

Design, synthesis and evaluation of multi-pharmacophore-containing spiropolycyclic harmaline-based hybrids as anticancer agents

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1. General information

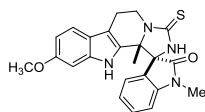
Reactions were monitored by thin layer chromatography using UV light to visualize the course of reaction. Purification of reaction products was carried out by flash chromatography on silica gel or just by simple filtration and washing. ^1H and ^{13}C NMR spectra were obtained using a Bruker DPX-400 spectrometer. ^1H NMR chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR chemical shifts are reported in ppm (δ) from tetramethylsilane (TMS) with the solvent resonance as the internal standard. Melting points were measured on an electrothermal digital melting point apparatus.

Human non-small cell lung cancer cells (A549), human chronic myeloid leukemia cells (K562), human prostate cancer cells (PC-3), human embryonic lung fibroblasts (MRC-5), and tubular epithelial cells (HK-2) were purchased from the Kunming Cell Bank of the Chinese Academy of Sciences. All of them were prepared into complete medium in RPMI-1640 medium, 10% fetal bovine serum, 1% glutamine, 1% penicillin and streptomycin; cell culture was performed at 37 °C and 5% CO_2 .

2. Synthesis of spirocyclic harmaline-based hybrids **3**

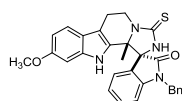
In a sealed tube equipped with a magnetic stirring bar, to 3.0 mL of toluene was added compound **1** (0.2 mmol), compound **2** (0.3 mmol) and catalyst Et_3N (10 mol %). The reaction mixture was stirred at rt for 1 h. After completion of the reaction, as indicated by TLC, purification by flash column chromatography (hexane/EtOAc, 5/1, v/v) was carried out to furnish the hybrids **3**. The major diastereomers **3** can be easily separated by silica column chromatography.

3. Characterization data of major diastereomers **3**

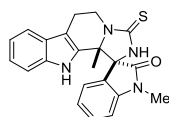


3aa (major): Light yellow solid, m.p. 159.9-160.4 °C; yield 87% (72.7 mg), 15:1 dr; ^1H NMR (DMSO- d_6 , 400 MHz) δ : 1.36 (s, 3H), 2.35-2.43 (m, 1H), 2.47-2.50 (m, 1H), 2.53 (s, 3H),

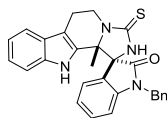
2.93-3.00 (m, 1H), 3.46 (s, 3H), 4.47-4.51 (m, 1H), 6.37-3.40 (m, 1H), 6.46 (d, $J = 2.0$ Hz, 1H), 6.82 (d, $J = 7.6$ Hz, 1H), 6.90-6.94 (m, 1H), 7.04 (d, $J = 8.8$ Hz, 1H), 7.21-7.25 (m, 1H), 7.40 (d, $J = 7.2$ Hz, 1H), 8.45 (br s, 1H), 9.95 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.3, 23.4, 26.3, 55.6, 66.4, 72.1, 95.3, 109.0, 109.2, 109.8, 119.2, 120.8, 122.4, 124.3, 127.3, 130.8, 131.5, 137.9, 145.3, 156.0, 174.2, 181.5; HRMS (ESI-TOF) m/z : Calcd. for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{NaO}_2\text{S}$ $[\text{M}+\text{Na}]^+$: 441.1356; Found: 441.1353; The chiral column and the method for HPLC analysis: using a Chiralpak IA column (60/40 hexane/*i*-PrOH; flow rate: 1.0 mL/min; $\lambda = 254$ nm; $\tau = 6.91$ min; $\tau = 10.81$ min).



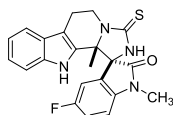
3ab (major): Light yellow solid, m.p. 144.5-145.4 °C; yield 79% (78.1 mg), 3:1 dr; ^1H NMR (DMSO- d_6 , 400 MHz) δ : 1.76 (s, 3H), 2.75-2.87 (m, 2H), 3.26-3.33 (m, 1H), 3.68 (s, 3H), 4.72-4.77 (m, 2H), 5.22 (d, $J = 16.0$ Hz, 1H), 6.53-6.64 (m, 4H), 6.70 (d, $J = 8.0$ Hz, 1H), 6.96-7.01 (m, 1H), 7.25-7.30 (m, 2H), 7.32-7.36 (m, 2H), 7.43 (d, $J = 7.2$ Hz, 2H), 9.30 (br s, 1H), 10.27 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.1, 21.9, 44.1, 55.5, 68.5, 70.5, 94.8, 108.6, 108.8, 109.7, 119.3, 120.5, 124.2, 127.8, 127.9, 128.1, 128.9, 129.9, 131.9, 136.3, 137.4, 142.0, 156.0, 173.0, 180.8; HRMS (ESI-TOF) m/z : Calcd. for $\text{C}_{29}\text{H}_{26}\text{N}_4\text{NaO}_2\text{S}$ $[\text{M}+\text{Na}]^+$: 517.1669; Found: 517.1673.



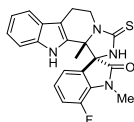
3ba (major): Light yellow solid, m.p. 156.5-157.3 °C; yield 81% (62.9 mg), 3:1 dr; ^1H NMR (DMSO- d_6 , 400 MHz) δ : 1.67 (s, 3H), 2.69-2.76 (m, 1H), 2.80 (s, 3H), 2.83-2.85 (m, 1H), 3.25-3.32 (m, 1H), 4.79-4.83 (m, 1H), 6.99-7.12 (m, 3H), 7.19-7.26 (m, 2H), 7.46 (d, $J = 7.6$ Hz, 1H), 7.50-7.54 (m, 1H), 7.70 (d, $J = 7.2$ Hz, 1H), 8.77 (br s, 1H), 10.44 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.3, 23.4, 26.3, 66.4, 72.1, 109.3, 109.8, 111.9, 118.6, 122.5, 124.4, 126.4, 127.4, 130.8, 133.0, 137.1, 145.4, 174.2, 181.5; HRMS (ESI-TOF) m/z : Calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{NaOS}$ $[\text{M}+\text{Na}]^+$: 411.1250; Found: 411.1253.



3bb (major): Light yellow solid, m.p. 147.2-148.1 °C; yield 79% (73.3 mg), 2:1 dr; ¹H NMR (CDCl₃, 400 MHz) δ: 1.69 (s, 3H), 2.72-2.77 (m, 1H), 2.87-2.95 (m, 1H), 3.20-3.28 (m, 1H), 4.55 (d, *J* = 14.8 Hz, 1H), 4.82-4.87 (m, 1H), 5.23 (d, *J* = 14.8 Hz, 1H), 6.39 (s, 1H), 6.50 (s, 1H), 6.53-6.57 (m, 1H), 6.64-6.66 (m, 1H), 6.72-6.77 (m, 2H), 6.91-6.97 (m, 3H), 7.27-7.29 (m, 1H), 7.35-7.44 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz) δ: 20.8, 22.2, 40.0, 44.3, 69.5, 70.9, 108.9, 110.4, 110.7, 118.6, 119.8, 122.5, 123.2, 124.7, 125.9, 126.5, 128.1, 128.6, 129.5, 130.5, 131.4, 135.9, 136.3, 141.0, 172.9, 181.8; HRMS (ESI-TOF) *m/z*: Calcd. for C₂₈H₂₄N₄NaOS [M+Na]⁺: 487.1563; Found: 487.1563.

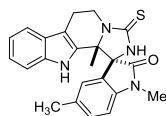


3bc (major): Light yellow solid, m.p. 185.3-186.2 °C; yield 76% (61.7 mg), 4:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 1.71 (s, 3H), 2.67-2.75 (m, 1H), 2.78 (s, 3H), 2.81-2.86 (m, 1H), 3.25-3.32 (m, 1H), 4.77-4.82 (m, 1H), 6.99-7.14 (m, 3H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.36-7.41 (m, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.68-7.71 (m, 1H), 8.77 (br s, 1H), 10.48 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 21.3, 23.0, 26.5, 66.5, 72.3, 109.4, 110.6 (d, *J*_{CF} = 8.2 Hz), 111.8, 115.3 (d, *J*_{CF} = 26.1 Hz), 117.1 (d, *J*_{CF} = 23.1 Hz), 118.7, 119.2, 121.8, 125.9 (d, *J*_{CF} = 8.1 Hz), 126.3, 132.7, 137.0, 141.6, 158.8 (d, *J*_{CF} = 235.4 Hz), 174.1, 181.6; HRMS (ESI-TOF) *m/z*: Calcd. for C₂₂H₁₉FN₄NaOS[M+Na]⁺: 429.1156; Found: 429.1155.

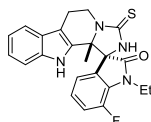


3bd (major): Light yellow solid, m.p. 152.6-152.9 °C; yield 80% (65.0 mg), 2:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 1.73 (s, 3H), 2.81-2.83 (m, 2H), 3.28-3.30 (m, 1H), 4.73 (d, *J* = 14.4 Hz, 1H), 6.41 (d, *J* = 7.2 Hz, 1H), 6.54-6.59 (m, 1H), 6.89-6.98 (m, 3H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 8.95 (br s, 1H), 10.52 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 21.0, 22.1, 29.7, 29.8, 68.5, 70.3, 108.7, 111.5, 117.7 (d, *J*_{CF} = 20.2 Hz), 118.7, 119.0, 120.1, 121.9, 123.4, 126.0, 129.5, 131.3, 133.0, 136.6, 146.9 (d, *J*_{CF} = 240.3 Hz), 172.4, 180.3; HRMS

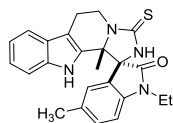
(ESI-TOF) m/z : Calcd. for $C_{22}H_{19}FN_4NaOS$ $[M+Na]^+$: 429.1156; Found: 429.1158.



3be (major): Light yellow solid, m.p. 198.7-199.5 °C; yield 81% (65.1 mg), 3:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 1.63 (s, 3H), 2.38 (s, 3H), 2.62-2.70 (m, 1H), 2.72 (s, 3H), 2.76-2.80 (m, 1H), 3.20-3.27 (m, 1H), 4.72-4.76 (m, 1H), 6.94-7.03 (m, 3H), 7.18 (d, $J = 8.0$ Hz, 1H), 7.28 (d, $J = 8.0$ Hz, 1H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.51 (s, 1H), 8.73 (br s, 1H), 10.39 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 14.6, 21.2, 23.3, 26.3, 60.2, 66.3, 72.1, 109.3, 109.5, 111.9, 118.6, 119.1, 121.7, 124.3, 126.3, 128.0, 131.0, 131.4, 133.0, 137.0, 143.0, 174.1, 181.5; HRMS (ESI-TOF) m/z : Calcd. for $C_{23}H_{22}N_4NaOS$ $[M+Na]^+$: 425.1407; Found: 425.1412.

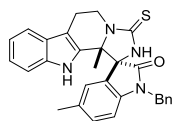


3bf (major): Light yellow solid, m.p. 146.9-147.5 °C; yield 82% (68.9 mg), 3:1 dr; 1H NMR (CDCl₃, 400 MHz) δ : 1.28-1.32 (m, 3H), 1.71 (s, 3H), 2.73-2.78 (m, 1H), 2.94-3.02 (m, 1H), 3.22-3.29 (m, 1H), 3.89-4.00 (m, 2H), 4.81-4.85 (m, 1H), 6.51-6.59 (m, 3H), 6.79-6.84 (m, 1H), 6.97-7.02 (m, 3H), 7.07 (s, 1H), 7.35 (d, $J = 7.2$ Hz, 1H); ^{13}C NMR (CDCl₃, 100 MHz) δ : 14.1, 20.7, 22.3, 37.8, 40.3, 69.6, 70.7, 110.9, 118.6 (d, $J_{CF} = 19.2$ Hz), 120.0, 121.1, 122.8, 123.8 (d, $J_{CF} = 6.4$ Hz), 126.1, 131.5, 136.2, 146.9 (d, $J_{CF} = 244.3$ Hz), 172.7, 181.8; HRMS (ESI-TOF) m/z : Calcd. for $C_{23}H_{21}FN_4NaOS$ $[M+Na]^+$: 443.1312; Found: 443.1315.

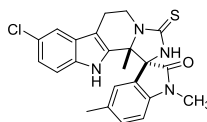


3bg (major): Light yellow solid, m.p. 178.7-179.3 °C; yield 78% (64.9 mg), 4:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 0.61-0.65 (m, 3H), 1.64 (s, 3H), 2.38 (s, 3H), 2.61-2.69 (m, 1H), 2.76-2.80 (m, 1H), 3.10-3.15 (m, 1H), 3.19-3.26 (m, 1H), 3.41-3.43 (m, 1H), 4.72-4.77 (m, 1H), 6.93-7.03 (m, 3H), 7.14 (d, $J = 7.6$ Hz, 1H), 7.27 (d, $J = 8.0$ Hz, 1H), 7.40 (d, $J = 8.0$ Hz, 1H), 7.51 (s, 1H), 8.71 (br s, 1H), 10.38 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 12.4, 21.2, 21.4, 23.0, 33.9, 66.2, 72.4, 109.3, 109.4, 111.9, 118.6, 119.0, 121.7, 124.1, 126.2, 128.2, 131.1, 131.2, 132.7, 136.8, 142.0, 173.5, 181.7; HRMS (ESI-TOF) m/z : Calcd. for $C_{24}H_{24}N_4NaOS$ $[M+Na]^+$:

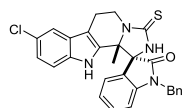
439.1563; Found: 439.1560.



3bh (major): Light yellow solid, m.p. 157.5-157.9 °C; yield 81% (77.4 mg), 5:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 1.77 (s, 3H), 1.81 (s, 3H), 2.79-2.95 (m, 2H), 3.28-3.33 (m, 1H), 4.72-4.77 (m, 2H), 5.20 (d, *J* = 12.4 Hz, 1H), 6.36 (s, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.90-7.00 (m, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.25-7.35 (m, 3H), 7.40-7.43 (m, 3H), 9.31 (br s, 1H), 10.39 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 20.8, 21.0, 21.9, 44.0, 68.7, 70.7, 108.9, 109.4, 111.4, 118.6, 119.0, 121.8, 125.1, 126.1, 127.7, 127.8, 128.9, 130.0, 131.1, 133.2, 136.3, 136.5, 139.9, 173.0, 181.3; HRMS (ESI-TOF) *m/z*: Calcd. for C₂₉H₂₆N₄NaOS[M+Na]⁺: 501.1720; Found: 501.1723.

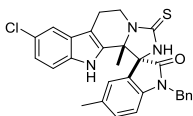


3ca (major): Light yellow solid, m.p. 158.9-159.5 °C; yield 85% (74.1 mg), 5:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 1.68 (s, 3H), 2.43 (s, 3H), 2.66-2.73 (m, 1H), 2.77 (s, 3H), 2.81-2.86 (m, 1H), 3.24-3.31 (m, 1H), 4.77-4.82 (m, 1H), 7.00-7.08 (m, 2H), 7.25 (d, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.52-7.56 (m, 2H), 8.79 (br s, 1H), 10.65 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 21.2, 23.2, 26.3, 55.4, 66.2, 72.1, 109.4, 109.6, 113.4, 118.0, 121.6, 123.8, 124.1, 127.5, 128.0, 131.1, 131.5, 134.8, 135.5, 143.0, 174.1, 181.7; HRMS (ESI-TOF) *m/z*: Calcd. for C₂₃H₂₁ClN₄NaOS[M+Na]⁺: 459.1017; Found: 459.1014.

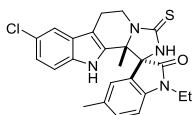


3cb (major): Light yellow solid, m.p. 147.6-148.5 °C; yield 75% (74.7 mg, HPLC purity >99%), 3:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 1.78 (s, 3H), 2.83-2.85 (m, 2H), 3.29-3.33 (m, 1H), 4.72-4.76 (m, 2H), 5.23 (d, *J* = 16.0 Hz, 1H), 6.54-6.59 (m, 2H), 6.70 (d, *J* = 8.0 Hz, 1H), 6.94-6.99 (m, 2H), 7.05 (d, *J* = 8.8 Hz, 1H), 7.27-7.35 (m, 3H), 7.42-7.45 (m, 3H), 9.36 (br s, 1H), 10.70 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 21.0, 21.8, 44.1, 68.4, 70.4, 108.8, 109.8, 113.0, 118.1, 121.7, 122.4, 123.7, 124.1, 127.2, 127.8, 127.9, 128.9, 130.0, 135.0,

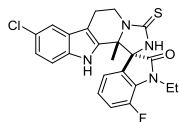
135.1, 136.2, 142.0, 172.8, 180.9; HRMS (ESI-TOF) m/z : Calcd. for $C_{28}H_{23}ClN_4NaOS [M+Na]^+$: 521.1173; Found: 521.1172.



3cc (major): Light yellow solid, m.p. 162.1-162.7 °C; yield 77% (78.8 mg), 4:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 1.76 (s, 3H), 1.82 (s, 3H), 2.80-2.87 (m, 2H), 3.28-3.34 (m, 1H), 4.70-4.75 (m, 2H), 5.18 (d, $J = 16.4$ Hz, 1H), 6.33 (s, 1H), 6.59 (d, $J = 8.0$ Hz, 1H), 6.79 (d, $J = 7.6$ Hz, 1H), 6.95-6.98 (m, 1H), 7.06 (d, $J = 8.8$ Hz, 1H), 7.27-7.36 (m, 3H), 7.38-7.44 (m, 2H), 7.46 (d, $J = 2.0$ Hz, 1H), 9.34 (br s, 1H), 10.62 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 20.7, 21.8, 44.0, 68.6, 70.7, 109.0, 109.5, 112.9, 118.0, 121.8, 123.6, 125.0, 127.2, 127.6, 127.8, 128.9, 130.1, 131.2, 134.9, 135.1, 136.3, 139.8, 172.9, 181.3; HRMS (ESI-TOF) m/z : Calcd. for $C_{29}H_{25}ClN_4NaOS[M+Na]^+$: 535.1330; Found: 535.1326.

