SUPPLEMENTARY INFORMATION

Synthesis and biological evaluation of new spiro oxindoles with embedded pharmacophores

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Experimental Section

General

All the reagent grade chemicals used for the synthesis were purchased from commercial sources and are used without further purification. Melting points were determined on Boetius micro heating table and Mettler-FP5 melting point apparatus and are uncorrected. IR spectra were obtained by using Shimadzu FTIR-8201 spectrophotometer (Shimadzu, Japan) as KBr pellets. 1D and 2D NMR experiments were recorded in DMSO-d_6/$CDCl_3$ on a Varian MR-400 FT-NMR spectrometer (Varian, Palo Alto, CA, USA), Bruker Avance III spectrometer at 300 MHz and 400MHz and TMS is used as an internal standard. The chemical shifts are quoted in parts per million (ppm). ESI-MS spectral studies were performed by ESI FT-MS on a Bruker APEX II mass spectrometer (Bruker, Bremen, Germany). Activated silica gel (60-120 mesh), Merck, Darmstadt, Germany was used for column chromatography to purify the crude products. X-ray diffraction data were collected from APEX2, Program for Data Collection and Integration on Area Detectors, BRUKER AXS Inc., 5465 East Cheryl Parkway, Madison, WI 53711-5373, USA. Micro analyses were carried out on a Vario EL III model CHNS analyser (Vario, Germany).

Abbreviations

s- singlet

d- doublet

dd- doublet of doublet

t- triplet

q- quartet

m- multiplet

bs- broad singlet

td - triplet of doublet

Experimental Procedure

General procedure for synthesis of *(E)*-3-((2-methoxyquinolin-3-yl)methylene) indolin-2-one 3a-d:

A mixture of indolin-2-one **1** (1 mmol) and substituted 2-methoxy quinoline-3-carbaldehyde **2** (1 mmol) were dissolved in 20 mL of ethanol, which was heated for 5 mins in a boiling water bath followed by the addition of 5-8 drops of pyrrolidine base slowly and stirred for 6 hrs at room temperature. The reaction was monitored by using TLC. The obtained solid from the reaction mixture was filtered, dried. This resulting residue was further purified by recrystallisation from the mixture of solvents chloroform/methanol (1:1) to afford *(E)*-3-((2-methoxyquinolin-3-yl)methylene) indolin-2-one as the pure products **3a-d** in good yields (70-85 %). Spectral data of all the compounds are given below.

***(E)*-3-((2-methoxyquinolin-3-yl)methylene)indolin-2-one (3a)**

Yellow coloured powder, yield (79 %); M.pt: 252-256 ˚C; IR(KBr) υ_{max}: 3187, 3077, 1716, 1613 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 4.02 (s, 3H, -OCH_3), 6.81 (t, 1H, *J*= 7.6 Hz, ArH), 6.87 (d, 1H, *J*= 7.6 Hz, ArH), 7.22 (t, 1H, *J*= 7.6, Hz, ArH), 7.31 (d, 1H, *J*= 7.6, Hz, ArH), 7.48 (t, 1H, *J*= 6.8 and 7.6 Hz, ArH), 7.57 (s, 1H, -CH=C<), 7.73 (t, 1H, *J*= 7.2 Hz, ArH), 7.82 (d, 1H, *J*= 8.4 Hz, ArH), 7.95 (d, 1H, *J*= 8.4 Hz, ArH), 8.61 (s, 1H, quin-C-4-H), 10.70 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 54.22, 110.69, 119.50, 121.70, 121.80, 122.88, 124.67, 125.24, 127.04, 128.80, 129.90, 129.95, 130.90, 131.32, 139.09, 143.80, 146.80, 159.80, 168.50. Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27; O, 10.58 %. Found C, 75.38; H, 4.59; N, 9.35; O, 10.66 %.

***(E)*-3-((2, 6-dimethoxyquinolin-3-yl)methylene)indolin-2-one (3b)**

Orange coloured powder, yield (85 %); M.pt:182-190 ˚C; IR(KBr) υ_{max}: 3179, 3071, 3020, 2938, 1700, 1605 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6):δ 3.88 (s, 3H, -OCH_3), 4.08 (s, 3H, -OCH_3), 6.88 (d, 1H, *J*= 7.2 Hz, ArH), 7.08 (t, 1H, *J*= 7.5 Hz, ArH), 7.36 -7.95 (m, 6H, -CH=C< and ArH), 8.72 (s, 1H, quin-C-4-H), 10.52 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.10, 55.87, 107.20, 110.35, 121.098, 121.95, 122.53, 124.37, 126.24, 126.75, 128.30, 129.86, 134.85, 136.63, 138.01, 141.62, 142.86, 156.42, 158.34, 169.20. Mass (ESI) *m/z* 333.20 $[M+H]^+$. Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.28; H, 4.85; N, 8.43; O, 14.44 %. Found C, 72.39; H, 4.96; N, 8.34; O, 14.51 %.

***(E)*-3-((2-methoxy-8-methylquinolin-3-yl)methylene)indolin-2-one (3c)**

Red coloured powder, yield (76 %); M.pt:182-190 ˚C; IR(KBr) υ_{max}: 3419, 3152, 3077, 3028, 1712, 1612 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.74 (s, 3H,-CH_3), 4.14 (s, 3H, -OCH_3), 6.85 (t, 1H, *J*= 7.6 and 8.0 Hz, ArH), 6.94 (d, 1H, *J*= 8 Hz, ArH), 7.25 (dd, 2H, *J*= 8.4, 7.6, Hz, ArH), 7.35 (t, 1H, *J*= 7.2 and 7.6 Hz, ArH), 7.52 (d, 1H, *J*= 7.6 Hz, ArH), 7.60 (q, 1H, *J*= 4.8, 7.2 and 8 Hz ArH) 7.94 (s, 1H, -CH=C<), 8.40 (s, 1H, quin-C-4-H), 8.64 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.62, 53.64, 110.33, 119.28, 121.88, 121.90, 122.79, 124.17, 124.28, 125.61, 128.70, 130.86, 132.06, 135.46, 138.54, 141.80, 145.90, 159.90, 170.00. Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86; O, 10.11 %. Found C, 75.99; H, 5.18; N, 8.79; O, 10.02 %.

***(E)*-3-((2-methoxy-6-methylquinolin-3-yl)methylene)indolin-2-one (3d)**

Red coloured powder, yield (70 %); M.pt:182-190 ˚C; IR(KBr) υ_{max}: 3220, 3144, 3064, 3014,1697,1606 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.46 (s, 3H,-CH_3), 4.03 (s, 3H, -OCH_3), 6.83 (d, 1H, *J*= 7.8 Hz, ArH), 7.25 (t, 3H, *J*= 7.2 and 7.5 Hz, ArH), 7.35 (d, 2H, *J*= 7.8 Hz, ArH), 7.59 (s, 1H, -CH=C<), 7.75 (d, 1H, *J*= 8.4 Hz, ArH), 8.54 (s, 1H, quin-C-4-H), 10.67 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (**75** MHz, DMSO- d_6): δ 21.31, 54.15, 110.71, 119.55, 121.83, 122.86, 124.62, 124.41, 126.89, 127.67, 130.15, 130.89, 133.28, 134.50, 138.52, 143.54, 145.01, 159.37, 168.72, 121.24. Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86; O, 10.11 %. Found C, 75.84; H, 5.21; N, 8.73; O, 10.19 %.

General procedure for the synthesis of dispiro pyrrolidine mono- and bis oxindole derivatives 6a-d, 8a-d and 10a-c:

A mixture of isatin/acenaphthoquinone/ninhydrin (**5, 7, 9)** (1 mmol), sarcosine **4** (1.5 mmol) and *(E)*-3-((2-methoxyquinolin-3-yl)methylene) indolin-2-one **3a-d** (0.5 mmol) was dissolved and refluxed in methanol for 8 hrs. Completion of reaction course was monitored by precoated TLC. The desired product **6a-d, 8a-d and 10a-c** were obtained from the reaction mixture was cooled to room temperature followed by filtration, dried and recrystallized from the mixture of solvents chloroform/methanol (4:1) in good yields (70-86 %). For exceptional cases, the target compound was obtained by silica gel column chromatography. Spectral data of all the compounds are given below.

1-Methyl-2, 3-bis (spiro-3'-indolino)- 4-(2-methoxy-quinolin-3-yl)-pyrrolidine (6a)

Yellowish white, yield (74 %); M.pt:220-224 ˚C; IR(KBr) υ_{max}: 3181, 3078, 1706, 1617 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.17 (s, 3H, N-CH_3), 3.28 (s, 3H, -OCH_3), 3.54 (t, 1H, *J*= 7.2 and 7.6 Hz, Pyrr-N-CH_2-H), 4.35 (t, 1H, *J*= 1.2 and 8.8 Hz, Pyrr-N-CH_2-H), 4.62 (t, 1H, *J*= 8.8 and 9.6 Hz, -CH), 6.25 - 6.19 (m, 2H, ArH), 6.53 (q, 2H, *J*= 2.4 and 5.2 Hz, ArH), 6.86 (td, 1H, *J*= 1.2, 2.00, 2.4, 5.6 and 6.00 Hz, ArH), 6.95 (t, 1H, *J*= 7.6Hz, ArH), 7.14 (t, 1H, *J*= 7.2 and 7.6 Hz, ArH), 7.45-7.41 (m, 2H, ArH), 7.58(m, 2H, ArH), 8.00 (d, 1H, *J*= 8.00 Hz, ArH), 8.41(s, 1H, ArH), 10.07(s, 1H, oxin-NH), 10.13(s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 35.22, 42.96, 52.66, 54.24, 62.85, 78.19, 108.64, 109.43, 120.14, 122.05, 124.17, 124.45, 125.21, 125.54, 126.25, 126.63, 126.81, 128.20, 128.33, 129.42, 129.93, 135.63, 143.59, 143.67, 144.90, 160.59, 176.79, 180.43. Anal. Calcd for $C_{29}H_{24}N_4O_3$: C, 73.09; H, 5.08; N, 11.76; O, 10.07%. Found C, 73.20; H, 5.16; N, 11.67; O, 10.15%.

1-Methyl-2, 3-bis (spiro-3'-indolino)- 4-(2, 6-dimethoxy-quinolin-3-yl)-pyrrolidine (6b)

Pale yellow solid, yield (80 %); M.pt: 204-210 ˚C; IR(KBr) υ_{max}: 3402, 3211, 1709, 1615 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.09 (s, 3H, N-CH_3), 3.17 (s, 3H, -OCH_3), 3.80 (t, 1H, *J*= 10.4 Hz, pyrr-N-CH_2-H), 3.83 (s, 3H, -OCH_3), 4.26 (t, 1H, *J*= 9.6 Hz, pyrr-N-CH_2-H), 4.50 (t, 1H, *J*= 9.2 Hz, -CH), 6.17 (m, 2H, -ArH), 6.49 (dd, 2H, *J*= 3.2 and 4.4 Hz, -ArH), 6.81 (td, 1H, *J*= 2.4 and 6.4 Hz, ArH), 6.90 (td, 1H, *J*= 7.6 Hz, ArH), 7.10 (td, 1H, *J*= 1.2 and 6.8 Hz, ArH), 7.17 (dd, 1H, *J*= 2.8 and 6.4 Hz, ArH), 7.38 (m, 2H, ArH), 7.46 (d, 1H, *J*= 8.8 Hz), 8.28 (s, 1H, quin-C-4-H), 10.02 (s, 1H, oxin-NH), 10.09 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 35.14, 42.95, 52.52, 54.11, 55.80, 62.84, 78.13, 107.29, 108.72, 109.51, 120.16, 120.70, 122.12, 124.15, 125.39, 125.83, 126.16, 126.56, 126.69, 127.88, 128.24, 129.97, 134.85, 140.03, 143.45, 143.51, 156.04, 159.19, 176.79, 180.58. Mass (ESI) *m/z* 507.19 $[M+H]^+$. Anal. Calcd for $C_{30}H_{26}N_4O_4$: C, 71.13; H, 5.17; N, 11.06; O, 12.63 %. Found C, 71.24; H, 5.25; N, 11.12; O, 12.55 %.

