Title: Synthesis of tryptanthrin appended dispiropyrrolidine oxindoles and their antibacterial evaluation

Authors: Sruthi Sudheendran Leena[1], Abdul Akhir[2], Deepanshi Saxena[2], Rahul Maitra[2], Sidharth Chopra[2,3,*] and Ani Deepthi[1,*]

Author Affiliations: [1]Department of Chemistry, University of Kerala, Kariavattom, Trivandrum–695581; [2]Division of Molecular Microbiology and Immunology, CSIR-Central Drug Research Institute, Sector 10, Janakipuram Extension, Sitapur Road, Lucknow-226031, Uttar Pradesh, India and [3]AcSIR: Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India.

Corresponding authors:

Dr. Ani Deepthi, Department of Chemistry, University of Kerala, Kariavattom, Trivandrum – 695581. Email: anideepthi@gmail.com

Dr. Sidharth Chopra, Division of Molecular Microbiology and Immunology, CSIR-Central Drug Research Institute, Sector 10, Janakipuram Extension, Sitapur Road, Lucknow-226031, Uttar Pradesh. Email: skchopra007@gmail.com

Table of Contents

General Procedure

NMR spectra were recorded on a Bruker AVANCE III HD 400 MHz spectrometer with TMS as an internal standard. IR spectra were recorded on an Agilient Cary 630 FTIR spectrometer. $CDCl_3$ was used as the solvent for NMR analysis. Chemical shifts are given in ppm and coupling constants (*J*) are given in Hz. The purity of the samples was ascertained using PE 2400 series II CHNS/O Analyser. The chemicals used were purchased from Sigma-Aldrich, TCI and Spectrochem Pvt. Ltd and were used without further purification. Melting points were recorded on an electrothermal digital melting point apparatus from Analab Scientific instruments Pvt. Ltd. Commercial grade solvents were used. Thin layer chromatography was performed on silica gel coated on aluminium sheets and was monitored using UV light of wavelength 254 nm. Column chromatography was performed on 100–200 mesh silica gel. Compounds were eluted by a mixture of hexane and ethyl acetate.

General procedure for the synthesis of tryptanthrin derivatives

A mixture of substituted isatin (1 mmol) and isatoic anhydride (1 mmol) were dissolved in toluene (5 mL), and triethyl amine (5 mmol) was added to it. The mixture was stirred at 110 ℃ for 30 minutes and the product was formed as a precipitate. The precipitate was filtered, washed with methanol and re-crystallized from ethanol.[1]

One-Pot, Three-Component Reaction of Tryptanthrins, isatilidene, and α-Amino Acids; General Procedure A

A mixture of corresponding tryptanthrin **1a** (0.3 mmol), *N*-substituted isatilidene **3** (0.3 mmol), and *α*-amino acid **4/6** (0.6 mmol) were suspended in a mixture of EtOH–toluene and was placed in a pre-heated oil bath at 80 °C. After heating to reflux for 12 h, the solvent was removed under vacuum and completion of the reaction was evidenced by thin layer chromatography. The residue was worked up and extracted with ethyl acetate five times. Ethyl acetate was removed under reduced pressure and the reaction mixture was then subjected to column chromatography with hexane-ethyl acetate mixture to attain the pure compound.

One-Pot, Three-Component Reaction of Tryptanthrins, isatilidene, and glycylglycine

8-Chloro tryptanthrin **1c** (0.3 mmol), *N*-ethyl isatilidene **3a** (0.3 mmol), and glycylglycine **7** (0.6 mmol) were taken in a mixture of EtOH–H_2O. Glacial acetic acid (0.2 mL, 3.0 mmol) was added dropwise and the reaction mixture was heated at reflux for 12 hours. The solvent was removed under reduced pressure. The residue is worked up and extracted with ethyl acetate five times. Ethyl acetate was removed under reduced pressure and the reaction mixture was then subjected to column chromatography with hexane-ethyl acetate mixture to attain the pure compound.

Spectral Data of the Compounds

1-Ethyl-1'-methyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5a

Yellow solid (85 mg, 85%); mp 265-267 ℃; IR (υ, cm^{-1}): 2810, 2216, 2122, 1730, 1685, 1515, 910. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.86 (t, 3H, *J* = 7.6 Hz, -$NCH_2\underline{CH_3}$), 2.04 (s, 3H, -NCH_3), 3.07 (d, 1H, *J* = 2 Hz, -CH_2), 3.13 (d, 1H, *J* = 0.4 Hz, -CH_2), 3.79 (q, 2H, *J* = 7.2 Hz, -$N\underline{CH_2}CH_3$), 7.40-7.44 (m, 2H, Ar-H), 7.65-7.69 (m, 2H, Ar-H), 7.76-7.80 (m, 3H, Ar-H), 7.83-7.85 (m, 2H , Ar-H), 7.90-8.03 (m, 2H, Ar-H), 8.41-8.62 (m, 1H, Ar-H) ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 (-NCH_3), 26.3 ($C(CN)_2$), 29.7 (CH_2), 56.0 (C_{spiro}), 82.0 (C_{spiro}), 117.9 (CN), 121.2 (C), 121.9 (CH), 123.7 (CH), 125.0 (CH), 125.4 (CH), 127.2 (CH), 127.5 (CH), 128.1 (CH), 130.2 (CH),

130.7 (CH), 131.6 (CH), 131.7 (CH), 135.1 (CH), 137.0 (CH), 137.2 (C), 138.2 (C), 144.3 (C), 144.5 (C), 146.3 (C), 146.6 (C), 158.0 (CH), 169.1 (C=O), 182.5 (C=O). Anal. Calcd (%) for $C_{30}H_{22}N_6O_2$: C, 72.28; H, 4.45; N, 16.86; Found: C, 72.25; H, 4.48; N, 16.86.

8''-Bromo-1-ethyl-1'-methyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5b

Yellow solid (73 mg, 83%); mp 258-260 ℃; IR (υ, cm^{-1}): 2820, 2218, 2194, 1715, 1685, 1650, 1425, 980, 818, 690. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.88 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.04 (s, 3H, -NCH_3), 2.52 (d, 1H, *J* = 2.4 Hz, -CH_2), 2.78 (d, 1H, *J* = 2.8 Hz, -CH_2), 4.09 (q, 2H, *J* = 6.8 Hz, -$N\underline{CH_2}CH_3$), 7.38-7.40 (m, 2H, Ar-H), 7.49-7.51 (m, 2H, Ar-H), 7.54-7.58 (m, 2H, Ar-H), 7.67-7.75 (m, 2H, Ar-H), 7.84-7.89 (m, 2H, Ar-H), 8.02-8.04 (m, 1H, Ar-H), 8.42-8.44 (m, 1H, Ar-H), 8,59 (d, 1H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 (-NCH_3), 29.7 ($C(CN)_2$), 31.9 (CH_2), 38.1 (CH_2), 60.4 (C_{spiro}), 86.2 (C_{spiro}), 114.0 (CN), 115.7 (CN), 117.4 (CH), 119.5 (CH), 120.7 (CH), 122.1 (CH), 123.3 (CH), 123.4 (CH), 125.0 (CH), 125.1 (CH), 127.6 (CH), 128.1 (CH), 128.6 (CH), 130.5 (C), 130.9 (C), 135.2 (C), 140.6 (C), 143.7 (C), 144.9 (C), 146.5 (C), 157.9 (C=O), 181.2 (C=O). Anal. Calcd (%) for $C_{30}H_{21}BrN_6O_2$: C, 62.40; H, 3.67; N, 14.55; Found: C, 62.43; H, 3.68; N, 14.52.

8''-Chloro-1-ethyl-1'-methyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5c

Yellow solid (77 mg, 82%); mp 235-237 ℃; IR (υ, cm^{-1}): 2218, 1718, 1614, 1560, 850. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.88 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.07 (s, 3H, -NCH_3), 2.76 (d, 1H, *J* = 16.8 Hz, -CH_2), 3.64 (d, 1H, *J* = 16.8 Hz, -CH_2), 4.48 (q, 2H, *J* = 7.2 Hz, -$N\underline{CH_2}CH_3$), 7.67-7.75 (m, 3H, Ar-H), 7.84-7.88 (m, 3H, Ar-H), 8.02-8.04 (m, 1H, Ar-H), 8.42-8.44 (m, 2H, Ar-H), 8.58-8.60 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.0 (-CH_3), 22.6 (-NCH_3), 24.7 ($C(CN)_2$), 29.6 (CH_2), 31.9 (CH_2), 63.3 (C_{spiro}), 93.2 (C_{spiro}), 118.0 (CN), 119.2 (CN), 123.1 (CH), 123.6 (CH), 125.2 (CH), 126.6 (CH), 127.6 (CH), 130.5 (CH), 130.9 (CH), 131.7 (CH), 133.2 (C), 135.3 (C), 137.7 (C), 139.2 (C), 143.9 (C), 144.5 (C), 146.5 (C), 157.9 (C=O), 181.4 (C=O). Anal. Calcd (%) for $C_{30}H_{21}ClN_6O_2$: C, 67.61; H, 3.97; N, 15.77; Found: C, 67.60; H, 3.99; N, 15.78.

1-Ethyl-8''-iodo-1'-methyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5d

Yellow solid (72 mg, 86%); mp 288-290 ℃; IR (υ, cm^{-1}): 2888, 2215, 1714, 1689, 1625, 1530, 860. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.89 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.04 (s, 3H, -NCH_3), 3.25 (d, 1H, *J* = 5.2 Hz, -CH_2), .3.63 (d, 1H, *J* = 7.2 Hz -CH_2), 4.48 (q, 2H, *J* = 7.2 Hz, -$N\underline{CH_2}CH_3$), 7.67-7.75 (m, 3H, Ar-H), 7.84-7.88 (m, 3H, Ar-H), 8.03 (d, 1H, *J* = 8Hz, Ar-H), 8.42 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.58 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.7 (-NCH_3), 29.7 ($C(CN)_2$), 31.9 (CH_2), 45.0 (CH_2), 60.4 (C_{spiro}), 82.9 (C_{spiro}), 99.9 (C), 114.1 (CH), 115.7 (CH), 117.1 (CH), 119.2 (CH), 122.3 (CH), 123.1 (CH), 123.3 (CH), 123.6 (CH), 125.2 (CH), 127.6 (CH), 128.6 (CH), 130.5 (CH), 130.9 (CH), 133.3 (CH), 135.3 (C), 135.4 (C), 137.7 (C), 143.9 (C), 144.5 (C), 146.5 (C), 157.9 (C=O), 181.4 (C=O). Anal. Calcd (%) for $C_{30}H_{21}IN_6O_2$: C, 57.70; H, 3.39; N, 13.46; Found: C, 57.71; H, 3.38; N, 13.44.

8''-Chloro-1-ethyl-2''-iodo-1'-methyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5e

Yellow solid (58mg, 72%); mp 281-283 °C; IR (υ, cm^{-1}): 2875, 2222, 1718, 1695, 1575, 1425, 1175, 975. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.86 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.03 (s, 3H, -NCH_3), 3.42 (d, 1H, *J* = 2.4 Hz, -CH_2), 3.65 (d, 1H, *J* = 8.8 Hz, -CH_2), 4.95 (q, 2H, *J* = 11.2 Hz, -$N\underline{CH_2}CH_3$), 7.75-7.81 (m, 2H, Ar-H), 7.96 (d, 2H, *J* = 9.2 Hz, Ar-H), 8.08-8.22 (m, 2H, Ar-H), 8.38-8.40 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 (-NCH_3), 24.8 ($C(CN)_2$), 29.7 (CH_2), 31.9 (CH_2), 40.2 (C_{spiro}), 84.8 (C_{spiro}), 91.3 (C), 118.9 (CN), 119.7 (CH), 123.5 (CH), 124.7 (CH), 125.5 (CH), 126.6 (CH), 127.2 (CH), 128.8 (CH), 129.4 (CH), 132.1 (CH), 132.9 (C), 134.1 (CH), 135.2 (C), 135.6 (C), 136.9 (C), 139.7 (C), 145.2 (C), 146.5 (C), 156.8 (C=O), 180.7 (C=O). Anal. Calcd (%) for $C_{30}H_{20}ClIN_6O_2$: C, 54.69; H, 3.06; N, 12.76; Found: C, 54.69; H, 3.05; N, 12.78.

1-Ethyl-1'-methyl-8''-nitro-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5f

Yellow solid (72 mg, 78%); mp 265-267 °C; IR (υ, cm^{-1}): 2892, 2260, 1718, 1689, 1579, 1315, 910. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.88 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.62 (s, 3H, -NCH_3), 3.78 (d, 1H, *J* = 7.2 Hz, -CH_2), 4.08 (d, 1H, *J* = 0.8 Hz, -CH_2), 4.57 (q, 2H, *J* = 7.6 Hz, -$N\underline{CH_2}CH_3$), 7.72-7.76 (m, 2H, Ar-H), 7.90-7.94 (m, 1H, Ar-H), 8.06-8.08 (m, 2H, Ar-H), 8.66-8.48 (m, 1H, Ar-H), 8.67-8.70 (m, 2H, Ar-H), 8.76 (t, 1H, *J* = 2 Hz, Ar-H), 8.84-8.87 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.7 (-NCH_3), 29.7 8 ($C(CN)_2$), 32.3 (CH_2), 59.5 (C_{spiro}), 86.7 (C_{spiro}), 116.1 (CN), 117.6 (CN), 118.5 (CH), 120.8 (CH), 122.3 (CH), 123.2 (CH), 127.9 (CH), 131.1 (CH), 131.2 (CH), 132.1 (CH), 132.9 (CH), 135.9 (CH), 136.9 (C), 137.8 (C), 140.2 (C), 144.0 (C), 146.2 (C), 149.3 (C), 150.8 (C), 158.0 (C=O), 180.6 (C=O). Anal. Calcd (%) for $C_{30}H_{21}N_7O_4$: C, 66.29; H, 3.89; N, 18.04; Found: C, 66.29; H, 3.88; N, 18.01.