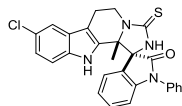


3cd (major): Light yellow solid, m.p. 165.5-166.4 °C; yield 84% (75.6 mg), 2:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 0.66-0.70 (m, 3H), 1.69 (s, 3H), 2.43 (s, 3H), 2.64-2.72 (m, 1H), 2.81-2.85 (m, 1H), 3.16-3.30 (m, 2H), 3.42-3.47 (m, 1H), 4.77-4.82 (m, 1H), 7.01-7.08 (m, 2H), 7.20 (d, $J = 8.8$ Hz, 1H), 7.31-7.33 (m, 1H), 7.52-7.56 (m, 2H), 8.78 (br s, 1H), 10.65 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 12.4, 21.2, 21.3, 22.9, 34.0, 66.0, 72.3, 109.5, 113.4, 118.0, 121.6, 123.8, 124.0, 127.4, 128.2, 131.2, 131.3, 134.6, 135.3, 142.0, 173.5, 181.8; HRMS (ESI-TOF) m/z : Calcd. for $C_{24}H_{23}ClN_4NaOS[M+Na]^+$: 473.1173; Found: 473.1176.

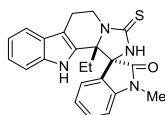


3ce (major): Light yellow solid, m.p. 155.4-155.7 °C; yield 74% (67.2 mg), 3:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 1.23-1.26 (m, 3H), 1.73 (s, 3H), 2.77-2.87 (m, 2H), 3.26-3.34 (m, 1H), 3.70-3.79 (m, 1H), 3.97-4.06 (m, 1H), 4.69-4.73 (m, 1H), 6.34-6.37 (m, 1H), 6.58-6.63 (m, 1H), 6.95-7.09 (m, 3H), 7.45 (s, 1H), 9.11 (br s, 1H), 10.60 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 14.0, 20.8, 22.0, 37.5, 55.4, 68.5, 70.4, 109.0, 113.0, 117.9 (d, $J_{CF} = 20.2$ Hz), 118.1, 120.5,

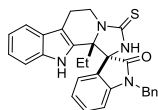
121.9, 123.4 (d, $J_{CF} = 8.4$ Hz), 123.7, 127.2, 128.7 (d, $J_{CF} = 9.0$ Hz), 131.1, 131.2, 134.8, 135.0, 146.6 (d, $J_{CF} = 241.3$ Hz), 172.4, 180.8; HRMS (ESI-TOF) m/z : Calcd. for $C_{23}H_{20}ClFN_4NaOS[M+Na]^+$: 477.0923; Found: 477.0927.



3cf (major): Light yellow solid, m.p. 164.7-165.6 °C; yield 75% (72.6 mg), 3:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 1.86 (s, 3H), 2.85-2.97 (m, 2H), 3.34-3.40 (m, 1H), 4.77-4.82 (m, 1H), 6.63-6.71 (m, 3H), 7.00-7.03 (m, 1H), 7.08-7.15 (m, 2H), 7.52-7.57 (m, 2H), 7.61-7.63 (m, 2H), 7.68-7.72 (m, 2H), 9.18 (br s, 1H), 10.77 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.0, 22.0, 55.4, 68.6, 70.3, 109.2, 109.5, 113.1, 118.2, 121.8, 123.0, 123.8, 124.6, 127.3, 127.5, 127.8, 128.7, 128.7, 130.0, 130.1, 134.7, 135.1, 135.2, 143.0, 172.3, 180.7; HRMS (ESI-TOF) m/z : Calcd. for $C_{27}H_{21}ClN_4NaOS[M+Na]^+$: 507.1017; Found: 507.1014.

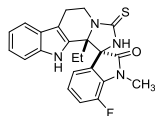


3da (major): Light yellow solid, m.p. 160.3-161.5 °C; yield 85% (68.3 mg), 15:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 0.65-0.69 (m, 3H), 1.89-1.95 (m, 1H), 2.43-2.48 (m, 1H), 2.66 (s, 3H), 2.68-2.73 (m, 1H), 2.80-2.84 (m, 1H), 3.25-3.32 (m, 1H), 4.90-4.94 (m, 1H), 6.95-7.04 (m, 3H), 7.15-7.23 (m, 2H), 7.43-7.49 (m, 2H), 7.65 (d, $J = 7.2$ Hz, 1H), 8.78 (br s, 1H), 10.44 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 9.9, 21.0, 26.2, 30.4, 43.3, 69.4, 73.8, 109.7, 111.5, 111.9, 118.6, 119.0, 122.4, 126.2, 127.5, 130.2, 130.9, 137.2, 145.3, 174.5, 183.9; HRMS (ESI-TOF) m/z : Calcd. for $C_{23}H_{22}N_4NaOS [M+Na]^+$: 425.1407; Found: 425.1413.

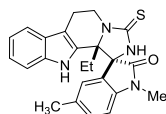


3db (major): Light yellow solid, m.p. 172.5-173.3 °C; yield 80% (76.5 mg), 3:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 0.85-0.89 (m, 3H), 2.06-2.12 (m, 1H), 2.58-2.67 (m, 1H), 2.80-2.93 (m, 2H), 3.42-3.43 (m, 1H), 4.70 (d, $J = 16.4$ Hz, 1H), 4.93-4.97 (m, 1H), 5.26 (d, $J = 16.0$ Hz, 1H), 6.50-6.54 (m, 1H), 6.61 (d, $J = 8.0$ Hz, 1H), 6.67 (d, $J = 6.8$ Hz, 1H), 6.86-6.97 (m, 3H), 7.07 (d, $J = 8.0$ Hz, 1H), 7.27-7.43 (m, 6H), 9.33 (br s, 1H), 10.52 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100

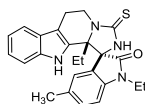
MHz) δ : 10.3, 21.0, 29.8, 42.1, 44.1, 71.7, 71.8, 109.6, 111.5, 118.6, 118.9, 121.7, 122.3, 123.6, 126.0, 127.8, 127.9, 128.8, 128.9, 129.7, 131.7, 136.2, 136.7, 141.5, 172.8, 181.8; HRMS (ESI-TOF) m/z : Calcd. for $C_{29}H_{26}N_4NaOS$ $[M+Na]^+$: 501.1720; Found: 501.1723.



3dc (major): Light yellow solid, m.p. 172.5-173.3 °C; yield 80% (67.2 mg), 3:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 0.83-0.87 (m, 3H), 1.99-2.05 (m, 1H), 2.52-2.56 (m, 1H), 2.76-2.82 (m, 1H), 2.86-2.91 (m, 1H), 3.34-3.39 (m, 1H), 4.89-4.94 (m, 1H), 6.46 (d, $J = 6.8$ Hz, 1H), 6.51-6.56 (m, 1H), 6.87-6.97 (m, 3H), 7.09 (d, $J = 8.0$ Hz, 1H), 7.37 (d, $J = 7.6$ Hz, 1H), 8.95 (br s, 1H), 10.54 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 10.2, 21.0, 29.7, 29.9, 42.1, 71.5, 71.8, 109.7, 111.5, 117.5(d, $J_{CF} = 19.6$ Hz), 118.6, 118.9, 119.7, 121.8, 123.2 (d, $J_{CF} = 6.3$ Hz), 125.8, 128.9 (d, $J_{CF} = 8.1$ Hz), 131.3, 131.9, 136.7, 146.7 (d, $J_{CF} = 241.1$ Hz), 172.3, 181.4; HRMS (ESI-TOF) m/z : Calcd. for $C_{23}H_{21}FN_4NaOS$ $[M+Na]^+$: 443.1312; Found: 443.1317.

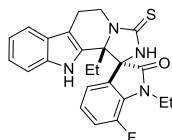


3dd (major): Light yellow solid, m.p. 153.7-154.4 °C; yield 80% (66.6 mg), 2:1 dr; 1H NMR (CDCl $_3$, 400 MHz) δ : 0.73-0.76 (m, 3H), 1.75-1.81 (m, 1H), 2.35 (s, 3H), 2.52-2.57 (m, 1H), 2.59 (s, 3H), 2.77-2.82 (m, 1H), 2.85-2.93 (m, 1H), 3.29-3.37 (m, 1H), 4.96-5.01 (m, 1H), 6.16 (s, 1H), 6.65 (d, $J = 8.0$ Hz, 1H), 7.00-7.10 (m, 3H), 7.16-7.19 (m, 1H), 7.29 (s, 1H), 7.37 (s, 1H), 7.43 (d, $J = 7.2$ Hz, 1H); ^{13}C NMR (CDCl $_3$, 100 MHz) δ : 8.3, 19.7, 20.2, 25.1, 29.0, 42.3, 68.6, 73.0, 107.6, 109.7, 112.3, 118.1, 118.8, 121.5, 125.3, 127.2, 127.3, 130.4, 131.3, 135.4, 141.4, 173.2, 183.6; HRMS (ESI-TOF) m/z : Calcd. for $C_{24}H_{24}N_4NaOS$ $[M+Na]^+$: 439.1563; Found: 439.1557.

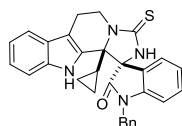


3de (major): Light yellow solid, m.p. 158.4-158.9 °C; yield 81% (69.7 mg), 7:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 0.45-0.48 (m, 3H), 0.67-0.71 (m, 3H), 1.90-1.95 (m, 1H), 2.38 (s, 3H), 2.44-2.48 (m, 1H), 2.65-2.73 (m, 1H), 2.79-2.83 (m, 1H), 3.02-3.07 (m, 1H), 3.26-3.36 (m, 2H), 4.89-4.94 (m, 1H), 6.91-7.03 (m, 3H), 7.16 (d, $J = 8.0$ Hz, 1H), 7.25 (d, $J = 7.6$ Hz, 1H), 7.43 (d, J

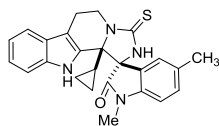
= 7.6 Hz, 1H), 7.48 (s, 1H), 8.75 (br s, 1H), 10.39 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 9.8, 12.3, 21.0, 21.2, 30.0, 33.8, 43.5, 69.2, 74.2, 109.3, 111.6, 111.9, 118.5, 118.9, 121.7, 123.8, 126.1, 128.3, 129.9, 131.0, 131.1, 137.0, 142.0, 173.8, 184.3; HRMS (ESI-TOF) m/z : Calcd. for $\text{C}_{25}\text{H}_{26}\text{N}_4\text{NaOS}$ $[\text{M}+\text{Na}]^+$: 453.1720; Found: 453.1725.



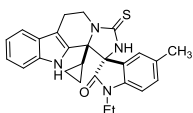
3df (major): Light yellow solid, m.p. 157.9-158.9 °C; yield 79% (68.6 mg), 4:1 dr; ^1H NMR (DMSO- d_6 , 400 MHz) δ : 0.85-0.88 (m, 3H), 1.25-1.29 (m, 3H), 2.04-2.10 (m, 1H), 2.54-2.60 (m, 1H), 2.83-2.93 (m, 2H), 3.36-3.38 (m, 1H), 3.72-3.81 (m, 1H), 4.03-4.08 (m, 1H), 4.92-4.97 (m, 1H), 6.48 (d, $J = 7.2$ Hz, 1H), 6.57-6.62 (m, 1H), 6.92-7.01 (m, 3H), 7.12 (d, $J = 8.0$ Hz, 1H), 7.41 (d, $J = 7.6$ Hz, 1H), 9.11 (br s, 1H), 10.48 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 10.2, 13.9, 20.9, 29.8, 37.6, 42.3, 71.5, 71.9, 109.9, 111.5, 117.7 (d, $J_{\text{CF}} = 20.2$ Hz), 118.6, 119.0, 120.1, 121.9, 123.2 (d, $J_{\text{CF}} = 6.3$ Hz), 125.9, 128.1 (d, $J_{\text{CF}} = 9.1$ Hz), 131.2, 132.0, 136.7, 146.7 (d, $J_{\text{CF}} = 240.4$ Hz), 172.3, 181.6; HRMS (ESI-TOF) m/z : Calcd. for $\text{C}_{24}\text{H}_{23}\text{FN}_4\text{NaOS}$ $[\text{M}+\text{Na}]^+$: 457.1469; Found: 457.1473.



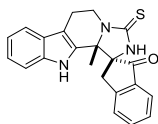
3ea (major): Light yellow solid, m.p. 155.6-155.9 °C; yield 79% (77.4 mg), 3:1 dr; ^1H NMR (CDCl_3 , 400 MHz) δ : 0.06-0.09 (m, 3H), 0.40-0.47 (m, 1H), 0.55-0.62 (m, 1H), 1.26-1.30 (m, 1H), 2.00-2.05 (m, 1H), 2.81-2.86 (m, 1H), 3.02-3.11 (m, 1H), 3.64-3.71 (m, 1H), 4.70 (d, $J = 15.2$ Hz, 1H), 4.89-4.94 (m, 1H), 5.32 (d, $J = 15.2$ Hz, 1H), 6.36 (s, 1H), 6.57-6.61 (m, 1H), 6.66 (s, 1H), 6.70-6.74 (m, 2H), 6.88 (d, $J = 7.6$ Hz, 1H), 7.01-7.08 (m, 3H), 7.39-7.51 (m, 4H), 7.59 (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 1.42, 3.28, 16.4, 20.5, 39.8, 44.5, 71.4, 74.3, 108.9, 110.6, 112.7, 118.6, 119.7, 122.7, 122.9, 125.1, 125.6, 126.5, 126.7, 128.4, 128.5, 129.3, 130.4, 135.7, 136.2, 141.5, 173.2, 181.2; HRMS (ESI-TOF) m/z : Calcd. for $\text{C}_{30}\text{H}_{26}\text{N}_4\text{NaOS}$ $[\text{M}+\text{Na}]^+$: 513.1720; Found: 513.1720.



3eb (major): Light yellow solid, m.p. 144.7-145.9 °C; yield 80% (68.5 mg), 2:1 dr; ¹H NMR (CDCl₃, 400 MHz) δ: 0.16-0.20 (m, 1H), 0.38-0.43 (m, 1H), 0.47-0.51 (m, 1H), 0.70-0.77 (m, 1H), 1.55-1.61 (m, 1H), 2.43 (s, 3H), 2.77 (s, 3H), 2.84-2.89 (m, 1H), 2.95-3.03 (m, 1H), 3.44-3.51 (m, 1H), 4.94-4.99 (m, 1H), 6.26 (s, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 7.09-7.19 (m, 3H), 7.28 (d, *J* = 6.0 Hz, 1H), 7.44 (s, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.64 (s, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ: 2.51, 3.16, 17.1, 20.7, 21.2, 26.2, 41.4, 70.1, 73.2, 108.5, 110.8, 112.7, 119.2, 119.9, 122.7, 122.8, 126.2, 127.7, 128.7, 131.4, 132.4, 136.2, 142.1, 173.9, 183.6; HRMS (ESI-TOF) *m/z*: Calcd. for C₂₅H₂₄N₄NaOS [M+Na]⁺: 451.1563; Found: 451.1567.

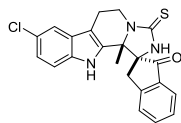


3ec (major): Light yellow solid, m.p. 142.3-143.2 °C; yield 78% (69.0 mg), 3:1 dr; ¹H NMR (CDCl₃, 400 MHz) δ: 0.19-0.24 (m, 1H), 0.40-0.44 (m, 1H), 0.50-0.57 (m, 1H), 0.70-0.77 (m, 4H), 1.56-1.60 (m, 1H), 2.43 (s, 3H), 2.84-2.89 (m, 1H), 2.95-3.04 (m, 1H), 3.06-3.12 (m, 1H), 3.42-3.48 (m, 1H), 3.52-3.57 (m, 1H), 4.95-4.99 (m, 1H), 6.23 (s, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 7.08-7.16 (m, 3H), 7.26-7.27 (m, 1H), 7.43 (s, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.65 (s, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ: 2.53, 3.15, 12.3, 17.0, 20.7, 21.2, 34.5, 41.6, 69.8, 73.4, 108.5, 110.8, 112.6, 119.2, 119.9, 122.7, 122.8, 126.2, 127.8, 128.9, 131.4, 132.2, 136.0, 141.3, 173.3, 183.8; HRMS (ESI-TOF) *m/z*: Calcd. for C₂₆H₂₆N₄NaOS [M+Na]⁺: 465.1720; Found: 465.1723.

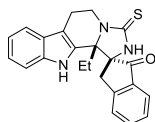


3fa (major): Light yellow solid, m.p. 164.7-165.5 °C; yield 82% (61.2 mg), 3:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 1.62 (s, 3H), 2.77-2.92 (m, 3H), 3.12 (d, *J* = 14.2 Hz, 1H), 3.22-3.29 (m, 1H), 4.69-4.73 (m, 1H), 7.01-7.11 (m, 3H), 7.25 (d, *J* = 7.6 Hz, 1H), 7.51-7.58 (m, 2H), 7.69-7.73 (m, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 8.81 (br s, 1H), 10.28 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 21.2, 21.6, 38.5, 68.5, 74.3, 109.6, 111.8, 118.8, 119.4, 122.1, 124.9, 126.3, 126.6, 128.3, 133.1, 135.6, 136.5, 136.6, 151.1, 180.3, 201.5; HRMS (ESI-TOF) *m/z*: Calcd. for

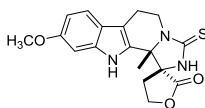
$C_{22}H_{19}N_3NaOS[M+Na]^+$: 396.1141; Found: 396.1145.