1-Methyl-2, 3-bis (spiro-3'-indolino)-4-(8-methyl 2-methoxy-quinolin-3-yl)-pyrrolidine (6c)

Yellow coloured powder, yield (70 %); M.pt:162-168 ˚C; IR(KBr) υ_{max}: 3405, 3211, 1709, 1615 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.02 (s, 3H, N-CH_3), 2.46 (s, 3H, -CH_3), 3.96 (s, 3H, -OCH_3), 3.50 (t, 1H, *J*= 7.6 and 8.8 Hz, Pyrr-N-CH_2-H), 4.32 (t, 1H, *J*= 8.0 and 9.6 Hz, Pyrr-N-CH_2-H), 4.58 (t, 1H, *J*= 9.2 and 9.6 Hz, -CH), 6.24–6.18 (m, 2H, ArH), 6.54-6.49 (dd, 2H, *J*= 3.2, 7.6 and 8.0 Hz, ArH), 6.80 (t, 1H, *J*= 7.6 Hz, ArH), 6.90 (t, 1H, *J*= 7.6 Hz, ArH), 7.10 (t, 1H, *J*= 7.6 Hz, ArH), 7.27 (t, 1H, *J*= 7.6 Hz, ArH), 7.33 (d, 1H, *J*= 7.6 Hz, ArH), 7.44 (dd, 1H, *J*= 6.8 and 13.2Hz, ArH), 7.69 (1H, d, *J*= 8 Hz, ArH), 8.06 (s, 1H, quin-C-4-H), 10.19 (s, 1H, oxin-NH), 10.26 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 17.36, 34.94, 48.78, 53.51, 58.90, 63.21, 78.10, 109.30, 109.68, 121.14, 121.58, 122.10, 124.41, 125.04, 125.25, 125.36, 125.94, 126.42, 128.68, 128.78, 129.89, 134.30, 134.99, 135.69, 143.08, 143.44, 143.86, 158.59, 177.14, 179.39. Anal. Calcd for $C_{30}H_{26}N_4O_3$: C, 73.45; H, 5.34; N, 11.42; O, 9.78 %. Found: C, 73.42; H, 5.23; N, 11.35; O, 9.84 %.

1-Methyl-2, 3-bis (spiro-3'-indolino)-4-(6-methyl 2-methoxy-quinolin-3-yl)-pyrrolidine (6d)

Orange coloured powder, yield (71 %); M.pt:180-184 ˚C; IR(KBr) υ_{max}: 3413, 3240, 1712, 1617 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.16 (s, 3H, N-CH_3), 2.45 (s, 3H, -CH_3), 3.26 (s, 3H, -OCH_3), 3.53 (t, 1H, *J*= 7.2 and 8.4 Hz, Pyrr-N-CH_2-H), 4.34 (t, 1H, *J*= 8.4 and 10 Hz, Pyrr-N-CH_2-H), 4.60 (t, 1H, *J*= 9.6 and 11.2Hz, -CH), 6.24-6.18 (m, 2H, ArH), 6.52-6.56 (dd, 2H, *J*= 2.0, 2.8, and 5.6 Hz, ArH), 6.85 (td, 1H, *J*= 0.8, 2, and 6 Hz, ArH), 6.95 (t, 1H, *J*= 7.6 and 8.0 Hz, ArH), 7.14 (t, 1H, *J*= 7.2 and 7.6Hz, ArH), 7.45-7.40 (m, 2H, ArH), 7.49 (d, 1H, *J*= 8.4Hz, ArH), 7.75 (s, 1H, ArH), 8.31(s, 1H, quin-C-4-H), 10.07 (s, 1H, oxin-NH), 10.12 (s, 1H, oxin-NH); ^{13}C NMR(100 MHz, DMSO-d_6): δ 21.39, 35.19, 42.78, 52.50, 58.72, 62.81, 78.17, 108.59, 109.45, 120.13, 122.01, 124.04, 125.13, 125.51, 126.21, 126.46, 126.70, 126.78, 127.33, 128.17, 129.90, 131.27, 133.52, 135.06, 143.18, 143.60, 143.70, 160.23, 176.76, 180.46. Anal. Calcd for $C_{30}H_{26}N_4O_3$: C, 73.45; H, 5.34; N, 11.42; O, 9.78 %. Found: C, 73.55; H, 5.43; N, 11.39; O, 9.86 %.

1-Methyl-2-(spiro-acenaphthene-1'-one)-3-(spiro-3'-indolino)-4-(2-methoxy-quinolin-3-yl)-pyrrolidine (8a)

Yellow coloured crystal, yield (81 %); M.pt:188-192 ˚C; IR(KBr) υ_{max}: 3410, 3153, 1710, 1616 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.10 (s, 3H, N-CH_3), 3.22 (s, 3H, -OCH_3), 3.65 (t, 1H, *J*= 8.8 Hz Pyrr-N-CH_2-H) 4.44 (t, 1H, *J*= 8.4 and 8.8 Hz, Pyrr-N-CH_2-H), 4.67 (t, 1H, *J*= 9.2 Hz, -CH), 5.93 (d, 1H, *J*= 7.6 Hz, ArH), 6.06 (t, 1H, *J*= 7.6 Hz, ArH), 6.27 (d, 1H, *J*= 7.6 Hz, ArH), 6.60 (t, 1H, *J*= 7.6 and 8 Hz, ArH), 7.45-7.40 (m, 2H, ArH), 7.55-7.49 (m, 3H, ArH), 7.70 (t, 1H, *J*= 7.2 and 8 Hz, ArH), 7.79 (d, 1H, *J*= 7.2 Hz, ArH), 7.89 (d, 1H, *J*= 8.4 Hz, ArH), 8.04 (d, 2H, *J*= 8 Hz, ArH), 8.50 (s, 1H, quin-C-4-H), 10.02 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.04, 43.33, 49.02, 52.60, 52.60, 55.04, 63.78, 80.90, 119.80, 120.16, 123.21, 124.02, 124.49, 125.23, 125.45, 125.83, 126.60, 127.98, 128.81, 128.45, 129.22, 129.49, 130.09, 131.50, 132.02, 135.40, 135.58, 142.45, 143.48, 144.87, 160.49, 180.52, 206.52. Mass (ESI) *m/z* 512.20 $[M+H]^+$. Anal. Calcd for $C_{33}H_{25}N_3O_3$: C, 77.48; H, 4.93; N, 8.21; O, 9.38 %. Found C, 77.57; H, 4.81; N, 8.32; O, 9.46 %.

1-Methyl-2-(spiro-acenaphthene-1'-one)-3-(spiro-3'-indolino)-4-(2, 6-dimethoxy-quinolin-3-yl)-pyrrolidine (8b)

Yellow coloured powder, yield (76 %); M.pt:186-190 ˚C; IR(KBr) υ_{max}: 3427, 3300, 1706, 1619 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.15 (s, 3H, N-CH_3), 3.22 (s, 3H, -OCH_3), 3.70 (t, 1H, *J*= 8 Hz, Pyrr-N-CH_2-H), 3.93 (s, 3H, -OCH_3), 4.47 (t, 1H, *J*= 8.4 and 8.8 Hz, Pyrr-N-CH_2-H), 4.70 (t, 1H, *J*= 8.8 and 9.6 Hz, -CH), 7.24 (dd, 1H, *J*= 2.72 Hz, ArH), 7.51 (m, 3H, ArH), 7.59 (m, 2H, ArH), 7.75 (t, 1H, *J*= 7.6 and 8 Hz, ArH), 7.84 (d, 1H, *J*= 9.2 Hz, ArH), 7.90 (t, 1H, *J*= 8.0 and 7.6 Hz, ArH), 7.94 (d, 1H, *J*= 9.2 Hz, ArH), 8.10 (d, 1H, *J*= 8.4 Hz, ArH), 8.53-8.46 (m, 3H, ArH), 10.05 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.39, 43.36, 52.50, 63.97, 81.09, 108.76, 119.82, 120.18, 123.24, 124.13, 125.53, 125.85, 125.97,

127.27, 127.99, 128.50, 129.26, 130.15, 131.10, 131.58, 132.05, 134.87, 135.54, 140.20, 142.54, 143.51, 156.15, 159.26, 166.65, 180.65, 206.71, 127. 92, 127.70, 134.75, Anal. Calcd for $C_{34}H_{27}N_3O_4$: C, 75.40; H, 5.02; N, 7.76; O, 11.82 %. Found C, 75.29; H, 5.15; N, 7.70; O, 11.76 %.

1-Methyl-2-(spiro-acenaphthene-1'-one)-3-(spiro-3'-indolino)-4-(8-methyl 2-methoxy-quinolin-3-yl)-pyrrolidine (8c)

Yellow coloured crystals, yield (86 %); M.pt:182-190 ˚C; IR(KBr) υ_{max}: 3403, 3258, 1715, 1617 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.14 (s, 3H, N-CH_3), 2.45 (s, 3H, CH_3), 3.70 (t, 1H, *J*= 8.4 Hz, pyrr-N-CH_2-H), 4.49 (t, 1H, *J*= 8.4 and 9 Hz, pyrr-N-CH_2-H), 4.72 (t, 1H, *J*= 9 and 9.3 Hz, -OH), 5.99(d, 1H, *J*= 7.8 Hz, ArH), 6.12 (t, 1H, *J*= 7.5 and 7.8 Hz, ArH), 6.31 (d, 1H, *J*= 7.5 Hz, ArH), 6.65 (m, 1H, ArH), 7.36 (t, 1H, *J*= 7.5 Hz, ArH), 7.58-7.44 (m, 3H, ArH), 7.75 (t, 1H, *J*= 7.2 and 8.1 Hz, ArH), 7.84 (d, 1H, *J*= 5.9 Hz, ArH), 7.93 (t, 2H, *J*= 8.4 and 9 Hz, ArH), 8.08 (d, 1H, *J*= 8.1 Hz, ArH), 8.50 (s, 1H, quin-C-4-H), 10.07 (s, 1H, oxin-NH, D_2O exchangeable proton) 3.28 (s, 3H,-OCH_3); ^{13}C NMR(**75** MHz, DMSO-d_6): δ 17.58, 35.08, 43.26, 52.39, 55.19, 63.84, 80.99, 108.64, 119.85, 120.20, 123.25, 123.50, 124.13, 125.02, 125.55, 125.86,126.18, 127.30, 127.99, 128.49, 129.26, 129.70, 130.13, 131.55, 132.05, 134.07, 135.47, 135.93, 142.48, 143.51, 143.70, 159.67, 180.56, 206.61. Anal. Calcd for $C_{34}H_{27}N_3O_3$: C, 77.70; H, 5.18; N, 7.99; O, 9.13 %. Found C, 77.79; H, 5.09; N, 7.89; O, 9.07 %.

1-Methyl-2-(spiro-acenaphthene-1'-one)-3-(spiro-3'-indolino)-4-(6-methyl 2-methoxy-quinolin-3-yl)-pyrrolidine (8d)

Dark yellow coloured powder, yield (72 %); M.pt:140-146 ˚C; IR(KBr) υ_{max}: 3405, 3248, 1715, 1614 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.14 (s, 3H, N-CH_3), 2.40 (s, 3H, -CH_3), 4.70 (t, 1H, *J*= 9.0 Hz, -CH) 4.50 (t, 1H, *J*= 8.4 Hz, Pyrr-N-CH_2-H), 3.70 (t, 1H, *J*= 8 Hz, Pyrr-N-CH_2-H), 3.23 (s, 3H, -OCH_3), 5.95 (d, 1H, *J*= 7.6 Hz, ArH), 6.09 (t, 1H, *J*= 6.8, ArH), 6.30 (d, 1H, *J*= 7.6 Hz, ArH), 6.64 (td, 1H, *J*= 1.2 and 8.0 Hz, ArH), 7.42 (dd, 1H, *J*= 1.6 and 7.2 Hz, ArH), 7.49 (d, 1H, *J*= 7.6Hz, ArH), 7.59-7.53 (m, 2H, ArH), 7.74 (t, 1H, *J*= 8.4 Hz, ArH), 7.83 (d, 1H, *J*= 6.4 Hz, ArH), 7.93 (t, 2H, *J*= 8.0 Hz, ArH), 8.08 (d, 1H, *J*= 8.0 Hz, ArH), 8.44 (s, 1H, quin-C-4-H), 10.07 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.79, 34.56, 42.80, 52.04, 54.53, 63.30, 80.40, 108.14, 119.29, 119.66, 122.71, 123.40, 124.64, 124.96, 125.34, 125.92, 126.03, 126.85, 127.97, 127.47, 128.74, 129.61, 130.88, 131.02, 131.53, 133.10, 134.54, 134.93, 141.96, 142.68, 142.98, 159.54, 180.05, 206.00. Anal. Calcd for $C_{34}H_{27}N_3O_3$: C, 77.70; H, 5.18; N, 7.99; O, 9.13 %. Found C, 77.80; H, 5.10; N, 7.90; O, 9.23 %.