1-Ethyl-8''-methoxy-1'-methyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5g

Yellow solid (66 mg, 70%); mp 248-250 °C; IR (υ, cm^{-1}): 2815, 2257, 1721, 1694, 1620, 1495, 1215. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.87 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.04 (s, 3H, -NCH_3), 3.24 (d, 1H, *J* = 5.6 Hz, -CH_2), 3.43 (d, 1H, J = 1.6 Hz, -CH_2), 4.46 (q, 2H, *J* = 7.2 Hz, -$N\underline{CH_2}CH_3$), 4.68 (s, 3H, -OCH_3), 7.31-7.35 (m, 2H, Ar-H), 7.65-7.70 (m, 3H, Ar-H), 7.72-7.75 (m, 2H, Ar-H), 7.82-7.87 (m, 1H, Ar-H), 8.00-8.37 (m, 2H, Ar-H), 8.39-8.58 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 12.5 (-CH_3), 23.0 (-NCH_3), 27.6 ($C(CN)_2$), 31.9 (CH_2), 52.4 (OCH_3), 58.3 (C_{spiro}), 84.3 (C_{spiro}), 113.6 (CN), 115.0 (CH), 117.1 (CH), 120.3 (CH), 121.1 (CH), 121.6 (CH), 122.1 (CH), 123.1 (CH), 125.5 (CH), 126.6 (CH), 128.5 (CH), 128.8 (CH), 131.2(C), 133.2 (C), 122.3 (C), 135.6 (C), 141.9 (C), 142.4 (C), 144.5 (C), 155.8 (C=O), 179.3 (C=O). Anal. Calcd (%) for $C_{31}H_{24}N_6O_3$: C, 70.44; H, 4.58; N, 15.90; Found: C, 70.43; H, 4.60; N, 15.89.

1,1'-Dimethyl-2,12''-dioxo-12''*H*-dispiro[indoline-3,4'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-3',3'-dicarbonitrile 5h

Yellow solid (82 mg, 84%); mp 258-260 °C; IR (υ, cm^{-1}): 2252, 1720, 1686, 1581, 1495, 1212, 980, 816. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 2.04 (s, 3H, -NCH_3), 2.77 (t, 1H, *J* = 8.4 Hz, -CH_2), 3.44 (t, 1H, *J* = 8.4 Hz, -CH_2), 3.66 (s, 3H, -NCH_3), 7.46-7.51 (m, 4H, Ar-H), 7.57-7.59 (m, 2H, Ar-H), 7.67-7.71 (m, 2H, Ar-H), 7.84-7.88 (m, 1H, Ar-H), 8.02-8.04 (m, 1H, Ar-H), 8.42-8.44 (m, 1H, Ar-H), 8.62-8.65 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.6 (-CH_3), 25.8 (-NCH_3), 27.7 ($C(CN)_2$), 29.7 (CH_2), 54.8 (C_{spiro}), 91.7 (C_{spiro}), 115.3 (CN), 117.4 (CN), 120.5 (CH), 121.5 (CH), 125.8 (CH), 126.1 (CH), 126.8 (CH), 127.3 (CH), 128.0 (CH), 128.2 (CH), 129.1 (CH),

129.6 (CH), 130.8 (CH), 131.5 (CH), 132.1 (C), 134.5 (C), 136.5 (C), 140.7 (C), 146.7 (C), 147.3 (C), 159.4 (C=O), 180.5 (C=O). Anal. Calcd (%) for $C_{29}H_{20}N_6O_2$: C, 71.89; H, 4.16; N, 17.35; Found: C, 71.87; H, 4.15; N, 17.35.

1'-Methyl-2,12''-dioxo-1-propyl-12''*H*-dispiro[indoline-3,3'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-4',4'-dicarbonitrile 5i

Yellow solid (83 mg, 81%); mp 291-293 ℃; IR (υ, cm^{-1}): 2262, 1714, 1612, 1520. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.85 (t, 3H, *J* = 4 Hz, -$NCH_2CH_2\underline{CH_3}$), 1.59-1.67 (m, 2H, -$NCH_2\underline{CH_2}CH_3$), 2.03 (s, 3H, -$NCH_3$), 2.33 (d, 1H, *J* = 7.6 Hz, -CH_2), 2.75 (d, 1H, *J* = 6.4 Hz, -CH_2), 4.29 (q, 2H, J = 8.4 Hz, -$N\underline{CH_2}CH_2CH_3$), 7.67-7.75 (m, 3H, Ar-H), 7.84-7.88 (m, 3H, Ar-H), 8.02-8.04 (m, 2H, Ar-H), 8.42-8.44 (m, 2H, Ar-H), 8.59 (d, 2H, *J* = 8.8 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 (-CH_2), 29.6 (-NCH_3), 31.9 ($C(CN)_2$), 42.8 (-CH_2), 52.8 (-CH_2), 89.2 (C_{spiro}), 109.8 (C_{spiro}), 111.2 (CN), 112.8 (CN), 114.0 (CH), 118.1 (CH), 121.8 (CH), 122.5 (CH), 123.2 (CH), 124.8 (CH), 126.4 (CH), 127.2 (CH), 128.2 (CH), 129.1 (CH), 130.1 (CH), 131.2 (CH), 131.3 (CH), 131.8 (C), 131.9 (C), 134.7 (C), 139.2 (C), 143.6 (C), 153.0 (C), 162.8 (C=O), 177.0 (C=O). Anal. Calcd (%) for $C_{31}H_{24}N_6O_2$: C, 72.64; H, 4.72; N, 16.40; Found: C, 72.64; H, 4.72; N, 16.41.

1-Ethyl-2,12''-dioxo-5',6',7',7a'-tetrahydro-1'H,12''H-dispiro[indoline-3,2'-pyrrolizine-3',6''-indolo[2,1-*b*]quinazoline]-1',1'-dicarbonitrile 5j

Yellow solid (95 mg, 90%); mp 270-272 ℃; IR (υ, cm^{-1}): 2262, 1721, 1695, 1613, 1518, 915. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 1.44 (t, 3H, *J* = 0.8 Hz, -$NCH_2\underline{CH_3}$), 1.97-2.06 (m, 3H, -CH_2), 2.28-2.37 (m, 1H, -CH_2), 3.54 (t, 2H, J = 7.2 Hz, -CH_2), 3.66 (m, 1H, -CH), 4.09 (q, 2H, *J* = 1.6 Hz, -$N\underline{CH_2}CH_3$), 7.67-7.75 (m, 5H, Ar-H), 7.84-7.89 (m, 2H, Ar-H), 8.02-8.04 (m, 1H, Ar-H), 8.42-8.45 (m, 1H, Ar-H), 8.59 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 (-CH_2), 24.7 ($C(CN)_2$), 25.3 (-CH_2), 29.7 (-CH_2), 30.2 (-CH_2), 31.9 (-CH), 55.3 (C_{spiro}), 78.9 (C_{spiro}), 112.2 (CN), 114.6 (CH), 117.9 (CH), 121.9 (CH), 123.7 (CH), 124.8 (CH), 125.4 (CH), 127.2 (CH), 127.3 (CH), 127.5 (CH), 127.6 (CH), 130.3 (C), 135.1 (C), 138.3 (C), 144.3 (C), 146.6 (C), 158.1 V, 159.9 (C=O), 182.5 (C=O). Anal. Calcd (%) for $C_{32}H_{24}N_6O_2$: C, 73.27; H, 4.61; N, 16.02; Found: C, 73.25; H, 4.60; N, 16.00.

8''-Bromo-2''-chloro-1-ethyl-2,12''-dioxo-5',6',7',7a'-tetrahydro-1'H,12''H-dispiro[indoline-3,2'-pyrrolizine-3',6''-indolo[2,1-*b*]quinazoline]-1',1'-dicarbonitrile 5k

Yellow solid (68mg, 78%); mp 280-282 ℃; IR (υ, cm^{-1}): 2218, 1724, 1671, 1575, 1520, 1485, 1240, 1018, 960. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 1.30 (t, 3H, *J* = 2.8 Hz, -$NCH_2\underline{CH_3}$), 2.00-2.07 (m, 2H, -CH_2), 2.28-2.37 (m, 2H, -CH_2), 3.60-3.66 (m, 2H, -CH_2), 3.73-3.82 (m, 1H, -CH), 4.30 (q, 2H, *J* = 6.8 Hz, -$N\underline{CH_2}CH_3$), 7.67-7.75 (m, 3H, Ar-H), 7.84-7.89 (m, 2H, Ar-H), 8.02 (t, 1H, *J* = 7.2 Hz, Ar-H), 8.42-8.44 (m, 2H, Ar-H), 8.59 (d, 2H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 ($C(CN)_2$), 29.3 (-CH_2), 29.7 (-CH_2), 34.7 (-CH_2), 37.1 (-CH), 51.3 (C_{spiro}), 66.7 (C_{spiro}), 92.5 (CH), 113.7 (CN), 119.2 (CH), 123.1 (CH), 123.6 (CH), 125.2 (CH), 127.6 (CH), 130.5 (CH), 130.9 (CH), 133.3 (CH), 135.3 (CH), 137.7 (CH), 138.9 (C), 140.8 (C), 143.9 (C), 144.5 (C), 146.5 (C), 149.9 (C), 155.9 (C), 157.9 (C=O), 181.4 (C=O). Anal. Calcd (%) for $C_{30}H_{18}BrClN_6O_2$: C, 57.08; H, 2.98; N, 13.78; Found: C, 57.08; H, 2.97; N, 13.79.

9''-Chloro-1-ethyl-2,12''-dioxo-5',6',7',7a'-tetrahydro-2'H,12''H-dispiro[indoline-3,1'-pyrrolizine-3',6''-indolo[2,1-*b*]quinazoline]-2',2'-dicarbonitrile 5l

Yellow solid (69mg, 70%); mp 270-272℃; IR (υ, cm^{-1}): 2195, 1720, 1685, 1560, 1468, 1232. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.88 (t, 3H, *J* = 7.2 Hz, -$NCH_2\underline{CH_3}$), 2.06-2.07 (m, 3H, -CH_2), 2.19-2.22 (m, 1H, -CH_2), 2.87-2.90 (m, 2H, -CH_2), 3.27-3.32 (m, 1H, -CH), 3.69 (q, 2H, *J* = 9.2 Hz, -$NCH_2\underline{CH_3}$), 6.57-6.59 (m, 1H, Ar-H), 6.96-7.03 (m, 4H, Ar-H), 7.17 (t, 1H, *J* = 6 Hz, Ar-H), 7.50-7.55 (m, 1H, Ar-H), 7.79-7.86 (m, 2H, Ar-H) 8.28-8.30 (m, 1H, Ar-H), 8.42 (d, 1H, *J* = 4.8 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.2 (-CH_3), 25.8 ($C(CN)_2$), 27.6 (-CH_2), 29.7 (-CH_2), 31.5 (-CH_2), 50.6 (-CH_2), 55.2 (-CH), 64.5 (C_{spiro}), 75.7 (C_{spiro}), 91.6 (C), 113.2 (CH), 113.4 (CH), 117.4 (CH), 117.6 (CH), 118.7 (CH), 118.8 (CH), 121.4 (CH), 126.8 (CH), 127.5 (CH), 127.8 (CH), 128.6 (C), 129.1 (C), 131.8 (C), 134.5 (C), 136.7 (C), 146.6 (C), 158.8 (C), 159.1 (C=O), 180.9 (C=O). Anal. Calcd (%) for $C_{32}H_{23}ClN_6O_2$: C, 68.75; H, 4.15; N, 15.03; Found: C, 68.74; H, 4.13; N, 15.05.

8''-Chloro-5-iodo-2,12''-dioxo-1',7a'-dihydro-3'H,7'H,12''H-dispiro[indoline-3,6'-pyrrolo[1,2-*c*]thiazole-5',6''-indolo[2,1-*b*]quinazoline]-7',7'-dicarbonitrile 5m

Yellow solid (95 mg, 80%); mp 279-281 ℃; IR (υ, cm^{-1}): 2874, 2214, 1710, 1680, 1528, 1125, 980, 915. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 3.22-3.26 (m, 1H, -CH_2), 3.31-3.36 (m, 1H, -CH_2), 4.26 (d, 1H, *J* = 6.4 Hz, -CH_2), 4.34-4.39 (m, 1H, -CH_2), 7.67-7.75 (m, 3H, Ar-H), 7.84-7.89 (m, 3H, Ar-H), 8.03 (d, 1H, *J* = 8 Hz, Ar-H), 8.42-8.44 (m, 1H, Ar-H), 8.59 (d, 2H, *J* = 8.8 Hz, Ar-H), 10.33 (s, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 22.7 ($C(CN)_2$), 29.3 (-CH_2), 31.9 (-CH_2), 37.1 (-CH), 57.9 (C_{spiro}), 91.3 (C_{spiro}), 119.2 (-C), 122.5 (-CN), 123.1 (-CH), 123.6 (-CH), 125.2 (-CH), 127.6 (-CH), 129.0 (-CH), 130.5 (-CH), 130.9 (-CH), 132.9 (-CH), 133.3 (-CH), 135.3 (-CH), 136.6 (-C), 137.7 (C), 138.2 (C), 141.1 (C), 142.1 (C), 143.9 (C), 144.5 (C), 146.5 (C), 157.9 (C=O), 181.4 (C=O). Anal. Calcd (%) for $C_{29}H_{16}ClISN_6O_2$: C, 51.61; H, 2.39; N, 12.45; Found: C, 51.61; H, 2.39; N, 12.44.