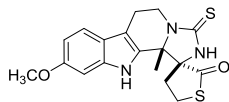
3ga (major): Light yellow solid, m.p. 175.4-175.7 °C; yield 83% (67.6 mg), 2:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 1.61 (s, 3H), 2.73-2.93 (m, 3H), 3.07 (d, $J = 17.2$ Hz, 1H), 3.22-3.29 (m, 1H), 4.68-4.72 (m, 1H), 7.06-7.12 (m, 2H), 7.25 (d, $J = 7.6$ Hz, 1H), 7.54-7.58 (m, 2H), 7.69-7.73 (m, 1H), 7.87 (d, $J = 7.6$ Hz, 1H), 8.84 (br s, 1H), 10.51 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.2, 21.5, 38.4, 68.4, 74.2, 109.7, 113.3, 118.2, 122.1, 124.1, 124.9, 126.5, 127.4, 128.3, 134.9, 135.1, 135.5, 136.6, 151.0, 180.3, 201.4; HRMS (ESI-TOF) m/z : Calcd. for $C_{22}H_{18}ClN_3NaOS[M+Na]^+$: 430.0751; Found: 430.0746.



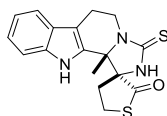
3ha (major): Light yellow solid, m.p. 172.9-173.5 °C; yield 75% (58.1 mg), 4:1 dr; 1H NMR (CDCl $_3$, 400 MHz) δ : 0.72-0.76 (m, 3H), 1.58-1.64 (m, 1H), 2.36-2.41 (m, 1H), 2.75-3.01 (m, 4H), 3.27-3.34 (m, 1H), 4.92-4.97 (m, 1H), 6.29 (s, 1H), 6.61 (s, 1H), 6.83-6.85 (m, 1H), 6.91 (d, $J = 7.6$ Hz, 1H), 7.05-7.07 (m, 2H), 7.43-7.47 (m, 2H), 7.52-7.55 (m, 1H), 7.82 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CDCl $_3$, 100 MHz) δ : 8.7, 20.3, 29.5, 38.0, 41.5, 71.5, 109.9, 111.8, 117.7, 119.1, 122.0, 123.3, 124.9, 125.4, 127.5, 128.2, 133.8, 134.7, 135.6, 149.0, 180.2, 199.7; HRMS (ESI-TOF) m/z : Calcd. for $C_{23}H_{21}N_3NaOS[M+Na]^+$: 410.1298; Found: 410.1295.



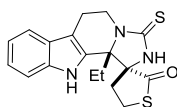
3ia (major): Light yellow solid, m.p. 155.6-156.3 °C; yield 90% (64.3 mg), 20:1 dr; 1H NMR (DMSO- d_6 , 400 MHz) δ : 1.61 (s, 3H), 2.10-2.16 (m, 1H), 2.23-2.28 (m, 1H), 2.70-2.74 (m, 2H), 3.13-3.20 (m, 1H), 3.77 (s, 3H), 4.14-4.21 (m, 1H), 4.29-4.34 (m, 1H), 4.54-4.58 (m, 1H), 6.68-6.70 (m, 1H), 6.86 (d, $J = 2.0$ Hz, 1H), 7.35 (d, $J = 8.4$ Hz, 1H), 8.95 (br s, 1H), 11.00 (br s, 1H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ : 21.2, 21.7, 32.2, 55.7, 65.3, 68.0, 69.4, 95.2, 109.7, 110.7, 119.5, 120.9, 130.6, 137.8, 156.6, 174.0, 180.7; HRMS (ESI-TOF) m/z : Calcd. for $C_{18}H_{19}N_3NaO_3S[M+Na]^+$: 380.1039; Found: 380.1042.



3ib (major): Light yellow solid, m.p. 142.2-143.1 °C; yield 88% (65.6 mg), 10:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 1.57 (s, 3H), 2.05-2.13 (m, 1H), 2.35-2.39 (m, 1H), 2.67-2.79 (m, 2H), 3.04-3.11 (m, 2H), 3.20-3.24 (m, 1H), 3.77 (s, 3H), 4.49-4.53 (m, 1H), 6.68-6.70 (m, 1H), 6.89 (d, *J* = 2.0 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 8.97 (br s, 1H), 10.80 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 20.9, 21.6, 26.4, 33.5, 55.7, 67.8, 77.7, 95.3, 109.7, 111.1, 119.5, 121.0, 130.7, 137.9, 156.6, 181.6, 204.9; HRMS (ESI-TOF) *m/z*: Calcd. for C₁₈H₁₉N₃NaO₂S₂ [M+Na]⁺: 396.0811; Found: 396.0815.

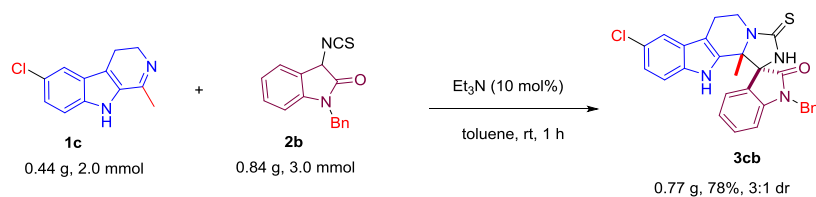


3ja (major): Light yellow solid, m.p. 147.7-148.8 °C; yield 88% (60.4 mg), 8:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 1.59 (s, 3H), 2.05-2.13 (m, 1H), 2.34-2.39 (m, 1H), 2.72-2.85 (m, 2H), 3.00-3.06 (m, 1H), 3.09-3.24 (m, 2H), 4.52-4.57 (m, 1H), 7.02-7.06 (m, 1H), 7.12-7.16 (m, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 9.01 (br s, 1H), 11.0 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 21.0, 21.5, 26.5, 33.6, 67.8, 77.6, 111.1, 112.2, 118.9, 119.6, 122.5, 126.6, 132.2, 137.1, 181.6, 204.8; HRMS (ESI-TOF) *m/z*: Calcd. for C₁₇H₁₇N₃NaOS₂ [M+Na]⁺: 366.0705; Found: 366.0708.



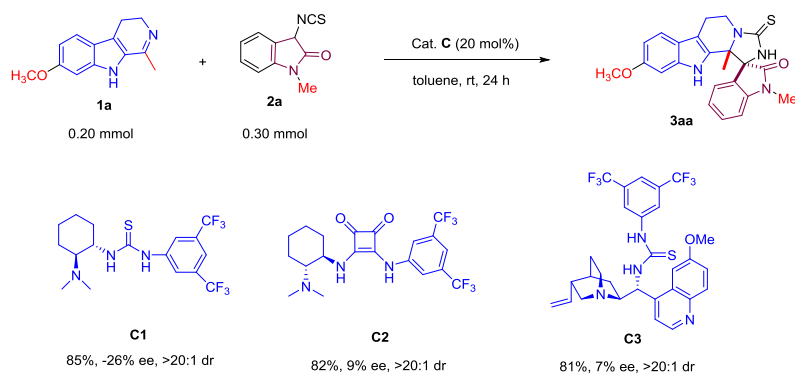
3ka (major): Light yellow solid, m.p. 160.4-161.3 °C; yield 89% (63.5 mg), 20:1 dr; ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 0.64-0.67 (m, 3H), 1.90-2.04 (m, 2H), 2.16-2.37 (m, 3H), 2.62-2.70 (m, 1H), 2.90-3.00 (m, 2H), 3.11-3.17 (m, 1H), 4.77-4.81 (m, 1H), 7.03-7.07 (m, 1H), 7.13-7.16 (m, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 8.91 (br s, 1H), 11.21 (br s, 1H); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ : 9.52, 21.6, 26.1, 28.3, 34.2, 43.7, 70.3, 79.1, 112.2, 112.9, 118.9, 119.6, 122.5, 126.4, 129.5, 136.9, 182.1, 204.1; HRMS (ESI-TOF) *m/z*: Calcd. for C₁₈H₁₉N₃NaOS₂ [M+Na]⁺: 380.0862; Found: 380.0863.

4. Scheme S1: gram scale synthesis of the products 3cb



In a sealed tube equipped with a magnetic stirring bar, to 20 mL of toluene was added harmaline derivative **1c** (0.44 g, 2.0 mmol), 3-isothiocyanato oxindole **2b** (0.84 g, 3.0 mmol) and catalyst Et₃N (10 mol%). The reaction mixture was stirred at rt for 1 h. After completion of the reaction, as indicated by TLC, purification by flash column chromatography (hexane/EtOAc, 5/1, v/v) was carried out to furnish the hybrid **3cb** (0.77 g, 78%, 3:1 dr).

5. Catalytic asymmetric reaction



Compound **1a** (0.3 mmol) and compound **2a** (0.2 mmol) were dissolved in toluene (12 mL). Cat. **C** (10 mol %) was added to this solution at room temperature, and the mixture was stirred for 24 h. After the removal of solvent, purification by flash column chromatography (hexane/ethyl acetate = 6:1~4:1) was carried out to give product **3aa** as a light yellow solid.

6. X-ray crystal data for compounds 3ba and 3de

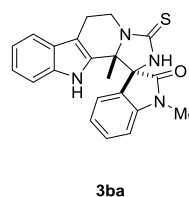
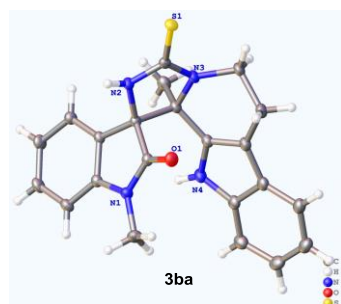


Table S1 Crystal data and structure refinement for 3ba

Identification code	3ba
Empirical formula	C ₂₃ H ₂₄ N ₄ O ₂ S
Formula weight	420.52
Temperature/K	150.00(10)
Crystal system	monoclinic
Space group	P2 ₁ /c
a/Å, b/Å, c/Å	16.1900(3), 9.48160(10), 14.9584(3)
α/°, β/°, γ/°	90, 113.201(2), 90
Volume/Å ³	2110.52(7)
Z	4
ρ _{calc} /cm ³	1.323
μ/mm ⁻¹	1.583
F(000)	888.0
Radiation	Cu Kα (λ = 1.54184)
Crystal size/mm ³	0.14 × 0.12 × 0.11
2θ range for data collection/°	5.94 to 142.766
Index ranges	-19 ≤ h ≤ 15, -8 ≤ k ≤ 11, -15 ≤ l ≤ 18
Reflections collected	10488
Independent reflections	4017 [R _{int} = 0.0165, R _{sigma} = 0.0181]
Data/restraints/parameters	4017/0/279
Goodness-of-fit on F ²	1.049
Final R indexes [I >= 2σ(I)]	R ₁ = 0.0394, wR ₂ = 0.1061
Final R indexes [all data]	R ₁ = 0.0409, wR ₂ = 0.1073
Largest diff. peak/hole / e Å ⁻³	0.49/-0.45

Crystal Data for C₂₃H₂₄N₄O₂S (*M* = 420.52 g/mol): monoclinic, space group P2₁/c (no. 14), *a* = 16.1900(3) Å, *b* = 9.48160(10) Å, *c* = 14.9584(3) Å, β = 113.201(2)°, *V* = 2110.52(7) Å³, *Z* = 4, *T* = 150.00(10) K, μ(Cu Kα) = 1.583 mm⁻¹, *D*_{calc} = 1.323 g/cm³, 10488 reflections measured (5.94° ≤ 2θ ≤ 142.766°), 4017 unique (*R*_{int} = 0.0165, *R*_{sigma} = 0.0181) which were used in all calculations. The final *R*₁ was 0.0394 (*I* > 2σ(*I*)) and *wR*₂ was 0.1073 (all data).

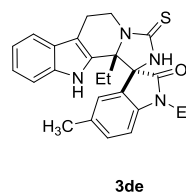
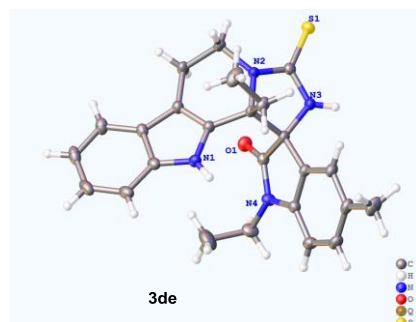


Table S2 Crystal data and structure refinement for 3de

Identification code	3de
Empirical formula	C ₂₅ H ₂₆ N ₄ OS
Formula weight	430.56
Temperature/K	295.55(10)
Crystal system	monoclinic
Space group	P2 ₁ /c
<i>a</i> /Å, <i>b</i> /Å, <i>c</i> /Å	13.01620(10), 15.4077(2), 11.23150(10)
α /°, β /°, γ /°	90, 98.5160(10), 90
Volume/Å ³	2227.64(4)
<i>Z</i>	4
ρ_{calc} /cm ³	1.284
μ /mm ⁻¹	1.478
<i>F</i> (000)	912.0
Radiation	Cu K α (λ = 1.54184)
Crystal size/mm ³	0.14 × 0.12 × 0.11
2 θ range for data collection/°	6.866 to 151.304
Index ranges	-16 ≤ <i>h</i> ≤ 15, -19 ≤ <i>k</i> ≤ 16, -14 ≤ <i>l</i> ≤ 9
Reflections collected	15536
Independent reflections	4442 [<i>R</i> _{int} = 0.0255, <i>R</i> _{sigma} = 0.0227]
Data/restraints/parameters	4442/0/283
Goodness-of-fit on <i>F</i> ²	1.172
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0422, <i>wR</i> ₂ = 0.1081
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0438, <i>wR</i> ₂ = 0.1092
Largest diff. peak/hole / e Å ⁻³	0.23/-0.39

Crystal Data for C₂₅H₂₆N₄OS (*M* = 430.56 g/mol): monoclinic, space group P2₁/c (no. 14), *a* = 13.01620(10) Å, *b* = 15.4077(2) Å, *c* = 11.23150(10) Å, β = 98.5160(10)°, *V* = 2227.64(4) Å³, *Z* = 4, *T* = 295.55(10) K, μ (Cu K α) = 1.478 mm⁻¹, *D*_{calc} = 1.284 g/cm³, 15536 reflections measured (6.866° ≤ 2 θ ≤ 151.304°), 4442 unique (*R*_{int} = 0.0255, *R*_{sigma} = 0.0227) which were used in all calculations. The final *R*₁ was 0.0422 (*I* > 2 σ (*I*)) and *wR*₂ was 0.1092 (all data).

7. MTT assay

Cells were trypsinogen in logarithmic phase, and 5×10^3 cells were resuspended in culture medium and inoculated into 96-well plates. After 24 h of culture, the cells were incubated with drugs for 48 h. MTT was added for 4 h, the supernatant culture medium was discarded, shaken with DMSO for 10 min, and the absorbance value was measured at 490 nm by a microplate reader. IBM SPSS Statistics 23 software was used to calculate half lethality (IC_{50}).

8. Apoptosis analysis

A549 cells were digested with pancreatin when they grew to logarithmic phase. Cell morphology, AO/EB staining, Hoechst 33258, reactive oxygen species, and JC-1 were all inoculated into 6-well plates at a medium dilution density of 1×10^5 cells. After 24 h, **3cb** were treated with drug addition (set up a control group, treated group (10, 20, 30 μ M) for 48 h. At the end of incubation, photographs were taken and recorded under an inverted fluorescence microscope and analyzed.

9. Colony formation assay

A549 cells were digested with trypsin when they grew to the logarithmic growth phase, and the medium diluted cell density was 200 cells/well seeded in 6-well plates. After 24 h of culture, **3cb** was treated and incubated for 48 h with the addition of drugs (set control group, and the additional group (1.25, 2.5, and 5 μ M). Fresh medium was replaced every 2 d for 14 d. Crystal violet staining was performed after the end of incubation. photographed and recorded and data analysis was performed.

10. Cell migration assay

Cell wound scratches were inoculated into 6-well plates at a density of 1×10^4 cells diluted in serum-free medium and allowed to grow confluent. Wound scratches were performed with a pipette tip and floating cells were washed off with PBS. Control group, **3cb** drug group (0.625, 2.5, 10 μ M) was established, and pictures recorded at 0 h and 24 h were collected under an inverted fluorescence microscope for analysis.

