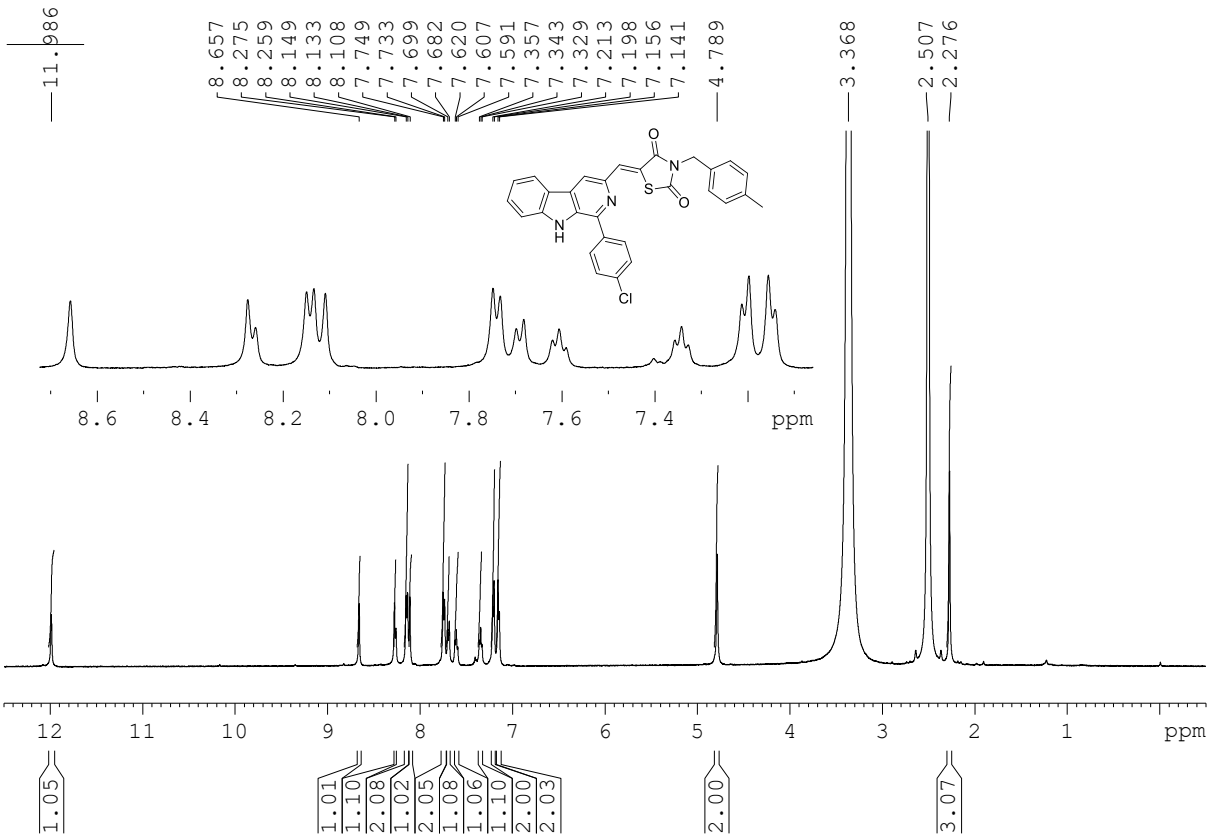
Compound **19i**: ^{13}C NMR (125 MHz, DMSO-d_6)



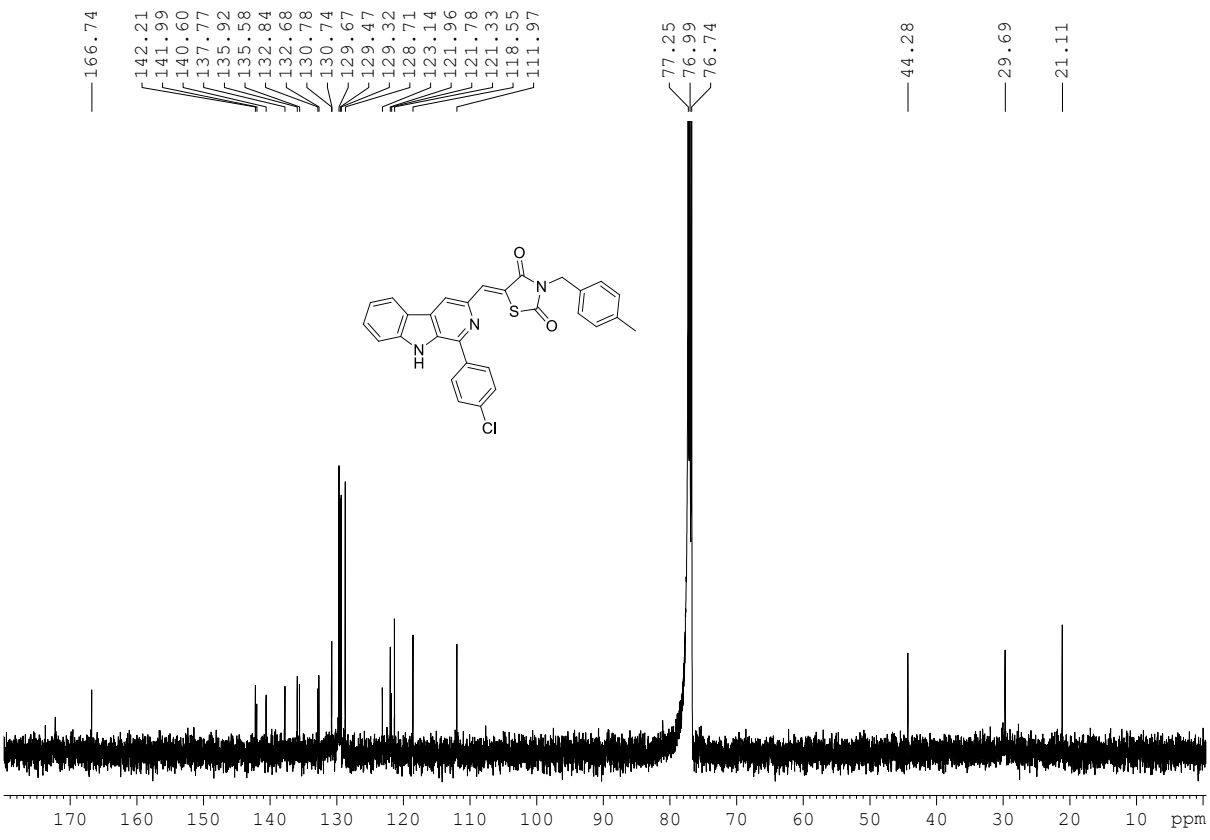
Compound **19j**: ^{1}H NMR (500 MHz, DMSO-d_6)



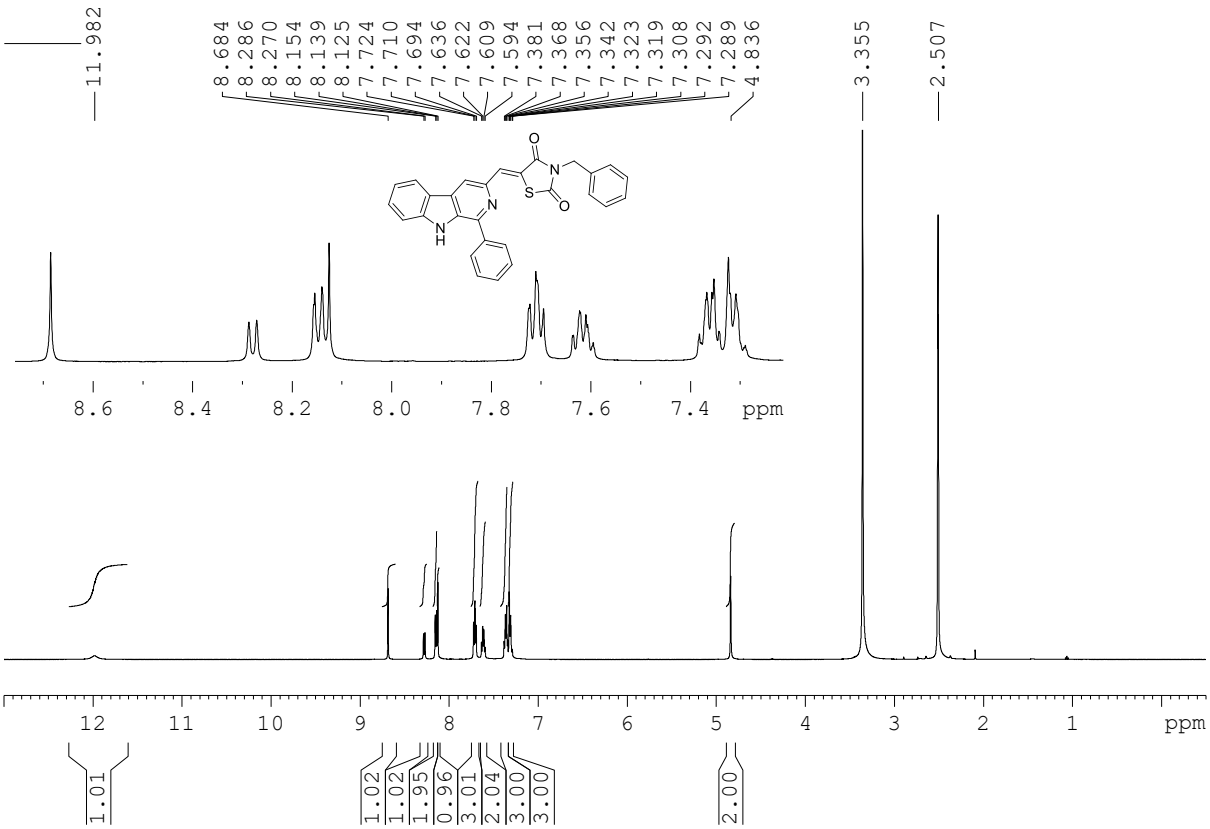
Compound **19j**: ^{13}C NMR (125 MHz, DMSO-d_6)



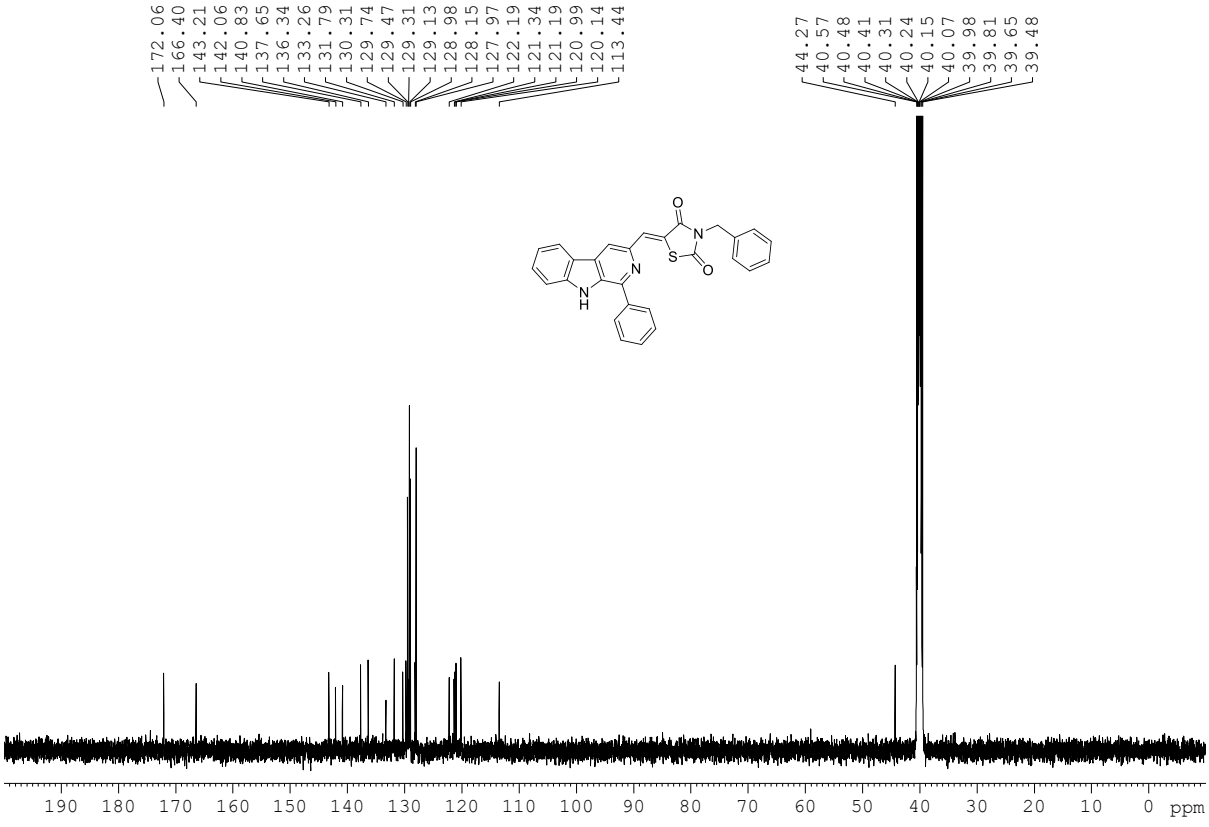
Compound **19k**: ^{1}H NMR (500 MHz, DMSO-d_6)