5'-Benzyl-8''-bromo-2,12''-dioxo-12''H-dispiro[indoline-3,3'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-4',4'-dicarbonitrile 5n

Yellow solid (75 mg, 75%); mp 295-297 ℃; IR (υ, cm^{-1}): 3216, 2896, 2216, 1714, 1606, 1598, 989. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 1.25 (t, 3H, *J* = 3.2 Hz, -$NCH_2\underline{CH_3}$), 2.03 (s, 1H, -NH), 3.02-3.05 (m, 1H, -CH_2), 3.17 (d, 1H, *J* = 4 Hz, -CH), 3.65 (q, 2H, *J* = 2.4 Hz, -CH_2), 3.89 (q, 1H, *J* = 4 Hz, -$NCH_2\underline{CH_3}$), 7.67-7.71 (m, 4H, Ar-H), 7.84-7.90 (m, 4H, Ar-H), 8.02-8.04 (m, 2H, Ar-H), 8.42-8.44 (m, 3H, Ar-H), 8.53 (d, 3H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.1 (-CH_3), 22.6 ($C(CN)_2$), 29.6 (-CH_2), 31.9 (-CH_2), 49.5 (-CH), 56.8 (C_{spiro}), 78.1 (C_{spiro}), 119.5 (-CN), 120.7 (-CN), 121.9 (-CH), 123.4 (-CH), 123.6 (-CH), 124.5 (-CH), 127.6 (-CH), 128.2 (-CH), 130.5 (-CH), 130.9 (-CH), 134.1 (-CH), 135.3 (-CH), 137.2 (-CH), 139.1 (-CH), 140.6 (-CH), 141.7 (-CH), 142.8 (-CH), 143.7 (-CH), 144.9 (-C), 146.5 (C), 147.5 (C), 150.3 (C), 152.7 (C), 154.4 (C), 157.9 (C), 159.1 (C), 163.0 (C), 168.6 (C=O), 181.2 (C=O). Anal. Calcd (%) for $C_{36}H_{25}BrN_6O_2$: C, 66.16; H, 3.86; N, 12.86; Found: C, 66.15; H, 3.85; N, 12.88.

5'-((1H-Indol-2-yl)methyl)-5,8''-dichloro-1-ethyl-2,12''-dioxo-12''H-dispiro[indoline-3,3'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazoline]-4',4'-dicarbonitrile 5o

Yellow solid (84 mg, 70%); mp 271-273 ℃; IR (υ, cm^{-1}): 3410, 3218, 2268, 1718, 1622, 1519, 1238, 881. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 1.57 (t, 3H, *J* = 1.2 Hz, -$NCH_2\underline{CH_3}$), 2.17 (s, 1H, -NH), 3.21 (q, 1H, *J* = 8 Hz, -CH_2), 3.37-3.42 (m, 1H, -CH_2), 3.96 (q, 1H, *J* = 8 Hz, -CH), 4.21 (q, 2H, *J* = 2.4 Hz, -$NCH_2\underline{CH_3}$), 7.39 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.53 (d, 3H, *J* = 9.2 Hz, Ar-H), 7.67-7.75 (m, 3H, Ar-H), 7.84-7.89 (m, 3H, Ar-H), 8.02-8.04 (m, 2H, Ar-H), 8.42-8.44 (m, 2H, Ar-H), 8.59 (d, 1H, *J* = 8.4 Hz, Ar-H), 10.68 (s, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$) (δ,

ppm): 14.1 (-CH_3), 25.2 (-CH_2), 29.7 ($C(CN)_2$), 36.1 (-CH_2), 51.6 (-CH), 55.3 (C_{spiro}), 68.9 (C_{spiro}), 112.2 (-CN), 113.0 (-CN), 114.6 (-CH), 117.6 (-CH), 117.9 (-CH), 121.3 (-CH), 121.9 (-CH), 122.6 (-CH), 123.7 (-CH), 124.8 (-CH), 125.4 (-CH), 126.1 (-CH), 127.2 (-CH), 127.3 (-CH), 127.5 (-CH), 127.6(-CH), 130.5 (-CH), 130.7 (-CH), 135.1 (-CH), 135.2 (C), 137.6 (C), 138.3 (C), 141.9 (C), 144.3 (C), 146.3 (C), 146.6 (C), 149.4 (C), 158.1 (C), 159.9 (C=O), 182.5 (C=O). Anal. Calcd (%) for $C_{38}H_{25}Cl_2N_7O_2$: C, 66.87; H, 3.69; N, 14.36; Found: C, 66.88; H, 3.67; N, 14.34.

(2-(8''-Chloro-4',4'-dicyano-1-ethyl-2,12''-dioxo-12''H-dispiro[indoline-3,3'-pyrrolidine-2',6''-indolo[2,1-*b*]quinazolin]-5'-yl)acetyl)glycine 8

Yellow solid (73.5 mg, 67%); mp 264-266 ℃; IR (υ, cm^{-1}): 3340, 3215, 3075, 2258, 1716, 1685, 1595, 1118. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm (*J*, Hz): 0.85 (t, 3H, *J* = 3.6 Hz, -$NCH_2\underline{CH_3}$), 2.04 (s, 1H, -NH), 3.43 (q, 2H, *J* = 8.4 Hz, -$NCH_2\underline{CH_3}$), 3.66 (s, 1H, -CH), 3.47 (s, 2H, -CH_2), 7.00 (s, 1H, -NH), 7.46-7.51 (m, 1H, Ar-H), 7.58 (t, 1H, = 3.6 Hz, Ar-H), 7.67-7.71 (m, 2H, Ar-H), 7.84-7.88 (m, 2H, Ar-H), 8.02-8.04 (m, 2H, Ar-H), 8.42-8.44 (m, 1H, Ar-H), 8.62-8.65 (m, 2H, Ar-H), 13.45 (s, 1H, -OH). ^{13}C NMR (100 MHz, $CDCl_3$) (δ, ppm): 14.0 (-CH_3), 23.0 ($C(CN)_2$), 29.7 (-CH_2), 32.0 (-CH_2), 50.1 (-CH), 61.1 (C_{spiro}), 86.0 (C_{spiro}), 111.9 (-CN), 112.2 (-CN), 116.7 (-CH), 119.6 (-CH), 119.7 (-CH), 123.3 (-CH), 123.4 (-CH), 123.7 (-CH), 124.7 (-CH), 124.9 (-CH), 125.8 (-CH), 127.5 (-CH), 130.5 (C), 135.2 (C), 137.7 (C), 139.4 (C), 142.4 (C), 144.3 (C), 146.5 (C), 157.8 (C=O), 167.3 (C=O), 176.1 (C=O), 182.2 (C=O). Anal. Calcd (%) for $C_{33}H_{24}ClN_7O_5$: C, 62.51; H, 3.82; N, 15.46; Found: C, 62.50; H, 3.80; N, 15.48.

Materials and Methods

Growth media and Reagents

All bacterial media and supplements including Mueller-Hinton cation supplemented broth II (MHBII), Mueller-Hinton agar (MHA) and Tryptic soy broth (TSB) were purchased from Becton-Dickinson (Franklin Lakes, NJ, USA). All other chemicals and antibiotics were procured from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute Medium (RPMI) and Fetal Bovine Serum (FBS) were purchased from Lonza (Lonza, USA). All methods were performed in accordance with the relevant guidelines and regulations.

Bacterial strains

Compounds was screened against a bacterial panel consisting of ESKAPE pathogens, namely *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumonia* BAA-1705, *Acinetobacter baumannii*BAA-1605 and *Pseudomonas aeruginosa* ATCC 27853. The panel was further expanded to include drug-resistant clinical *S. aureus* strains including those resistant to Vancomycin and other clinically-utilized antibiotics. These strains were procured from Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection (BEI/NARSA/ATCC, USA) and routinely cultivated on MHA and MHBII. Before starting the experiment, a single colony was picked from MHA plate, inoculated in MHBII and incubated overnight at 37 ℃ with shaking for 18–24 h to get the starter culture.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of compounds was conducted according to the CLSI guidelines using broth microdilution assay.[2] 10 mg/mL stock solutions of test compounds were prepared in DMSO. Bacterial cultures were inoculated in MHBII and optical density (OD) was measured at 600nm, followed by dilution to achieve ~10^6 CFU/mL. The compounds were tested from 64–0.5 mg/L in two-fold serial diluted fashion with 2.5 μL of each concentration added to well of a 96-well round bottom microtiter plate. Later, 97.5 μL of bacterial suspension was added to each well containing either test compound or appropriate controls. The plates were incubated at 37 ℃ for 18-24 h following which the MIC was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

Cell cytotoxicity against Vero cells

Cell toxicity was performed against Vero cells using the MTT assay.[3] ~10^3 cells/well were seeded in 96 well plate and incubated at 37℃ in an 5% CO_2 atmosphere. After 24 h, compound was added ranging from 100-12.5 µg/mL concentration and incubated for 72 h. After the incubation was over, MTT was added in each well, incubated at 37℃for further 4 h, residual medium was discarded, 0.1 mL of DMSO was added to solubilise the formazan crystals and OD was taken at 540 nm for the calculation of CC_{50}. CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as positive control and each experiment was repeated in triplicate.

Bacterial time kill kinetics with 5b

The presence or absence of bactericidal activity was assessed by the time-kill method as described earlier.[4] Briefly, *S. aureus* ATCC 29213 were diluted ~10^6 CFU/mL in MHBII and treated with 1X and 10X MIC of **5b** and Vancomycin and incubated at 37℃ with shaking for 24 h. 100 µL samples were collected at 0, 1, 6 and 24 h, serially diluted in PBS and plated on MHA followed by incubation at 37 ℃ for 18–20 h. The kill curves were constructed by counting the colonies from plates and plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of compound. Each experiment was repeated three times in duplicate and the mean data were plotted.

Drug interaction of 5b with FDA approved drugs

Interaction of **5b** with FDA approved drugs was tested by the checkerboard method. Serial two-fold dilutions of each drug were freshly prepared prior to testing. **5b** was two-fold diluted along the abscissa while the antibiotics were serially diluted along the ordinate in 96 well microtiter plate. 95 μL of ~10^5 CFU/mL was added to each well and plates were incubated at 37℃ for 24 h. After the incubation, the ΣFICs (fractional inhibitory concentrations) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone and FIC B is the MIC of drug B in the combination/MIC of drug B alone. The combination is considered synergistic when the ∑FIC is ≤0.5, indifferent when the ∑FIC is >0.5 to 4, and antagonistic when the ∑FIC is >4.[5]

References

1. V. M. Sharma, P. Prasanna, K. A. Seshu, B. Renuka, C. L. Rao, G. S. Kumar, R. Rajagopalan, *Bioorg. Med. Chem. Lett.*, 2002, **12,** 2303-2307.

2. CLSI.Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
3. P. R. Twentyman, M. Luscombe, A study of some variables in atetrazolium dye (MTT) based assay for cell growth and chemosensitivity, *Br. J. Cancer,* 1987, **56** 279-285, https://doi.org/10.1038/bjc.1987.190
4. R. Thakare, A. K. Singh, S. Das, N. Vasudevan, G. R. Jachak, D. S. Reddy, A. Dasgupta, S. Chopra, Repurposing Ivacaftor for treatment of Staphylococcus aureus infections, *Int. J. Antimicrob. Agents*, 2017, **50**, 389-392, https://doi.org/10.1016/j.ijantimicag.2017.03.020.
5. F. C. Odds, *J. Antimicrob. Chemoth*er., 2003, **52**, 1. https://doi.org/10.1093/jac/dkg301