Transwell migration was inoculated into the upper chamber of transwell inserts at a density of 3

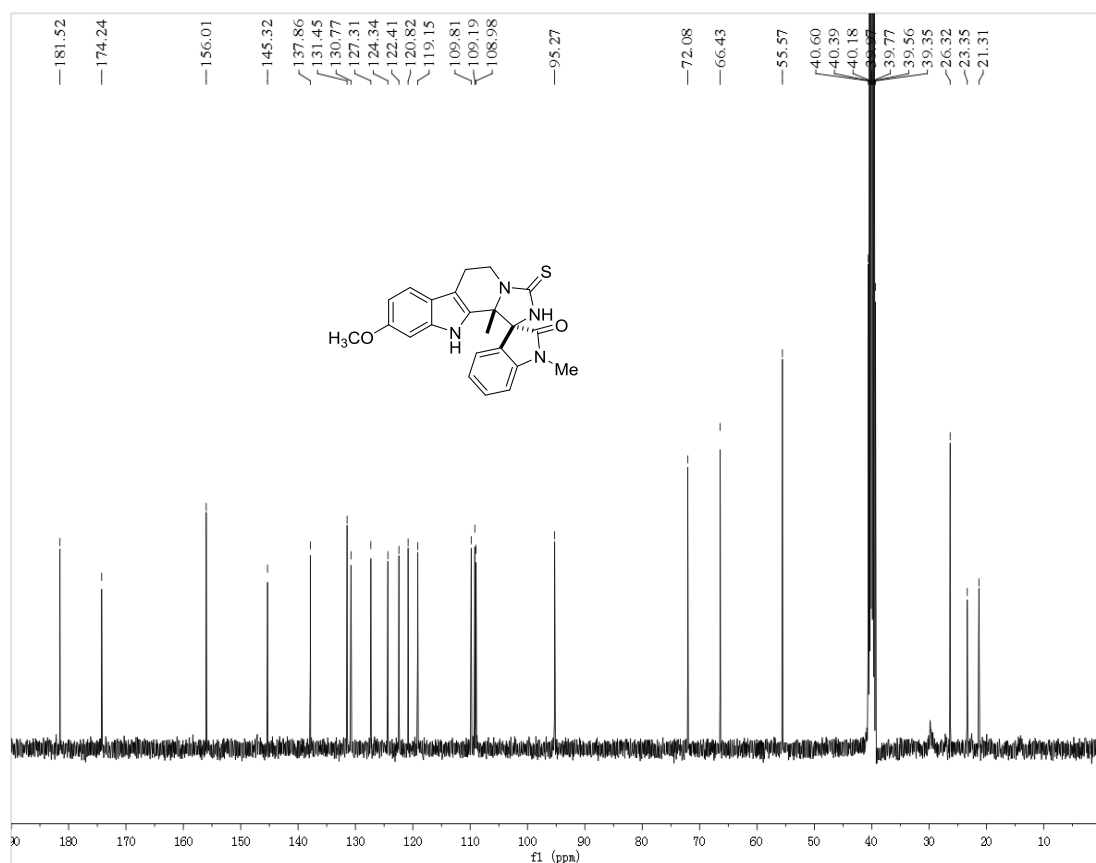
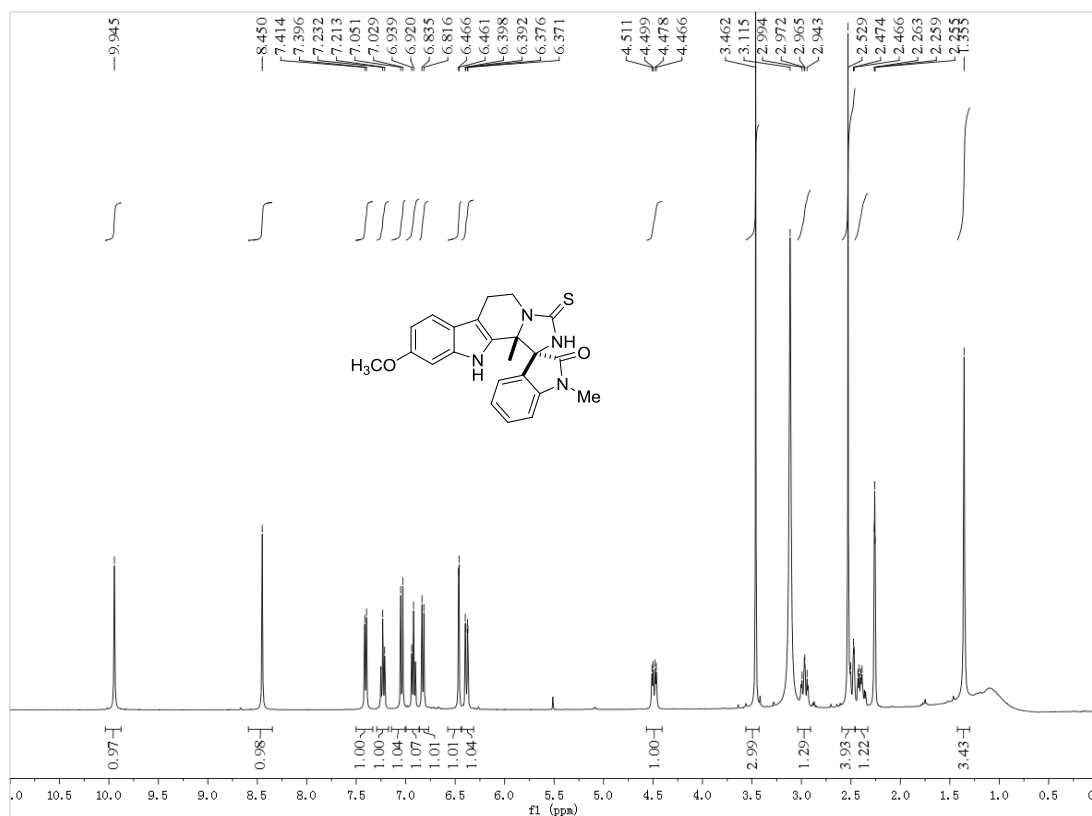
$\times 10^4$ cells/100 μL diluted in serum-free medium. Add 600 μL culture media containing 10% fetal bovine serum in the lower chamber, which is similar to the setting of **3cb** drug concentration in the upper chamber (the control group, drug group 2.5, 5 and 10 μM). Co-cultures were incubated for 24 h and washed twice with PBS at the end of the incubation. Co-cultures were incubated for 24 h and washed twice with PBS at the end of the incubation. Cells that did not migrate in the upper chamber were rubbed off and fixed in 4% paraformaldehyde for 2 min. Methanol was incubated for 20 min and crystal violet staining was performed for 15 min before photographic recording.

11. Westren Blot assay

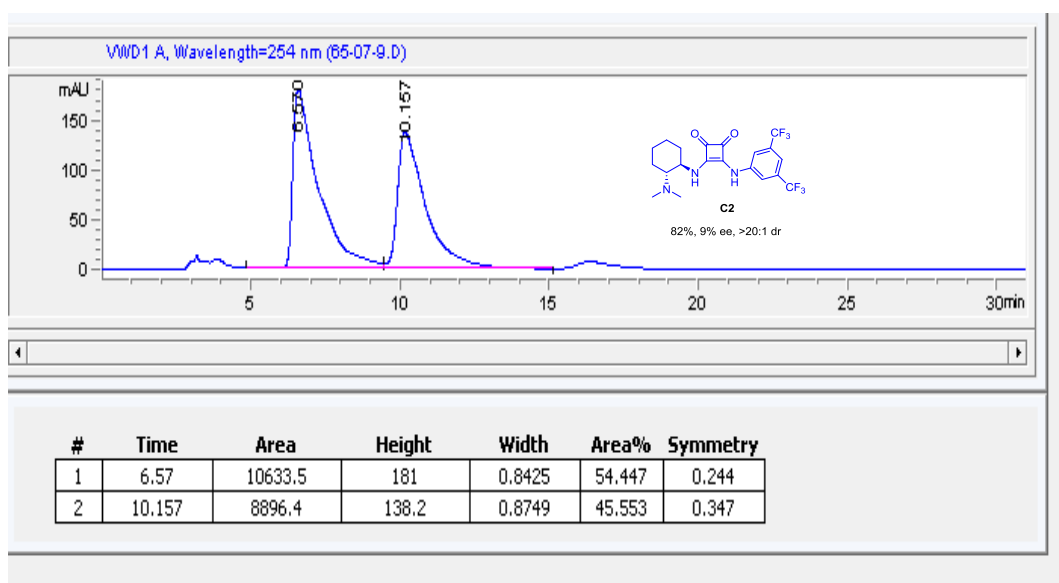
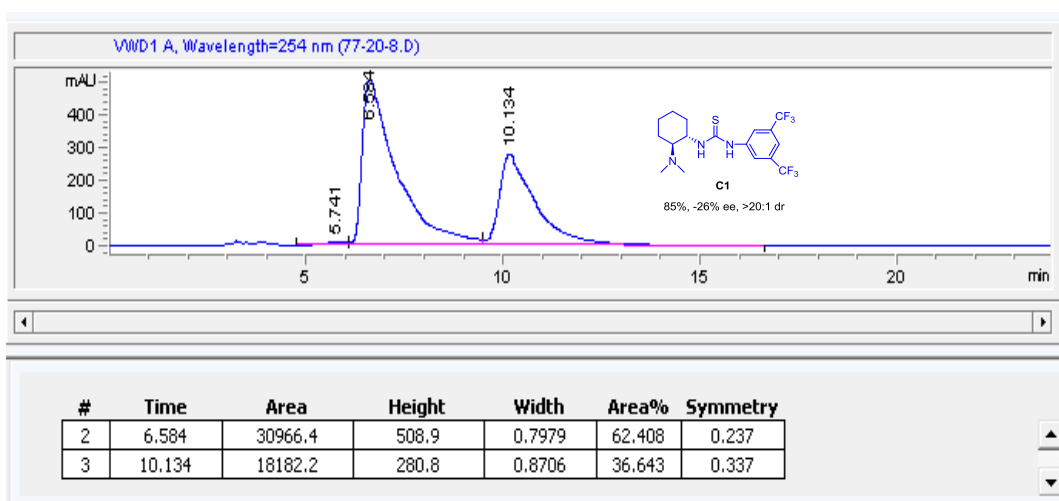
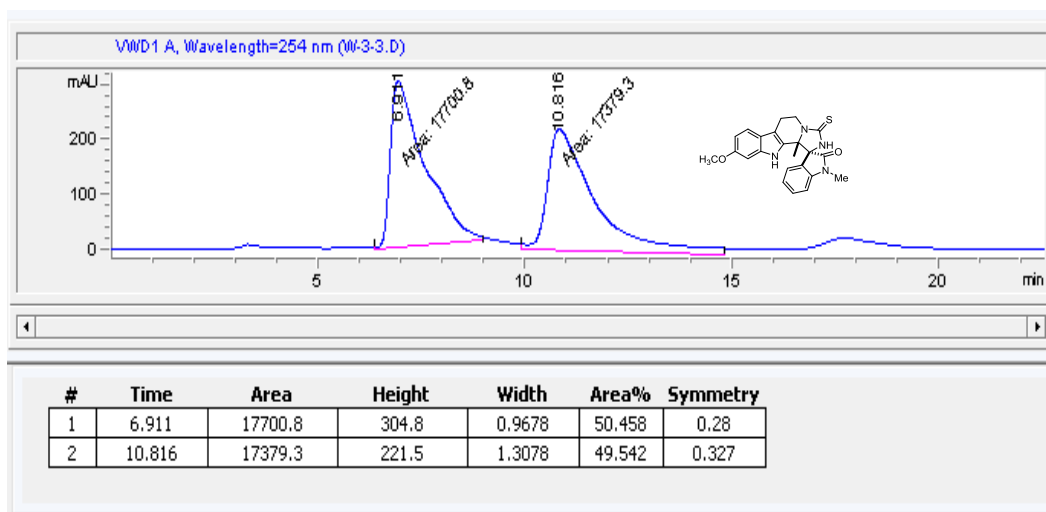
A549 cells were digested with trypsin when grew to log phase and 3×10^5 cells were diluted with medium and seeded in 6-well plates. After 24 h of incubation, the cells were incubated for 48 h with drug (the control group, **3cb** treatment group 10, 20, 30 μM). At the end of incubation, cell pellets were collected. Lysis was performed on ice for 30 min, supernatants were collected by centrifugation. Protein samples were boiled, and protein was quantified by BCA after denaturation. Electrophoresis was performed at 80 V for 30 min, 120 V for 1 h, transferred on ice at 90 V for 1 h, blocked with skim milk for 1 h, incubated overnight at 4 $^{\circ}\text{C}$ with primary antibodies, and after 1 h with secondary antibodies, ECL luminescence was developed. Protein band gray values were analyzed and recorded by Image Lab.

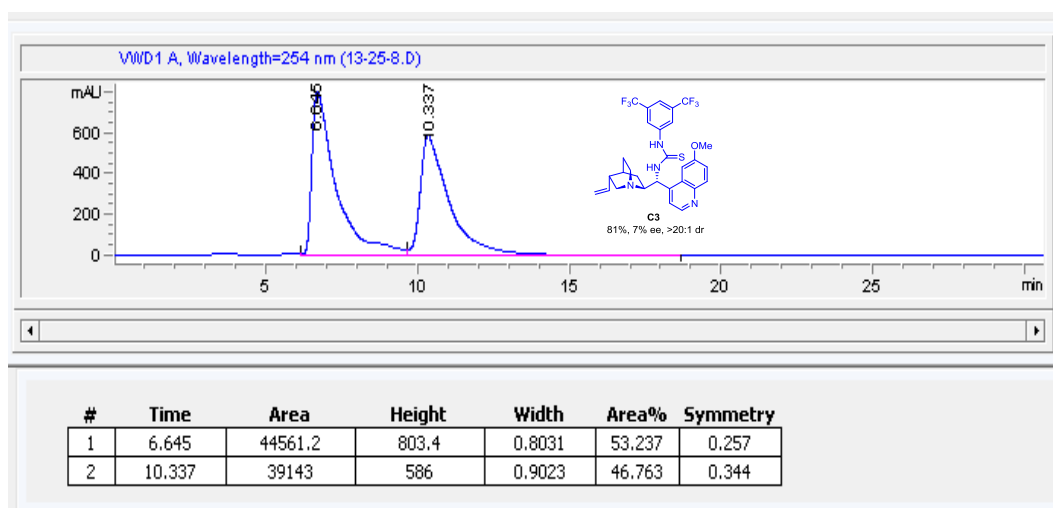
12. The copies of ^1H NMR, ^{13}C NMR and HPLC spectra for compounds 3

^1H and ^{13}C NMR of 3aa

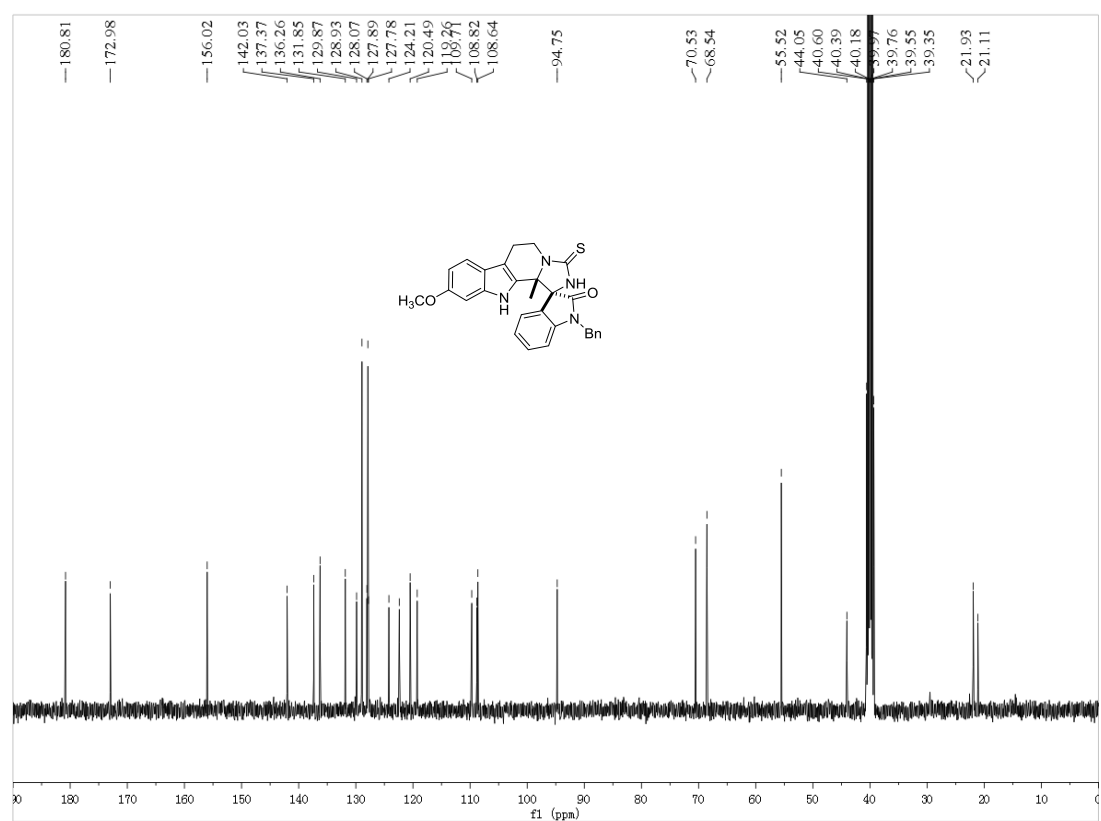
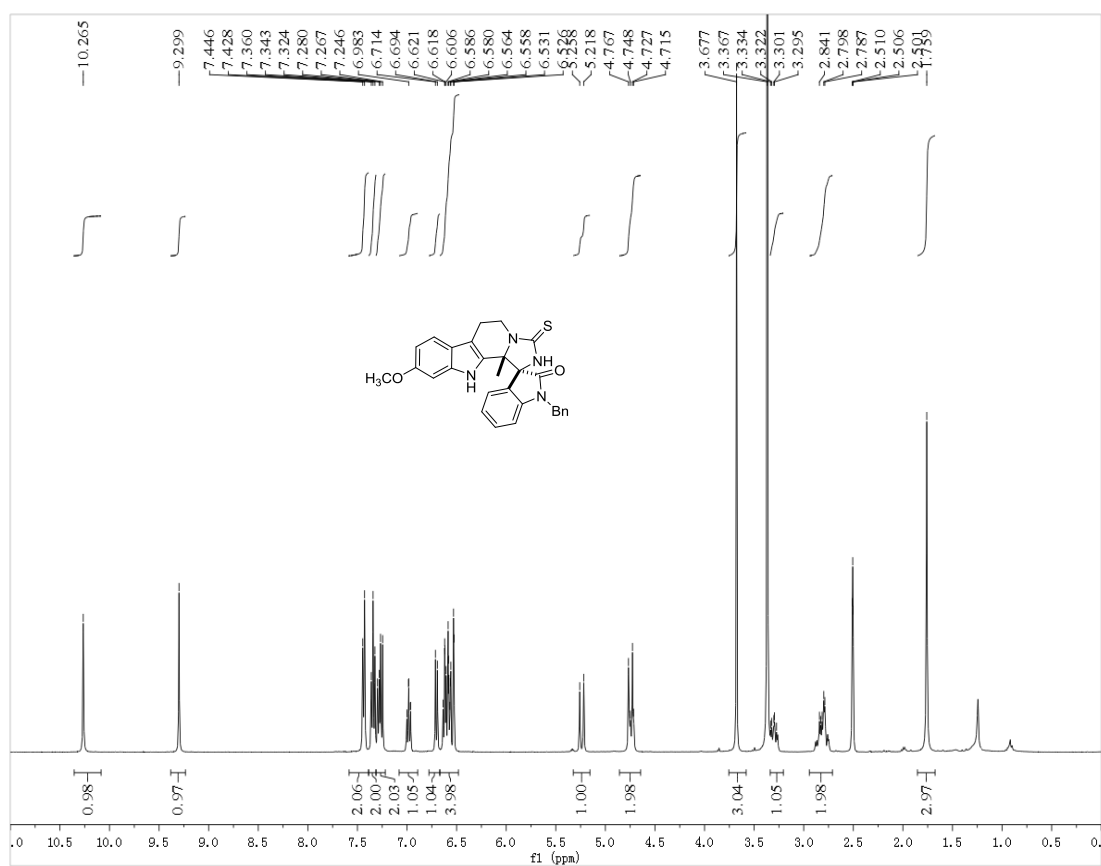