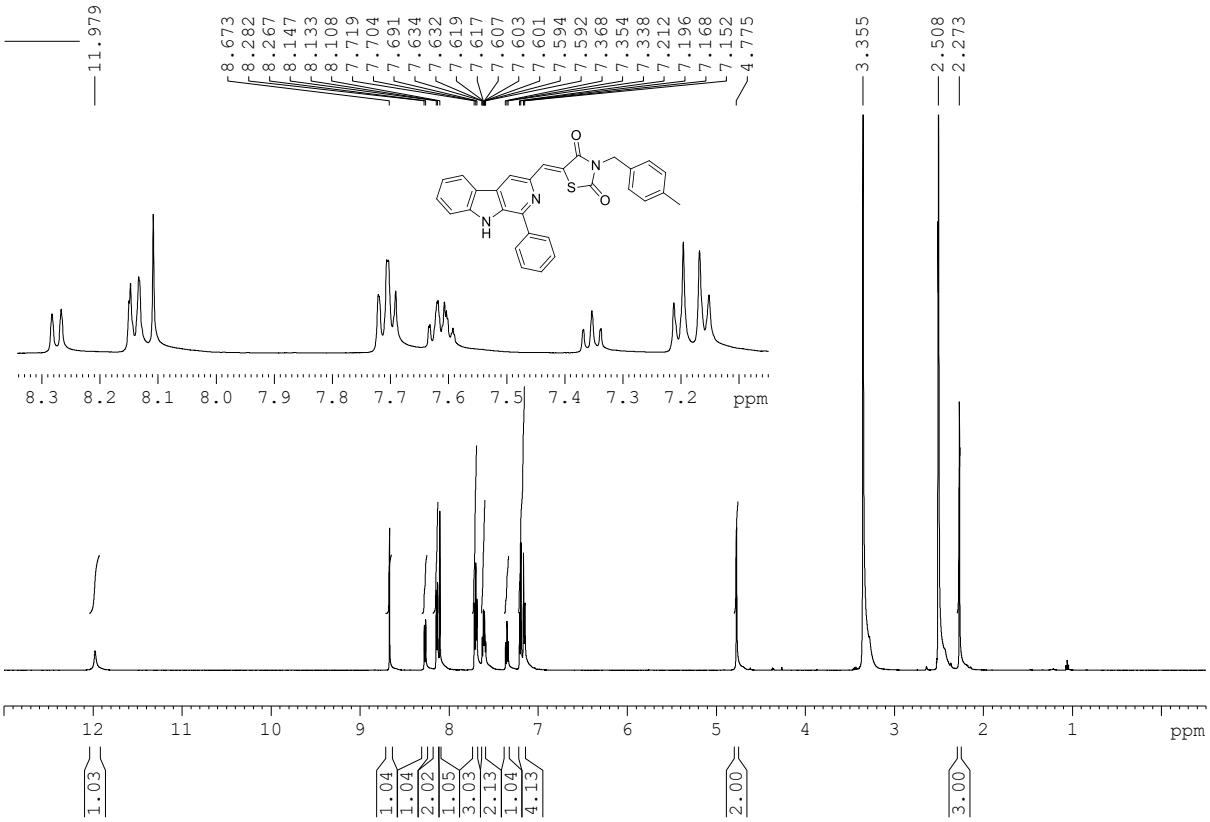
Compound **19k**: ^{13}C NMR (125 MHz, $CDCl_3$)



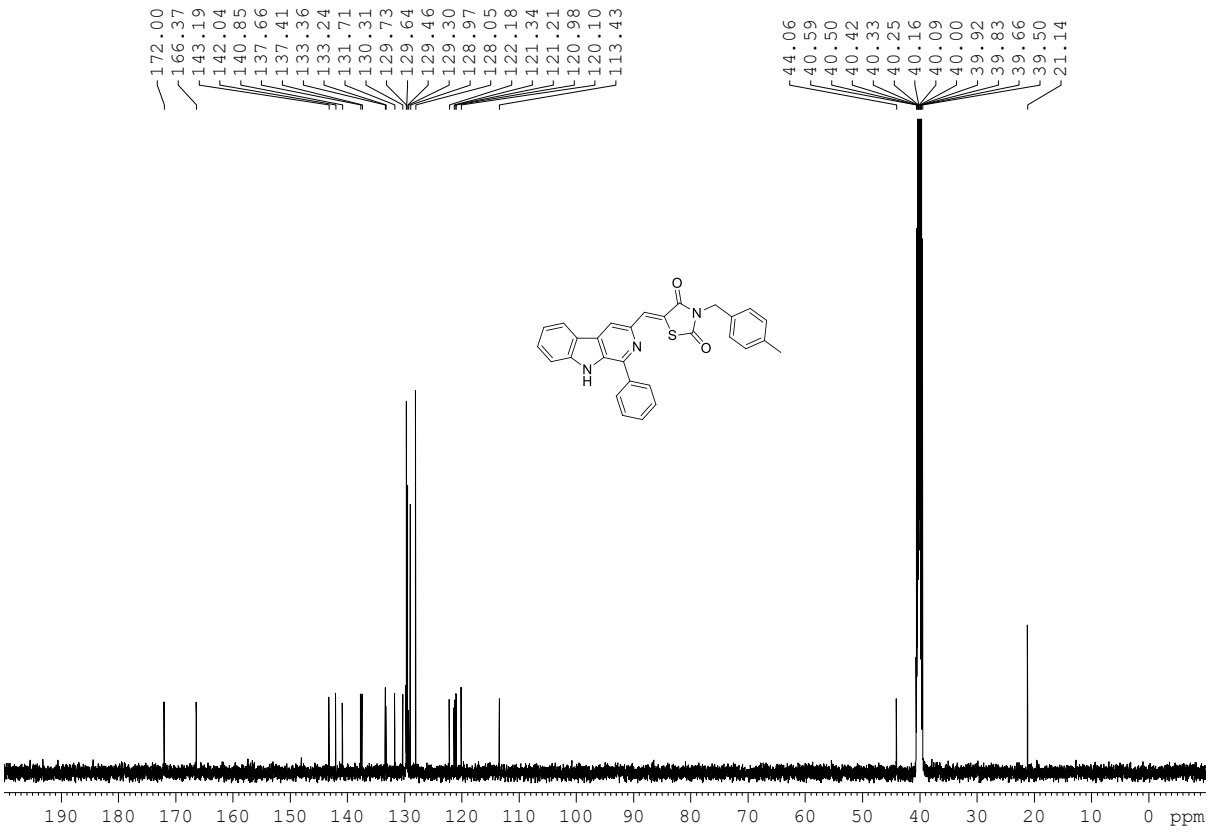
Compound **19l**: ^{1}H NMR (500 MHz, DMSO-d_6)



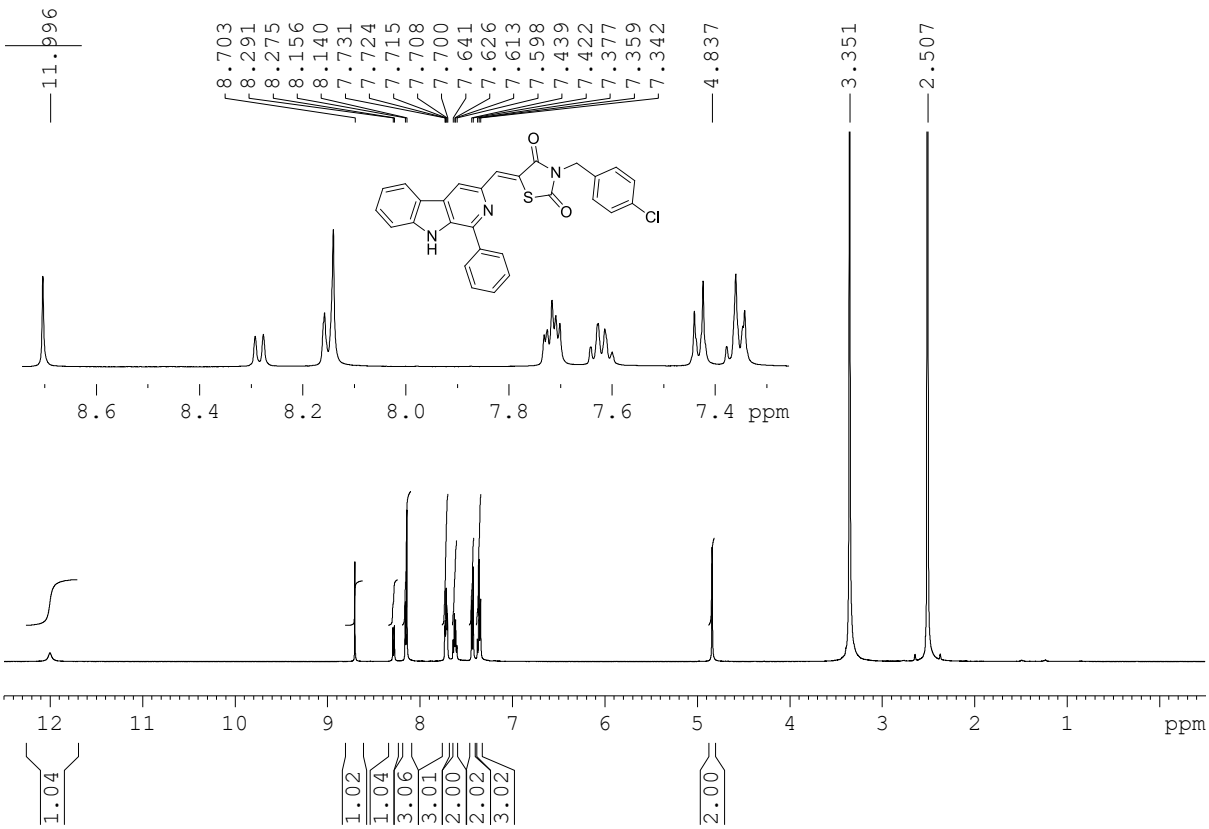
Compound **19l**: ^{13}C NMR (125 MHz, DMSO-d_6)



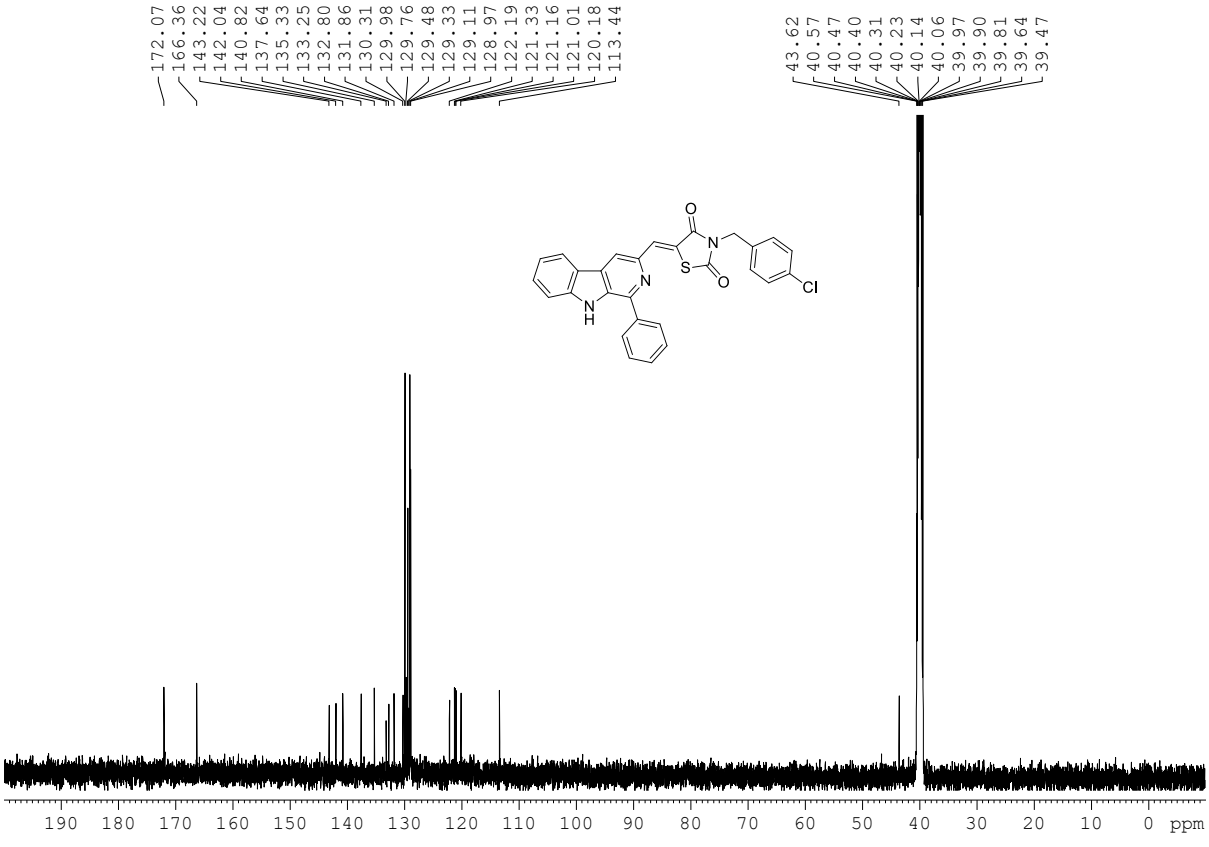
Compound **19m**: ^{1}H NMR (500 MHz, DMSO-d_6



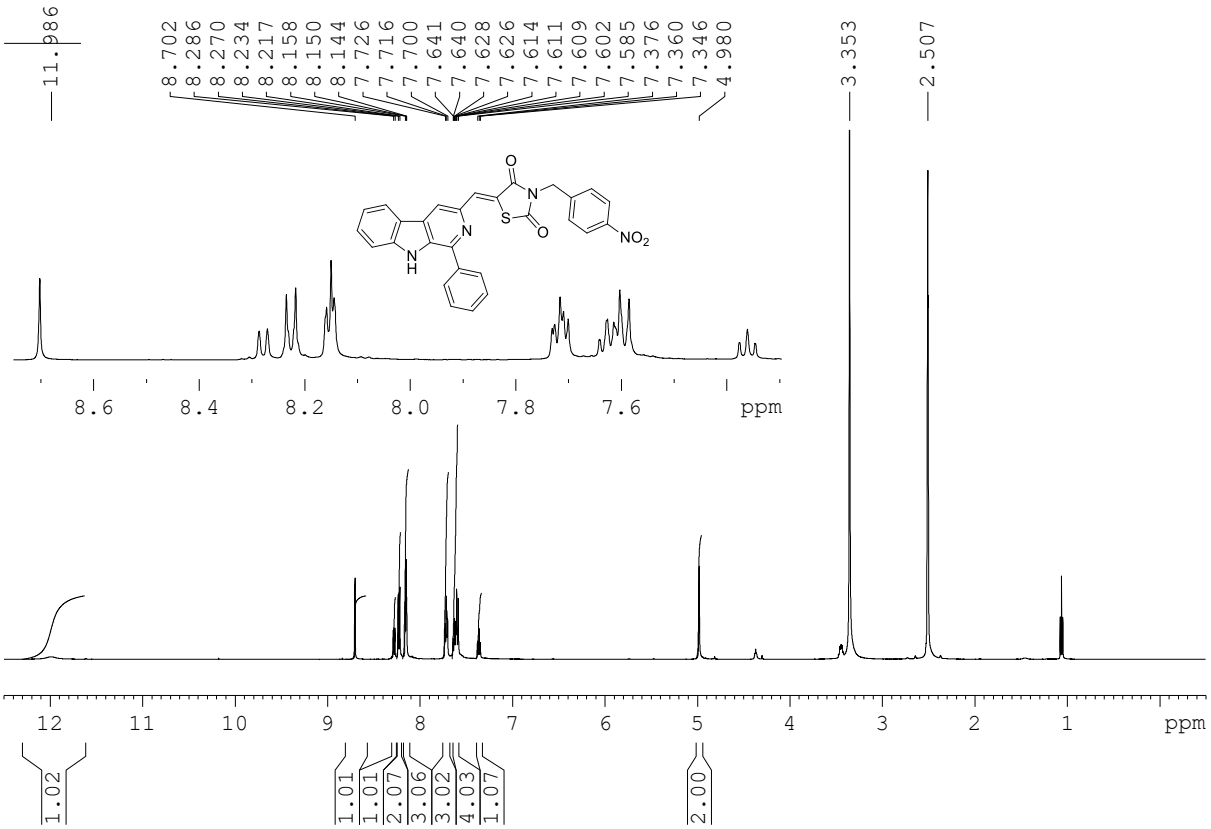
Compound **19m**: ^{13}C NMR (125 MHz, DMSO-d_6)