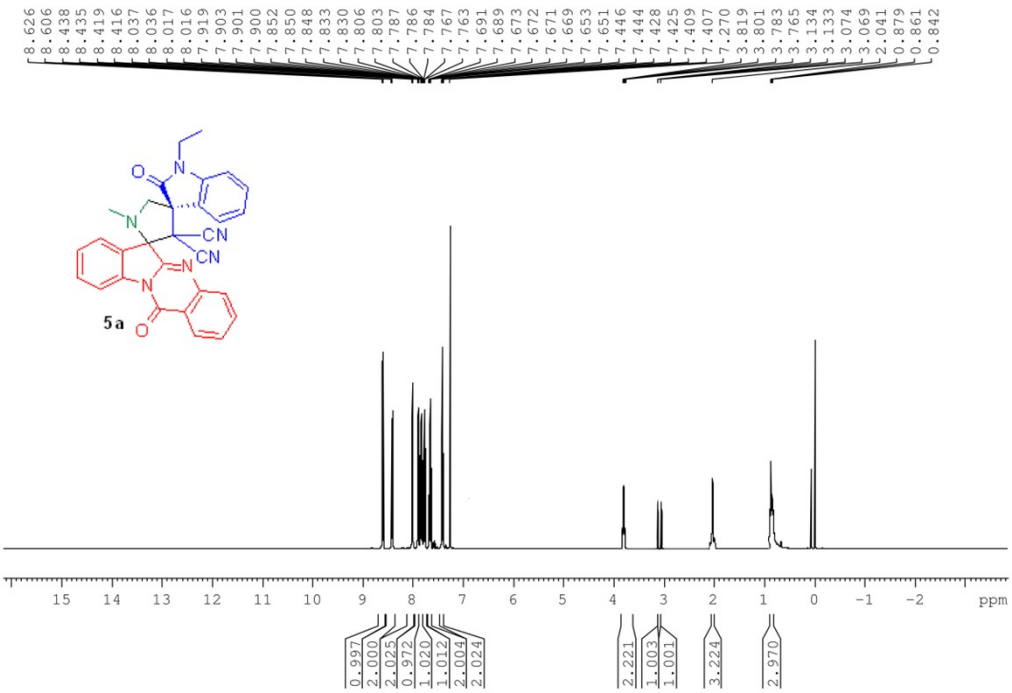

Figure S1 ^{1}H NMR spectrum of **5a**

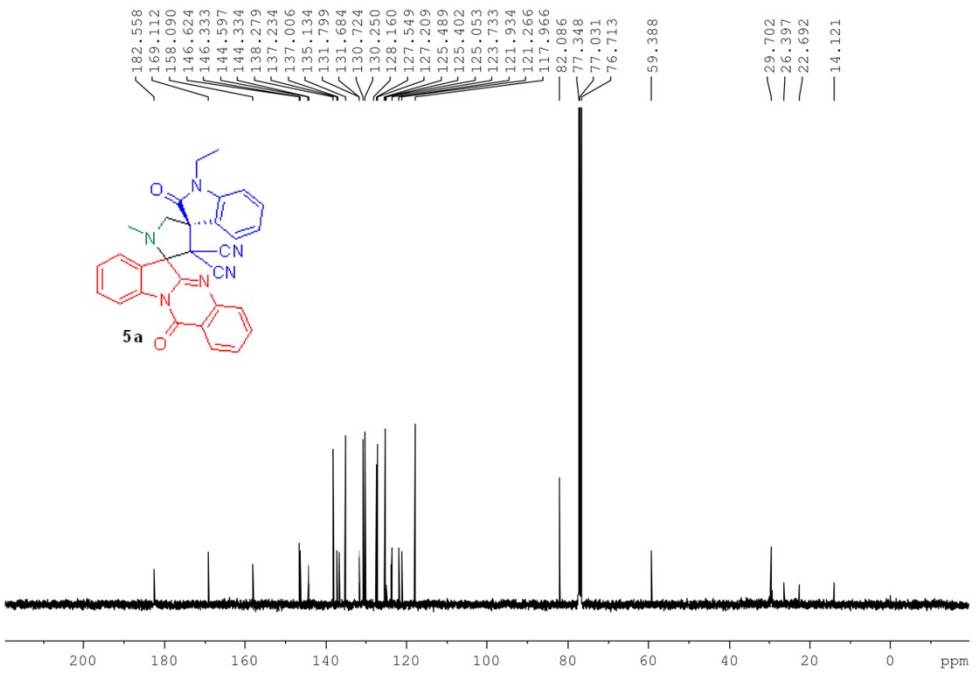

Figure S2 ^{13}C NMR spectrum of **5a**

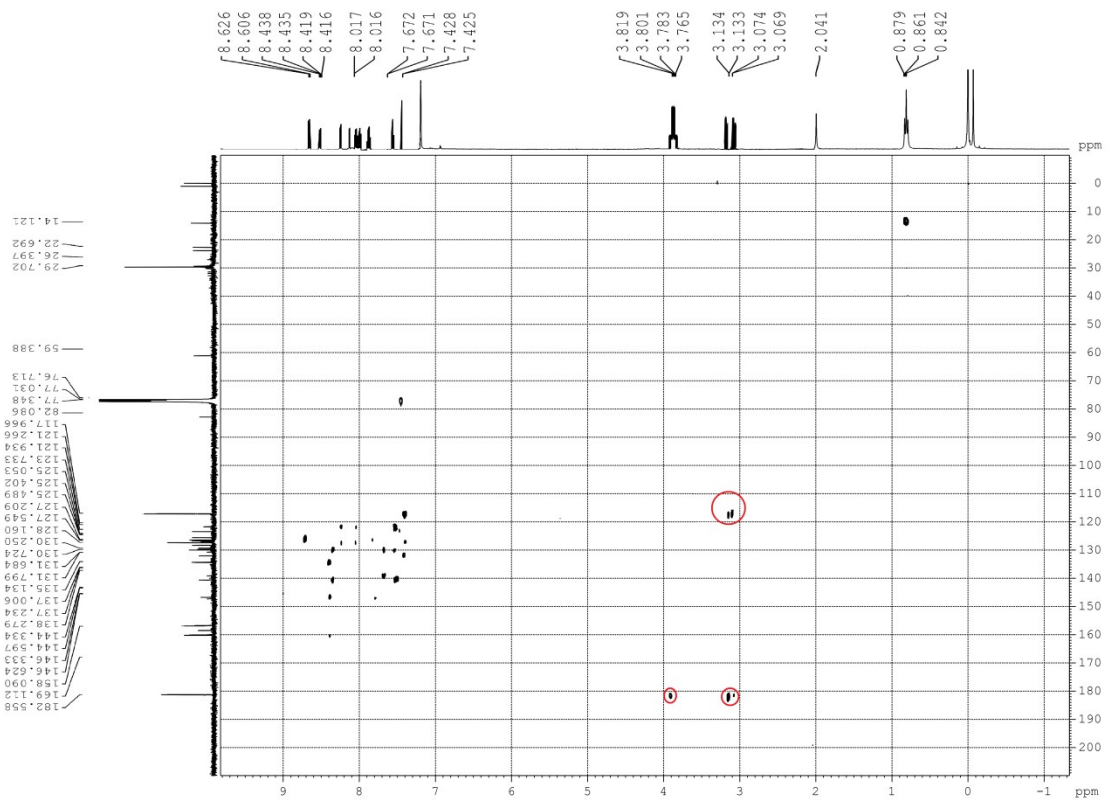

Figure S3 HMBC spectrum of **5a**

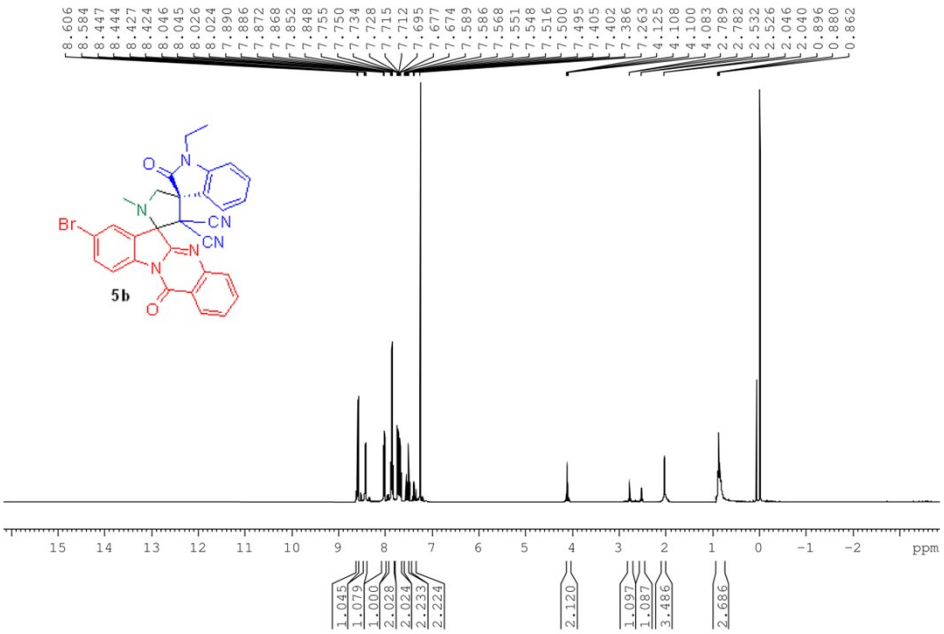

Figure S4 ^{1}H NMR spectrum of **5b**

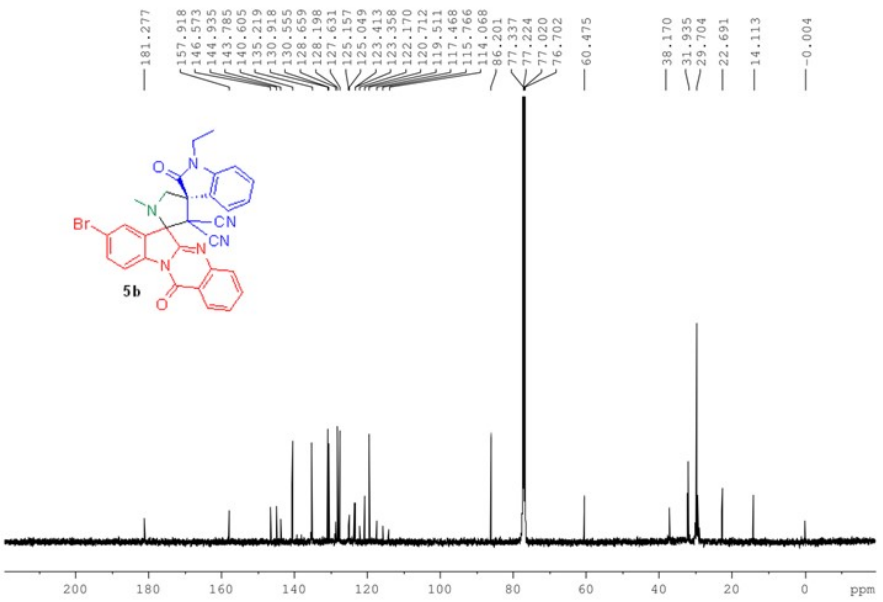

Figure S5 ^{13}C NMR spectrum of **5b**

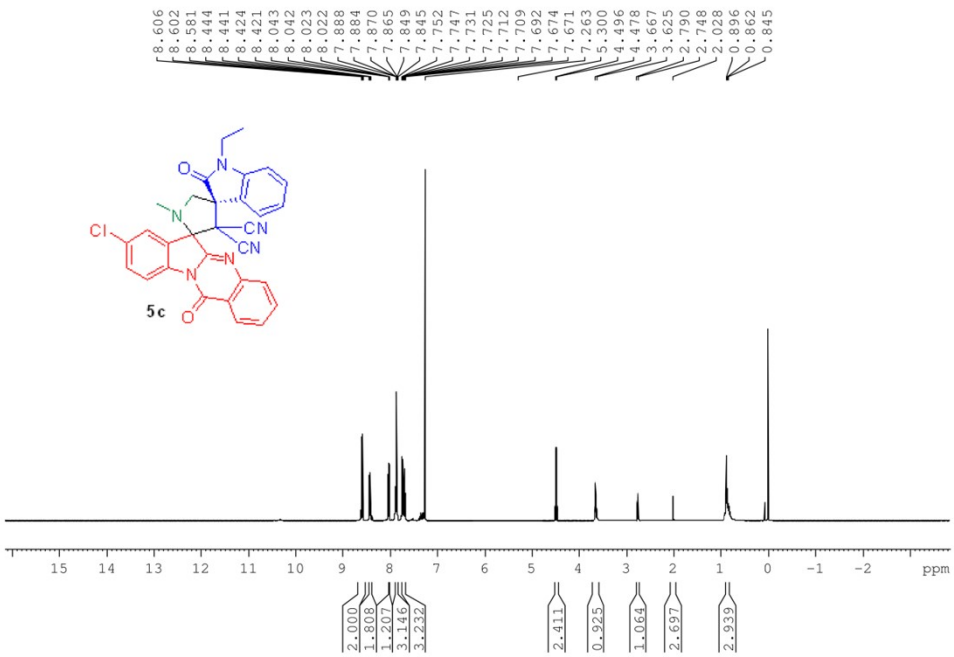

Figure S6 ^{1}H NMR spectrum of **5c**

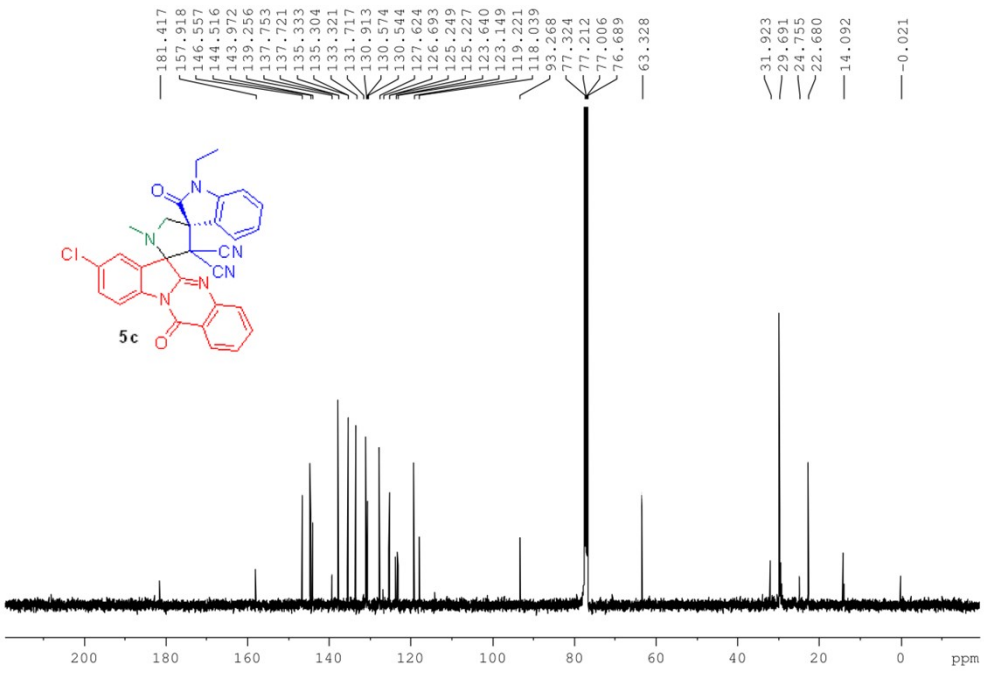