HPLC of 3aa

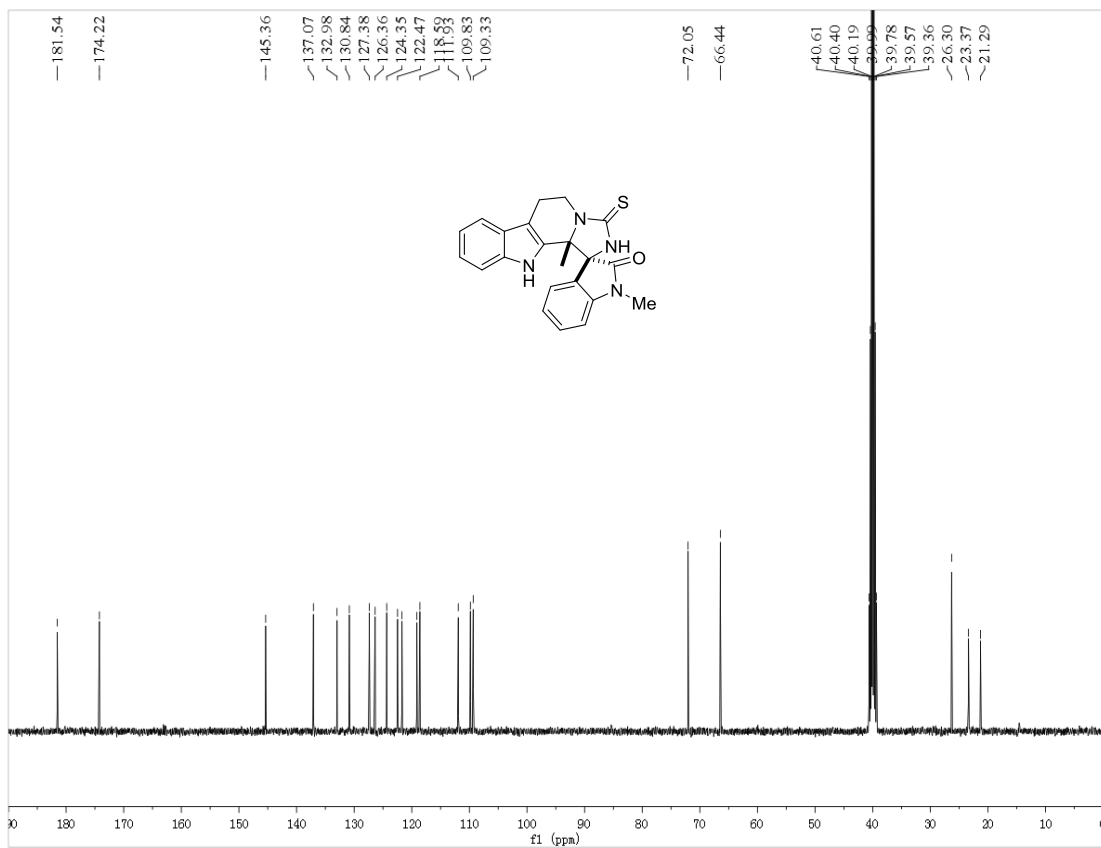
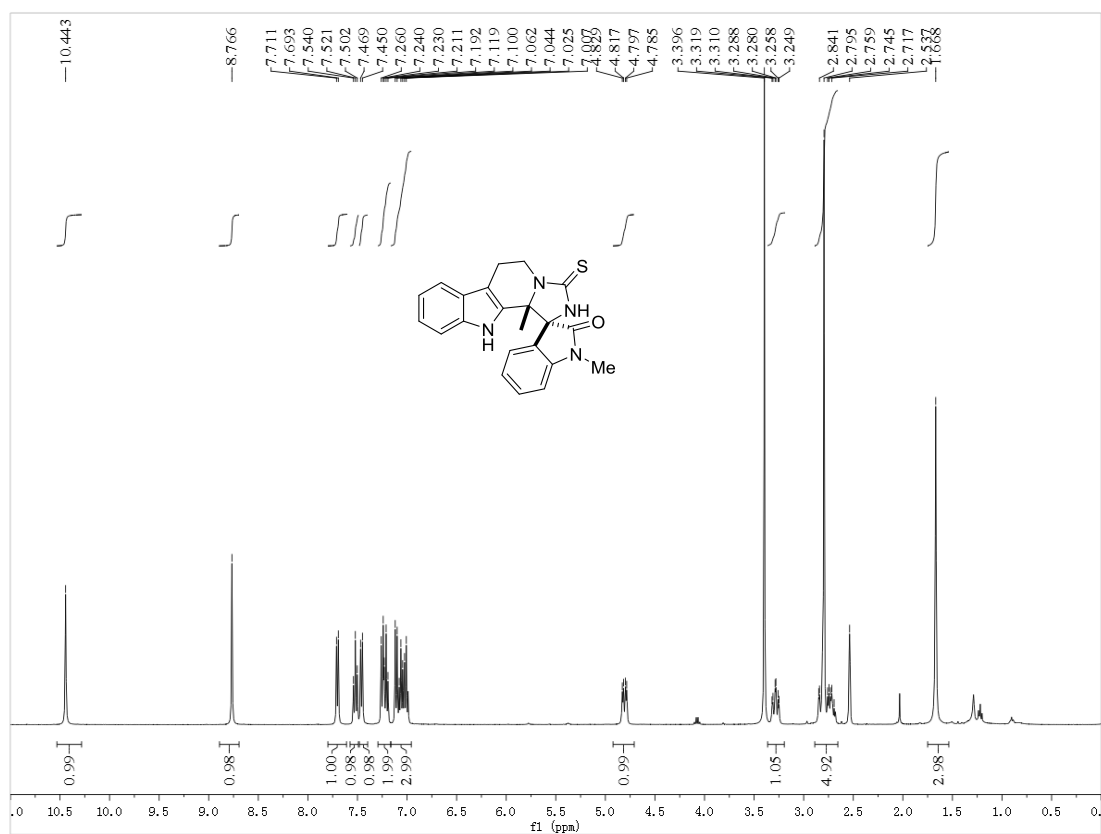




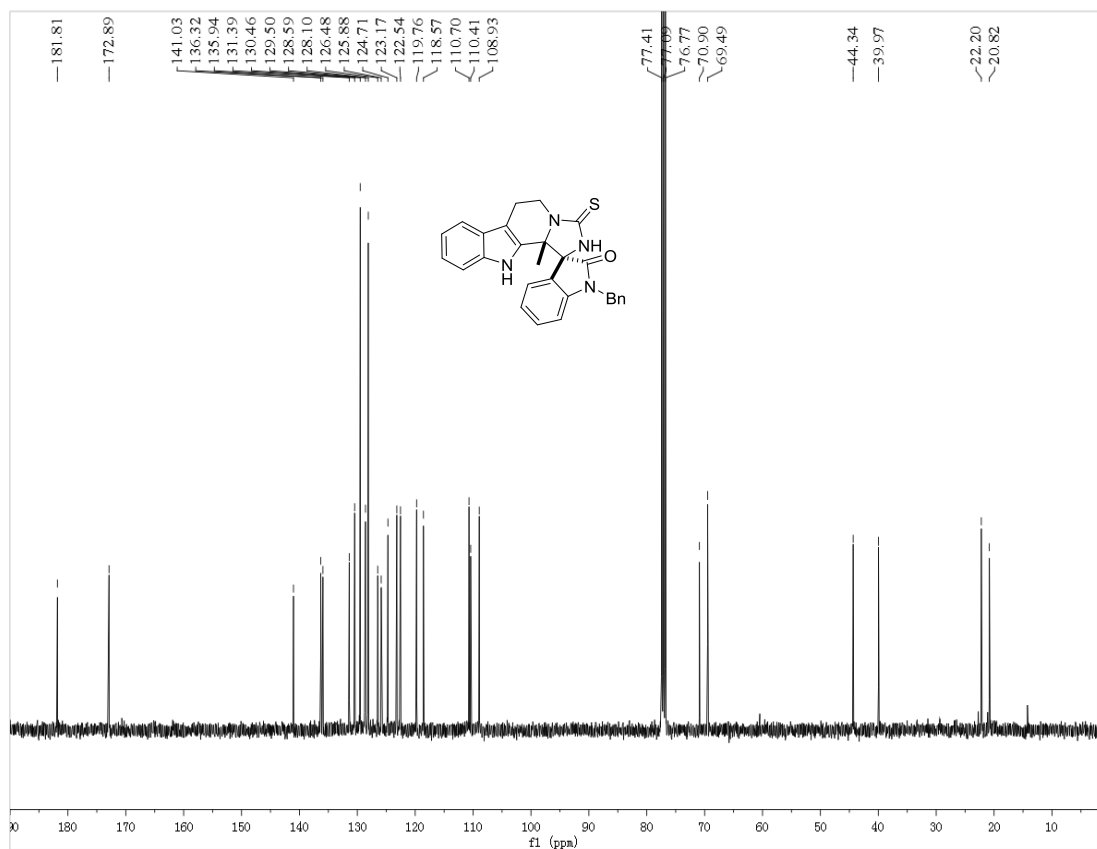
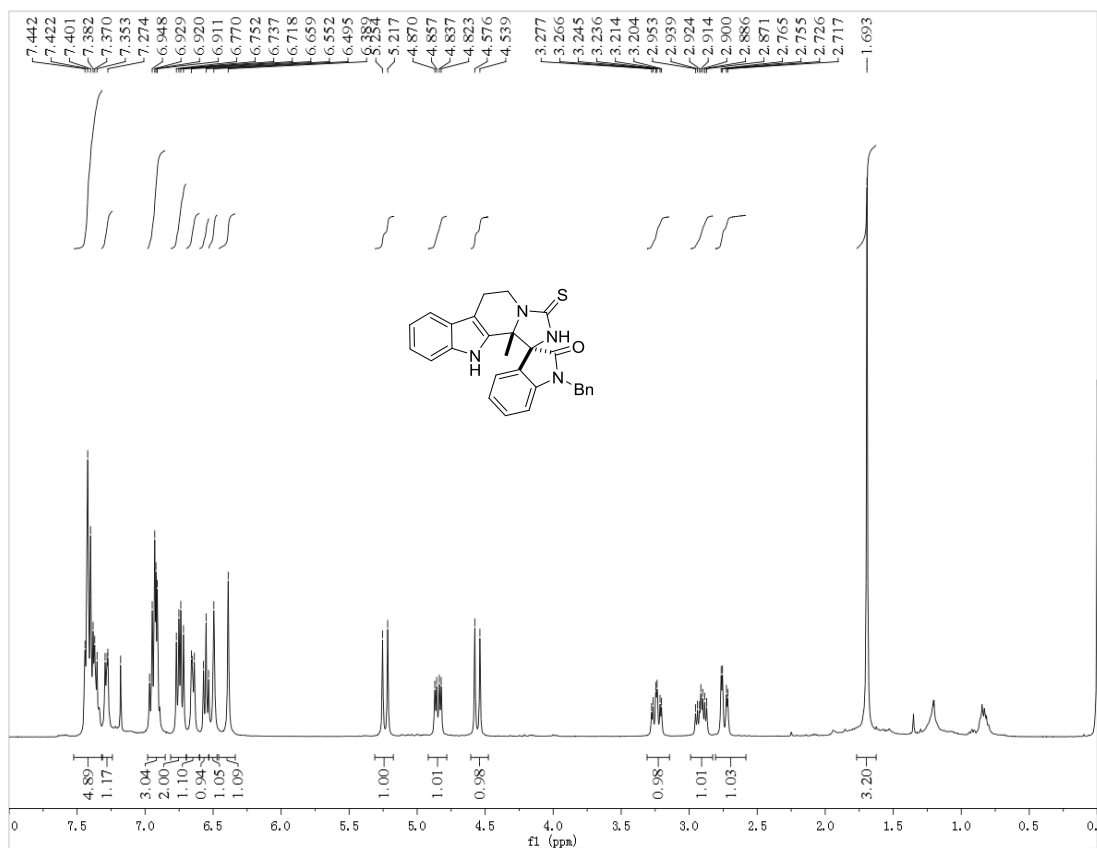
^1H and ^{13}C NMR of 3ab



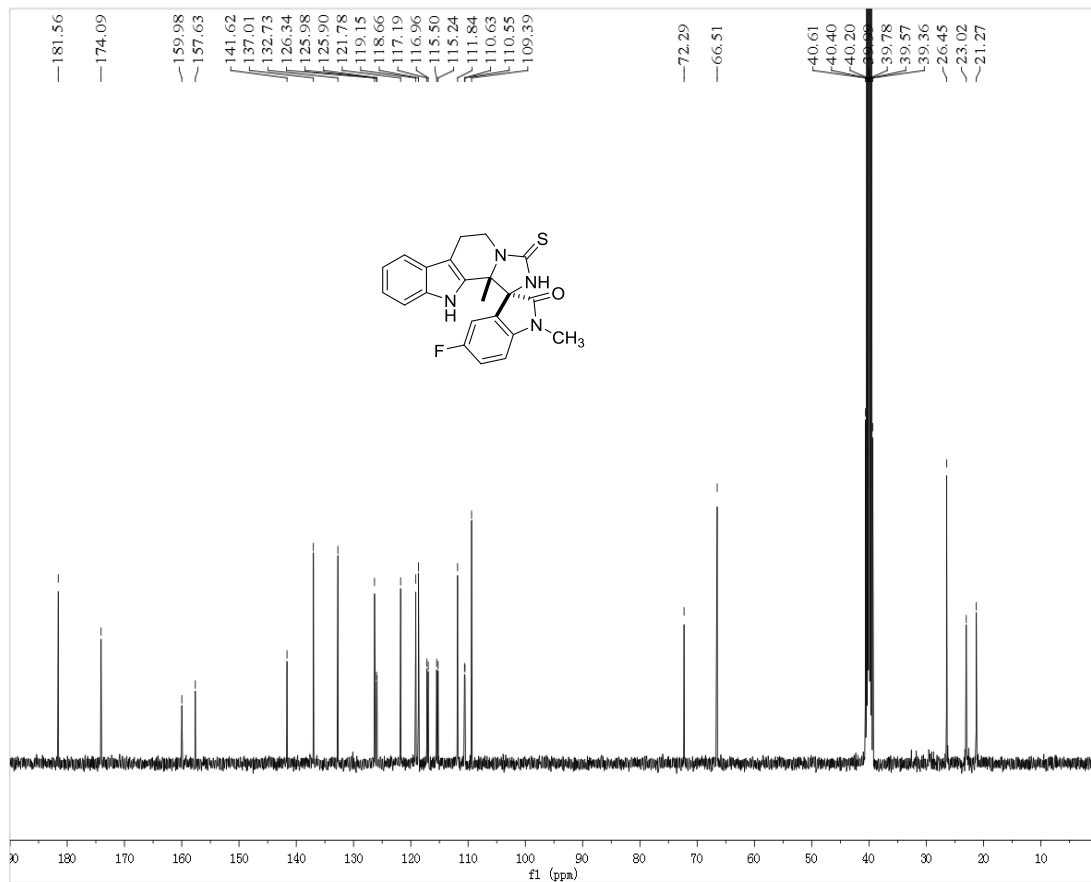
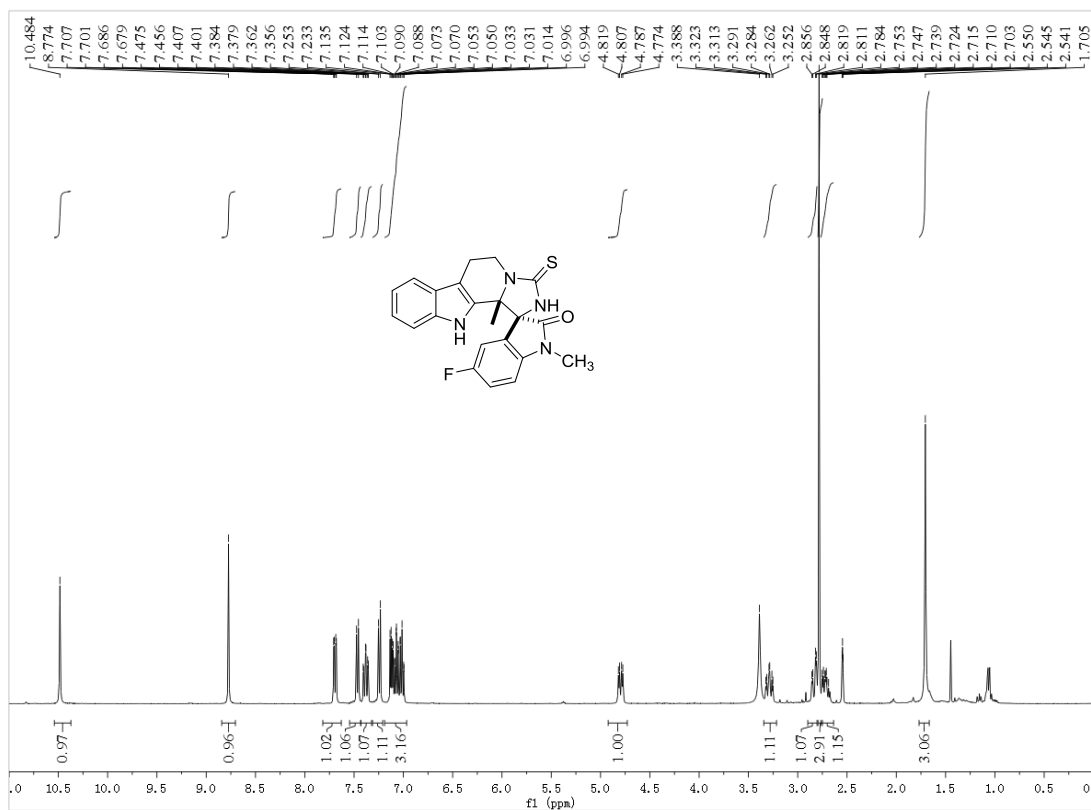
^1H and ^{13}C NMR of 3ba