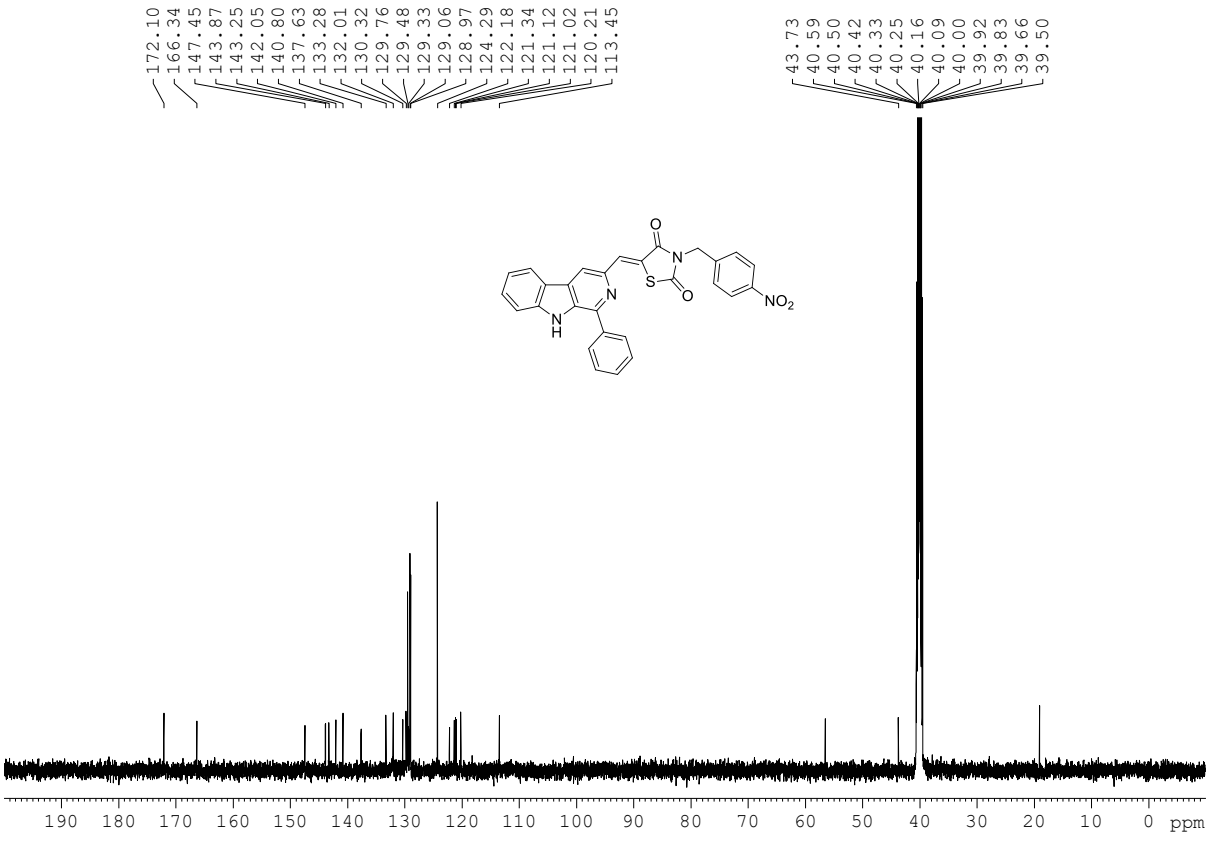
Compound **19n**: ^{1}H NMR (500 MHz, DMSO-d_6)



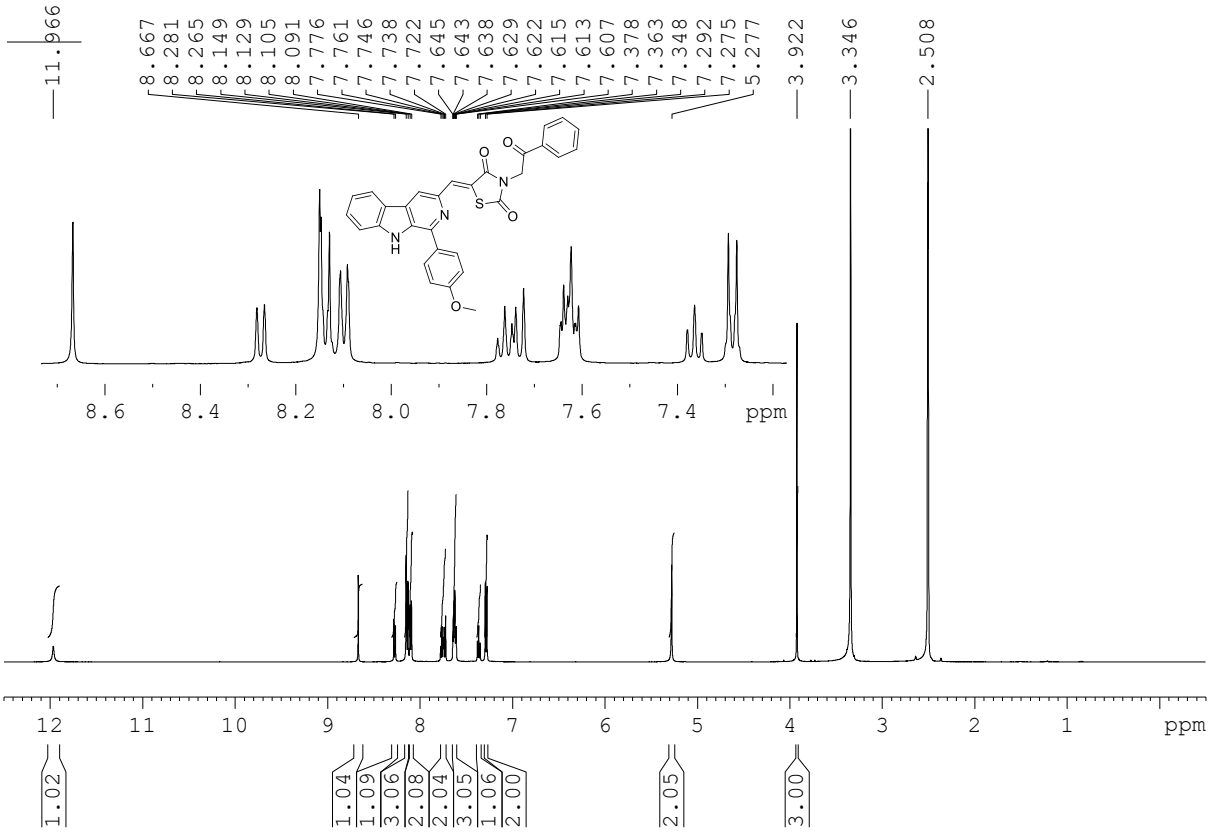
Compound **19n**: ^{13}C NMR (125 MHz, DMSO-d_6)



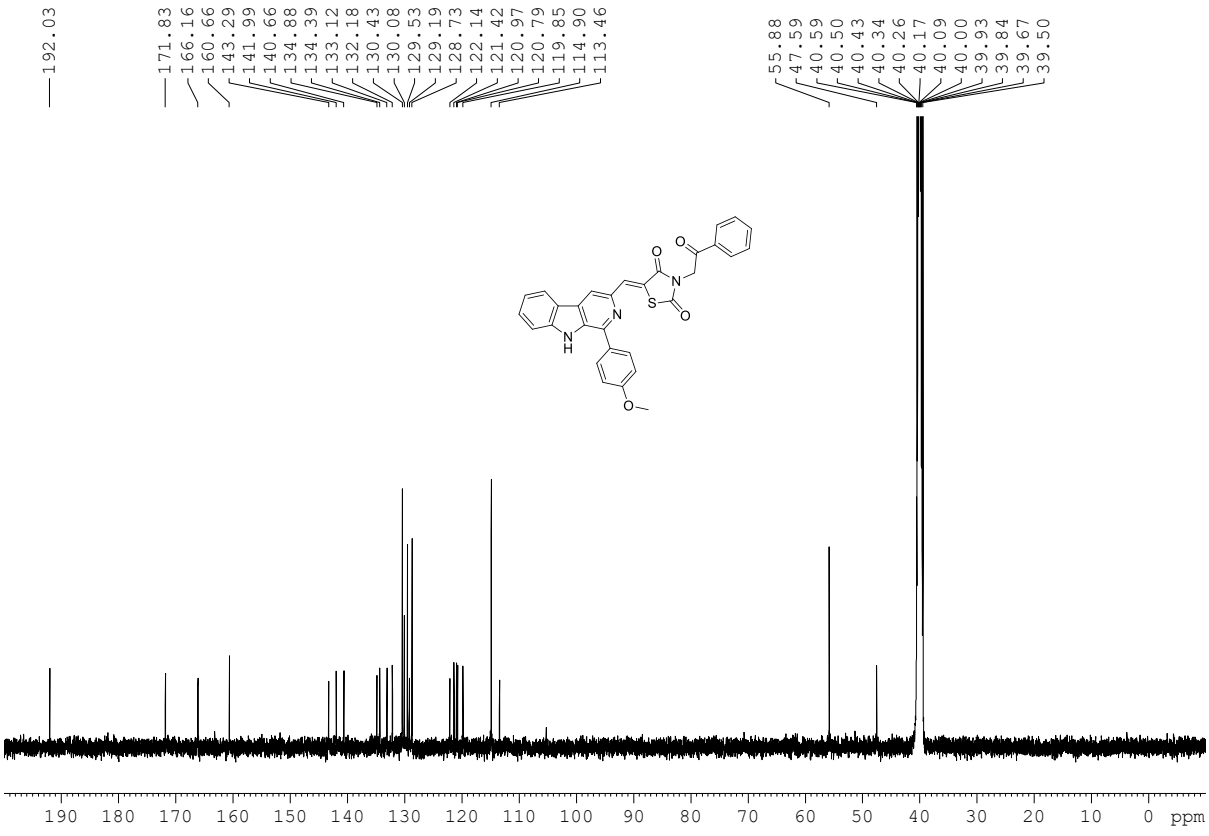
Compound **19o**: ^{1}H NMR (500 MHz, DMSO-d_6)



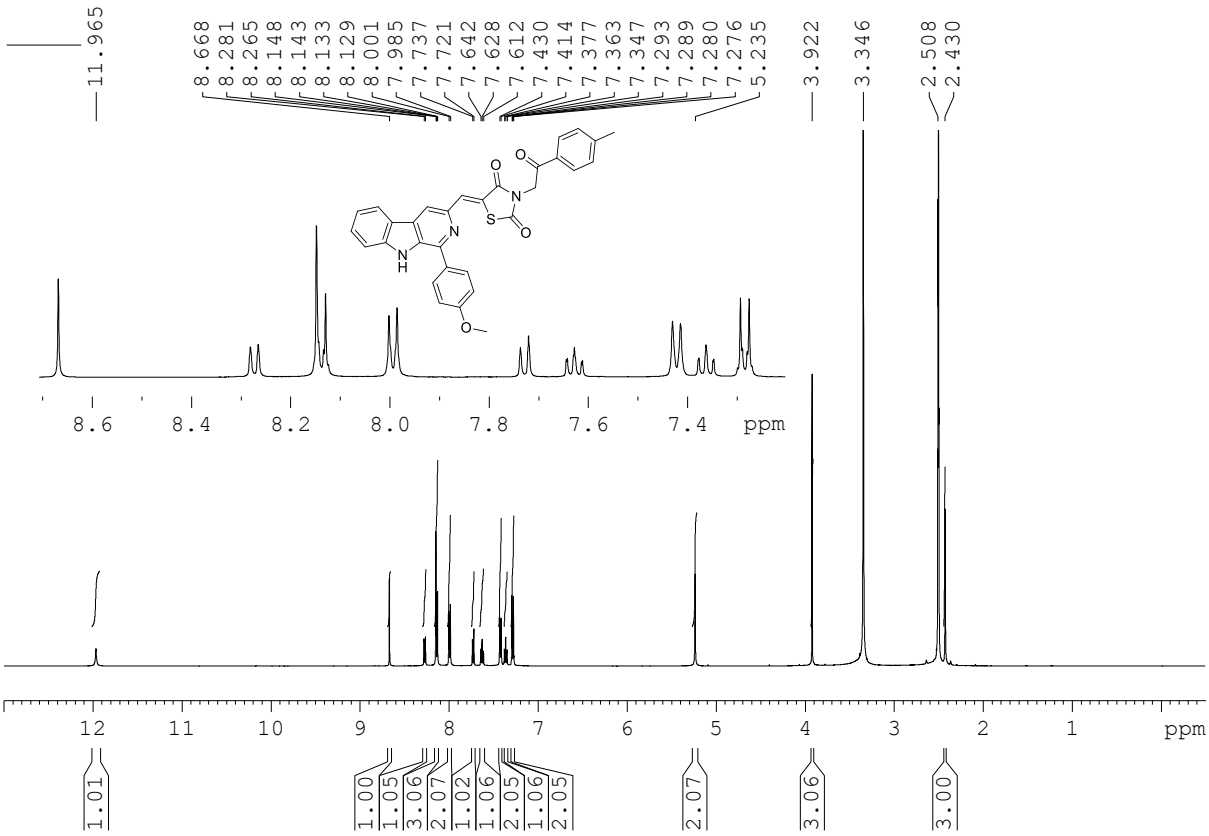
Compound **19o**: ^{13}C NMR (125 MHz, DMSO-d_6)