Figure S7 ^{13}C NMR spectrum of **5c**

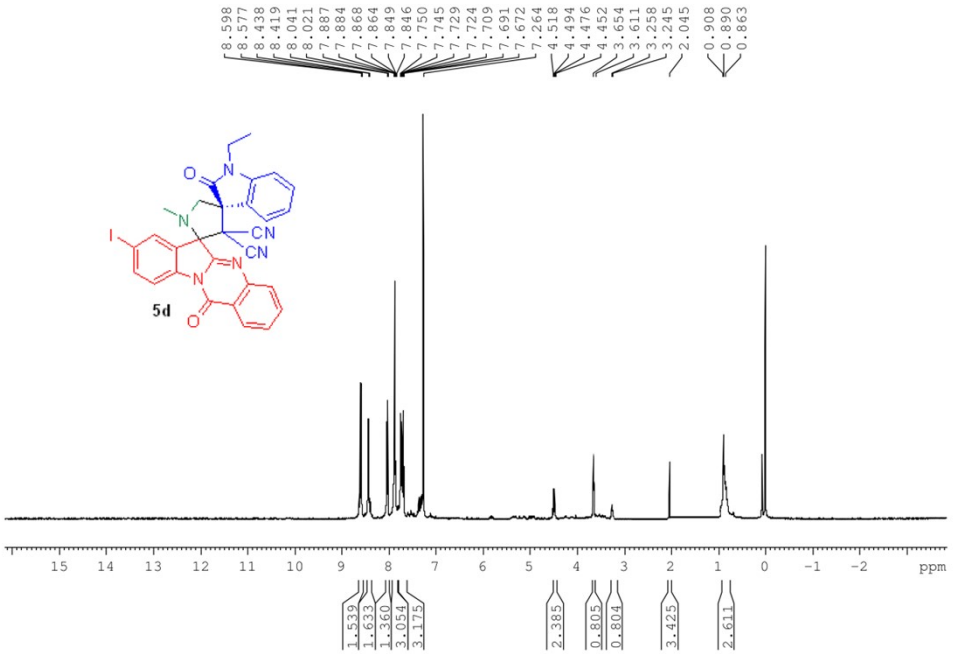

Figure S8 ^{1}H NMR spectrum of **5d**

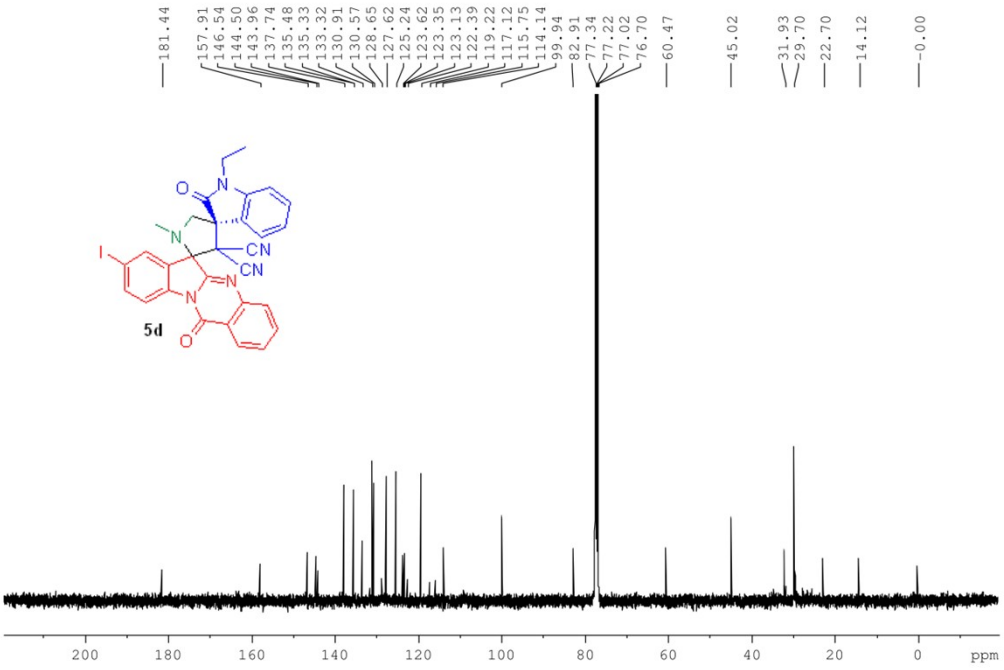

Figure S9 ^{13}C NMR spectrum of **5d**

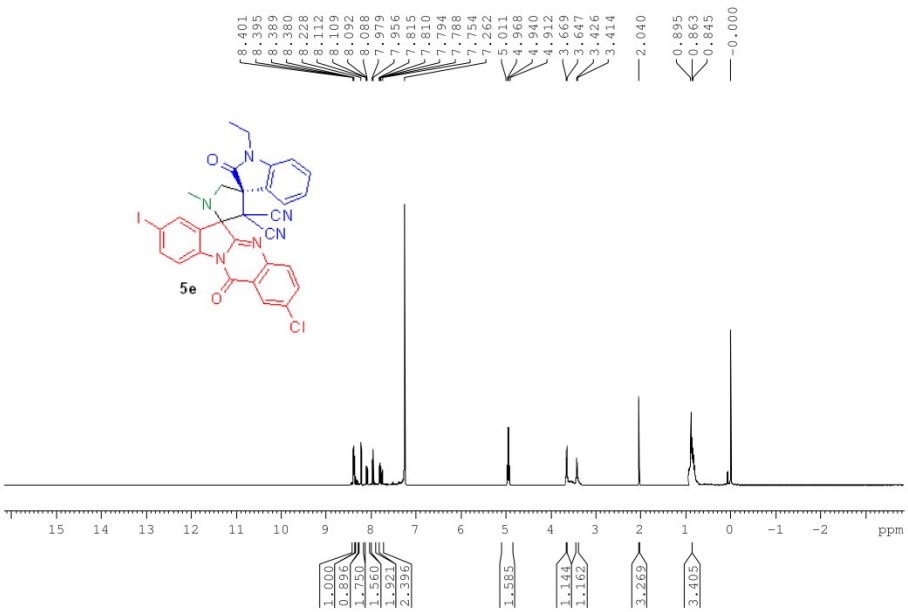

Figure S10 ^{1}H NMR spectrum of **5e**

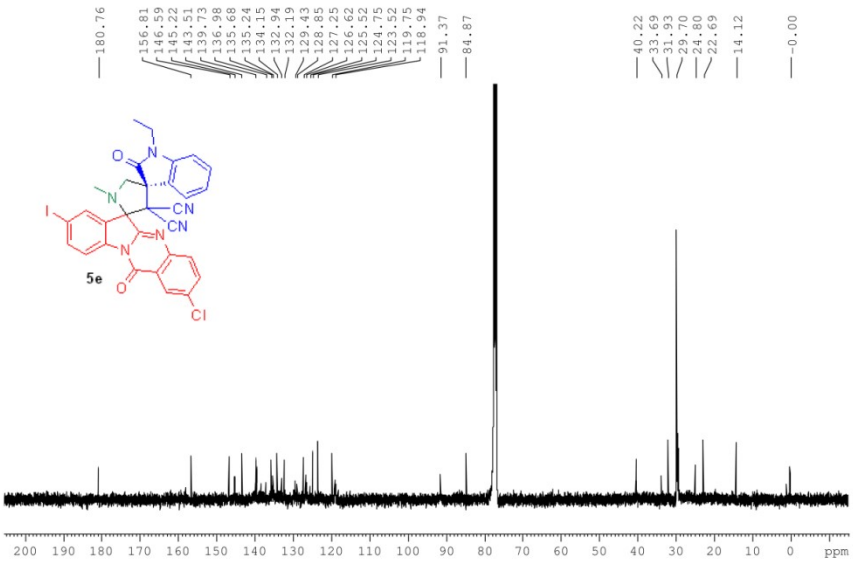

Figure S11^{13}C NMR spectrum of **5e**

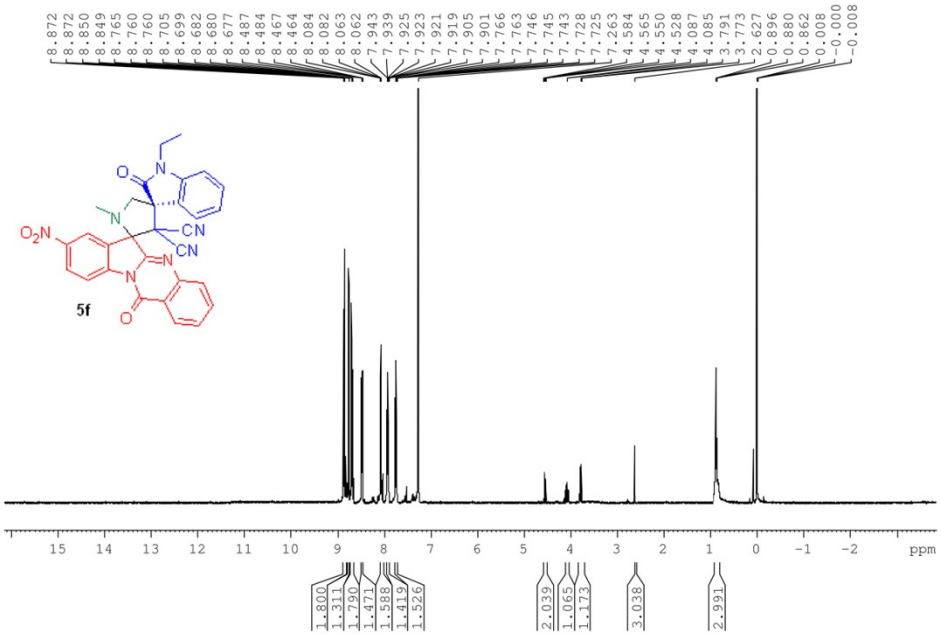

Figure S12 ^{1}H NMR spectrum of **5f**

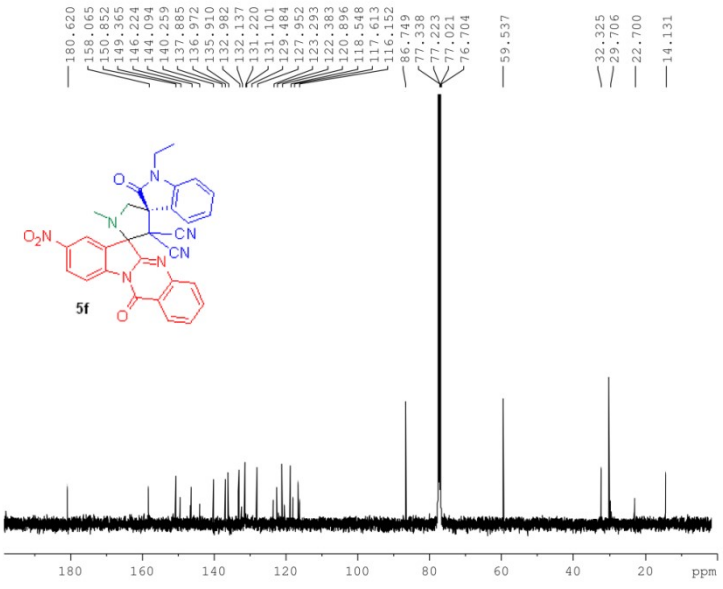

Figure S13 ^{13}C NMR spectrum of **5f**

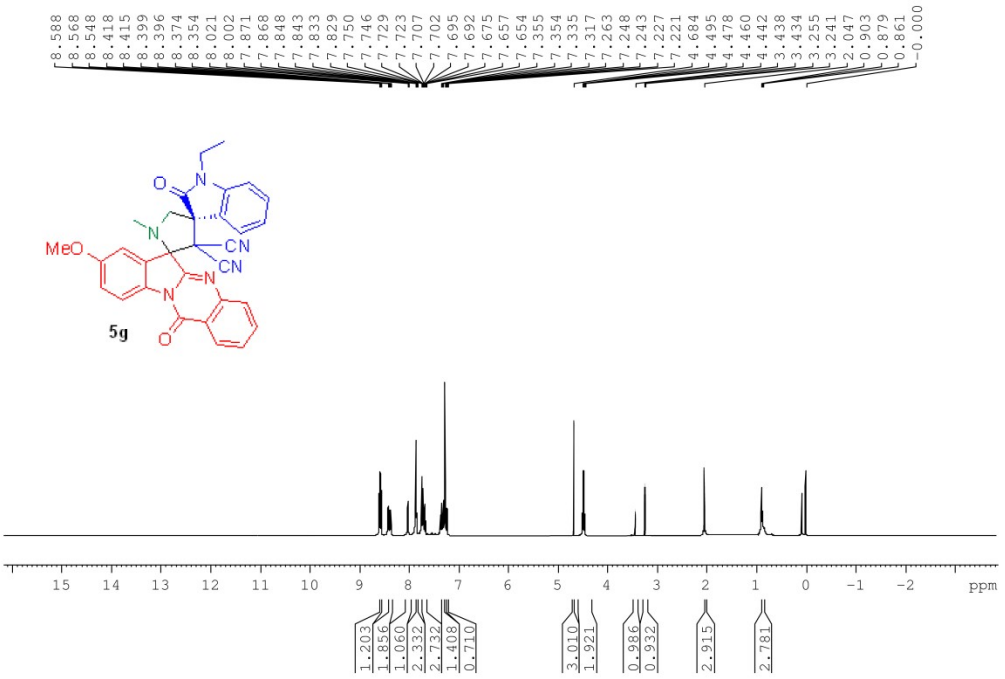