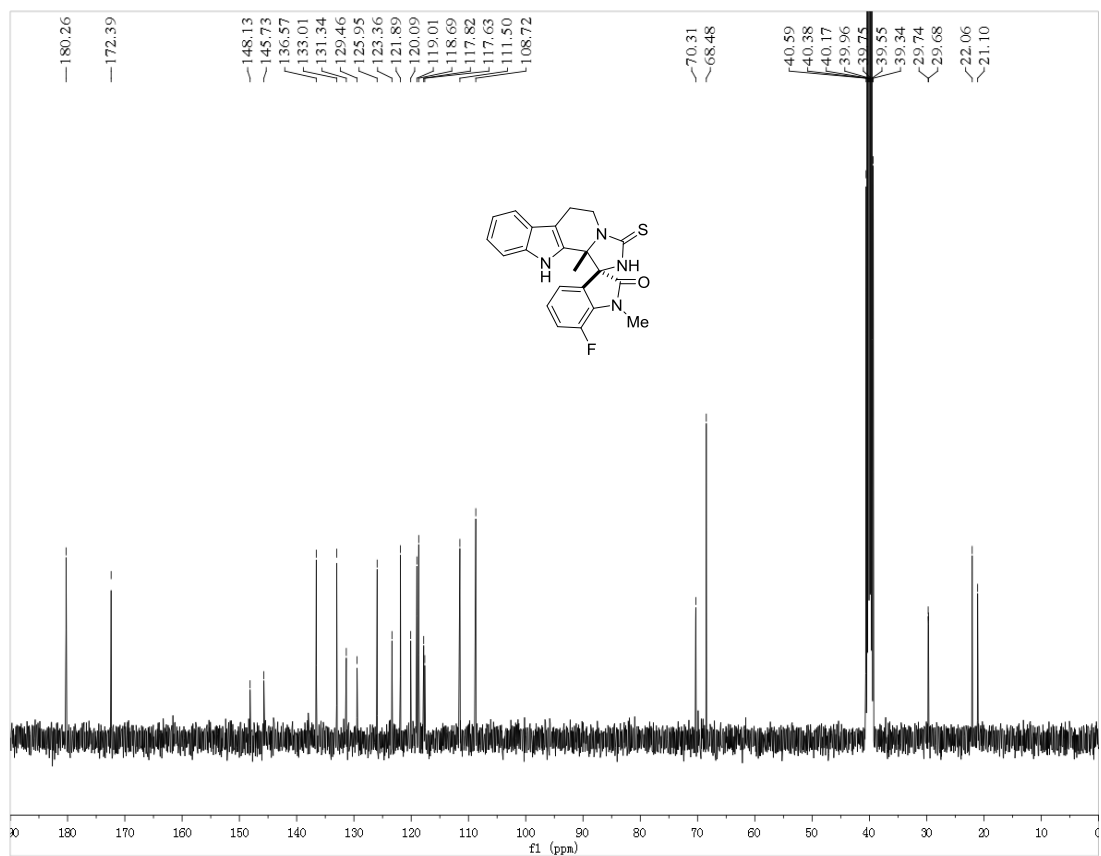
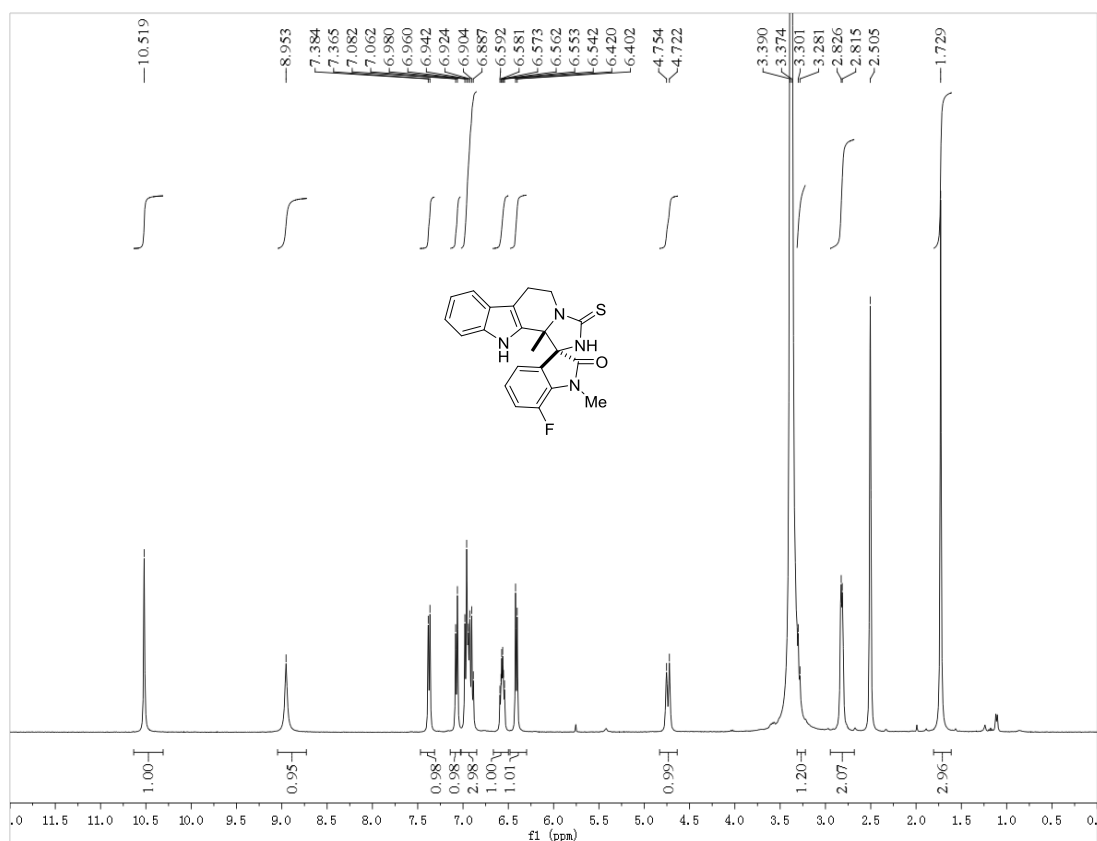
^1H and ^{13}C NMR of 3bb



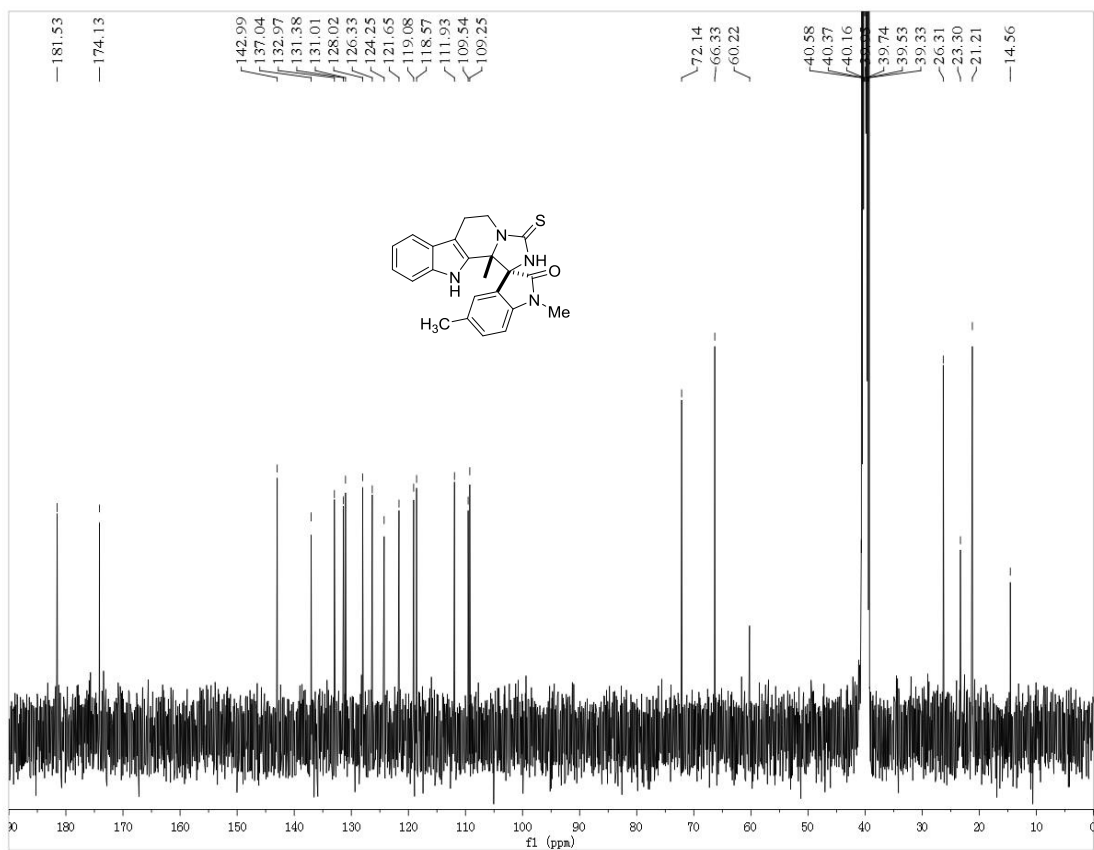
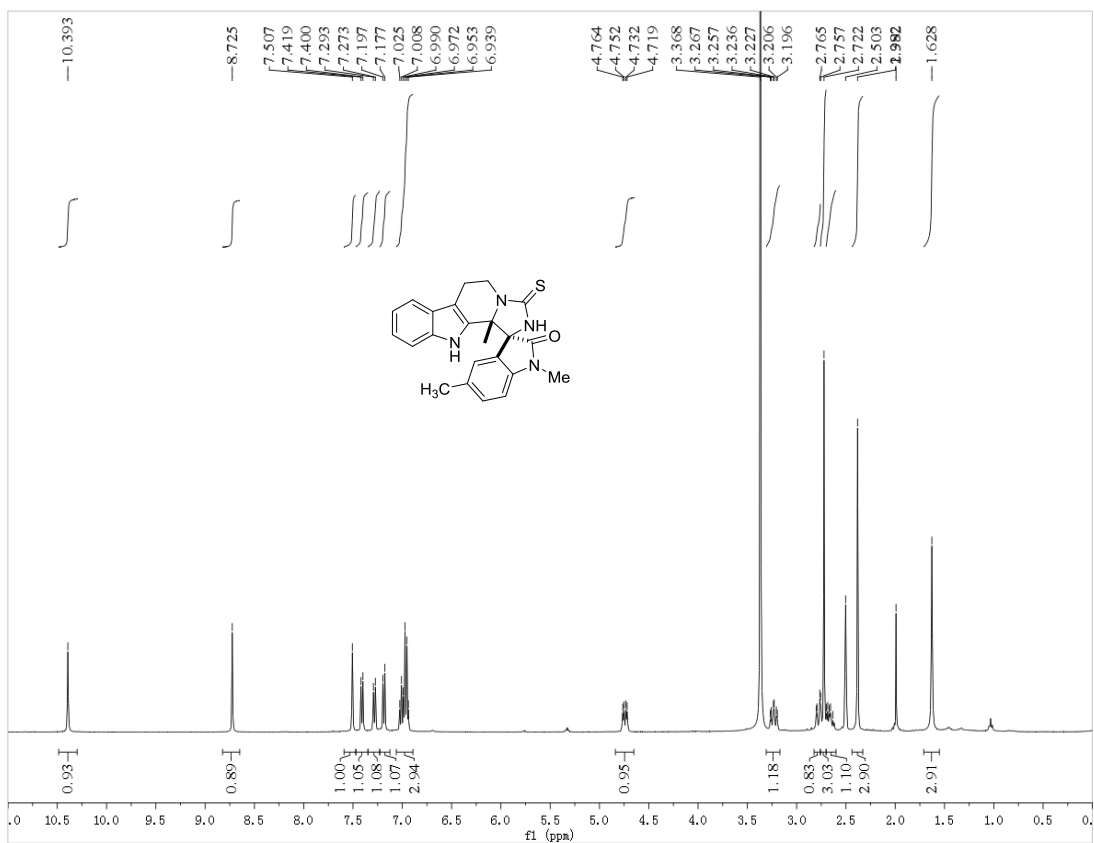
^1H and ^{13}C NMR of 3bc