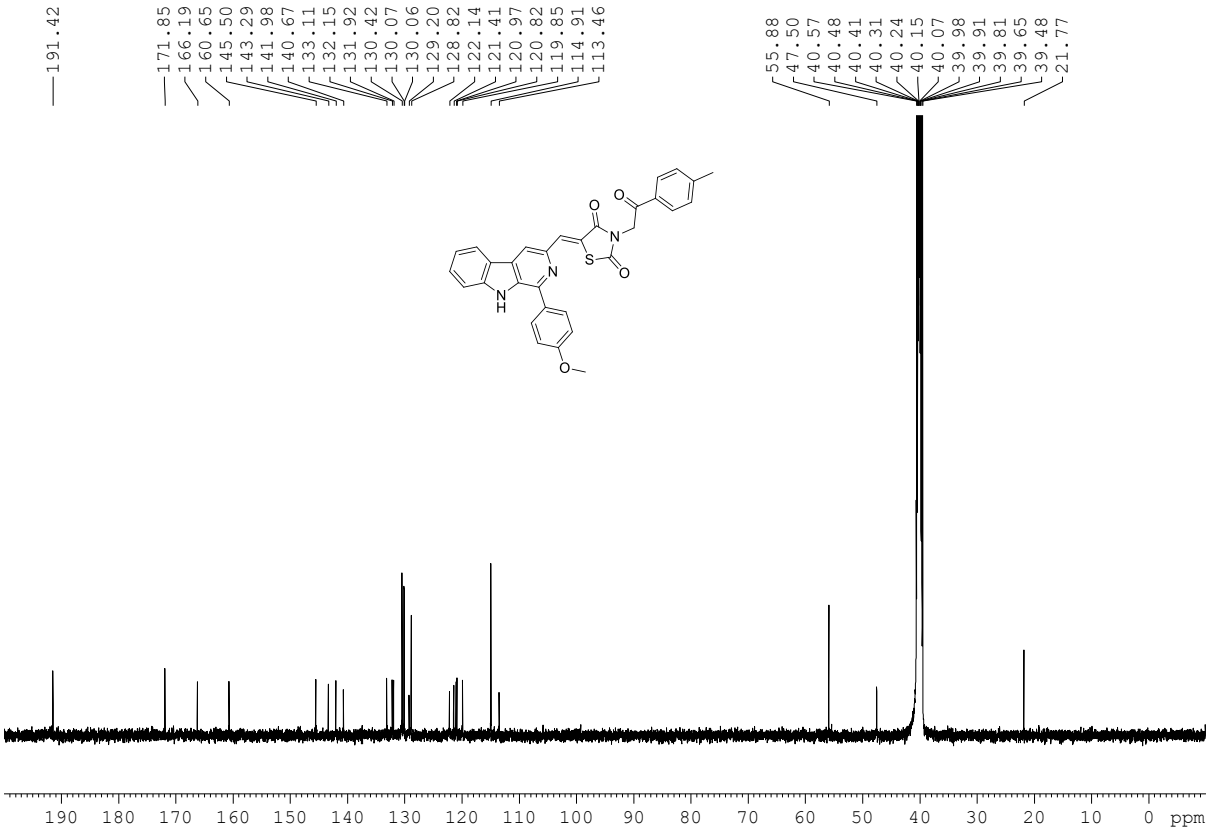
Compound **19p**: ^{1}H NMR (500 MHz, DMSO-d_6)



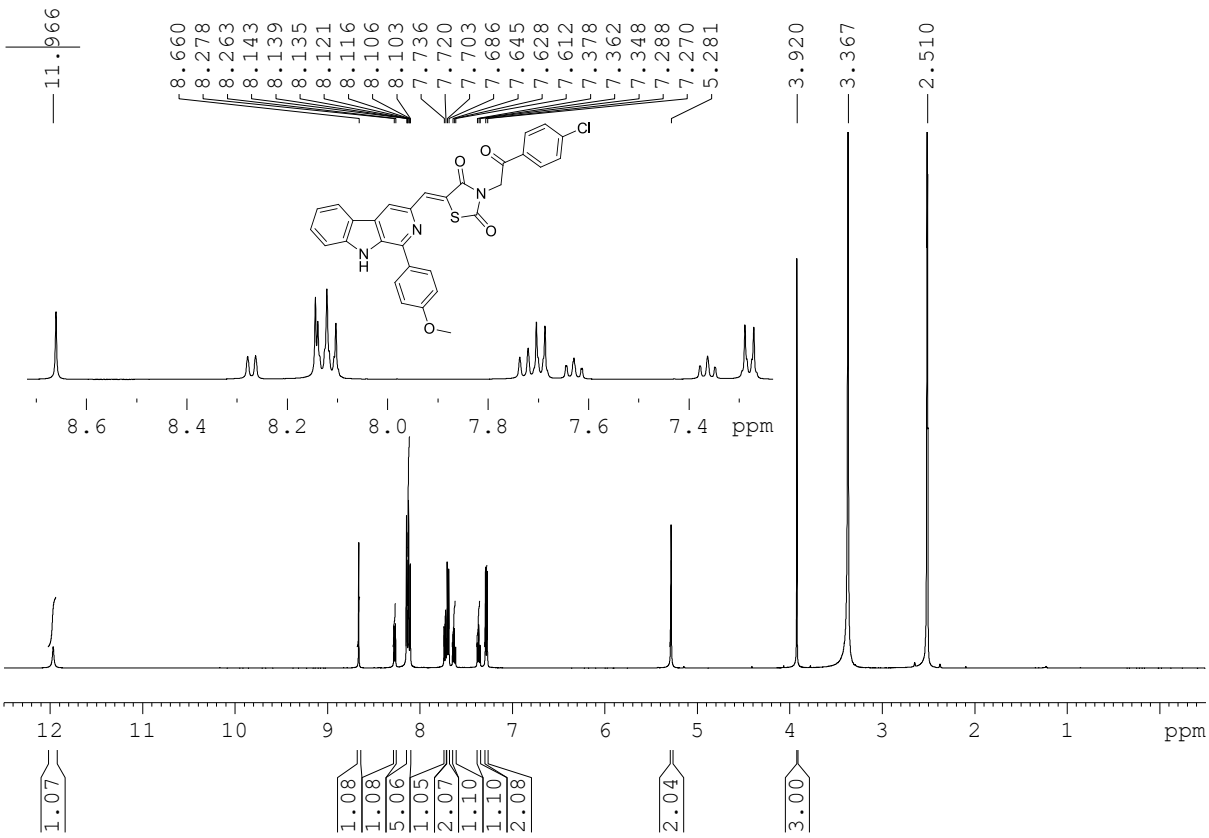
Compound **19p**: ^{13}C NMR (125 MHz, DMSO-d_6)



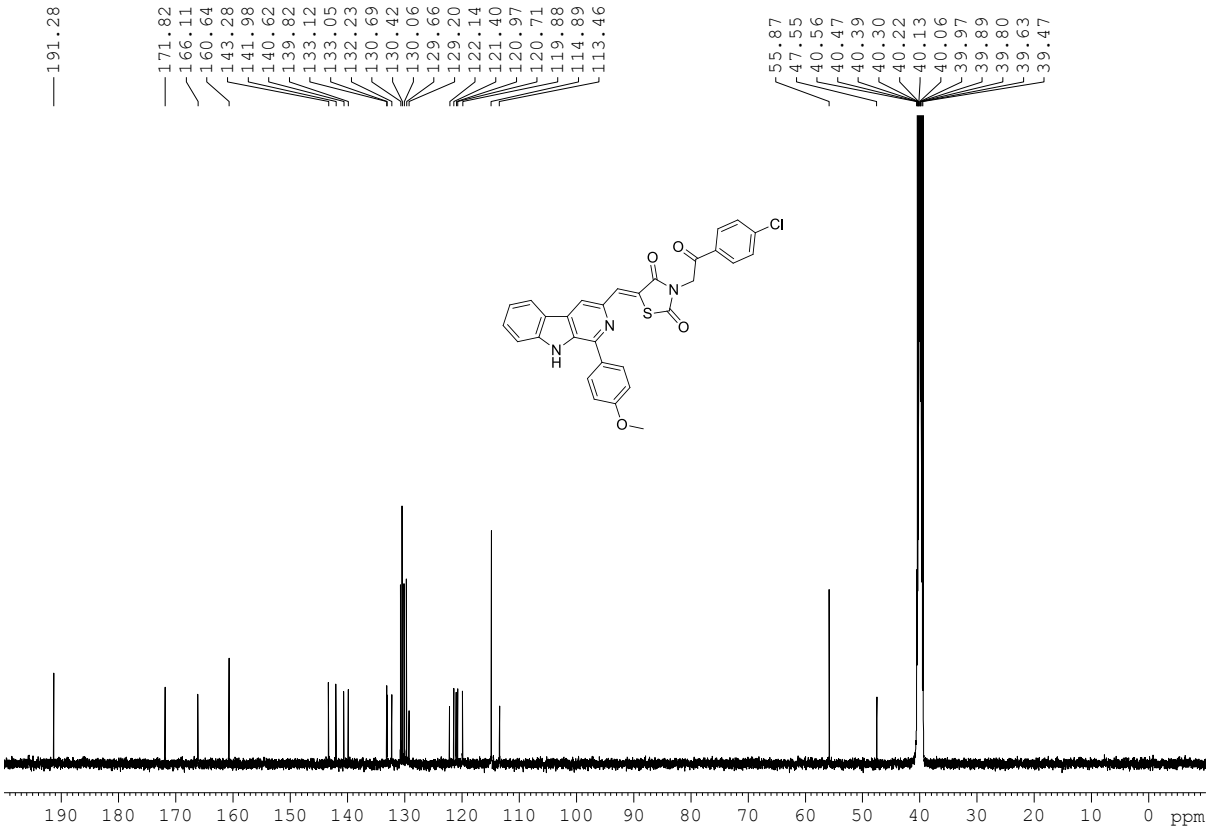
Compound **19q**: ^{1}H NMR (500 MHz, DMSO-d_6)



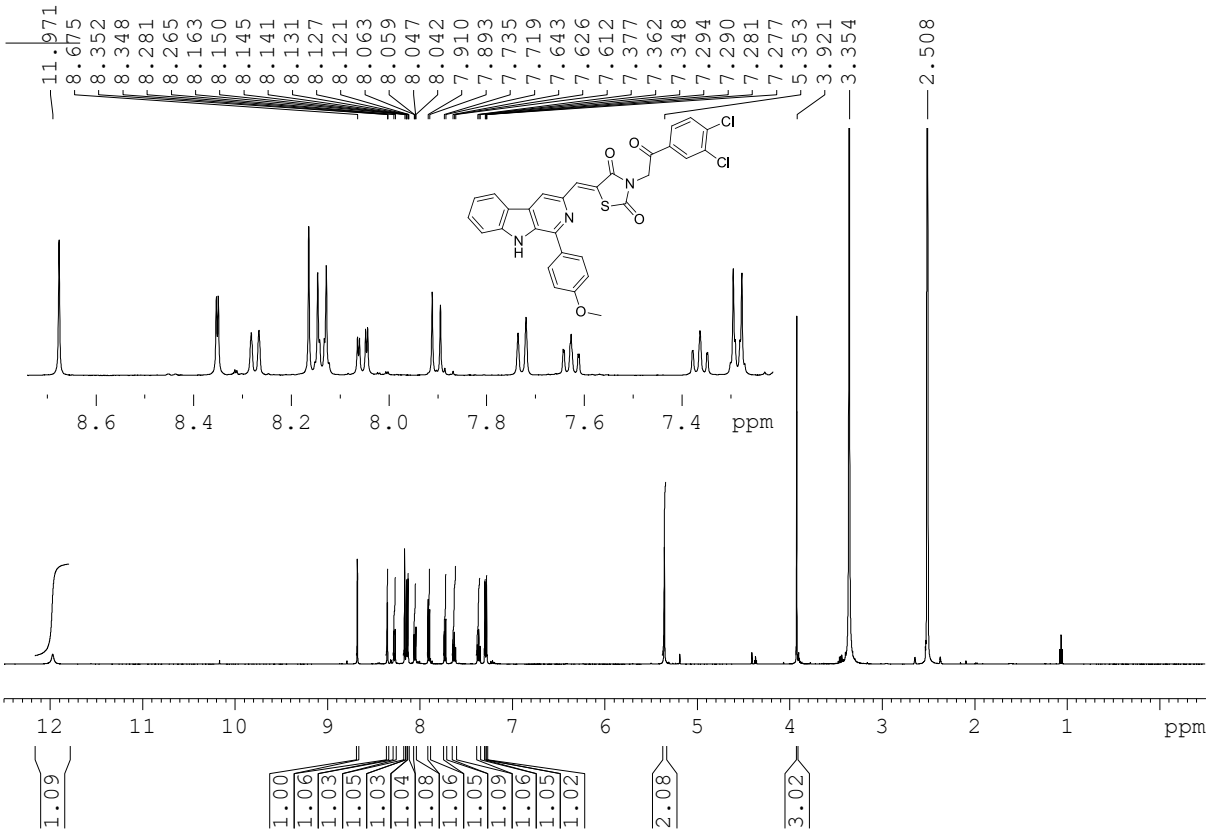
Compound **19q**: ^{13}C NMR (125 MHz, DMSO-d_6)