1-Methyl-2-(spiro-indan-1', 3'-dione)-3-(spiro-3'-indolino)-4-(2-methoxy-quinolin-3-yl)-pyrrolidine (10a)

Yellow coloured crystals, yield (78 %); M.pt:164-168 ˚C; IR(KBr) υ_{max}: 3422, 3205, 3076, 1709, 1619 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.38 (s, 3H, N-CH_3), 3.66 (s, 3H, -OCH_3), 3.81 (t, 1H, *J*= 9.3 Hz, pyrr-N-CH_2-H), 4.21 (t, 1H, *J*= 8.7, 8.7 Hz, pyrr-N-CH_2-H), 4.84 (t, 1H, *J*= 8.7 and

9 Hz, CH) 6.38 (d, 1H, *J*= 7.8 Hz, ArH), 6.71 (t, 1H, *J*= 7.5 and 9.5 Hz, ArH), 6.91-6.82 (m, 2H, ArH), 7.34-7.26 (m, 2H, ArH), 7.54-7.49 (m, 2H, ArH), 7.69 -7.61 (m, 2H, ArH), 7.83 -7.81 (m, 1H, ArH), 7.95-7.94 (m,1H, ArH), 8.04 (s, 1H, quin-C-4-H) 10.15 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (**75** MHz, DMSO-d_6): δ 35.69, 44.31, 52.99, 55.88, 65.33, 81.14, 109.11, 120.63, 121.81, 123.06, 124.44, 124.82, 125.26, 126.05, 126.42, 127.07, 128.05, 128.68, 128.84, 129.77, 136.43, 137.56, 140.82, 141.47, 141.88, 144.79, 160.35, 175.12, 196.52, 199.39. Anal. Calcd for $C_{30}H_{23}N_3O_4$: C, 73.61; H, 4.74; N, 8.58; O, 13.07 %. Found C, 73.50; H, 4.65; N, 8.64; O, 13.20 %.

1-Methyl-2-(spiro-indan-1', 3'-dione)-3-(spiro-3'-indolino)-4-(2, 6-dimethoxy-quinolin-3-yl)-pyrrolidine (10b)

Brown coloured powder, yield (73 %); M.pt: 146-152 ˚C; IR(KBr) υ_{max}: 3429, 3176, 3081, 1712, 1617 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, N-CH_3), 3.58 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH_3), 3.76 (t, 1H, *J*= 8.8 Hz, pyrr-N-CH_2-H), 4.16 (t, 1H, *J*= 8.8 Hz, pyrr-N-CH_2-H), 4.77 (t, 1H, *J*= 8.8 Hz, CH), 6.39 (d, 1H, *J*= 8.00 Hz, ArH), 6.67 (t, 1H, *J*= 7.6 Hz, ArH), 6.86 (t, 1H, *J*= 7.6 Hz, ArH), 7.18 (dd, 3H, *J*= 2.8 and 4.8 Hz, ArH), 7.48 (m, 1H, ArH), 7.63 (d, 1H, *J*= 7.6 Hz, ArH), 7.83 (m, 1H, ArH), 7.94 (m, 2H, ArH), 8.02 (s, 1H, quin-C-4-H), 10.16 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.16, 43.82, 51.39, 52.21, 55.25, 64.76, 80.70, 108.53, 120.03, 120.42, 121.52, 122.34, 122.51, 125.02, 125.50, 127.24, 128.02, 128.11, 135.90, 136.10, 136.78, 139.78, 139.57, 140.31, 140.96, 141.42, 155.44, 158.45, 174.75, 196.12, 198.68. Anal. Calcd for $C_{31}H_{25}N_3O_5$: C, 71.66; H, 4.85; N, 8.09; O, 15.40 %. Found C, 71.60; H, 4.78; N, 8.20; O, 15.50 %.

1-Methyl-2-(spiro-indan-1', 3'-dione)-3-(spiro-3'-indolino)-4-(8-methyl 2-methoxy-quinolin-3-yl)-pyrrolidine (10c)

Brown coloured crystal, yield (84 %); M.pt:172-180 ˚C; IR(KBr) υ_{max}: 3431, 3183, 3085, 1713, 1715, 1619 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, N-CH_3), 2.48 (s, 3H, -CH_3), 3.64 (s, 3H, -OCH_3), 3.79 (t, 1H, *J*= 6.9 and 9.6 Hz, pyrr-N-CH_2-H), 4.18 (t, 1H, *J*= 8.4 and 8.8 Hz, pyrr-N-CH_2-H), 4.82 (t, 1H, *J*= 8.8, Hz, CH), 6.36 (t, 1H, *J*= 8.76 Hz, ArH), 6.68 (t, 1H, *J*= 7.6 Hz, ArH), 6.85 (t, 1H, *J*= 7.6 Hz, ArH), 7.32-7.24 (m, 2H, ArH), 7.55-7.48 (m, 2H, ArH), 7.62 (d, 1H, *J*= 7.6 Hz, ArH), 7.69 (d, 1H, *J*= 8 Hz, ArH), 7.84-7.78 (m, 1H, ArH), 7.93-7.91 (m, 1H, ArH), 8.02 (s, 1H, quin-C-4-H), 10.65 (s, 1H, oxin-NH, D_2O exchangeable proton); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.5, 35.66, 44.30, 52.97, 54.24, 55.85, 65.31, 81.13, 109.09, 120.62, 121.79, 122.88, 123.04, 124.44, 124.80, 126.03, 126.40, 128.08, 128.66, 129.76, 136.42, 137.34, 137.53, 140.81, 141.44, 141.85, 144.77, 160.34, 175.11, 196.51, 199.38. Anal. Calcd for $C_{31}H_{25}N_3O_4$: C, 73.94; H, 5.00; N, 8.34; O, 12.71 %. Found C, 73.89; H, 5.13; N, 8.25; O, 12.80 %.

General procedure for the synthesis of dispiro thiapyrrolizidine mono- and bis oxindole derivatives 12a-d and 13a-d:

A mixture of isatin/acenaphthoquinone/ (**5, 7)** (1 mmol), thiaproline **11** (0.75 mmol) and *(E)*-3-((2-methoxyquinolin-3-yl)methylene) indolin-2-one **3a-d** (0.5 mmol) was dissolved and refluxed in methanol for 8 hrs. Completion of reaction course was monitored by pre coated TLC. The desired product **12a-d and 13a-d** was obtained from the reaction mixture which was cooled to room temperature followed by filtration, dried and recrystalized from the mixture of solvents chloroform/methanol (4:1) in good yields (70-80 %). For exceptional cases, the target compound was obtained by silica gel column chromatography. Spectral data of all the compounds are given below.

7-(2-methoxy-quinolin-3-yl)-5, 6-bis(spiro-3', 3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (12a)

Brown coloured powder, yield (77 %); M.pt: 184-188 ˚C; IR(KBr) υ_{max}: 3401, 3214, 1711, 1617 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.80 (t, 1H, *J*= 9.2 and 8.4 Hz, S-CH_2-H), 3.15 (t, 1H, *J*= 9.2 Hz, S-CH_2-H), 3.31 (s, 3H, -OCH_3 merged with moisture peak), 3.49 (d, 1H, *J*= 5.6 Hz, Thiapyrr-N-CH_2-H), 3.71 (d, 1H, *J*= 4.8 Hz, Thiapyrr- N-CH_2-H), 4.03 (d, 1H, *J*= 9.6 Hz, CH), 5.62 (m, 1H, N-CH), 6.27 (t, 1H, *J*= 7.6 Hz, ArH), 6.38 (d, 1H, *J*= 7.2Hz, ArH), 6.56 (q, 2H, *J*= 7.6 and 3.2Hz, ArH), 6.88 (t, 1H, *J*= 6.8 and 8 Hz, ArH), 6.98 (t, 1H, *J*= 8.4 and 7.2 Hz, ArH), 7.19 (t, 1H, *J*= 7.2 and 7.6 Hz, ArH), 7.46 (quin, 1H, *J*= 4.4 and 3.2 Hz, ArH), 7.54 (d, 1H, *J*= 7.2 Hz, ArH), 7.59 (d, 2H, *J*= 4.0 Hz, ArH), 8.05 (d, 1H, J=8 Hz, ArH), 8.48 (s, 1H, quin-C-4- H), 10.20 (s, 1H, oxin-NH), 10.21 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 32.01, 45.39, 47.32, 52.59, 67.42, 68.45, 73.41, 108.70, 109.78, 120.27, 122.11, 123.33, 124.48, 124.61, 125.29, 125.83, 126.61, 127.04, 127.07, 128.43, 128.54, 129.61, 130.24, 135.47, 143.38, 143.17, 144.95, 160.40, 176.16, 180.09. Anal. Calcd for $C_{30}H_{24}N_4O_3S$: C, 69.21; H, 4.65; N, 10.76; O, 9.22; S, 6.16 %. Found: C, 69.10; H, 4.58; N, 10.67; O, 9.28; S, 6.24 %.

7-(2, 6-dimethoxy-quinolin-3-yl)-5, 6-bis(spiro-3', 3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (12b)

White coloured powder, yield (80 %); M.pt: 202-208 ˚C; IR(KBr) υ_{max}: 3402, 3196, 3085, 1709, 1615 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.76 (t, 1H, *J*= 9.2 and 8.8 Hz, S-CH_2-H), 3.26 (t, 1H, *J*= 5.2 and 6.4 Hz, S-CH_2-H), 3.28 (s, 3H, -OCH_3), 3.45 (d, 1H, *J*= 5.2 Hz, Thiapyrr-N-CH_2-H), 3.66 (d, 1H, *J*= 5.2 Hz, Thiapyrr-N-CH_2-H), 3.85 (s, 3H, -OCH_3), 4.00 (t, 1H, *J*= 7.2 Hz, -CH), 5.54 (m, 1H, N-CH), 6.24 (t, 1H, *J*= 7.6 and 7.2 Hz, ArH), 6.34 (d, 1H, *J*= 7.6 Hz, ArH), 6.52 (q, 2H, *J*= 7.6 and 4.4 Hz, ArH), 6.84 (t, 1H, *J*= 7.6 and 8.4 Hz, ArH), 6.94 (t, 1H, *J*= 7.6 and 7.2 Hz, ArH), 7.18 (m, 2H, ArH), 7.50 (m, 3H, ArH), 8.37 (s, 1H, quin-C-4-H), 10.15 (s, 1H, oxin-NH), 10.17 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 32.02, 45.35, 47.21, 52.34, 55.77, 67.41, 68.42, 73.31, 107.47, 108.65, 109.69, 120.22, 120.85, 122.08, 123.33, 124.62, 125.96, 125.86, 126.99, 127.03, 127.86, 128.46, 130.24, 134.68, 140.15, 143.13, 143.32, 156.04, 158.96,

176.10, 180.05. Mass (ESI) *m/z* 551.10 $[M+H]^+$. Anal. Calcd for $C_{31}H_{26}N_4O_4S$: C, 67.62; H, 4.76; N, 10.18; O, 11.62; S, 5.82 %. Found C, 67.70; H, 4.88; N, 10.30; O, 11.70; S, 5.79 %.