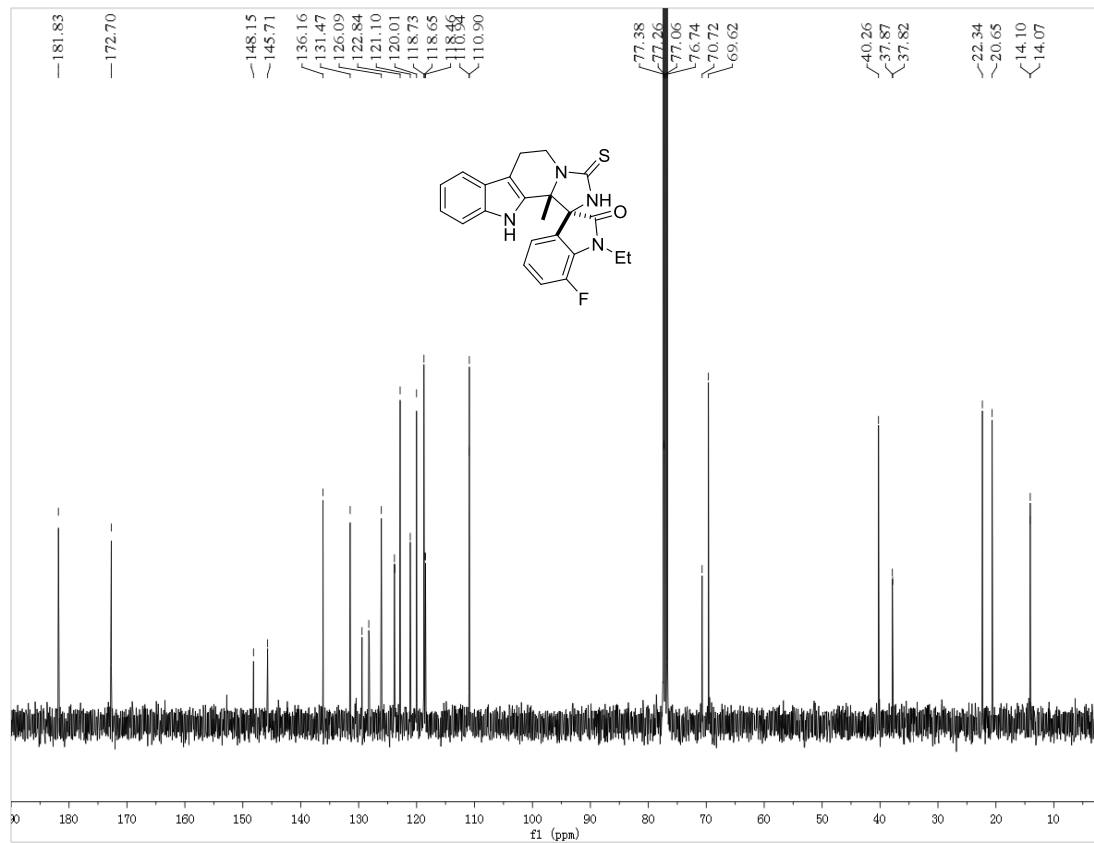
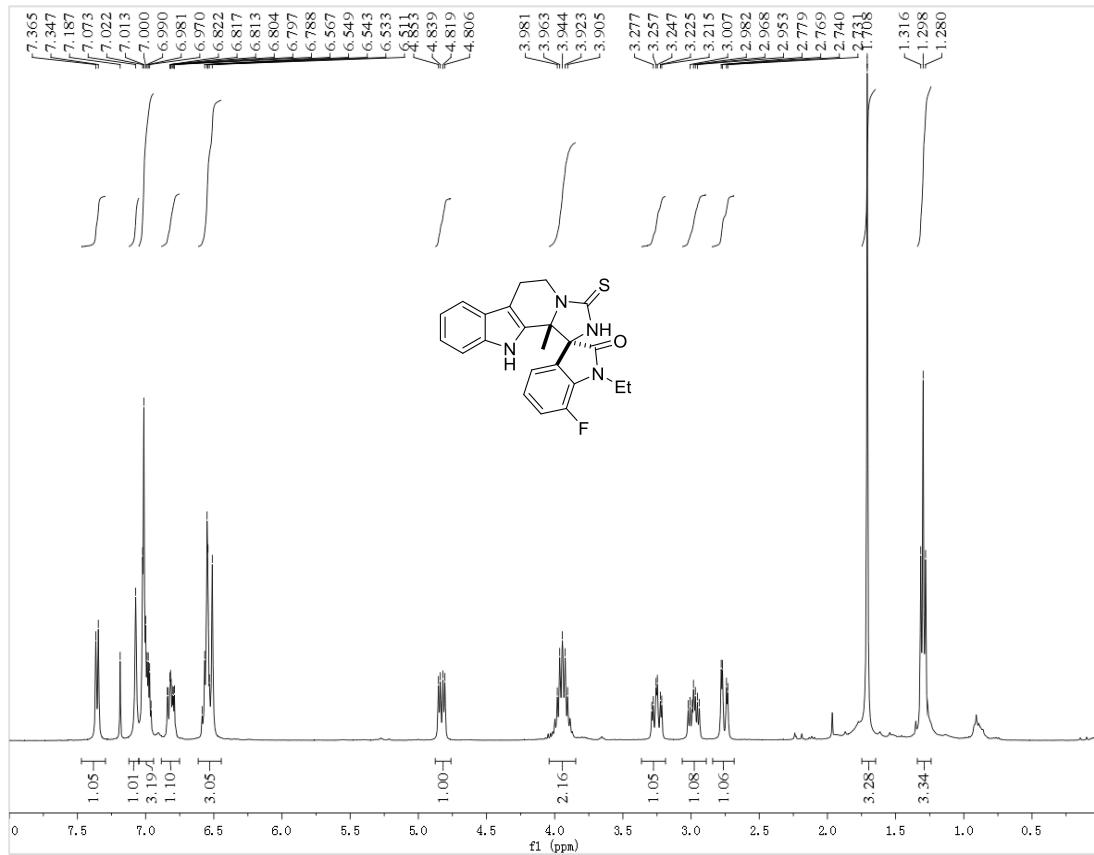
¹H and ¹³C NMR of 3bd



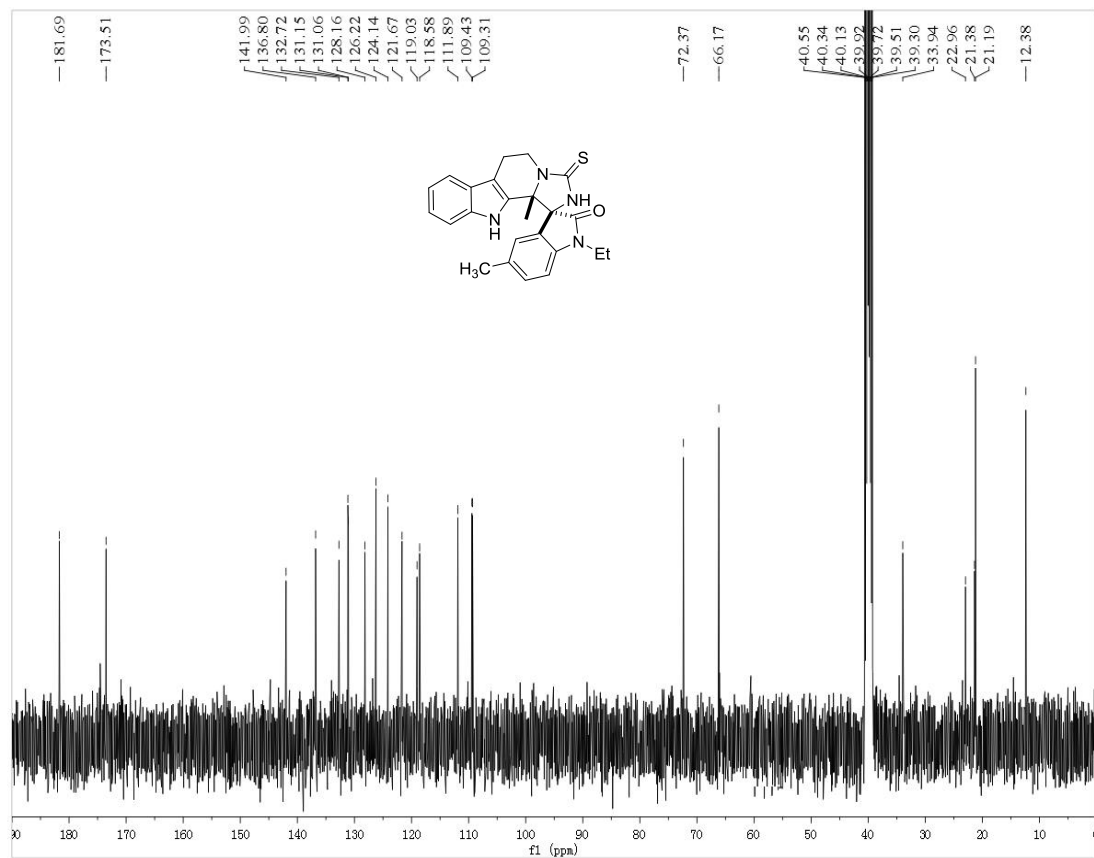
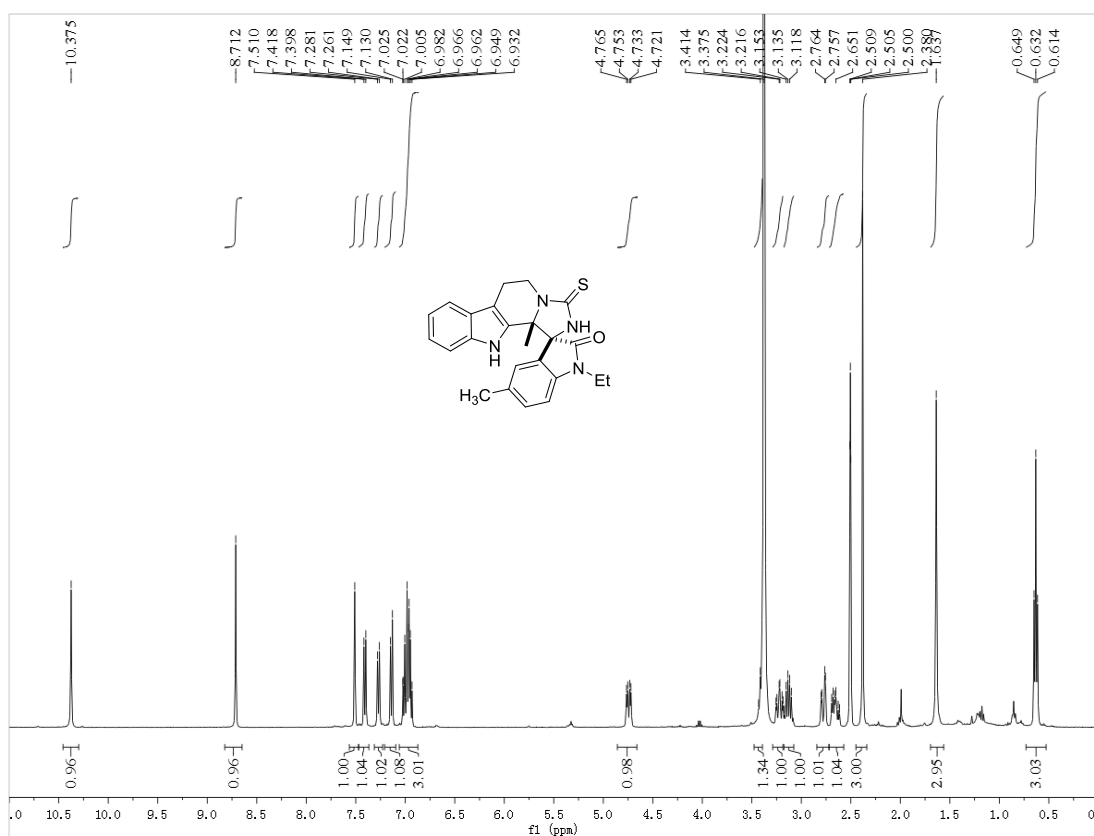
¹H and ¹³C NMR of 3be



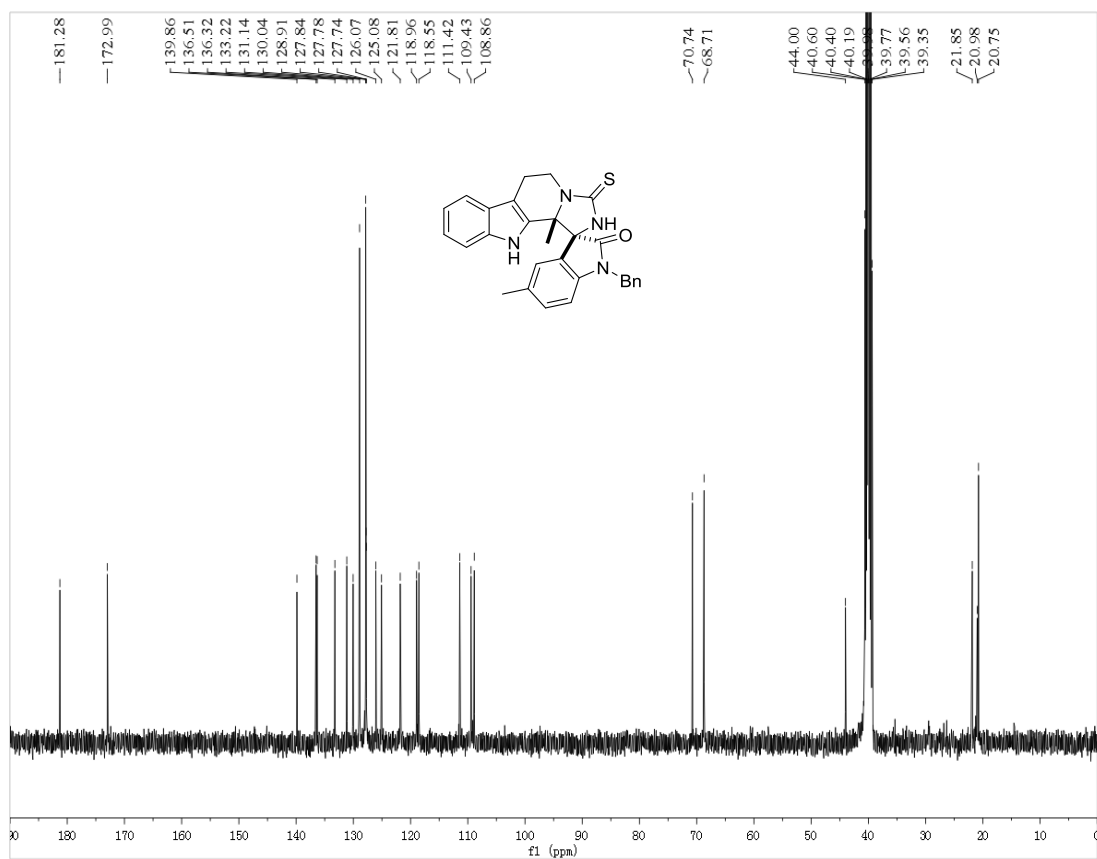
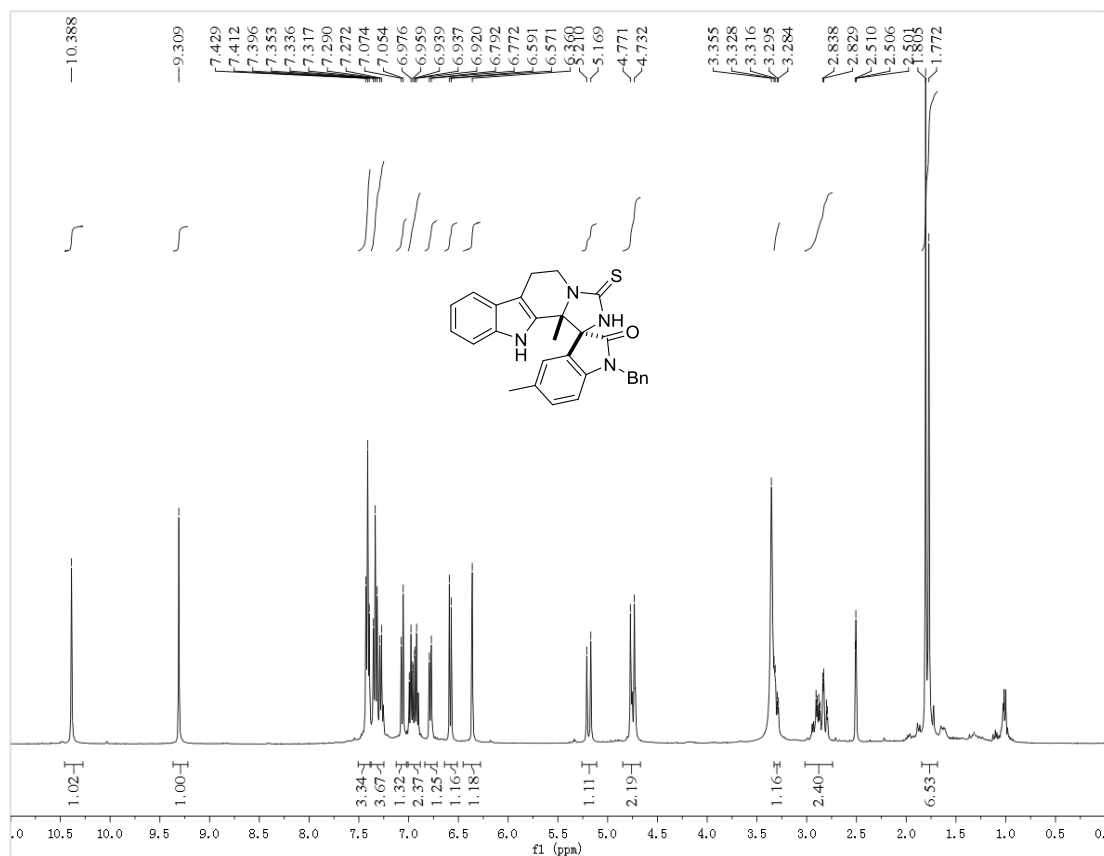
¹H and ¹³C NMR of 3bf