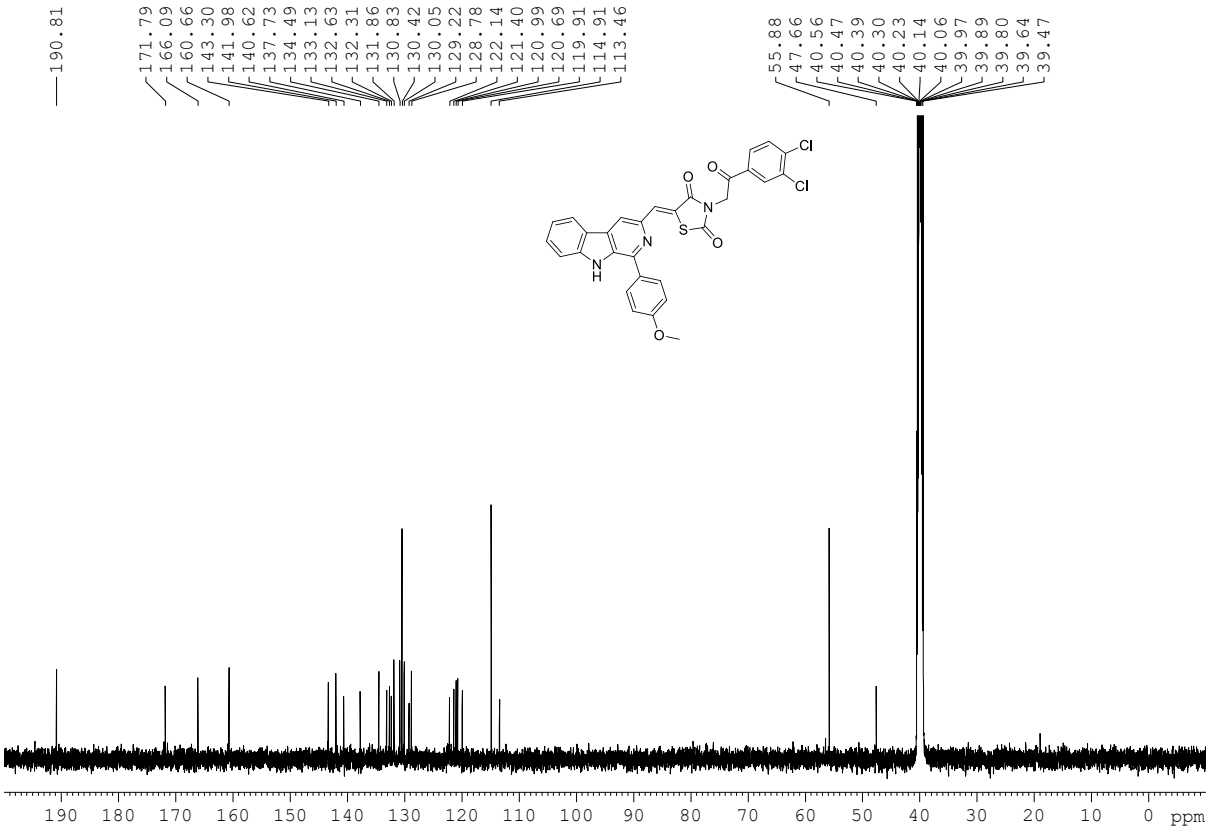
Compound **19r**: ^{1}H NMR (500 MHz, DMSO-d_6)



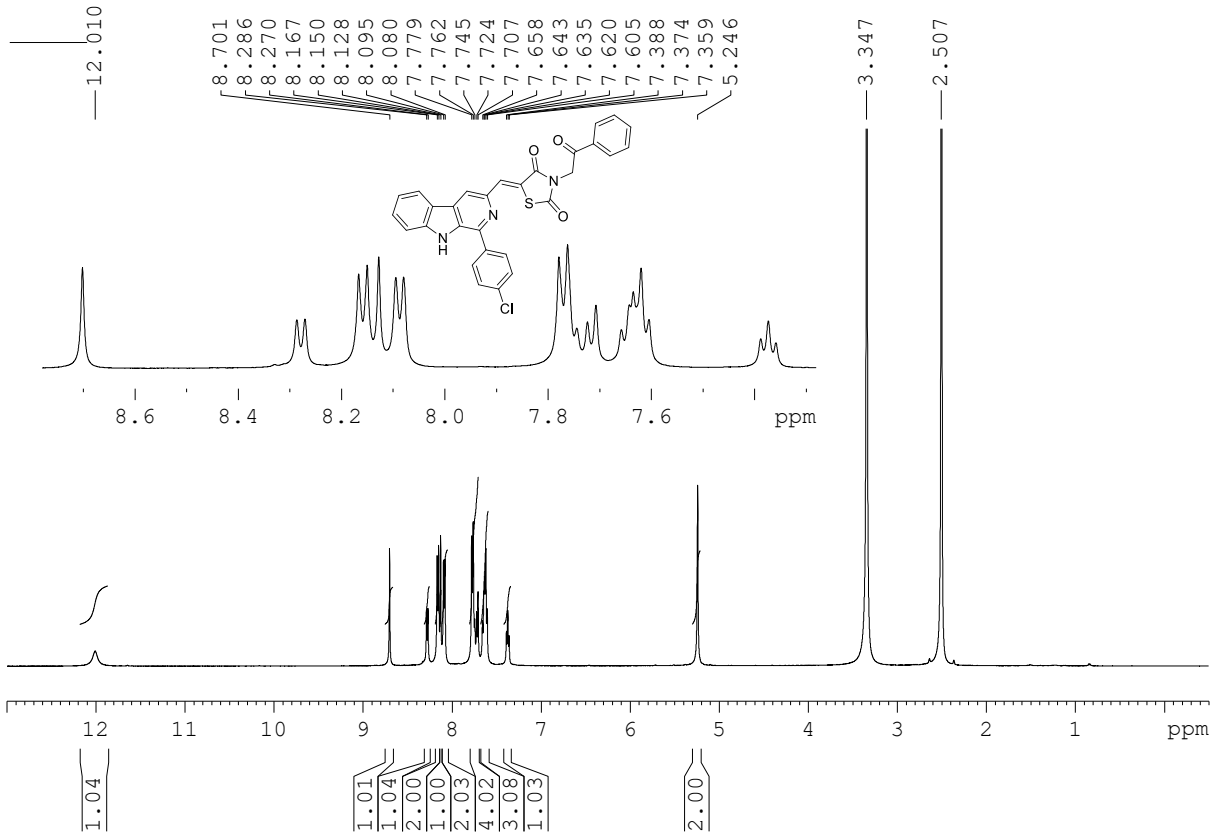
Compound **19r**: ^{13}C NMR (125 MHz, DMSO-d_6)



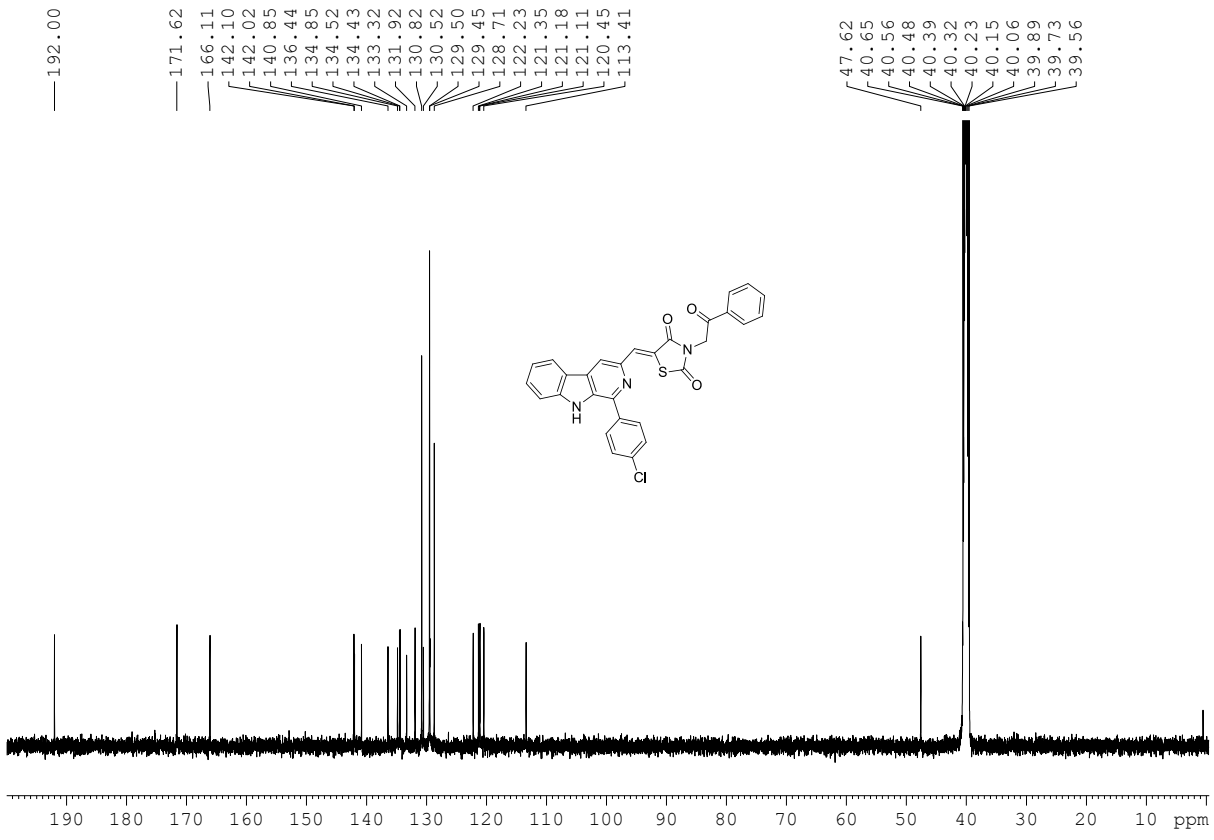
Compound **19s**: ^{1}H NMR (500 MHz, DMSO-d_6)



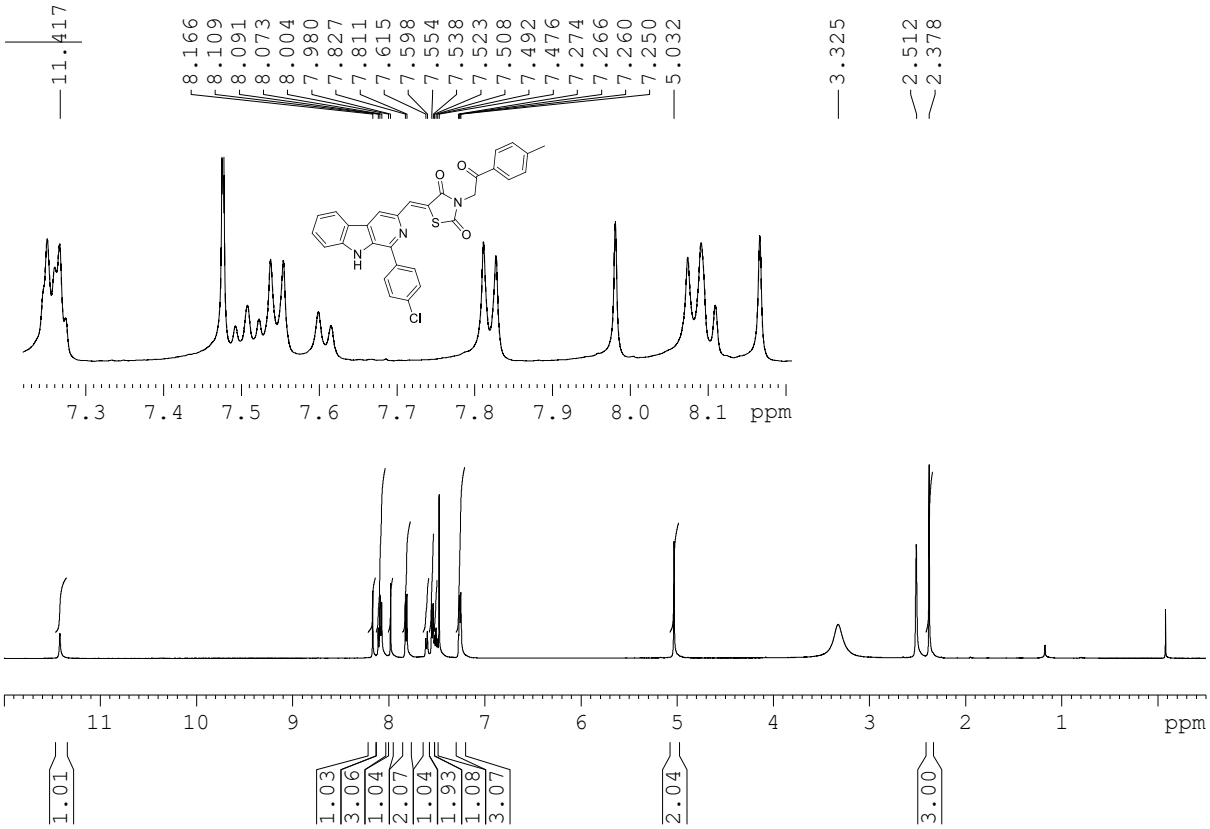
Compound **19s**: ^{13}C NMR (125 MHz, DMSO-d_6)



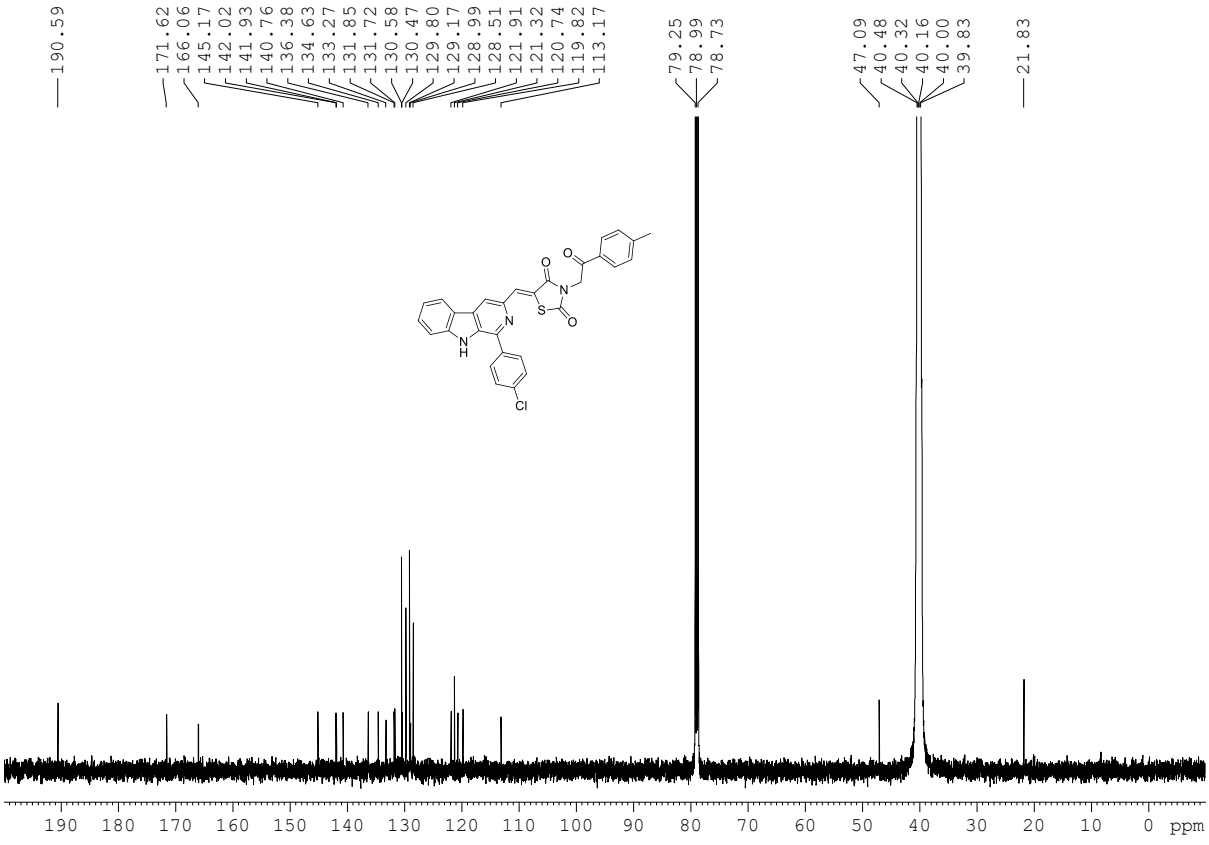
Compound **19t**: ^{1}H NMR (500 MHz, DMSO-d_6)