7-(8-methyl 2-methoxy-quinolin-3-yl)-5, 6-bis(spiro-3', 3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (12c)

Dark yellow coloured powder, yield (78 %); M.pt:176-184 ˚C; IR(KBr) υ_{max}: 3400, 3243, 1712, 1618 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.46 (s, 3H, -CH_3), 2.79 (t, 1H, *J*= 7.6 and 8.8 Hz, S-CH_2-H) , 3.29 (t, 1H, *J*= 9.21 Hz, s- CH_2-H), 3.35 (s, 3H, -OCH_3), 3.50 (d, 1H, *J*= 6.4 Hz, Thiapyrr- N-CH_2-H), 3.72 (d, 1H, *J*= 5.2 Hz, Thiapyrr-N-CH_2-H), 4.04 (d, 1H, *J* = 8.8 Hz, -CH), 5.62 (m, 1H, N-CH), 6.29 (t, 1H, *J*= 8.72 Hz, ArH) 6.41 (d, 1H, *J*= 7.2 Hz, ArH), 6.56 (q, 2H, *J*= 3.2 and 7.6 Hz, ArH), 6.88 (t, 1H, *J*= 7.6 Hz ArH), 6.98 (t, 1H, *J*= 8.48 Hz, ArH), 7.19 (t, 1H, *J*= 6.8 and 8.8 Hz, ArH), 7.34 (t, 1H, *J*= 7.6 Hz, ArH), 7.46 (d, 1H, *J*= 5.2 Hz, ArH), 7.55 (d, 1H, *J*= 7.2 Hz, ArH) 7.87 (d, 1H, *J*= 8.4 Hz, ArH), 8.44 (s, 1H, quin-C-4- H), 10.19 (s, 1H, oxin-NH), 10.21 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.69, 32.08, 45.50, 47.44, 52.31, 67.47, 68.45, 73.50, 108.70, 109.74, 120.32, 122.13, 122.81, 124.10, 124.67, 125.09, 125.93, 126.26, 127.06, 127.13, 128.51, 129. 80, 130.29, 134.18, 135.93, 143.18, 143.35, 143.75, 159.65, 176.18, 180.07. Anal. Calcd for $C_{31}H_{26}N_4O_3S$: C, 69.64; H, 4.90; N, 10.48; O, 8.98; S, 6.00%. Found C, 69.75; H, 4.99; N, 10.68; O, 8.99; S, 6.12 %.

7-(6-methyl 2-methoxy-quinolin-3-yl)-5,6-bis(spiro-3', 3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (12d)

Brown coloured powder, yield (71 %); M.pt: 194-200 ˚C; IR(KBr) υ_{max}: 3421, 3215, 1711, 1610 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.46 (s, 3H, -CH_3), 2.80 (t, 1H, *J*= 9.2 and 8.8 Hz, S-CH_2-H), 3.25 (t, 1H, *J*= 5.2 and 6.4 Hz, S-CH_2-H), 3.28 (s, 3H, -OCH_3), 3.46 (d, 1H, *J*= 5.2 Hz, Thiapyrr- N-CH_2-H), 3.68 (d, 1H, *J*= 5.2 Hz, Thiapyrr-N-CH_2-H), 4.00 (t, 1H, *J*= 8.8 Hz, -CH), 5.50 (m, 1H, N-CH), 6.25 (t, 1H, *J*= 7.6 and 7.2 Hz, ArH), 6.36 (d, 1H, *J*= 7.6 Hz, ArH), 6.56 (q, 2H, *J*= 7.6 and 4.4 Hz, ArH), 6.86 (t, 1H, *J*= 7.6 and 8.4 Hz, ArH), 6.96 (t, 1H, *J*= 7.6 and 7.2 Hz, ArH), 7.17 (t, 1H, *J*= 7.6 and 8.0 Hz, ArH), 7.53 (m, 3H, ArH), 7.79 (s, 1H, ArH), 8.36 (s, 1H, quin-C-4- H), 10.27 (s, 1H, oxin-NH), 10.29 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.13, 32.05, 45.90, 47.50, 51.98, 67.45, 68.50, 73.50, 108.54, 109.52, 120.22, 121.05, 122.08, 123.07, 124.52, 124.16, 125.90, 125.76, 126.88, 127.12, 127.91, 128.50, 129.82, 130.24, 134.17, 140.10, 143.19, 143.28, 158.59, 176.12, 179.63.Anal. Calcd for $C_{31}H_{26}N_4O_3S$: C, 69.64; H, 4.90; N, 10.48; O, 8.98; S, 6.00%. Found C, 69.70; H, 5.02; N, 10.57; O, 8.89; S, 6.08 %.

7-(2-methoxy-quinolin-3-yl)-5-(spiro-2'-acenaphthene-1'-one)-6-(spiro-3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (13a)

Pale orange coloured granules, yield (76 %); M.pt:176-184 ˚C; IR(KBr) υ_{max}: 3400, 3243, 1712, 1712 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.91 (t, 1H, *J*= 8.4 and 9.6 Hz, S-CH_2-H), 3.32 (s, 3H, -OCH_3), 3.42 (q, 1H, *J*= 3,4,6 and 9 Hz, S-CH_2-H), 3.52 (d, 1H, *J*= 5.2 Hz, Thiapyrr-N-CH_2-H) , 3.88 (d, 1H, *J*= 4.8 Hz, Thiapyrr N-CH_2-H) 4.18 (d, 1H, *J*= 8.4 Hz, -CH), 5.68 (d, 1H, *J*= 5.2

Hz, N-CH), 6.20 (m, 2H, ArH), 6.31 (d, 1H, *J*= 8 Hz, ArH), 6.69 (m, 1H, ArH), 7.44-7.48 (m, 1H, ArH), 7.55 (d, 1H, *J*= 7.2 Hz, ArH), 7.61-7.57 (m, 3H, ArH), 7.77 (t, 1H, *J*= 7.2 and 8.4 Hz, ArH), 7.94 (d, 1H, *J*= 7.6 Hz, ArH), 7.77 (d,1H, *J*= 8 Hz, ArH), 8.10 (d, 1H, *J*= 8 Hz, ArH) 8.14 (d, 1H, *J*= 8 Hz, ArH), 8.61 (s, 1H, quin-4-H), 10.13 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 32.47, 46.08, 48.22, 52.70, 68.25, 69.22,76.81, 108.90, 120.09, 121.06, 123.21, 123.79, 124.56, 125.15, 125.36, 126.24, 126.65, 127.89, 128.34, 128.62, 129.25, 129.78, 130.27, 131.06, 132.43, 134.18, 135.73, 142.15, 143.32, 145.02, 160.43, 180.26, 204.18. Mass (ESI) *m/z*; 556.14 $[M+H]^+$. Anal. Calcd for $C_{34}H_{25}N_3O_3S$: C, 73.49; H, 4.53; N, 7.56; O, 8.64; S, 5.77 %. Found C, 73.58; H, 4.62; N, 7.49; O, 8.69; S, 5.70 %.

7-(2, 6-dimethoxy-quinolin-3-yl)-5-(spiro-2'-acenaphthene-1'-one)-6-(spiro-3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (13b)

Red coloured powder, yield (74 %); M.pt:170-174 ˚C; IR(KBr) υ_{max}: 3403, 3199, 1712, 1616 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.80 (t, 1H, *J*= 8.4 and 9.6 Hz, S-CH_2-H), 3.26 (s, 3H, -OCH_3), 3.42 (m, 1H, S-CH_2-H), 3.50 (d, 1H, *J*= 5.6 Hz, Thiopyrr-N-CH_2-H), 3.85 (d, 1H, *J*= 5.2 Hz, Thiopyrr-N-CH_2-H), 3.91(s, 3H, -OCH_3), 4.14 (d, 1H, *J*= 9.6 Hz, -CH), 5.70 (m, 1H, N-CH), 6.20-6.12 (m, 2H, ArH), 6.29 (d, 1H, *J*= 7.6 Hz, ArH), 6.68-6.64 (td, 1H, *J*= 1.2, 6.4,and 1.6 Hz, ArH), 7.23-7.20 (dd, 1H, *J*= 2.8, 6.4 and 2.8 Hz, ArH), 7.53-7.47 (m, 1H, ArH), 7.60-7.56 (m, 2H, ArH), 7.76 (t, 2H, *J*= 7.2 and 7.2 Hz, ArH), 7.98-7.90 (dd, 2H, *J*= 15.6,and 6.8 Hz, ArH), 8.13 (d, 1H, *J*= 7.6 Hz, ArH), 8.54 (s, 1H, quin-C-4-H), 10.12 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 32.50, 46.00, 48.50, 51.88, 55.33, 67.50, 68.72, 76.08, 107.03, 120.01, 120.53, 122.71, 122.90, 124.66, 125.55, 125.55, 127.90, 127.70, 126.50, 127.40, 128.75, 129.74, 130.70, 134.50, 135.00, 139.90, 142.50, 143.50, 145.20, 155.63, 158.80, 180.00, 204.80. Anal. Calcd for $C_{35}H_{27}N_3O_4S$: C, 71.78; H, 4.65; N, 7.17; O, 10.93; S, 5.47 %. Found C, 71.70; H, 4.57; N, 7.28; O, 10.86; S, 5.53 %.

7-(8-methyl 2-methoxy-quinolin-3-yl)-5-(spiro-2'-acenaphthene-1'-one)-6-(spiro-3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (13c)

Dark red coloured powder, yield (80 %); M.pt:192-198 ˚C; IR(KBr) υ_{max}: 3400, 3191, 3045, 1709, 1616 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.44 (s, 3H, -CH_3), 2.89 (t, 1H, *J*= 8.7 and 8.8 Hz, S-CH_2-H), 3.20 (s, 3H,-OCH_3), 3.52 (d, 1H, *J*= 5.4 Hz, S-CH_2-H), 3.87 (d, 1H, *J*= 5.1 Hz, Thiapyrr-N-CH_2-H), 4.05 (d, 1H, *J*= 5.7 Hz, Thiapyrr-N-CH_2-H) 4.19 (d, 1H, *J*= 9.8 Hz, -CH), 5.70 (q, 1H, *J*= 6.8 and 8.4 Hz, -N-CH_2-H) 6.20 (d, 2H, *J*= 4.2 Hz, ArH), 6.31 (d, 1H, *J*= 7.8 Hz, ArH) 6.66 (m, 1H, ArH), 7.36 (t, 1H, *J*= 7.2 and 7.5 Hz, ArH), 7.45 (d, 1H, *J*= 6.6 Hz, ArH), 7.58-7.52 (m, 2H, ArH), 7.77 (t, 2H, *J*= 7.5 and 8.1 Hz, ArH) 7.98-7.89 (dd, 2H, *J*= 9, 7.8, 9.9 Hz, ArH), 8.12 (d, 1H, *J*= 7.5 Hz, ArH), 8.56 (s, 1H, quin-C-4-H), 10.13 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.55, 32.51, 46.00, 48.09, 52.31, 68.28, 69.18, 76.74, 108.74, 120.12, 121.05, 122.57, 123.80, 124.16, 125.10, 125.18, 126.27, 127.94, 128.30, 128.58, 129.22, 129.86, 130.24, 131.00, 132.29, 134.06, 134.22, 136.02, 141.98, 143.19, 143.76, 159.53, 180.16, 204.06,

134.09. Anal. Calcd for $C_{35}H_{27}N_3O_3S$: C, 73.79; H, 4.78; N, 7.38; O, 8.43; S, 5.63 %. Found C, 73.71; H, 4.70; N, 7.48; O, 8.47; S, 5.60 %.

7-(6-methyl 2-methoxy-quinolin-3-yl)-5-(spiro-2'-acenaphthene-1'-one)-6-(spiro-3'-indolino)-tetrahydro-1H-pyrolo [1, 2c] [1, 3] thiazole (13d)

Pale orange coloured granules, yield (72 %); M.pt:184-192 ˚C; IR(KBr) υ_{max}: 3404, 3206, 3090, 1713,1615 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.47 (s, 3H, -CH_3), 2.90 (t, 1H, *J*= 9.87 Hz, S-CH_2-H) 3.29 (s, 3H, -OCH_3), 3.51 (d, 1H, *J*=5.4 Hz, -S-CH_2-H), 3.87 (d, 1H, *J*= 5.4 Hz, Thiapyrr-N-CH_2-H), 4.05 (d, 1H, *J*= 5.7 Hz, Thiapyrr-N-CH_2-H), 4.16 (d, 1H, *J*= 9.3 Hz, -CH), 5.68 (q, 1H, *J*= 8.7, 5.7, 8.7 Hz, N-CH), 6.20 (m, 2H, ArH), 6.30 (d, 1H, *J*= 7.8 Hz, ArH), 6.68-6.63 (td, 1H, *J*= 1.8,5.4,1.2 Hz ArH), 7.60-7.39 (m, 3H, ArH), 7.77-7.71 (m, 2H, ArH), 7.84 (1H, brs, ArH), 7.98-7.88 (dd, 2H, *J*= 8.4, 5.4, 6.9 Hz, ArH), 8.13 (d, 1H, *J*= 7.8 Hz, ArH), 8.49 (s, 1H, quino-C-4-H), 10.13 (s, 1H, oxin-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.38, 32.45, 45.95, 48.14, 52.47, 68.17, 69.15, 76.64, 100.74, 120.07, 121.05, 121.86, 123.03, 123.77, 124.64, 125.28, 126.24, 126.43, 127.51, 127.91, 128.30, 128.60, 129.00, 130.24, 131.07, 131.56, 132.30, 133.64, 134.21, 135.12, 142.00, 143.26, 159.90, 180.24, 204.06. Anal. Calcd for $C_{35}H_{27}N_3O_3S$: C, 73.79; H, 4.78; N, 7.38; O, 8.43; S, 5.63 %. Found C, 73.68; H, 4.69; N, 7.45; O, 8.36; S, 5.52 %.