Figure S14 ^{1}H NMR spectrum of **5g**

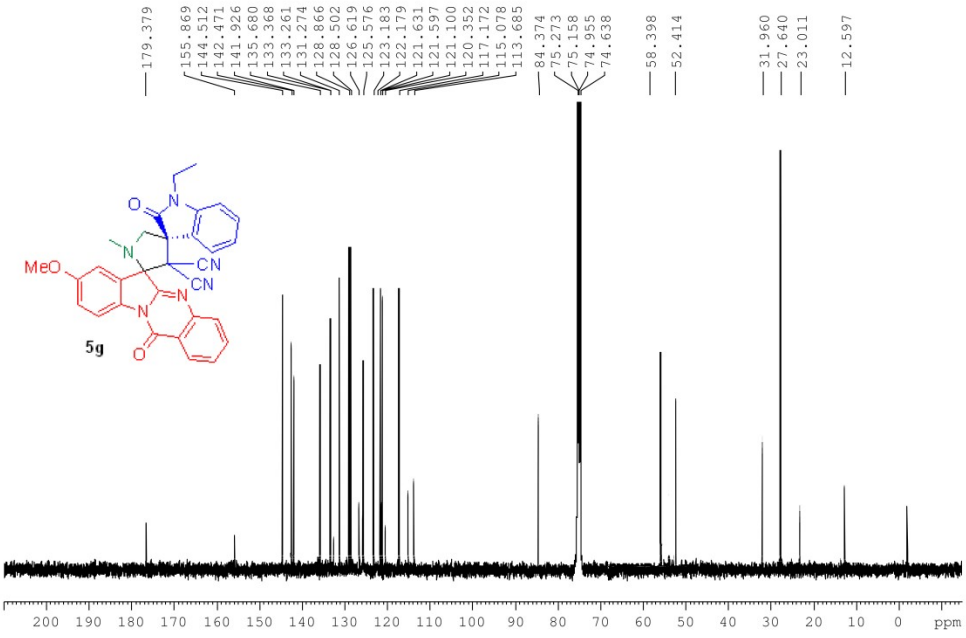

Figure S15 ^{13}C NMR spectrum of **5g**

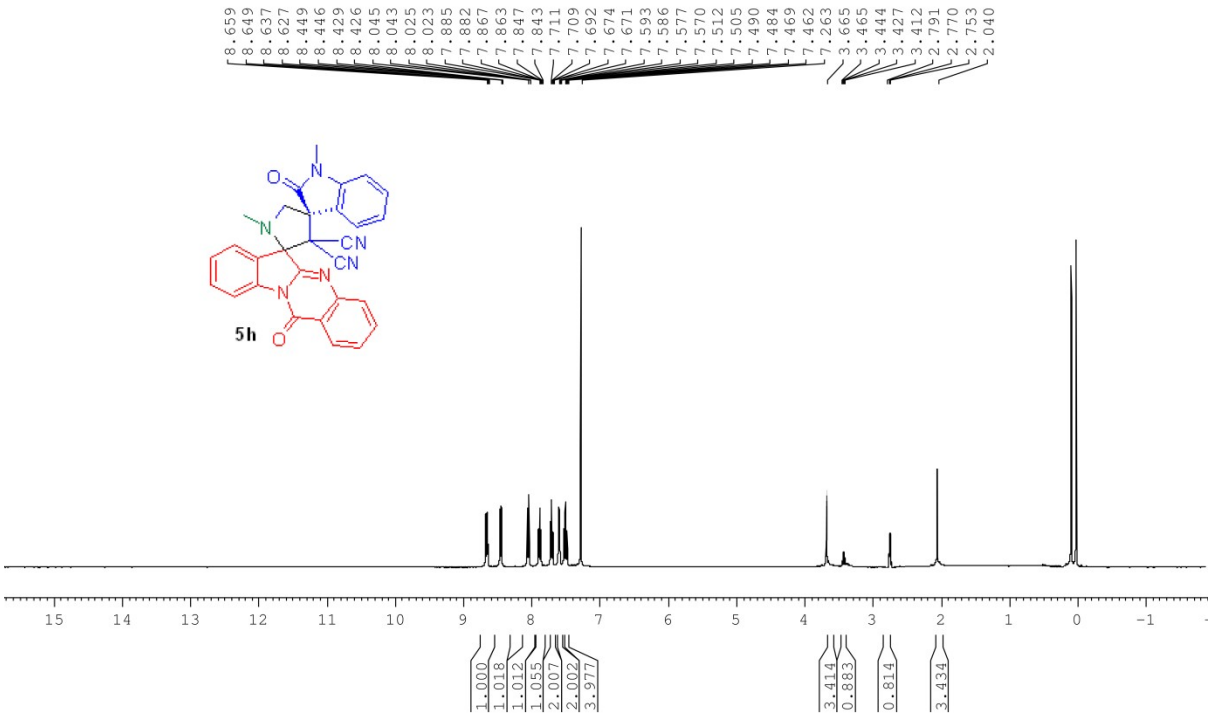

Figure S16[1]H NMR spectrum of **5h**

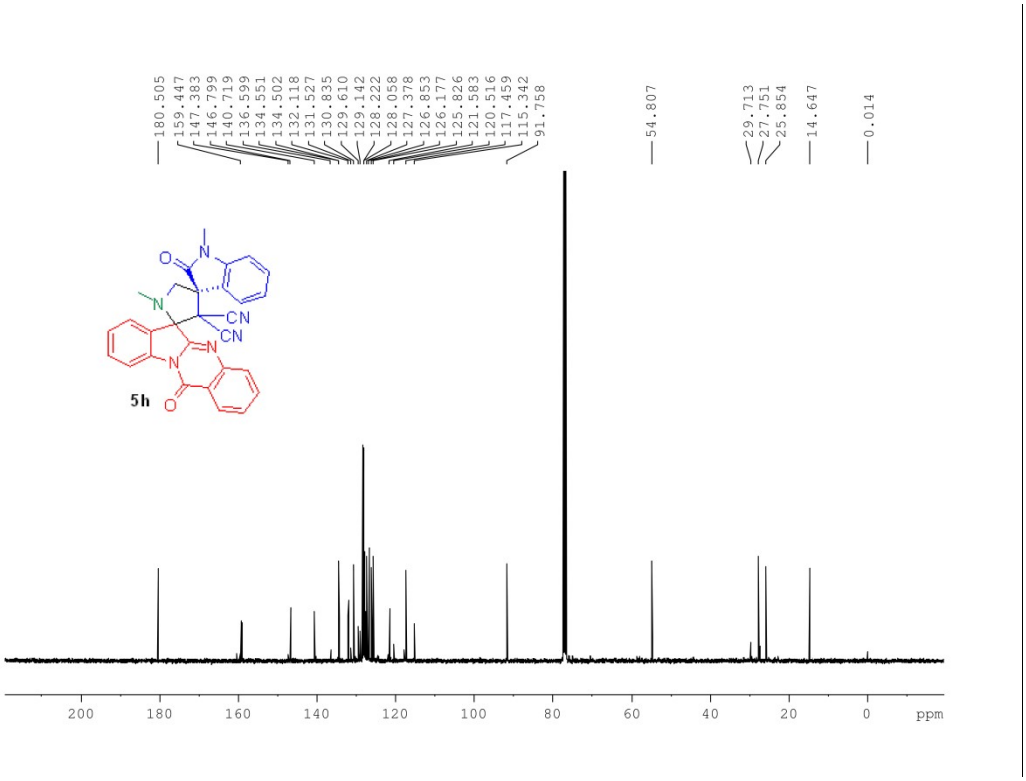

Figure S17 [13]C NMR spectrum of **5h**

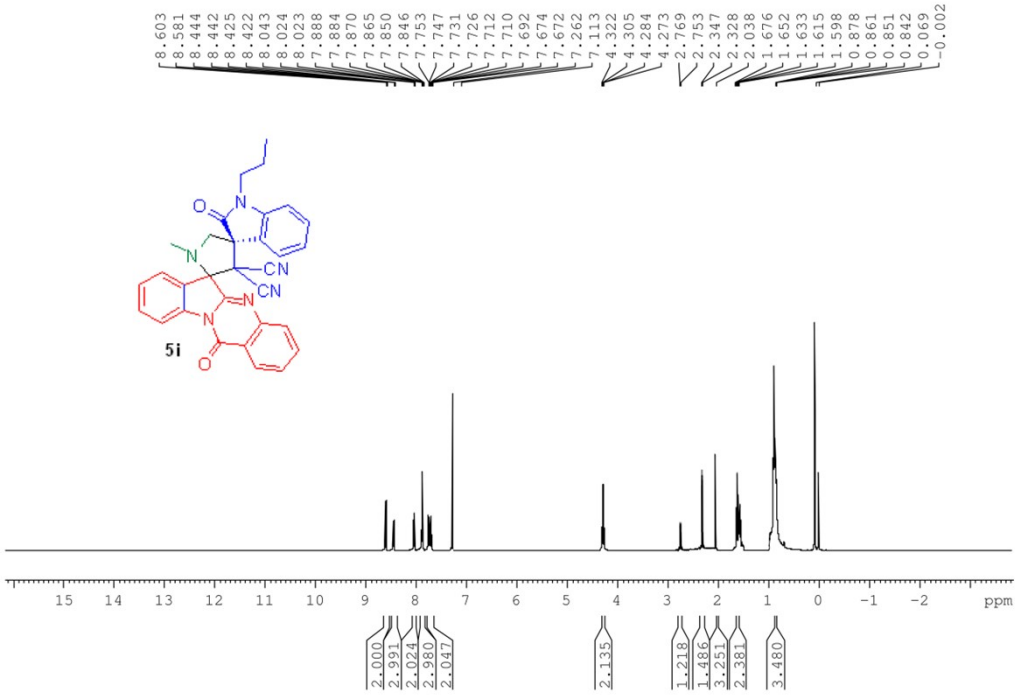

Figure S18 ^{1}H NMR spectrum of **5i**

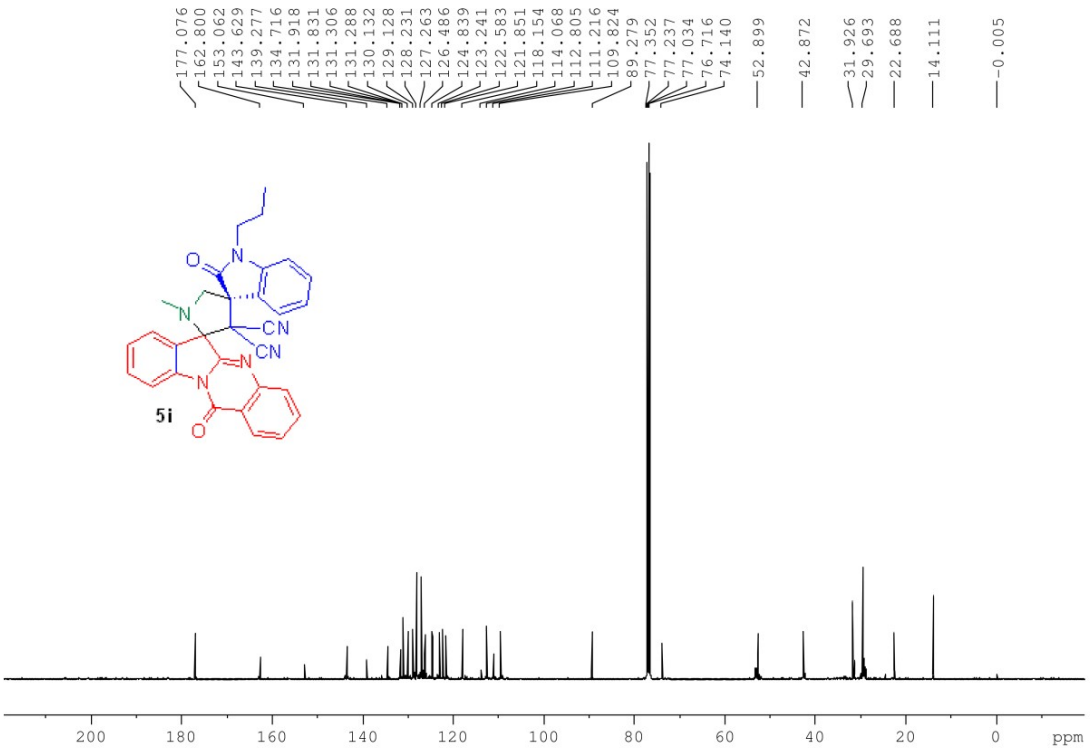

Figure S19 ^{13}CNMR spectrum of **5i**

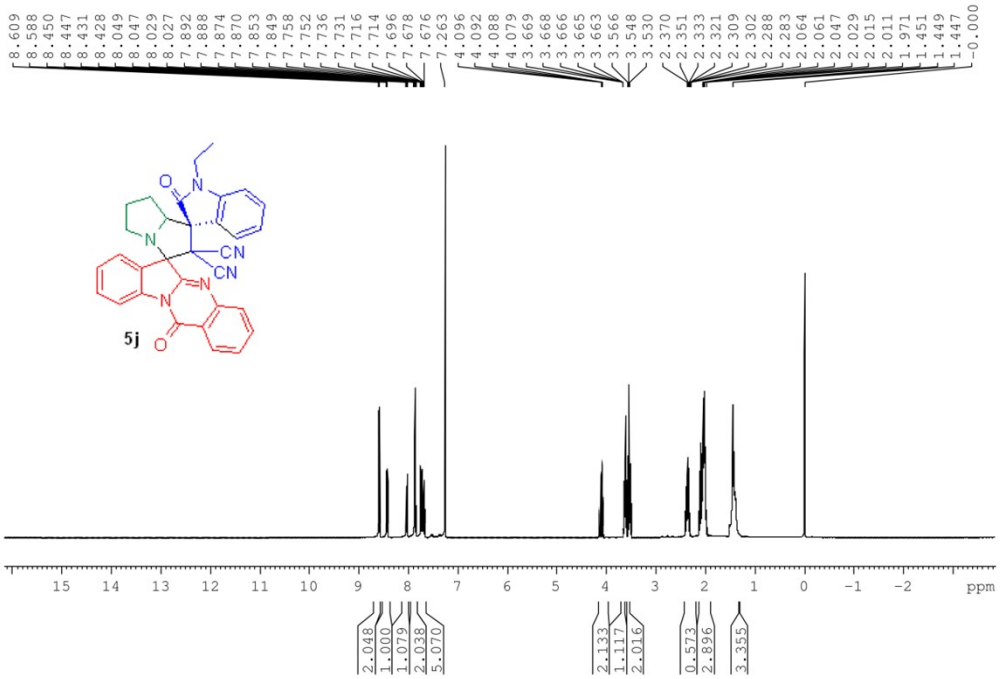

Figure S20 ^{1}H NMR spectrum of **5j**