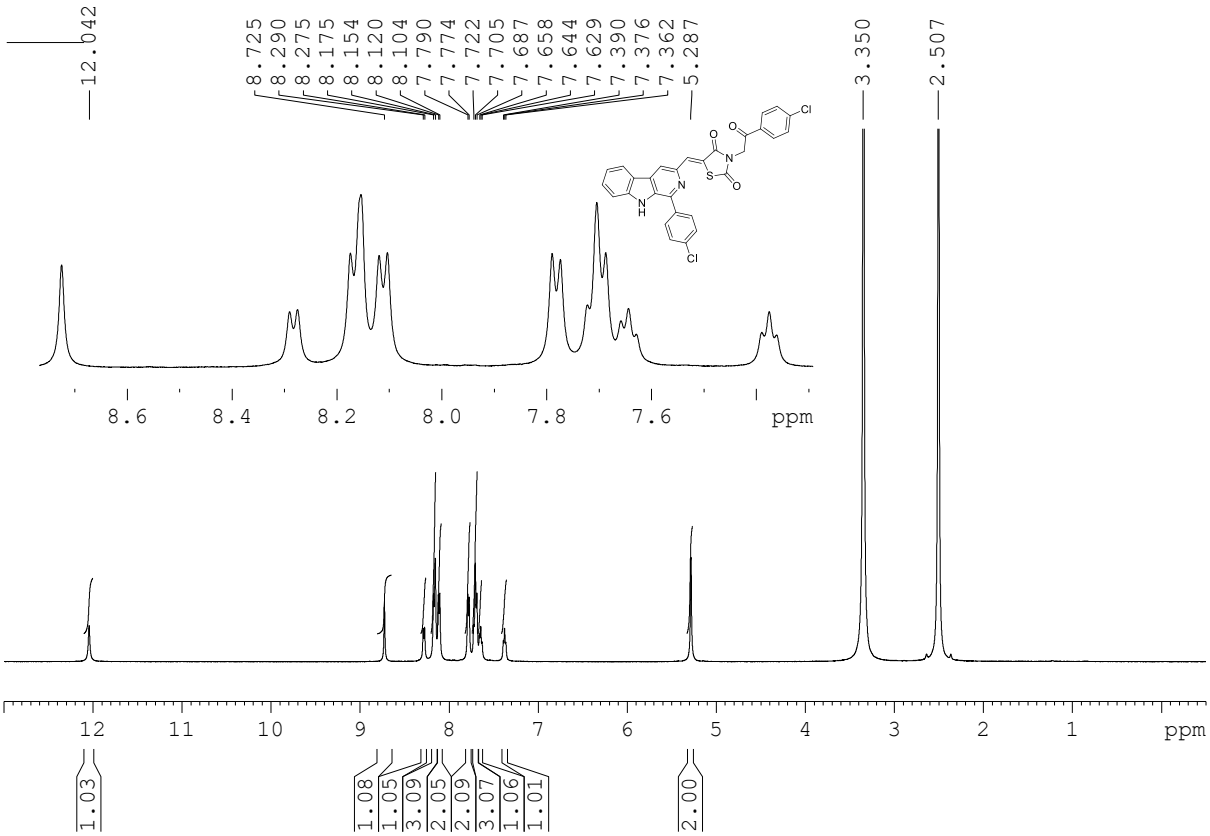
Compound **19t**: ^{13}C NMR (125 MHz, DMSO-d_6)



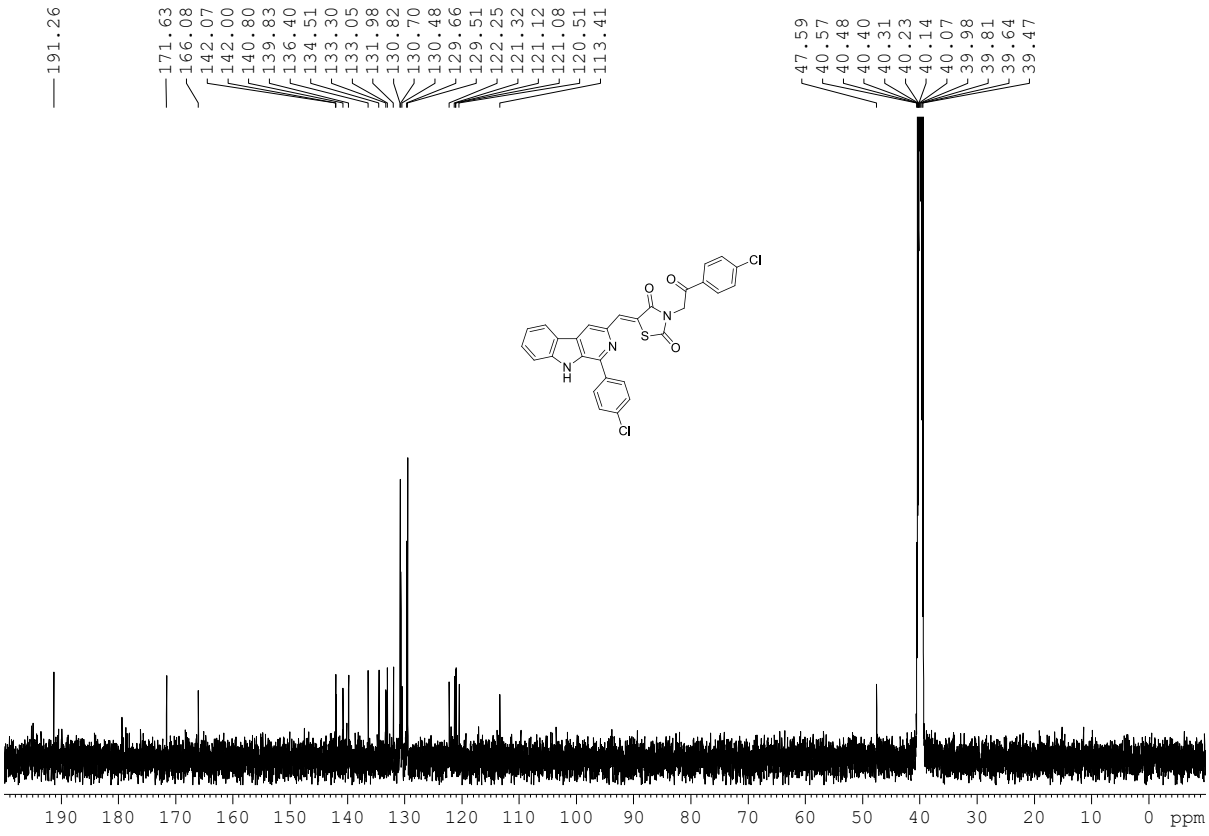
Compound **19u**: ^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6)



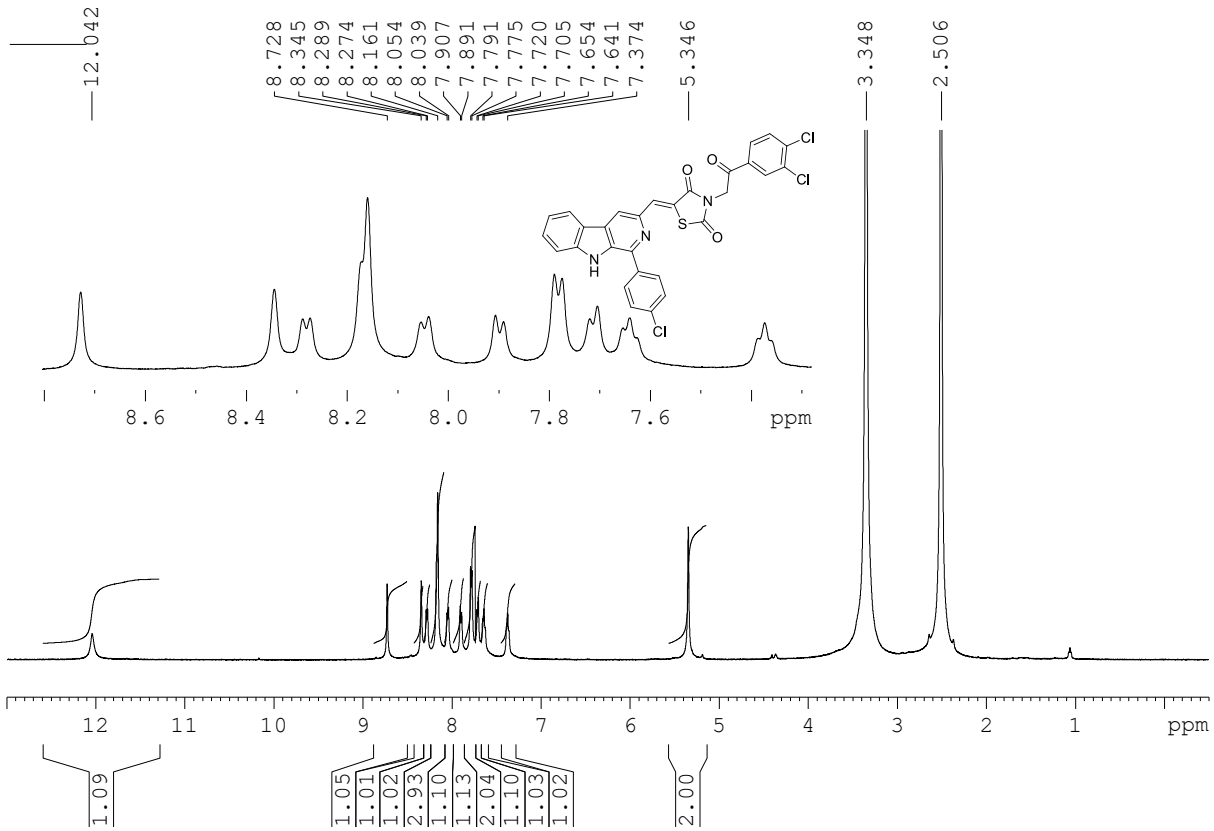
Compound **19u**: ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6)



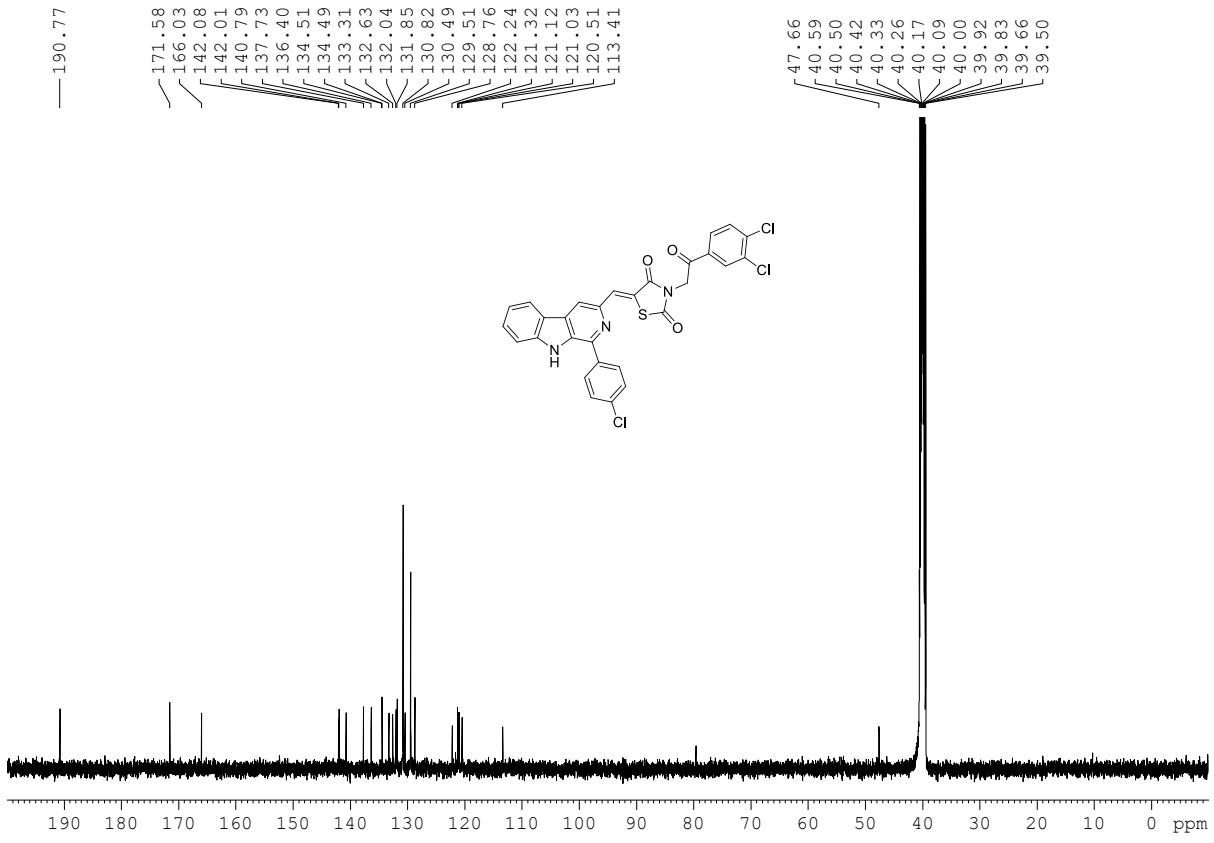
Compound **19v**: ^{1}H NMR (500 MHz, DMSO-d_6)