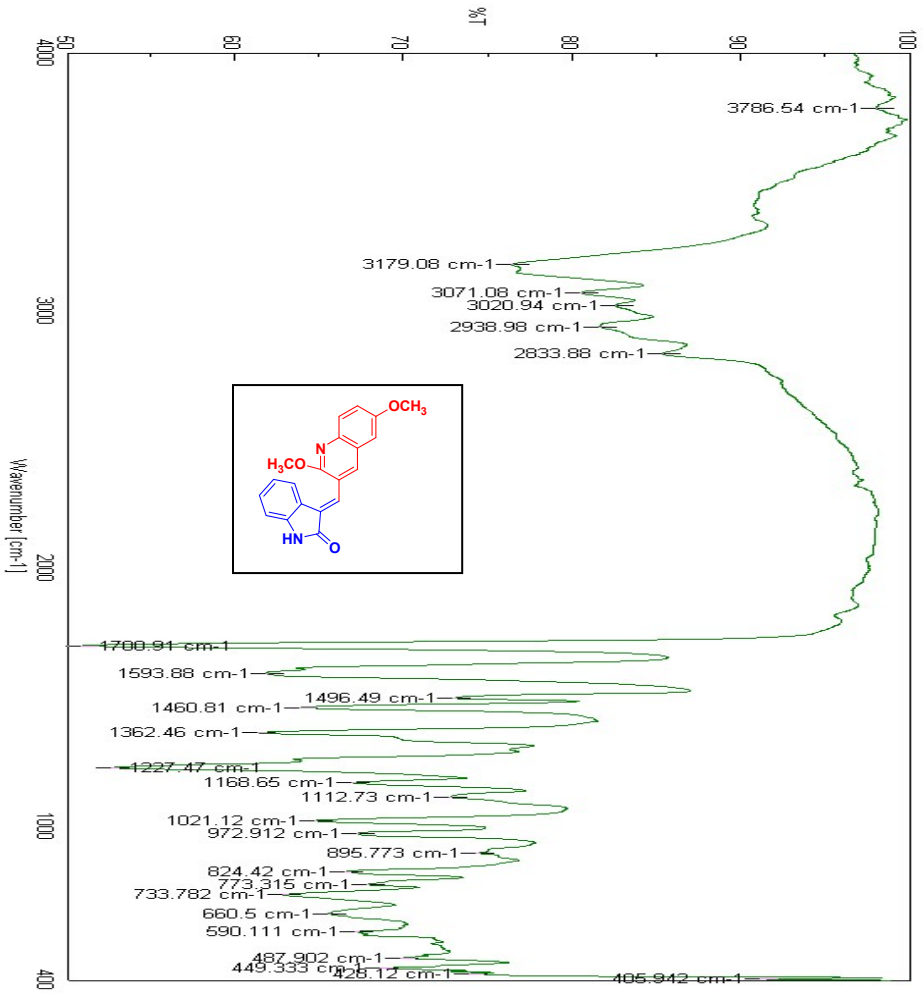

Fig S1. IR Spectrum of compound **3b**

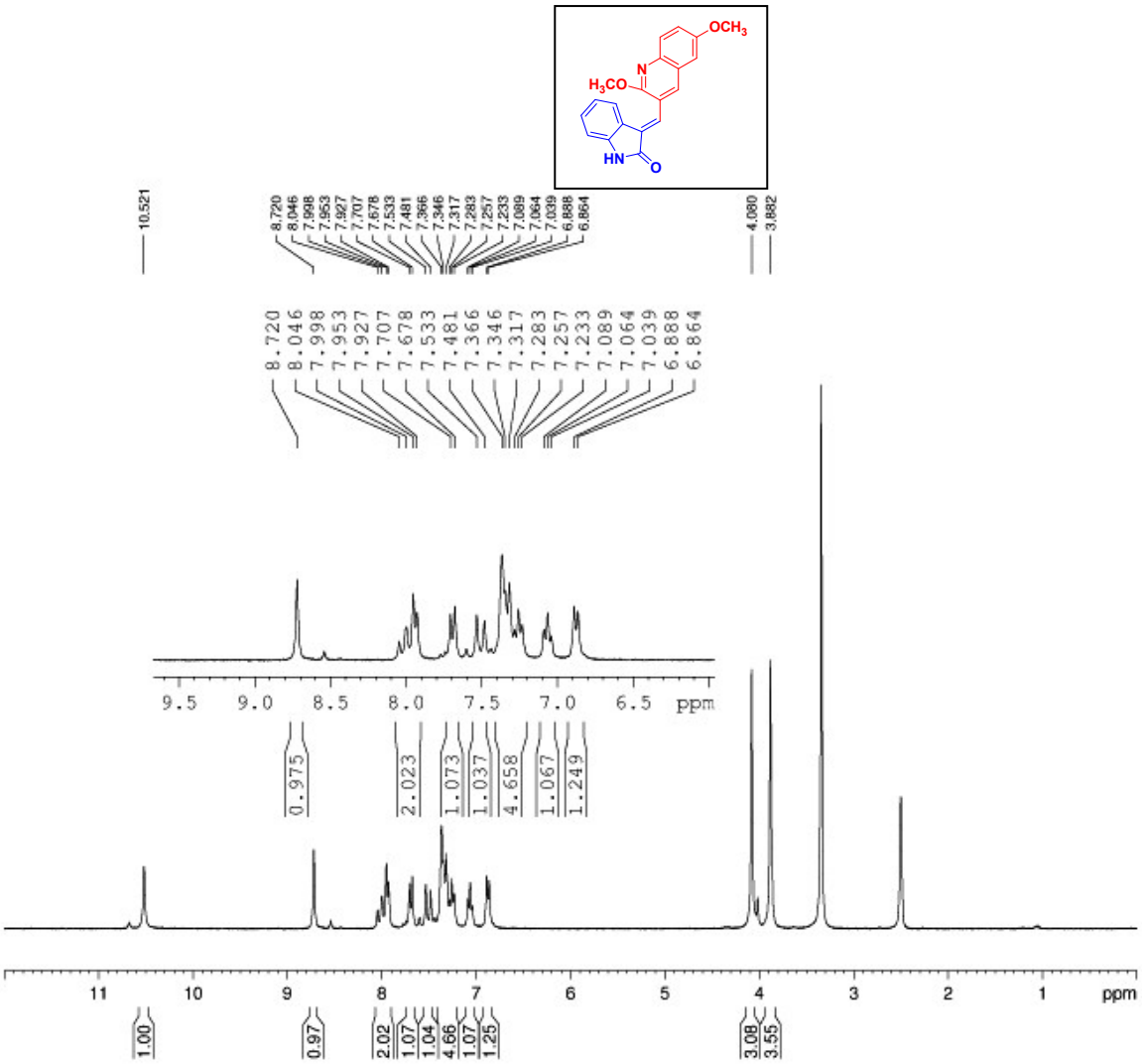

Fig S2. ^{1}H NMR Spectrum of compound **3b**

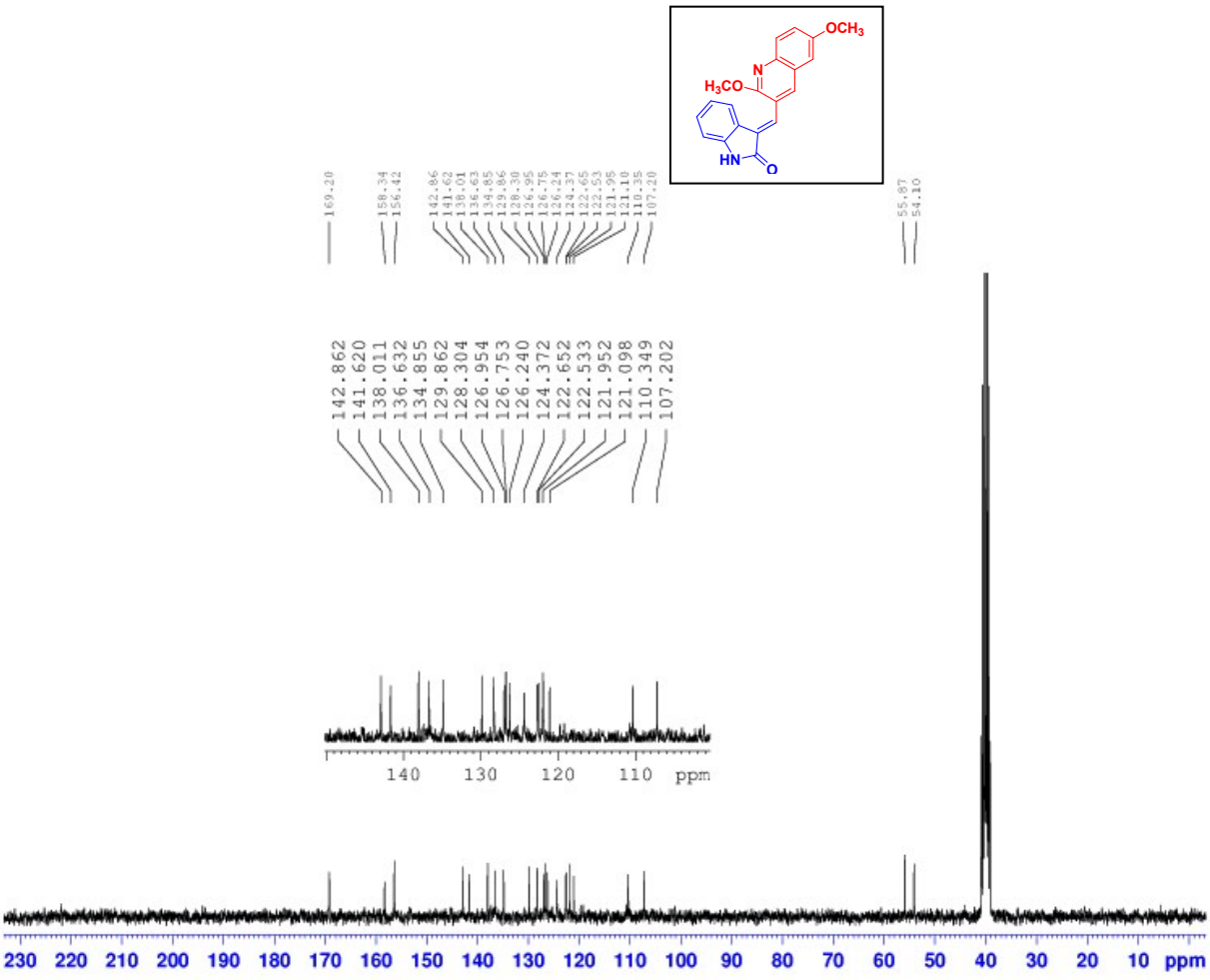

Fig S3. ^{13}C NMR Spectrum of compound **3b**

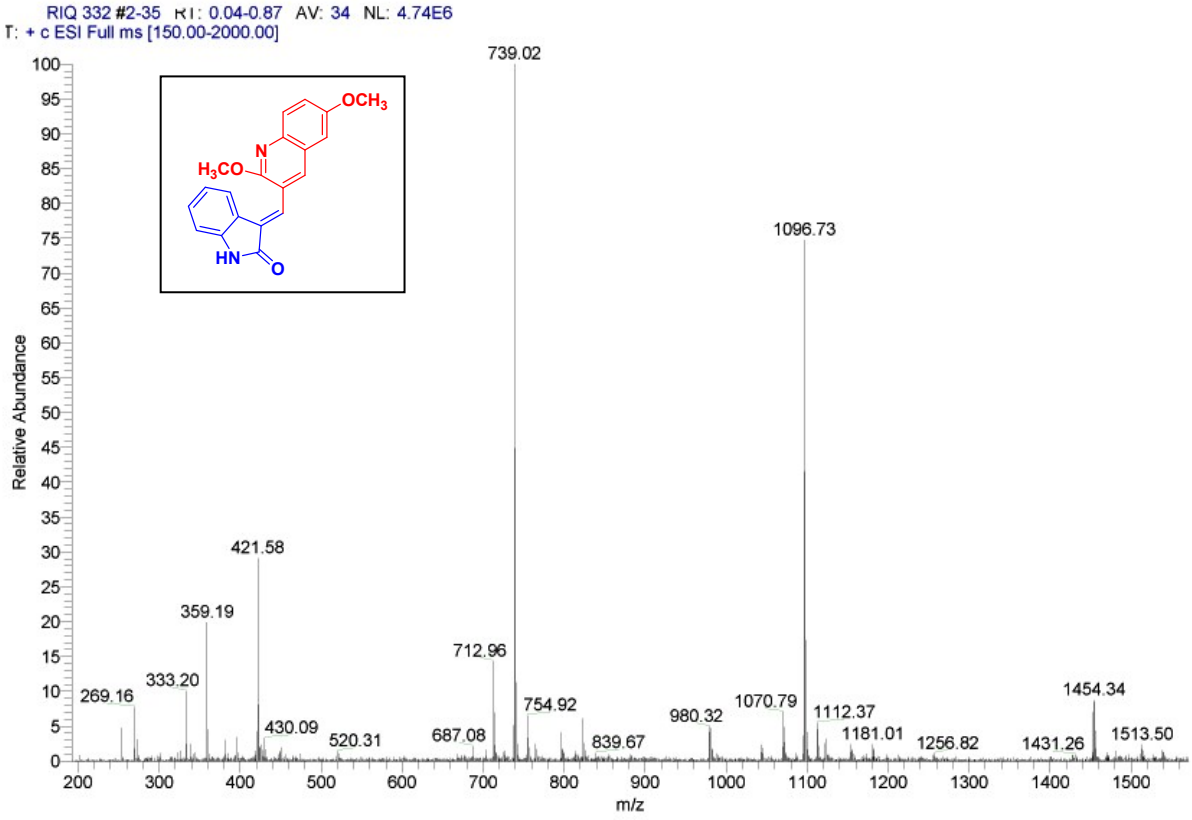

Fig S4. Mass spectrum of compound **3b**

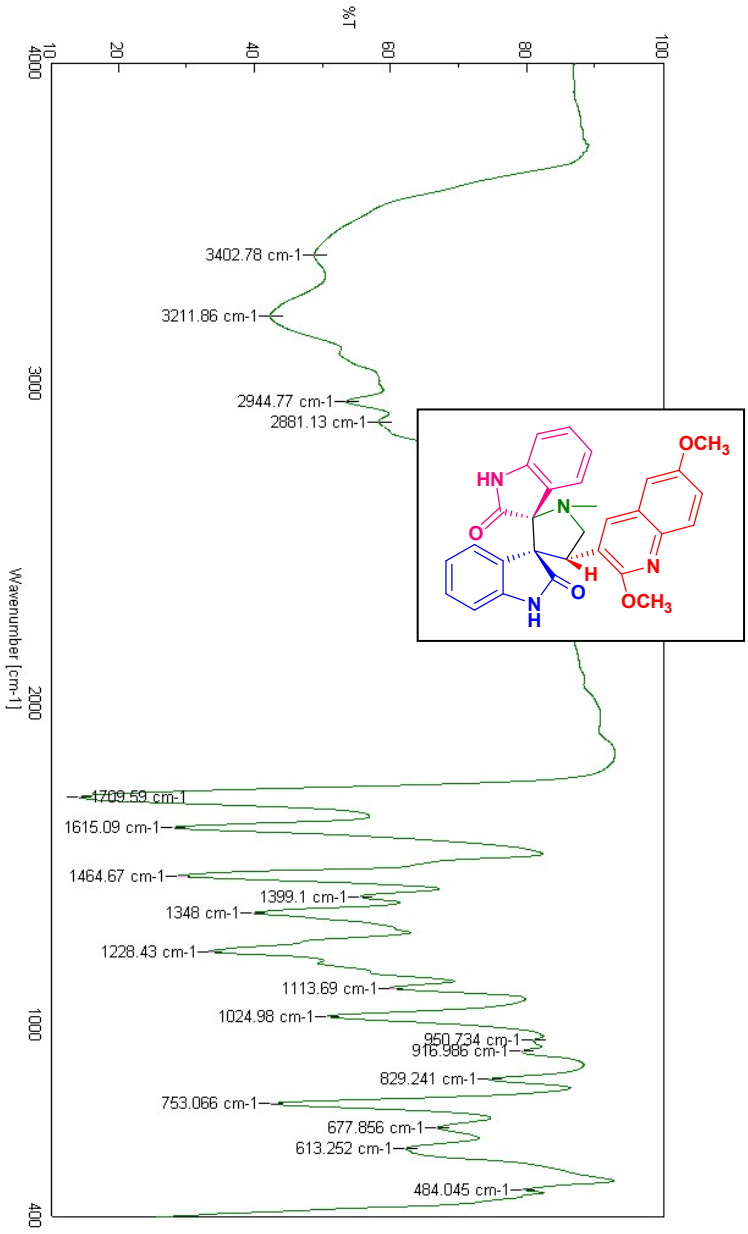

Fig S5. IR Spectrum of compound **6b**

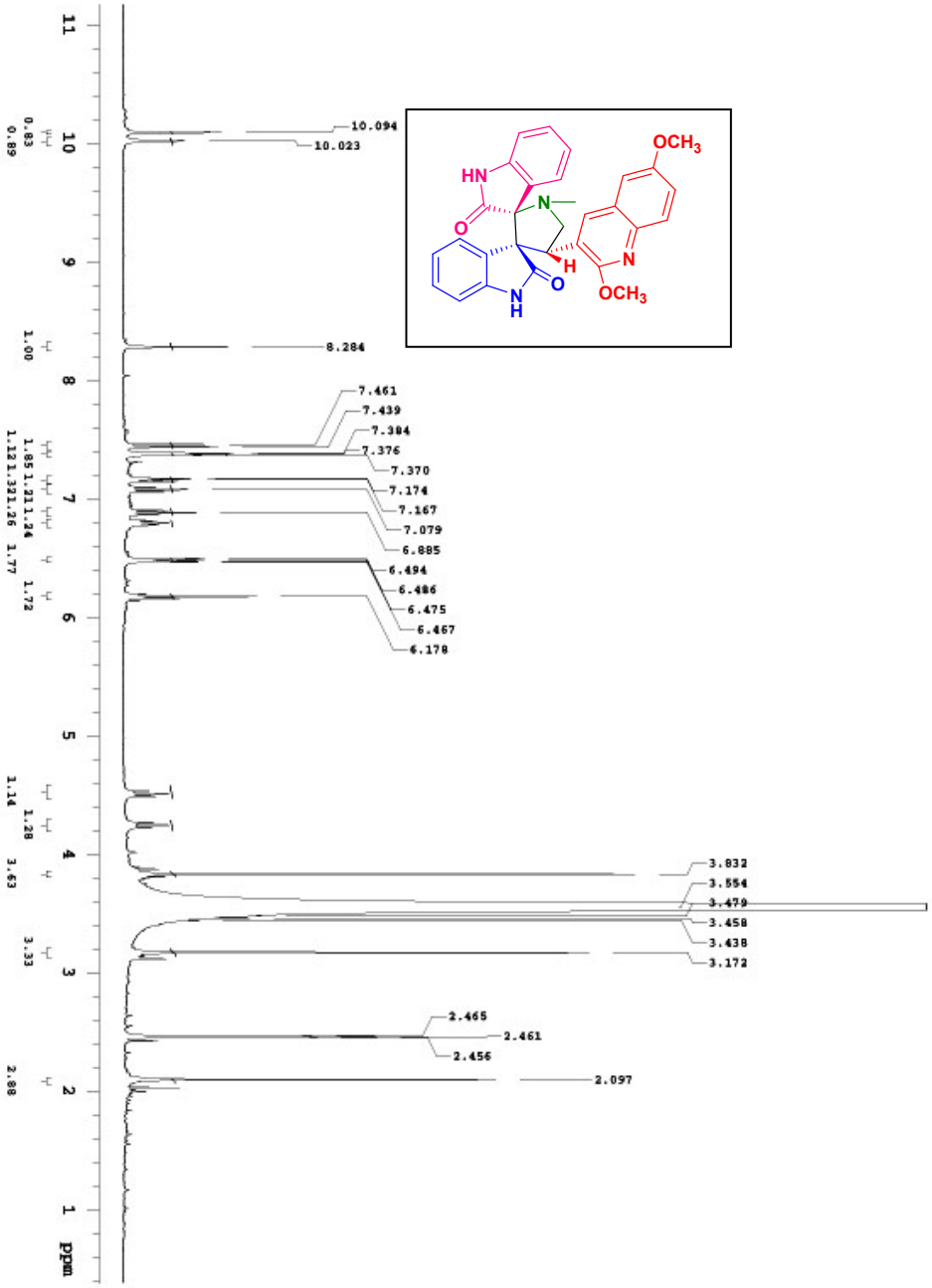

Fig S6. ^{1}H NMR Spectrum of compound **6b**

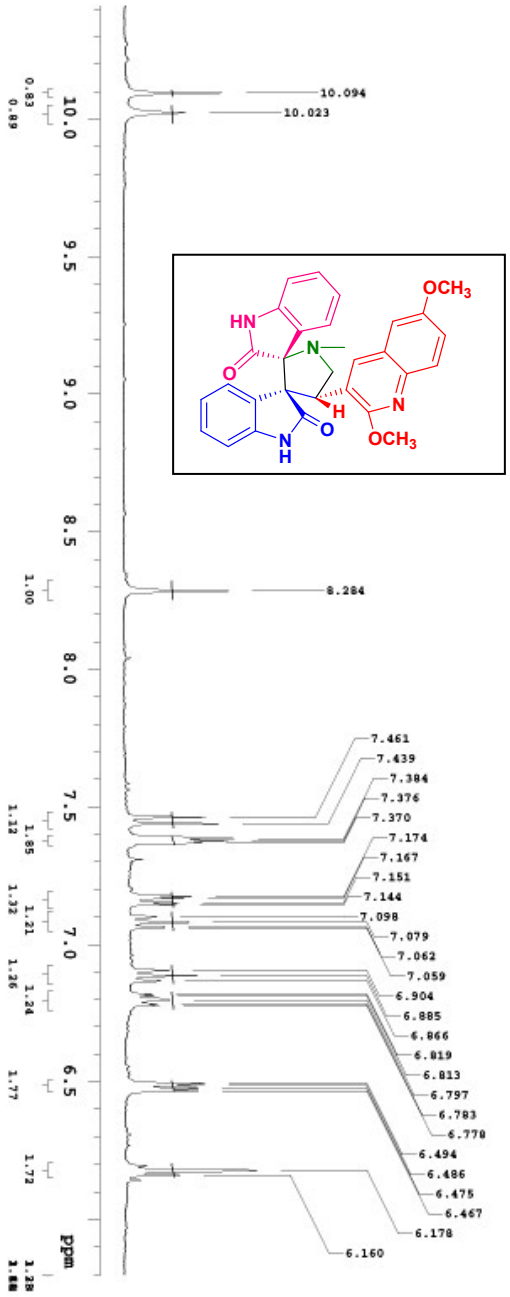

Fig S7. ^{1}H NMR expand-1 Spectrum of compound **6b**

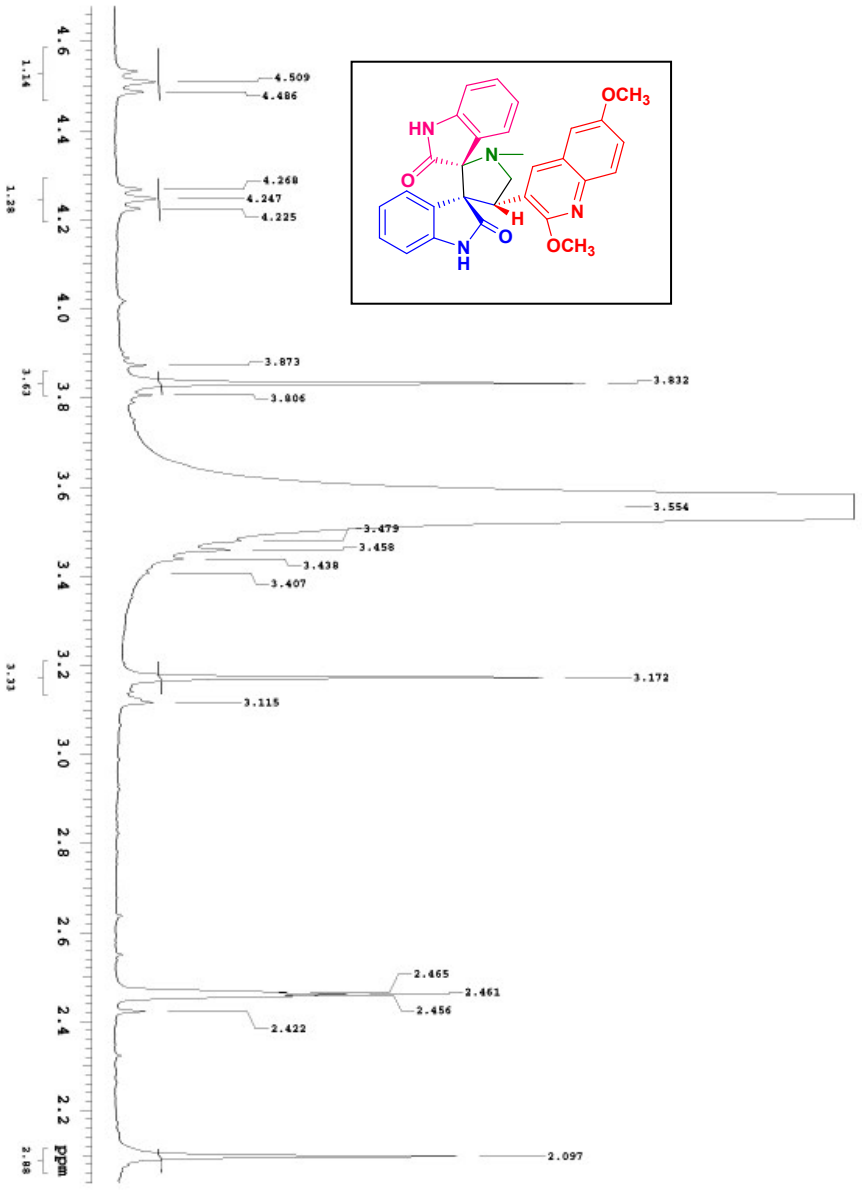

Fig S8. ^{1}H NMR expand-2 Spectrum of compound **6b**

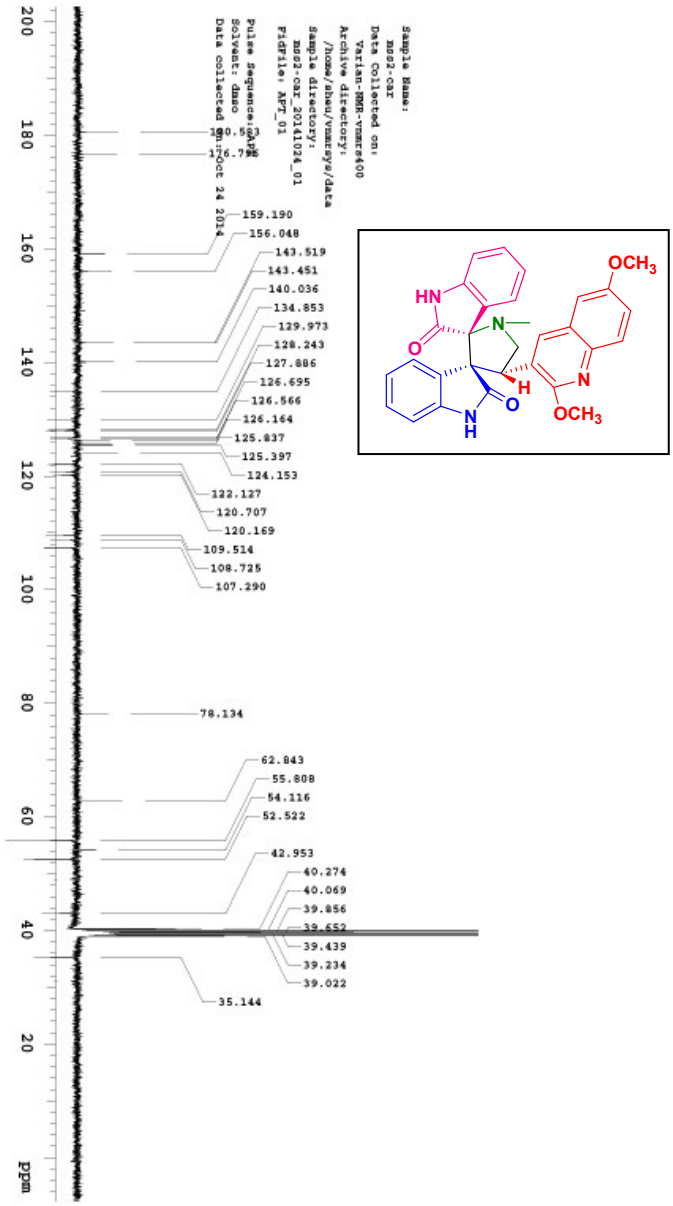

Fig S9. ^{13}C APT Spectrum of compound **6b**

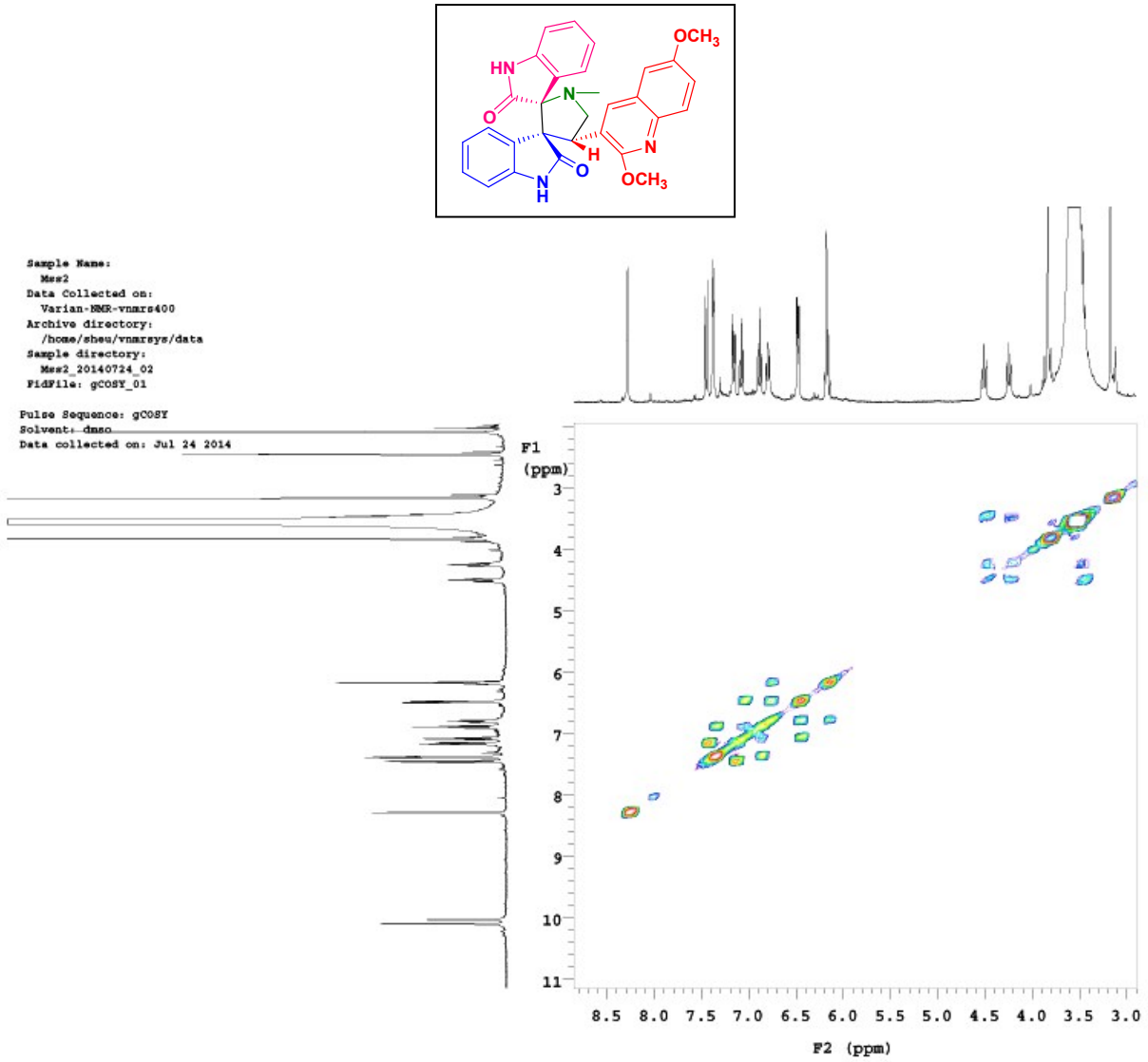

Fig S10. COSY Spectrum of compound **6b**

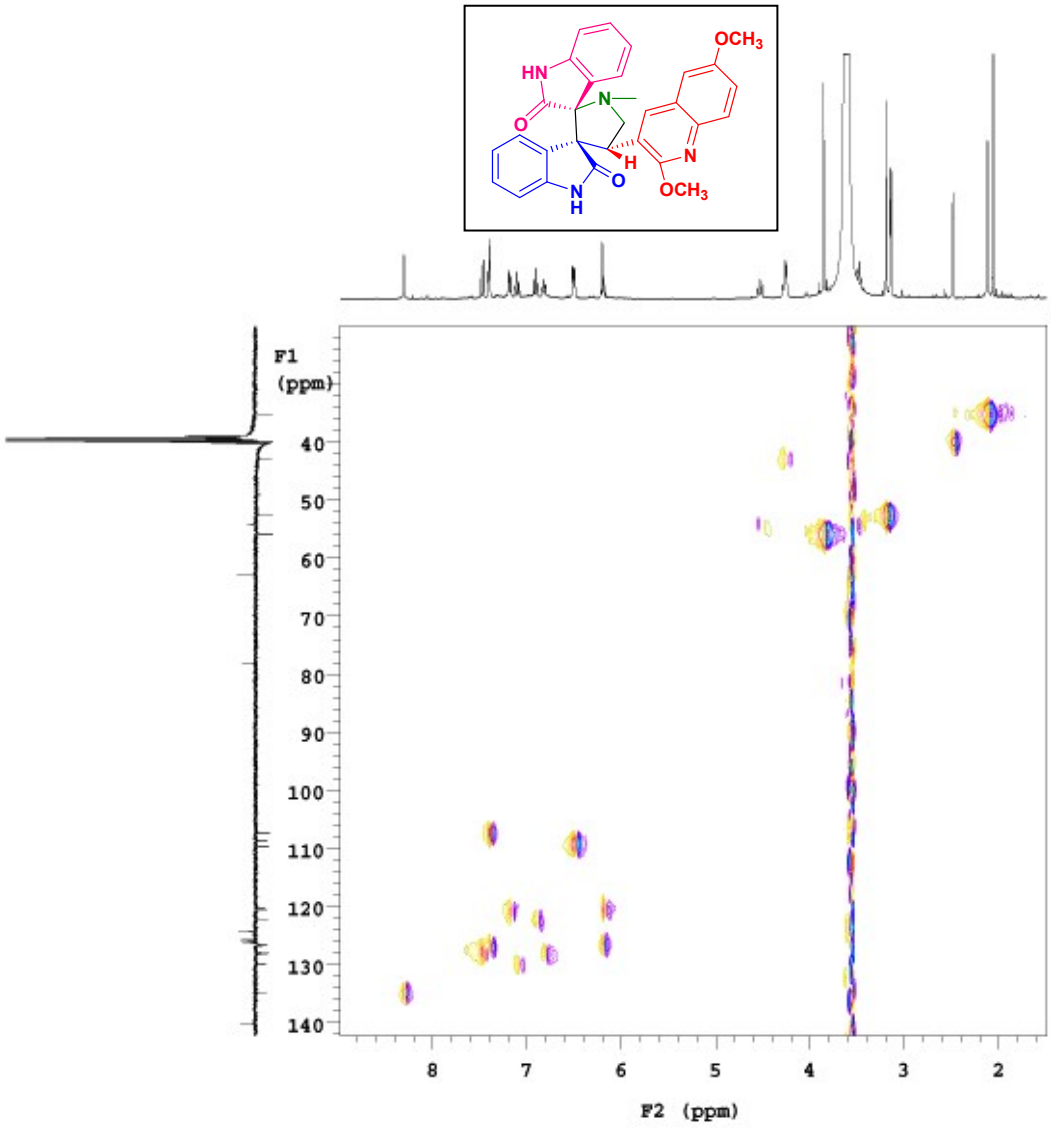

Fig S11. HSQC Spectrum of compound **6b**

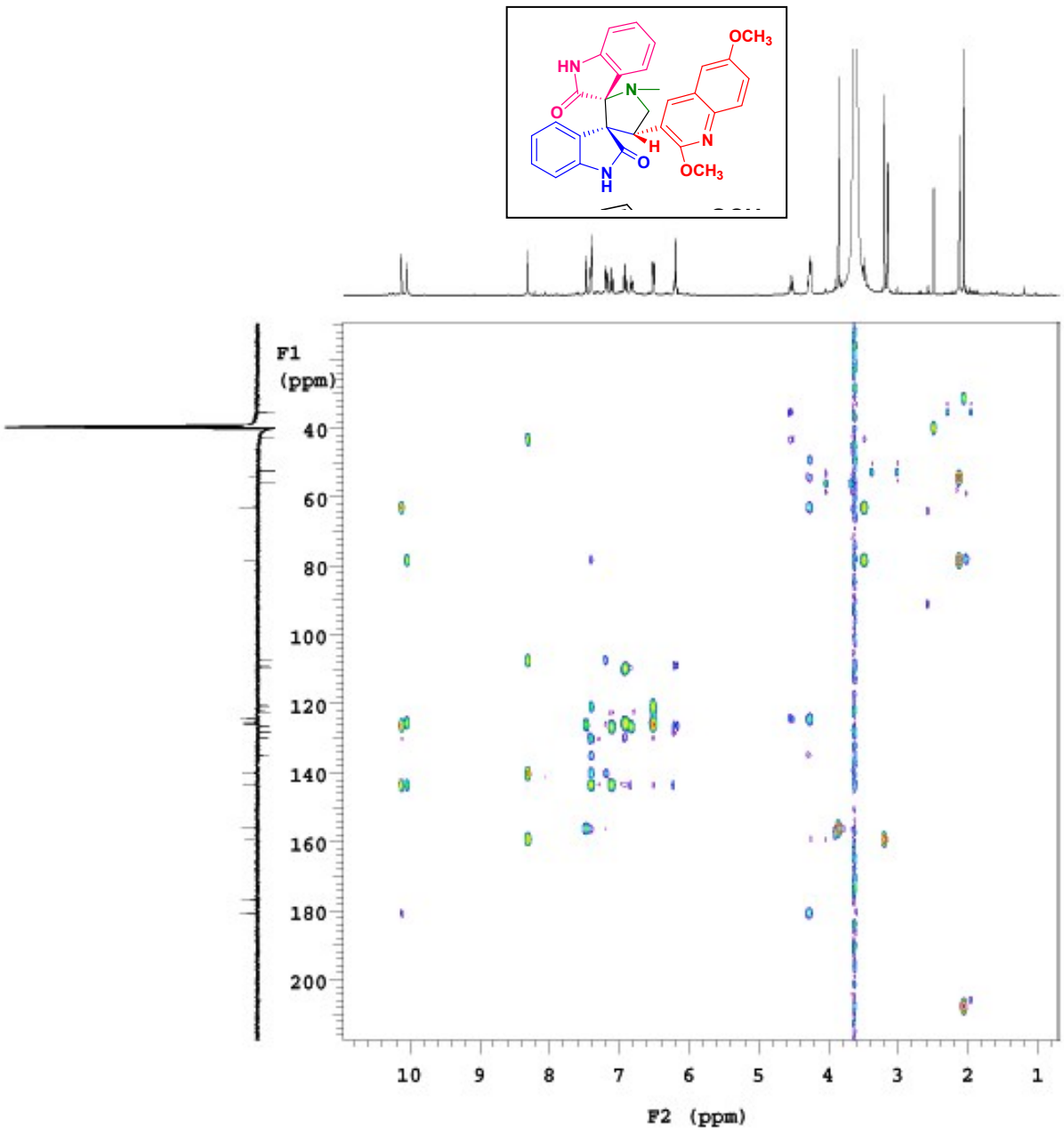

Fig S12. HMBC Spectrum of compound **6b**

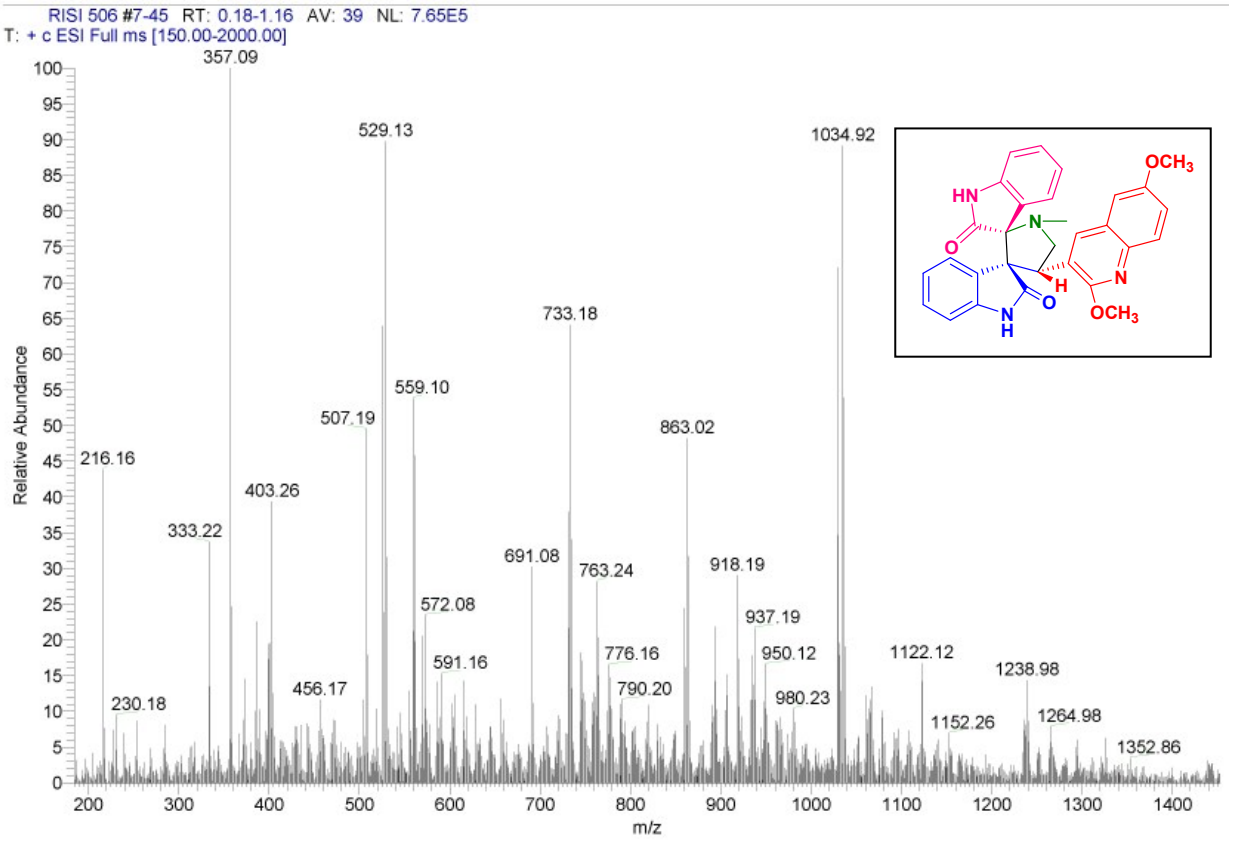

Fig S13. Mass spectrum of compound **6b**

Biological Studies

1. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The various concentrations of test compounds and standards are dissolved in DMSO and mixed with 0.1 mM DPPH in ethanol and 50 mM tris-HCl buffer (pH 7.4) containing in each solution. This reaction mixture was incubated in the dark at 37 ˚C for 30 mins and reduction of DPPH radicals were measured at 517 nm.[1] The percentage of radical scavenging activity was calculated using the following equation:

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,
Where A_B- absorption of blank sample, A_A- absorption of test sample

2. Metal Chelating activity

The ferrous ion chelating ability of compounds was estimated by the method of Dinis *et al*., [2] (1994). Various concentrations of test compounds and standards were mixed with 2 mM $FeCl_2$ individually followed by the addition of 5 mM ferrozine solution, the reaction was initiated. After 10 min incubation at room temperature absorbance was measured at 562 nm. The ability of compounds was calculated by the following equation:

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,
Where A_B- absorption of blank sample, A_A- absorption of test sample

3. Lipid peroxidation by Linoleic acid-thiocyanate method

The inhibition of lipid peroxidation was determined using by the procedure of Choi *et al*.,[3] (2002) according to the ferric thiocyanate method. Various concentrations of test compounds in methanol were added to the mixture of 20 mM of linoleic acid emulsion and 100 mM HCl (pH 7.5), 5 mM ascorbic acid. The reaction mixture was incubated for 30 mins in the dark at 37 ˚C to accelerate the peroxidation process by the addition of 4 mM $FeSO_4.7H_2O$ and terminated by the addition of 2 mL of ice cold trichloroacetic acid (TCA). Further addition of 1 mL of thiobarbituric acid (TBA) of 1% w/v in 50 mM NaOH) to the 1 mL of the reaction mixture, which was measured at 532 nm after heating at 95 ˚C for 60 min. The mixture prepared as above without any test sample

was used as control. The percentage of linoleic acid peroxidation inhibition was calculated as follows,

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,
Where A_B- absorption of blank sample, A_A- absorption of test sample

4. α-amylase potential inhibition activity

A mixture of 100 µL α-amylase solution (4.5 units/mL/min) and 100 µL of 0.02 mol/L sodium phosphate buffer (pH 6.9) were incubated at 25 ˚C for 10 min and mixed with 100 µL of test sample. After the addition of 100 µL of 1% freshly prepared starch solution followed by the addition of 1.0 mL dinitrosalicylic acid reagent (1 g of 3,5-dinitrosalicylic acid, 20 mL of 2 mol/L NaOH, and 30 g Rochelle salt dissolved in 50 mL of distilled water) for quench the reaction and again this