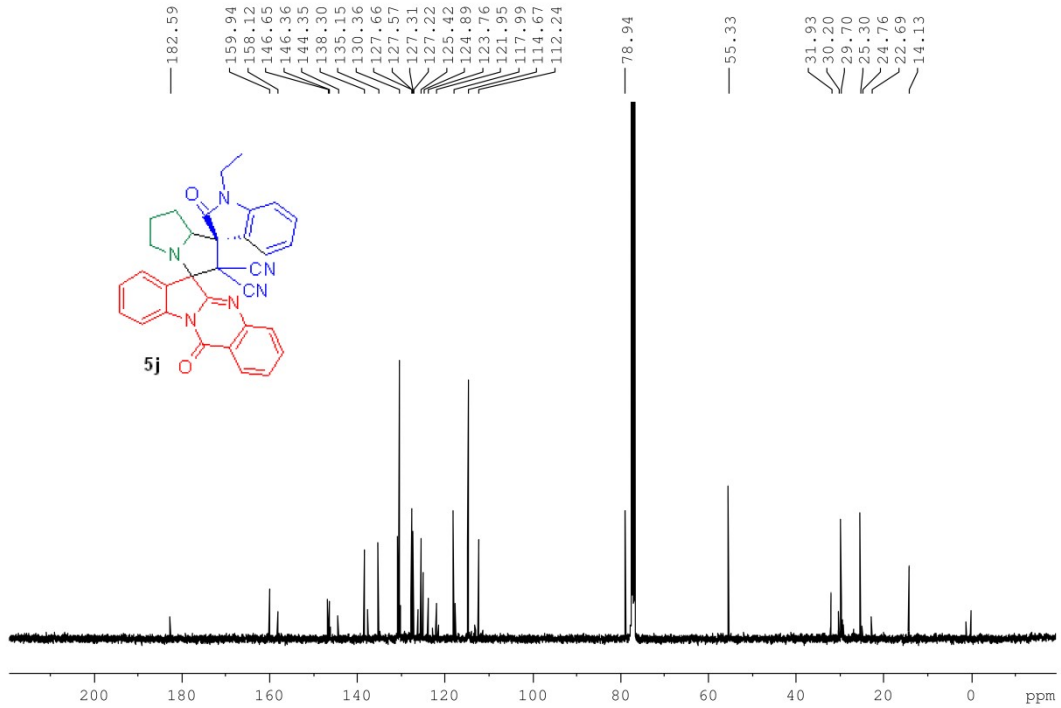

Figure S21 ^{13}C NMR spectrum of **5j**

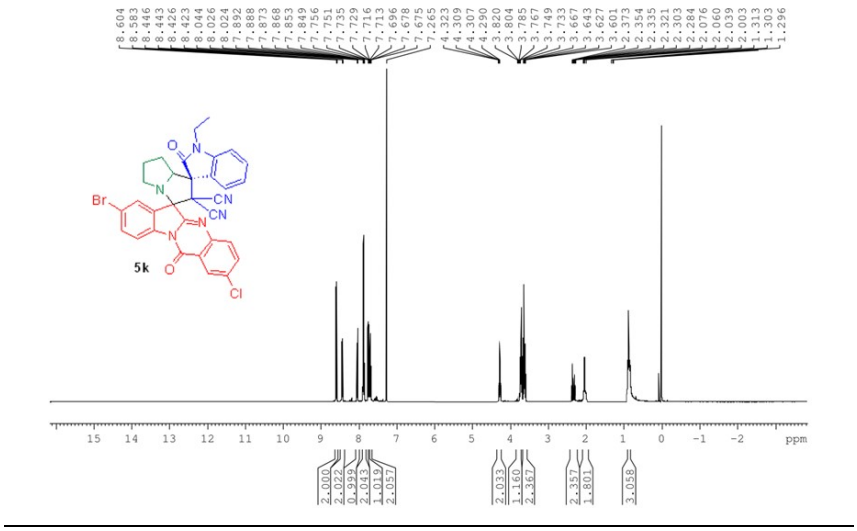

Figure S22 ^{1}H NMR spectrum of **5k**

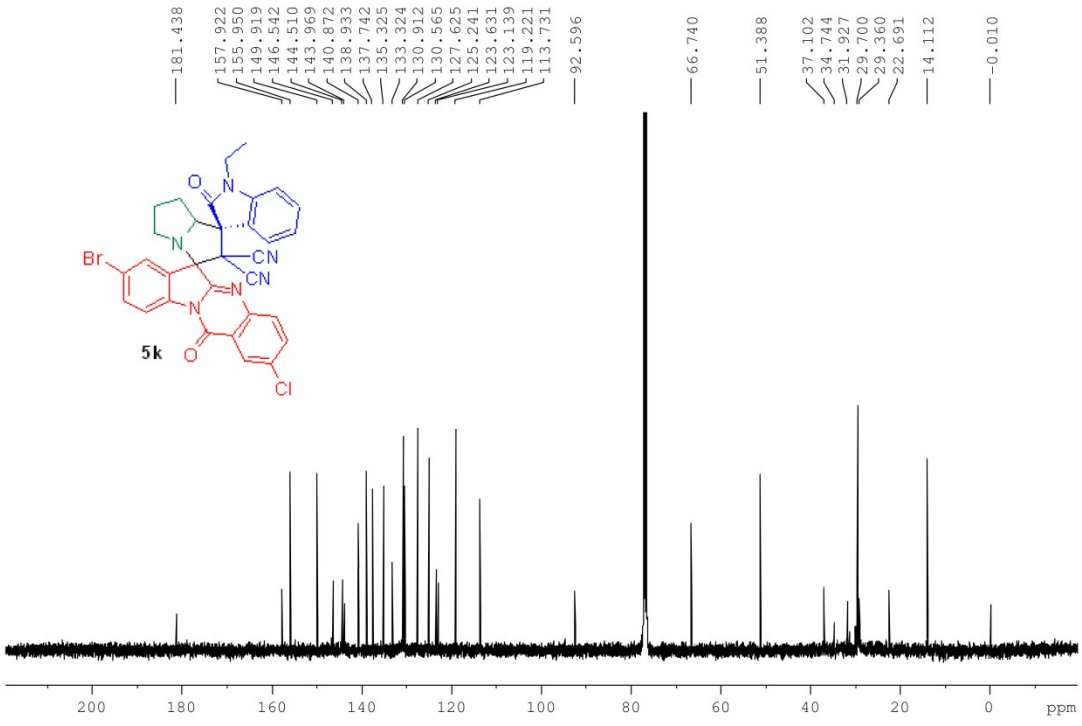

Figure S23 ^{13}C NMR spectrum of **5k**

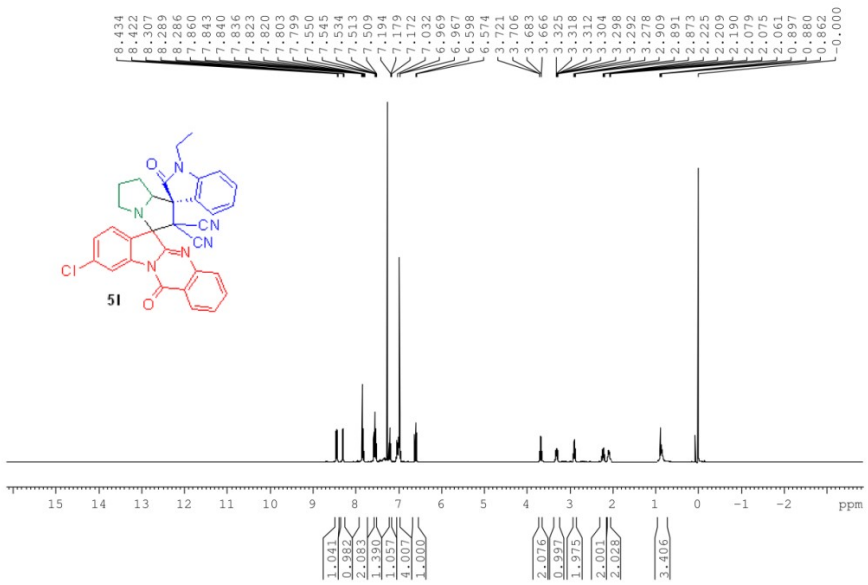

Figure S24 ^{1}H NMR spectrum of **5l**

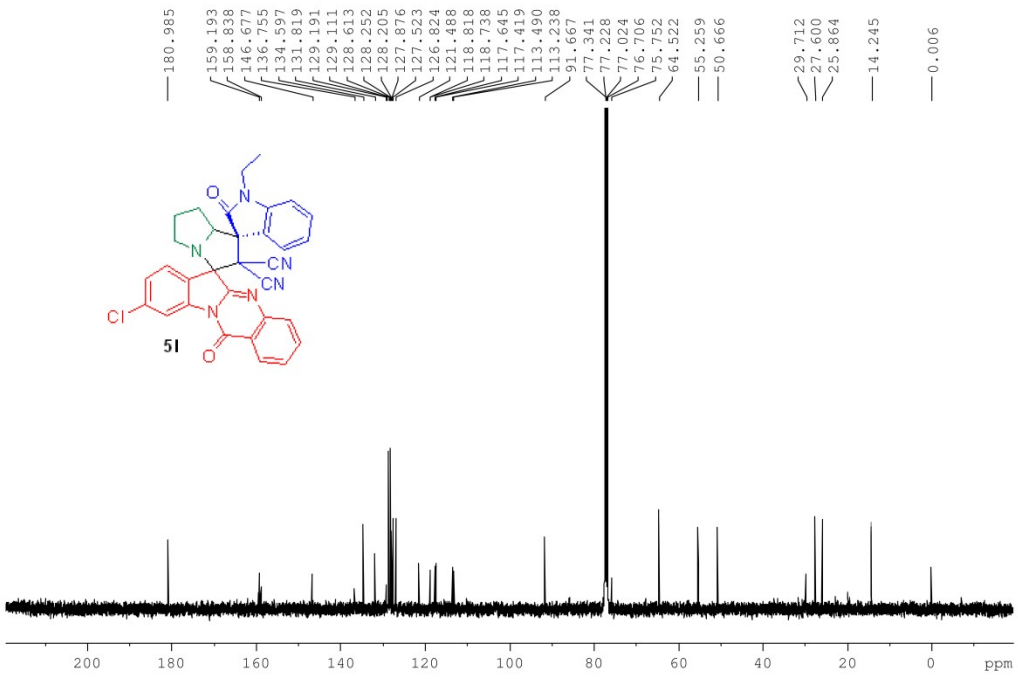

Figure S25 ^{13}C NMR spectrum of **5l**

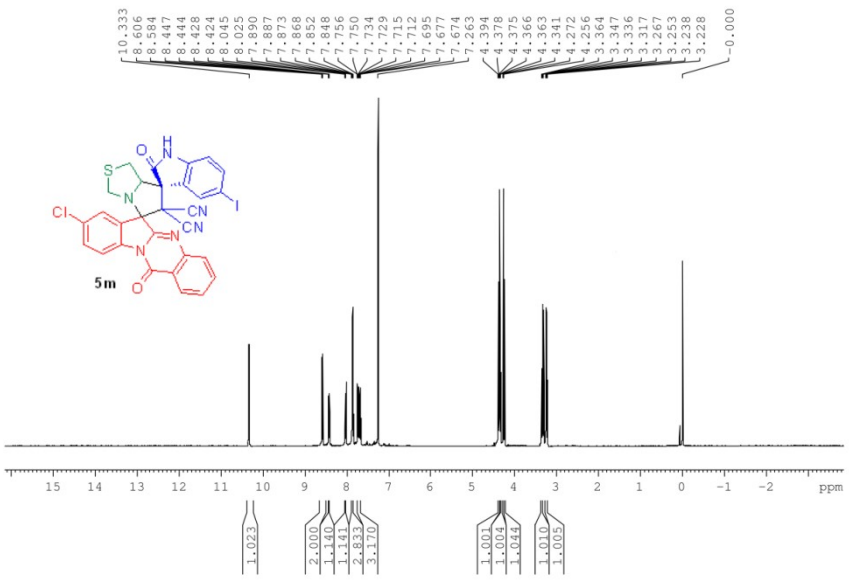

Figure S26 ^{1}H NMR spectrum of **5m**

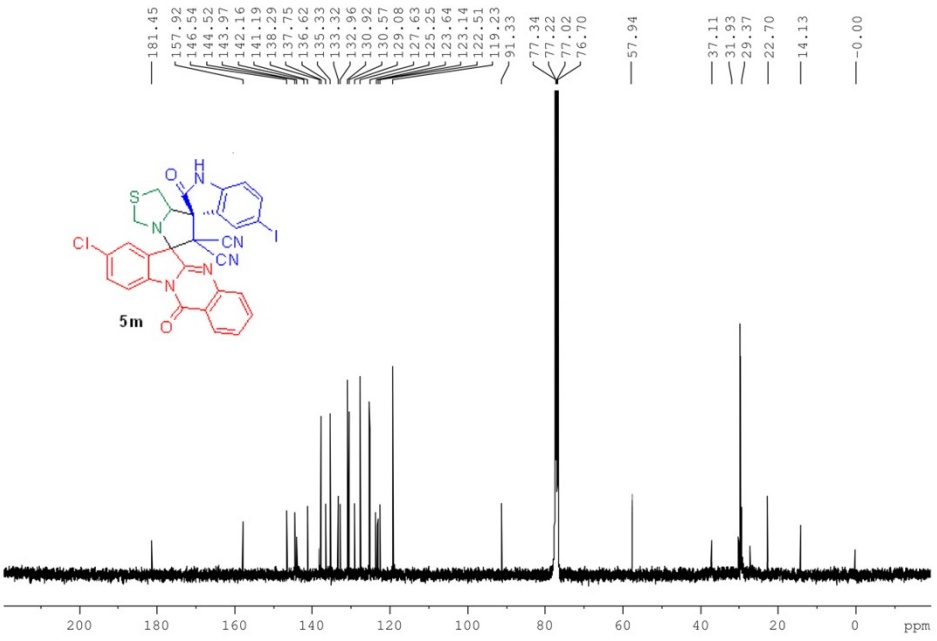