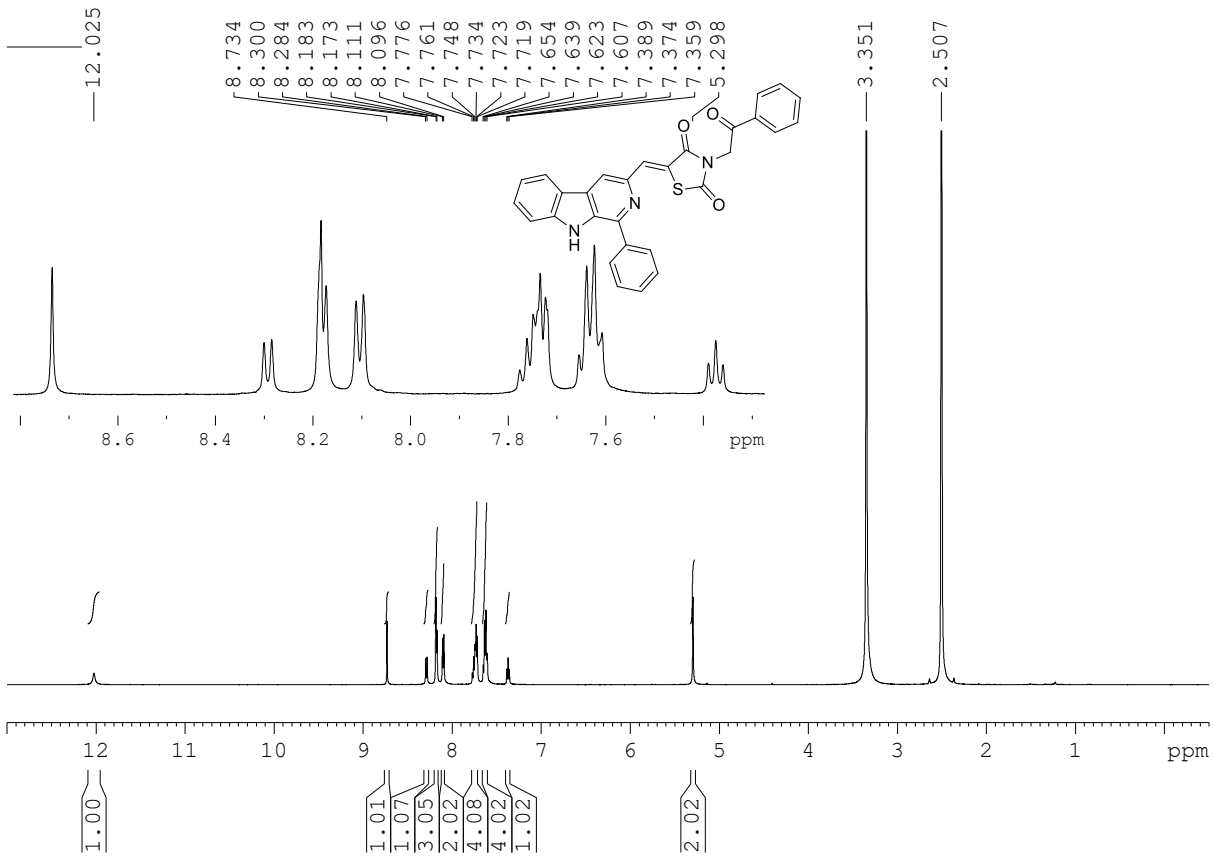
Compound **19v**: ^{13}C NMR (125 MHz, DMSO-d_6)



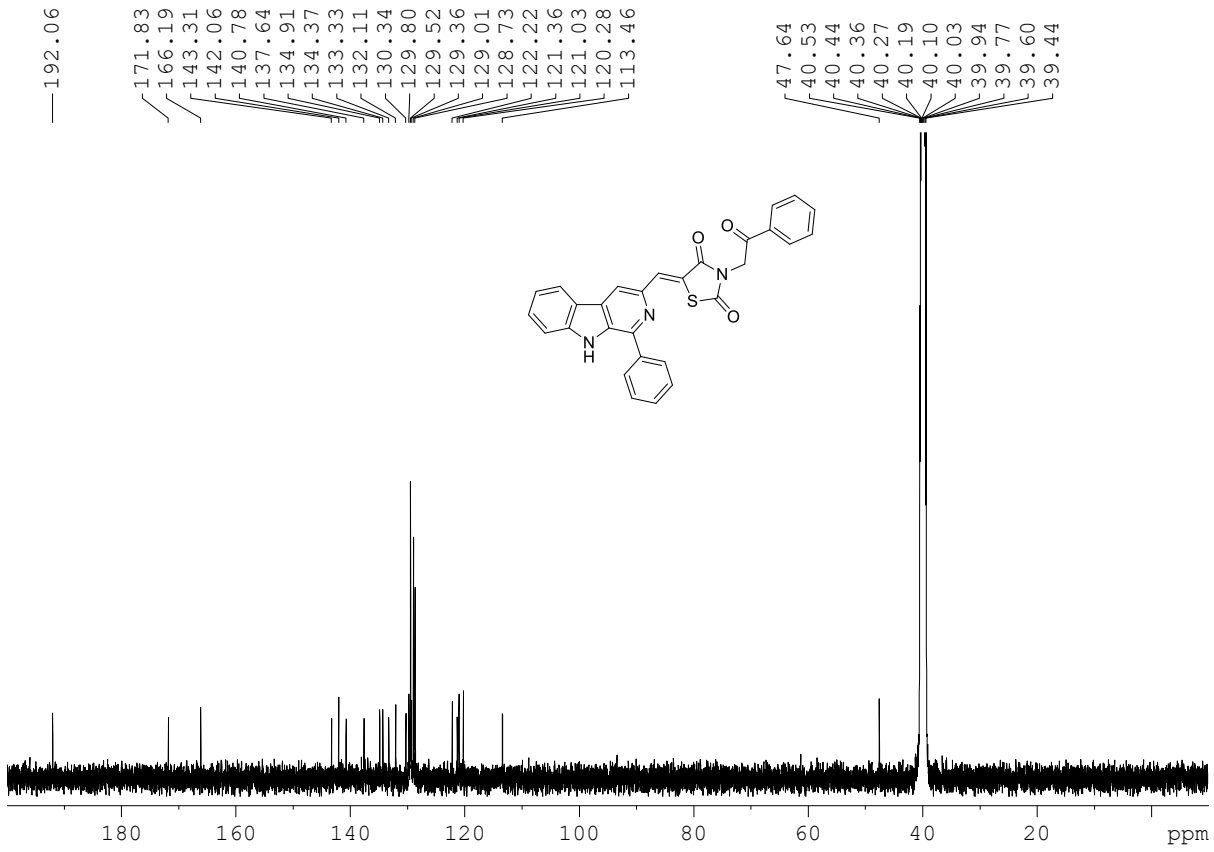
Compound **19w**: ^{1}H NMR (500 MHz, DMSO-d_6)



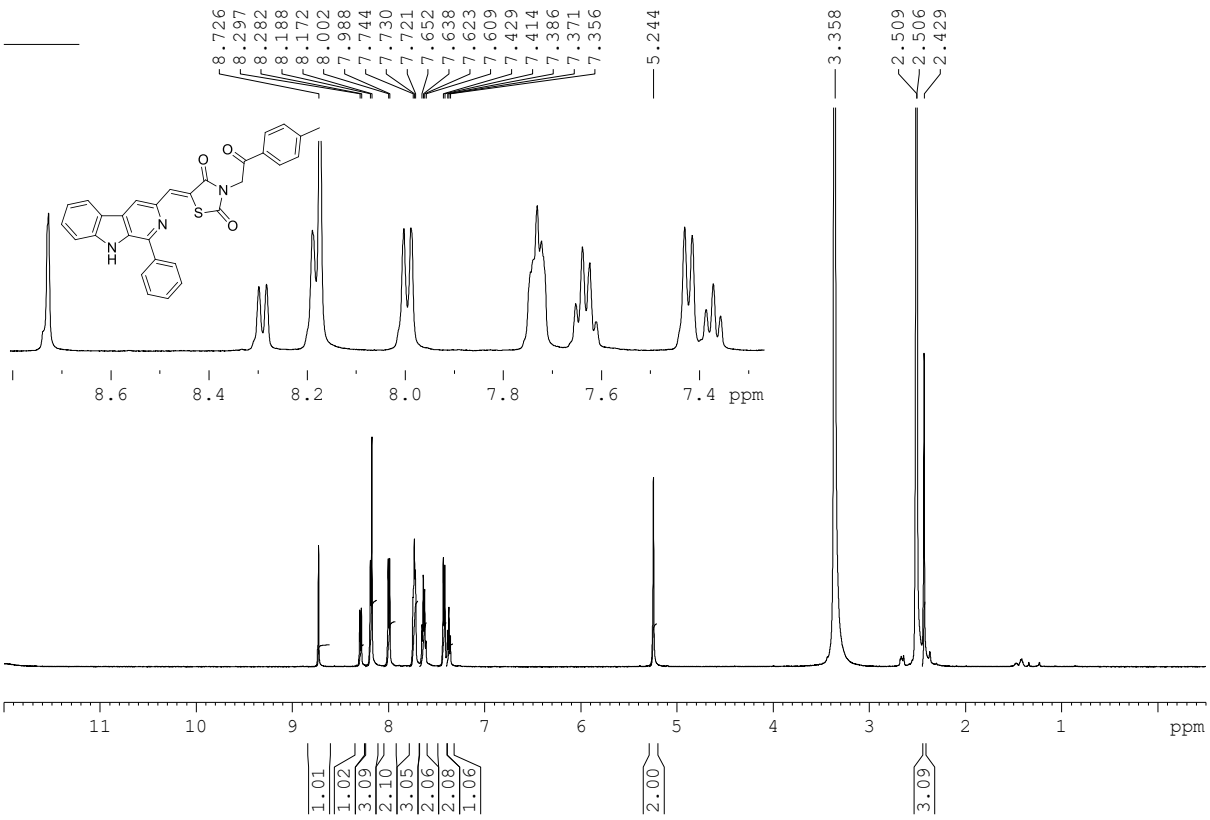
Compound **19w**: ^{13}C NMR (125 MHz, DMSO-d_6)



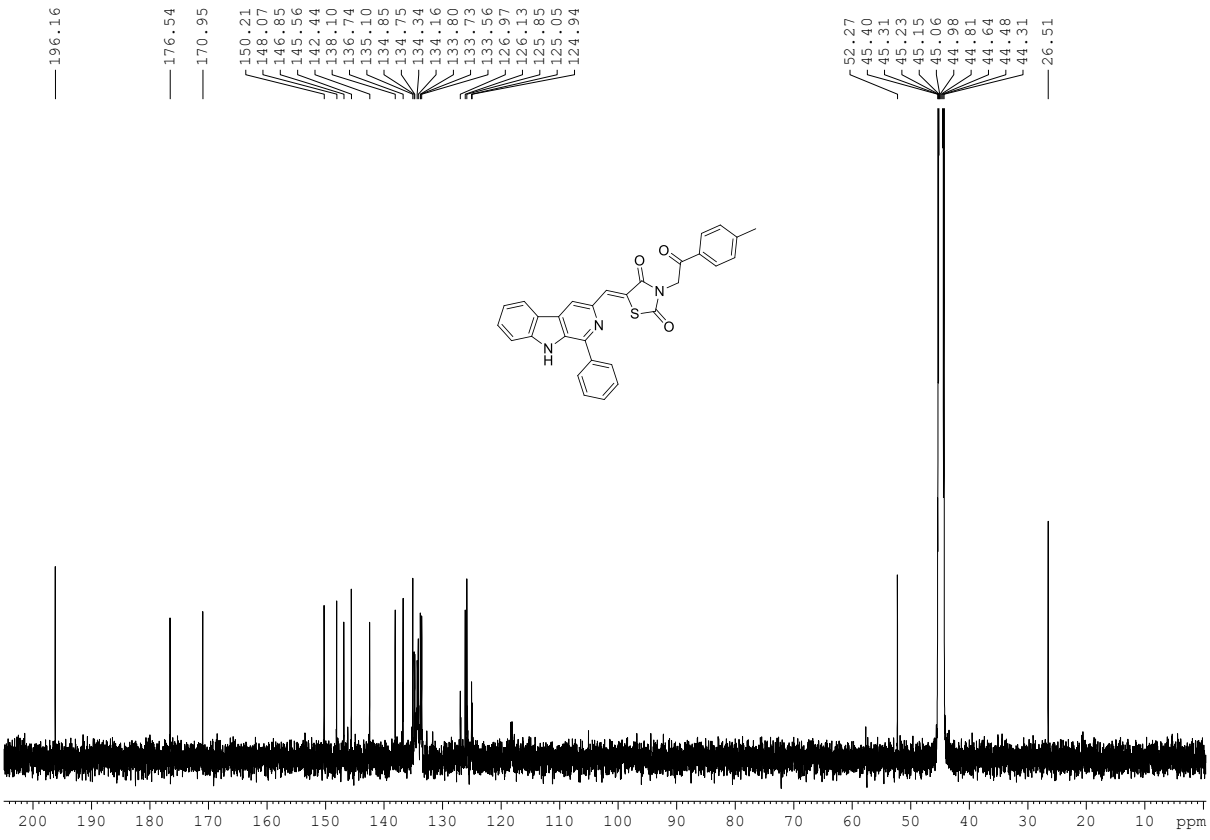
Compound **19x**: ^{1}H NMR (500 MHz, DMSO-d_6)