reaction mixture was incubated at 25 ˚C for 30 min. Aliquots were incubated for 5 min in a boiling water bath and cooled to room temperature, further dilution of 10-fold times with distilled water and the absorbance was measured at 540 nm.[4] Acarbose is used as a positive control.

5. Acetylcholinesterase inhibition assay

Ellman Method

The enzymatic activity was measured using Ellman's spectrophotometric method[5] with slight modification. Diverse concentrations of test compounds were mixed with a mixture of 275 µL of 50 mM tris-Hcl buffer (pH 8), 500 µL of 3 mM DTNB, and the addition of 10 µL of 0.28 U/ml acetylcholine esterase with a final Concentration 0.023 U/ml prepared in phosphate buffer, pH 8 (AChE True cholinesterase, EC3.1.1.7 Type VI-S: From Electric Eel) and incubated for 15 min at 25˚C followed by the addition of 100 µL of 15 mM Acetyl Choline Iodide for initiation of the reaction. The hydrolysis of aliquots was monitored for 5 min at 405 nm by spectrophotometrically. Physostigmine (Eserine) is used as a standard AChE inhibitor in this study. The inhibition rate (%) was calculated by the equation: % Inhibition = 100 - (100 x VT/ VK). VT= velocity of test solution, VK= velocity of control without inhibitor.

References

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