Figure S27 ^{13}C NMR spectrum of **5m**

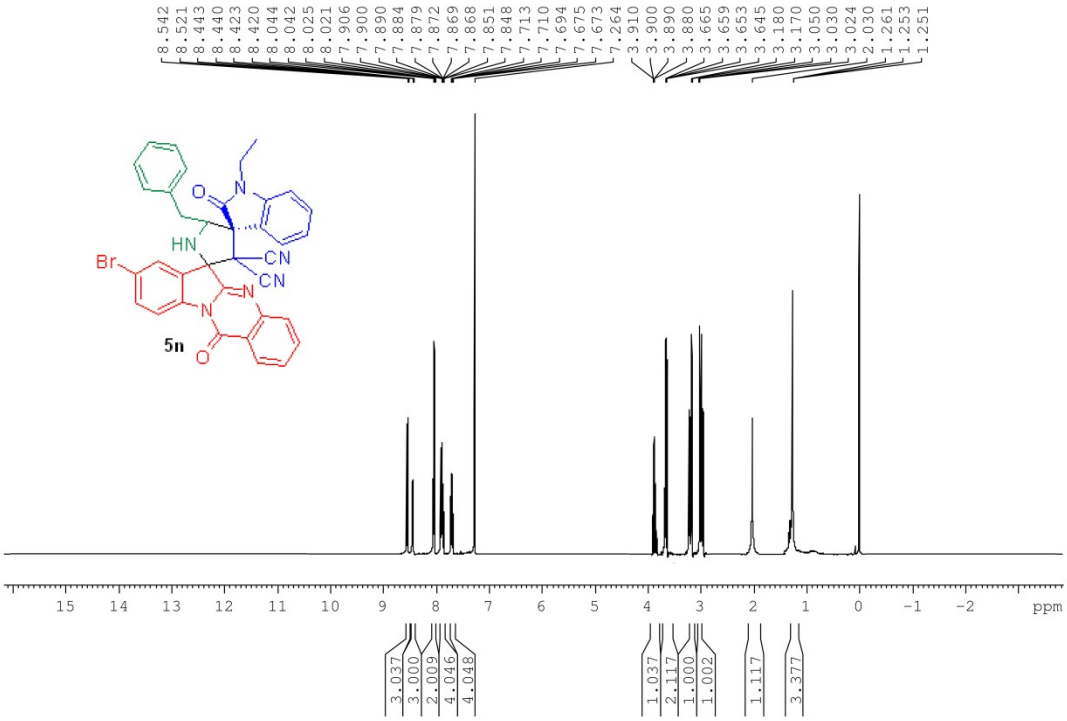

Figure S28 ^{1}H NMR spectrum of **5n**

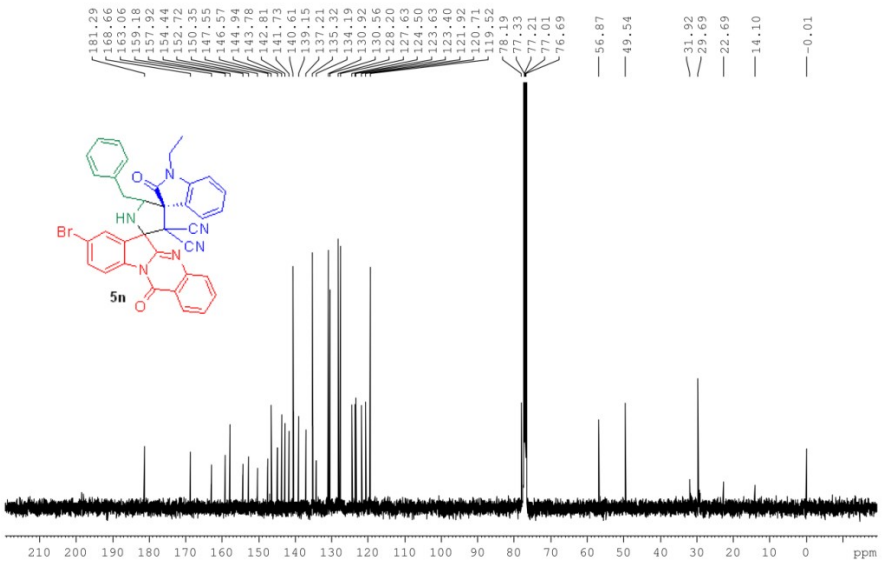

Figure S29 ^{13}C NMR spectrum of **5n**

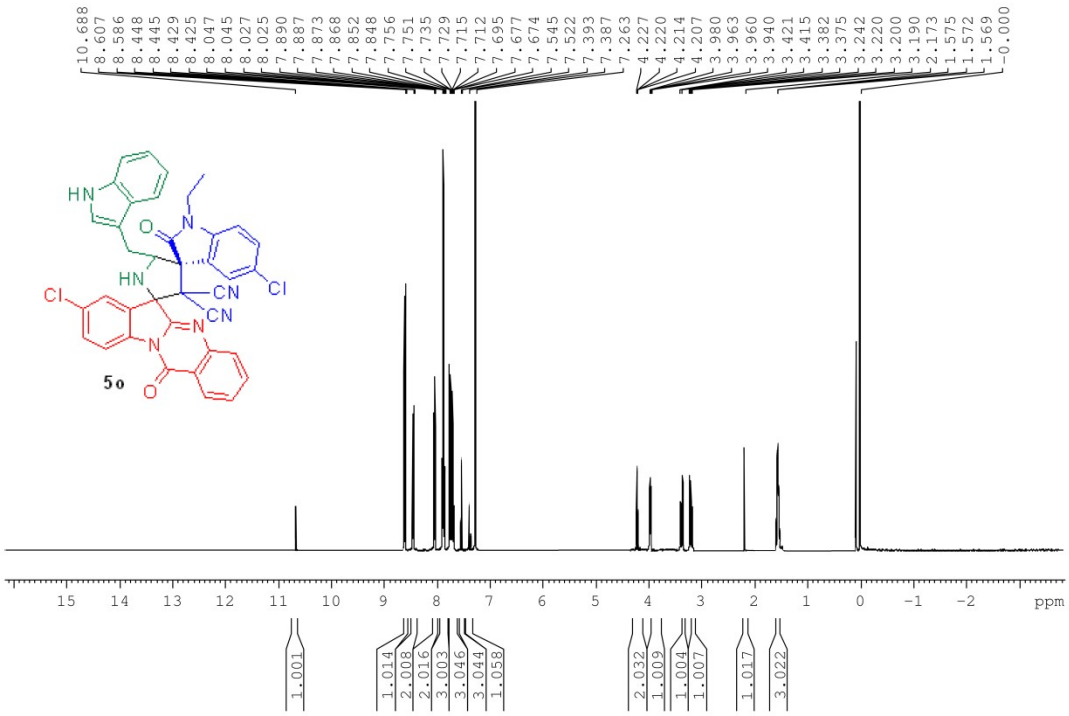

Figure S30 ^{1}H NMR spectrum of **5o**

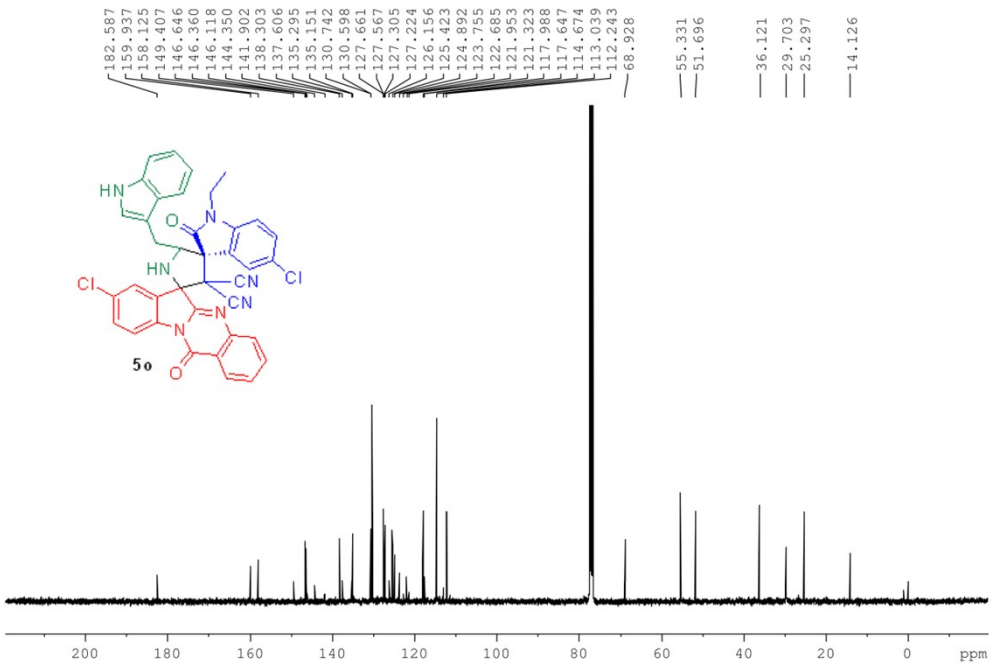

Figure S31 ^{13}C NMR spectrum of **5o**

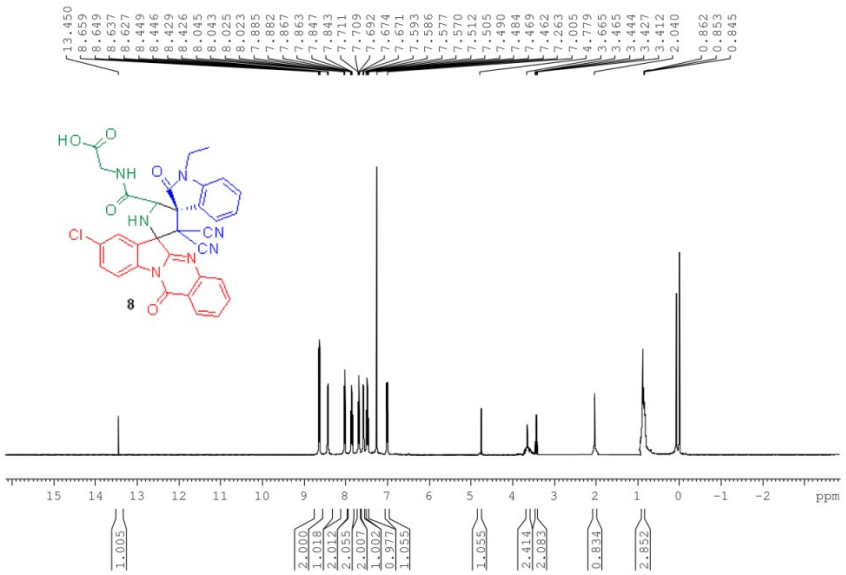

Figure S32 ^{1}H NMR spectrum of **8**

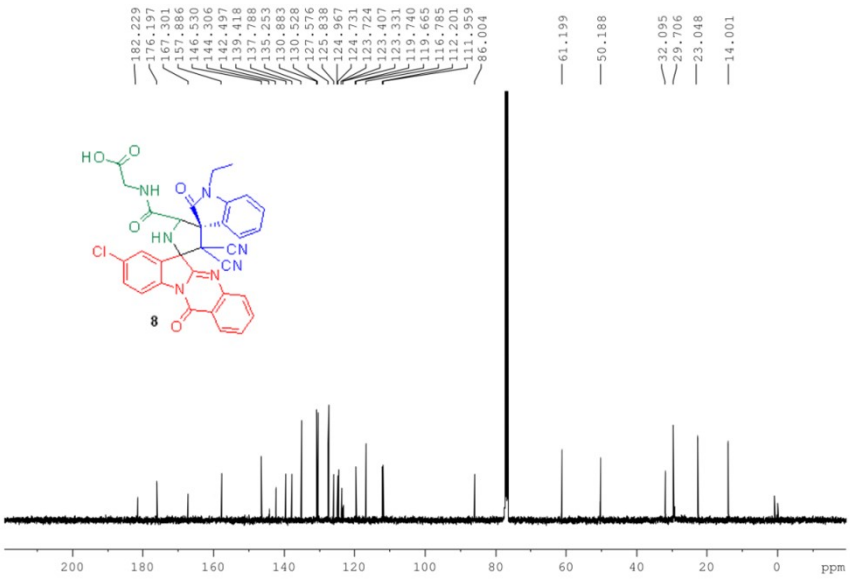

Figure S33 ^{13}C NMR spectrum of **8**