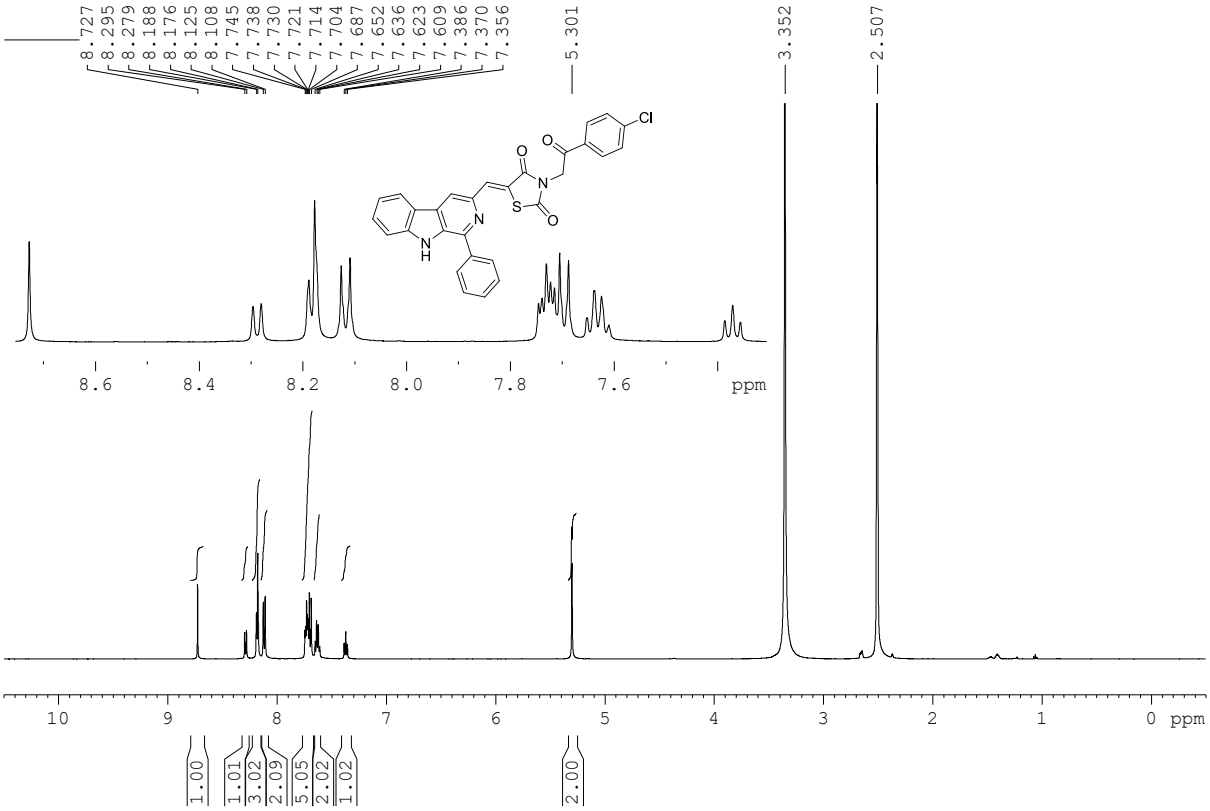
Compound **19x**: ^{13}C NMR (125 MHz, DMSO-d_6)



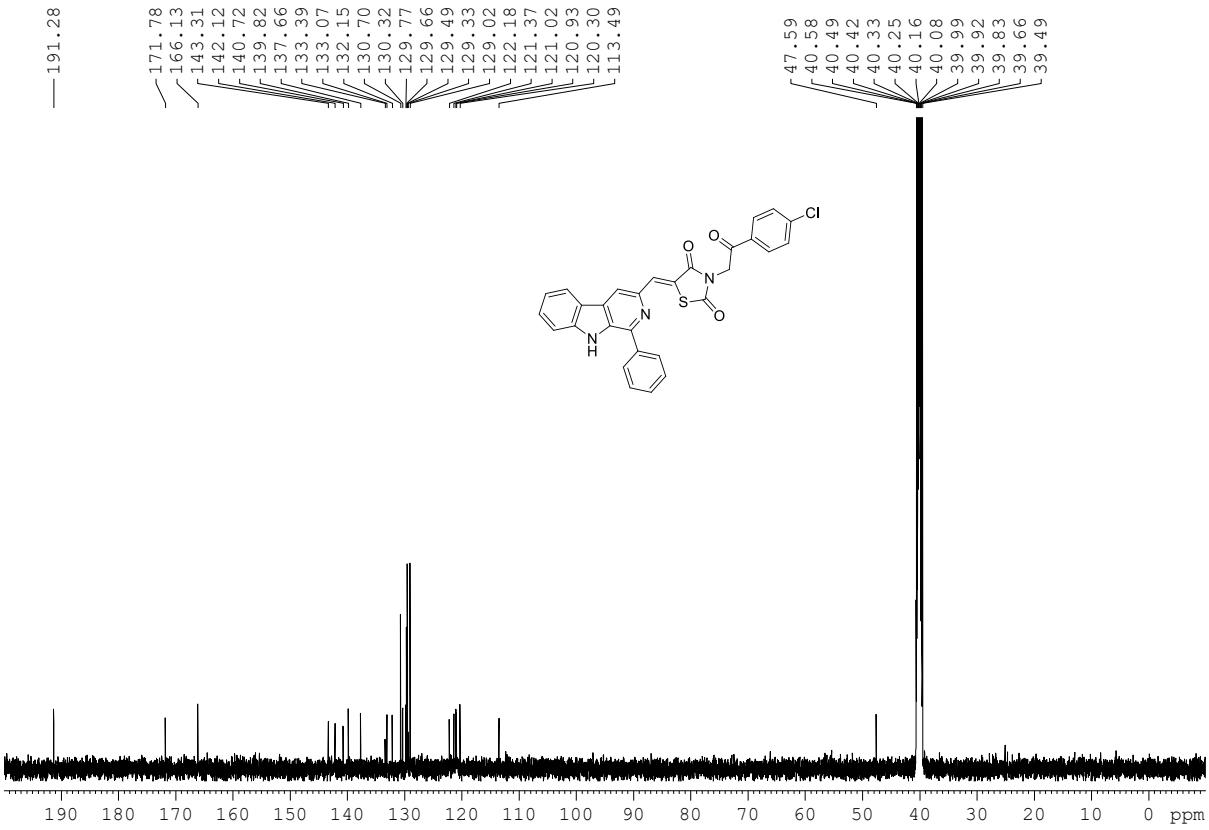
Compound **19y**: ^{1}H NMR (500 MHz, DMSO-d_6)



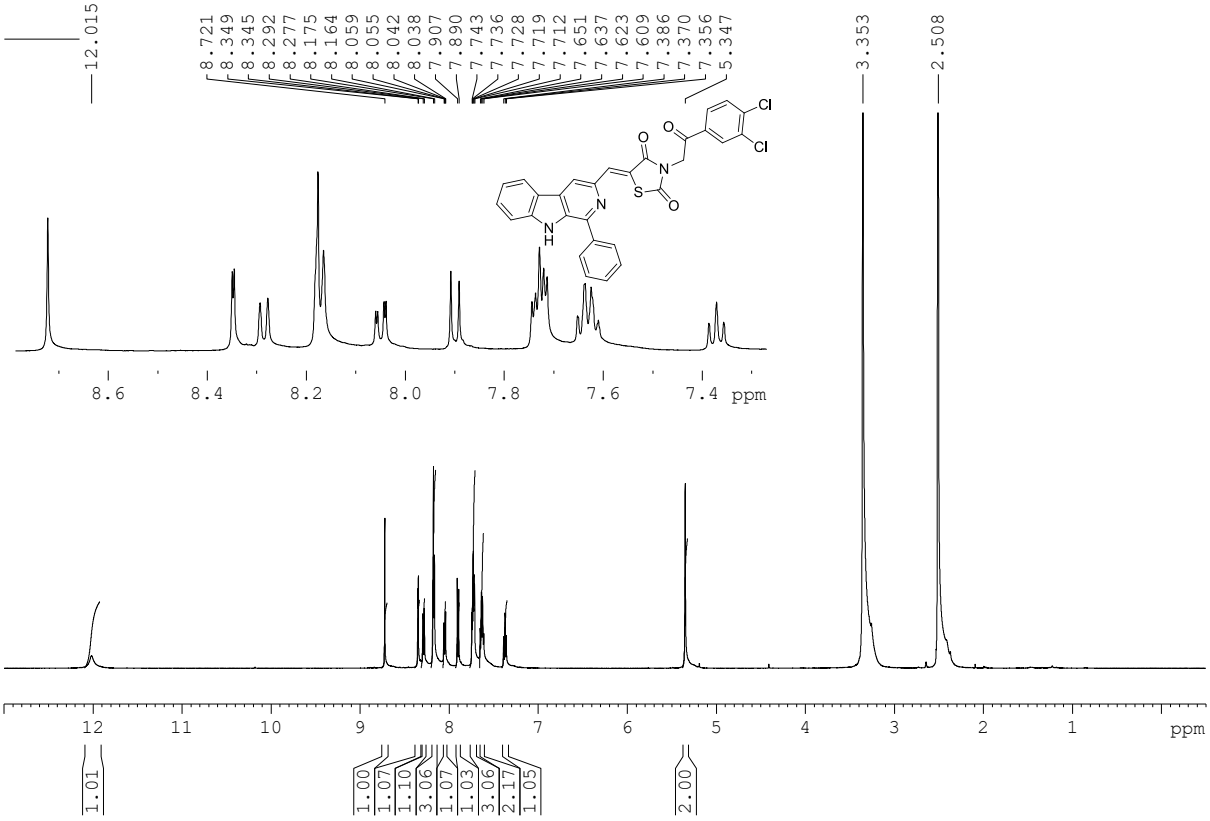
Compound **19y**: ^{13}C NMR (125 MHz, DMSO-d_6)



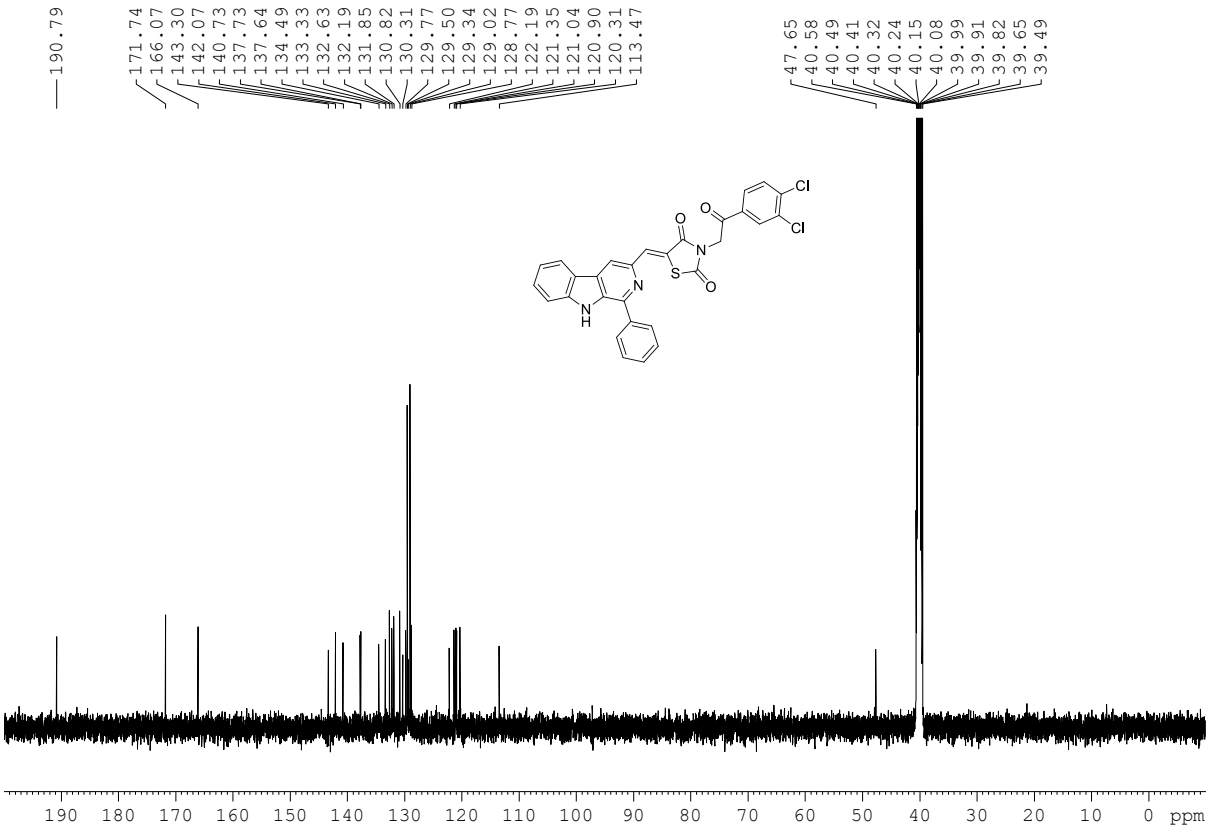
Compound **19z**: ^{1}H NMR (500 MHz, DMSO-d_6)



Compound **19z**: ^{13}C NMR (125 MHz, DMSO-d_6)



Compound **19aa**: ^{1}H NMR (500 MHz, DMSO-d_6)



Compound **19aa**: ^{13}C NMR (125 MHz, DMSO-d_6)

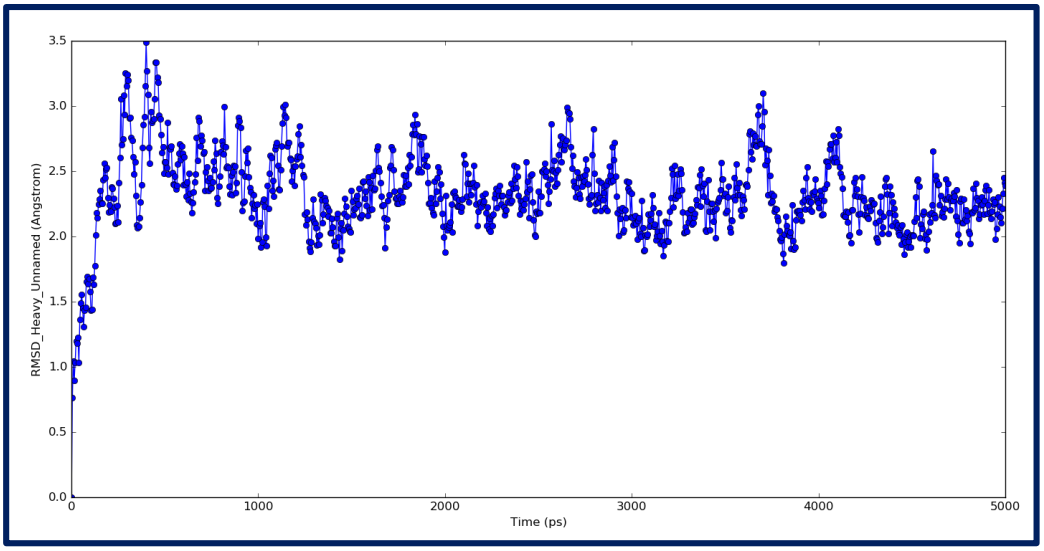


Figure 1. Plot of Root-mean-square-deviation (RMSD) of atoms of the backbone (Å) versus time; Molecular dynamics simulations for **19e**.