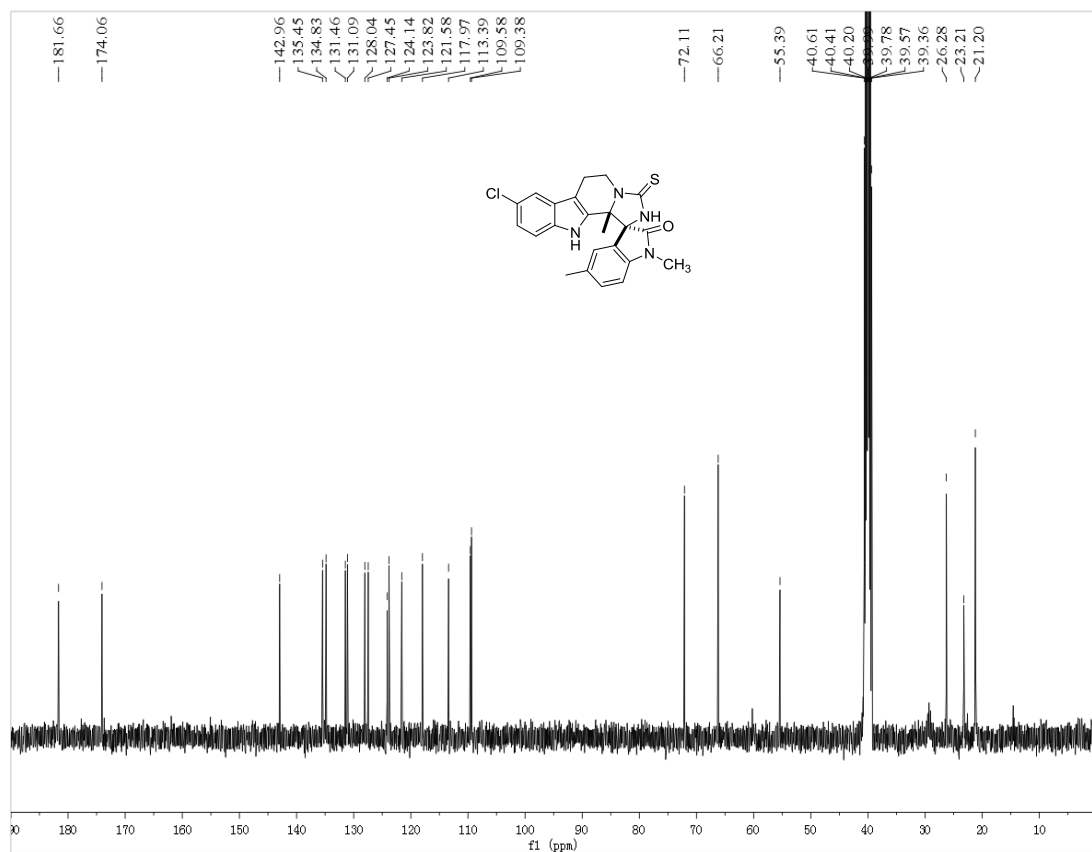
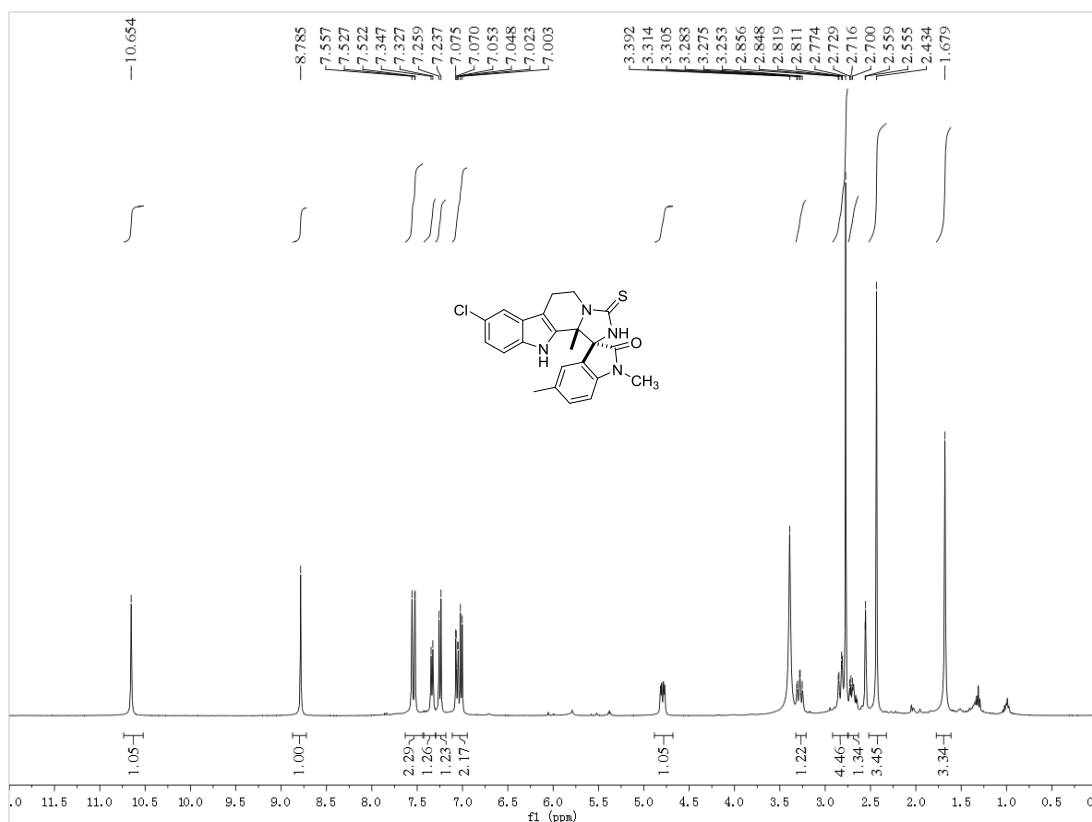
^1H and ^{13}C NMR of 3bg



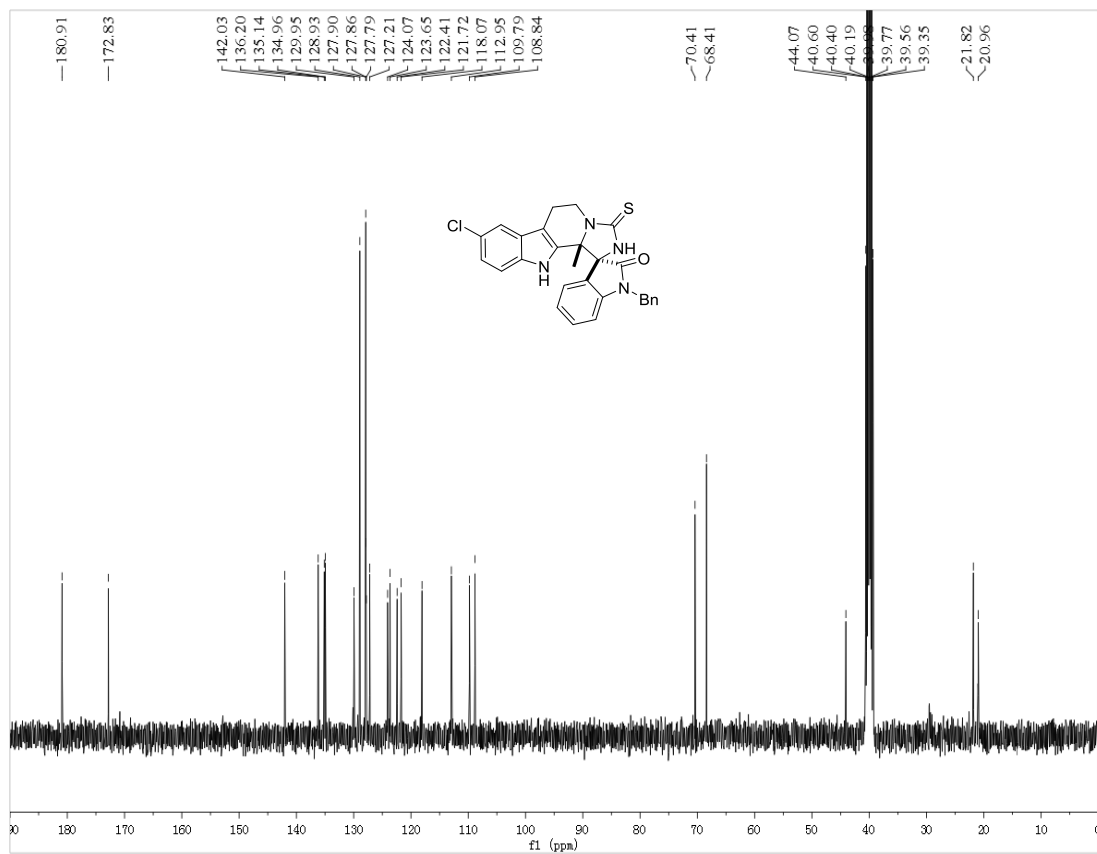
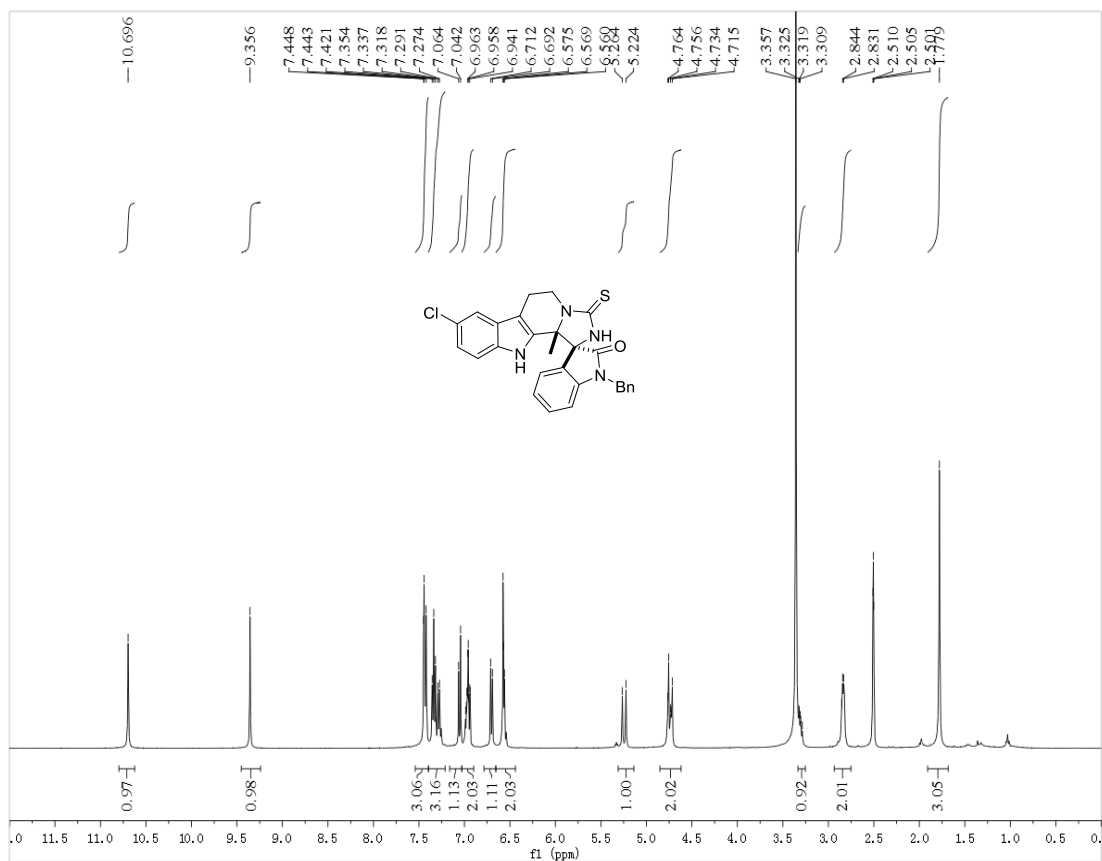
^1H and ^{13}C NMR of 3bh



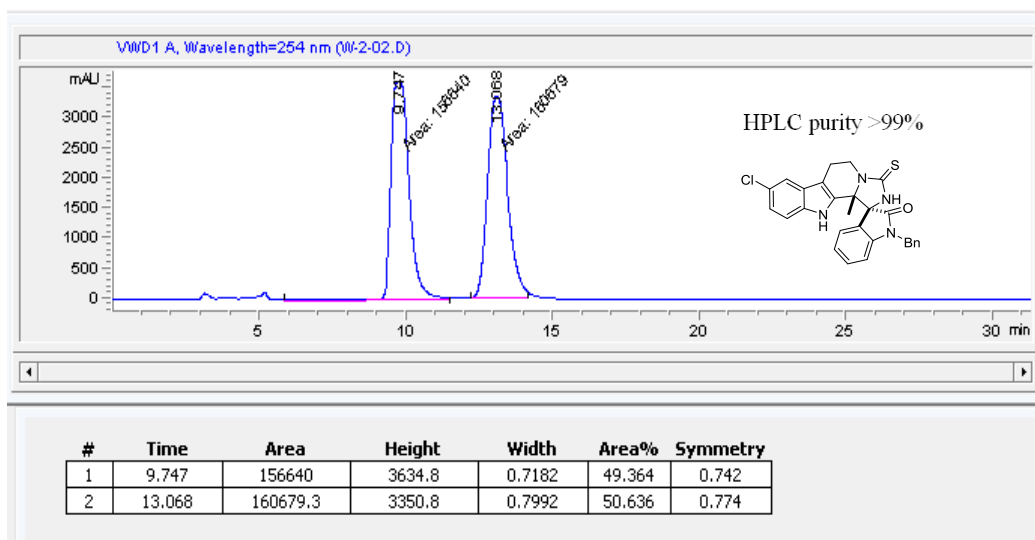
^1H and ^{13}C NMR of 3ca



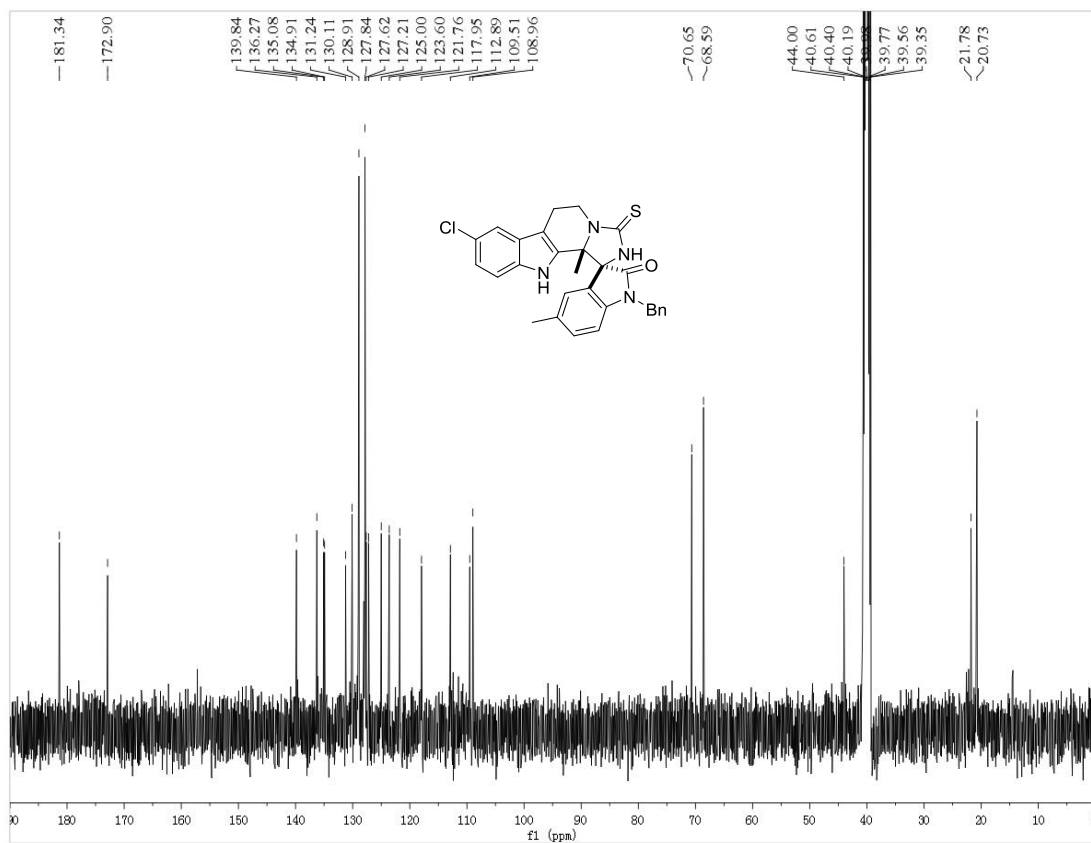
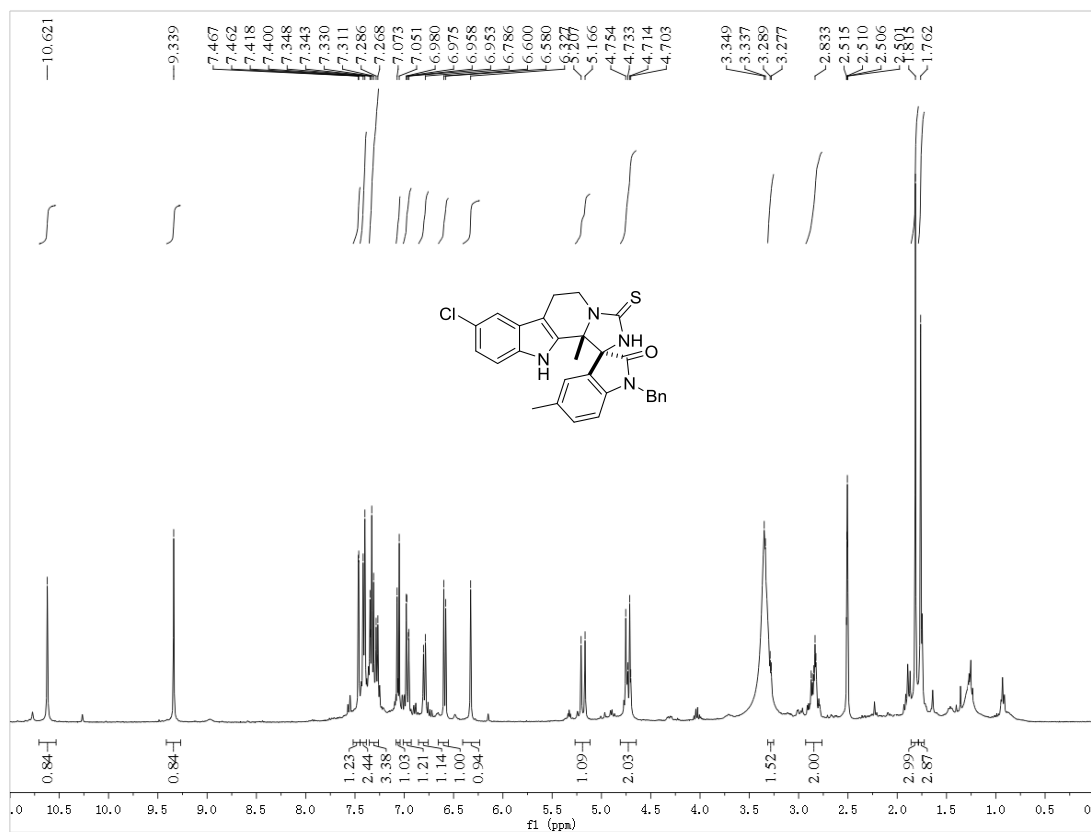
¹H and ¹³C NMR of 3cb



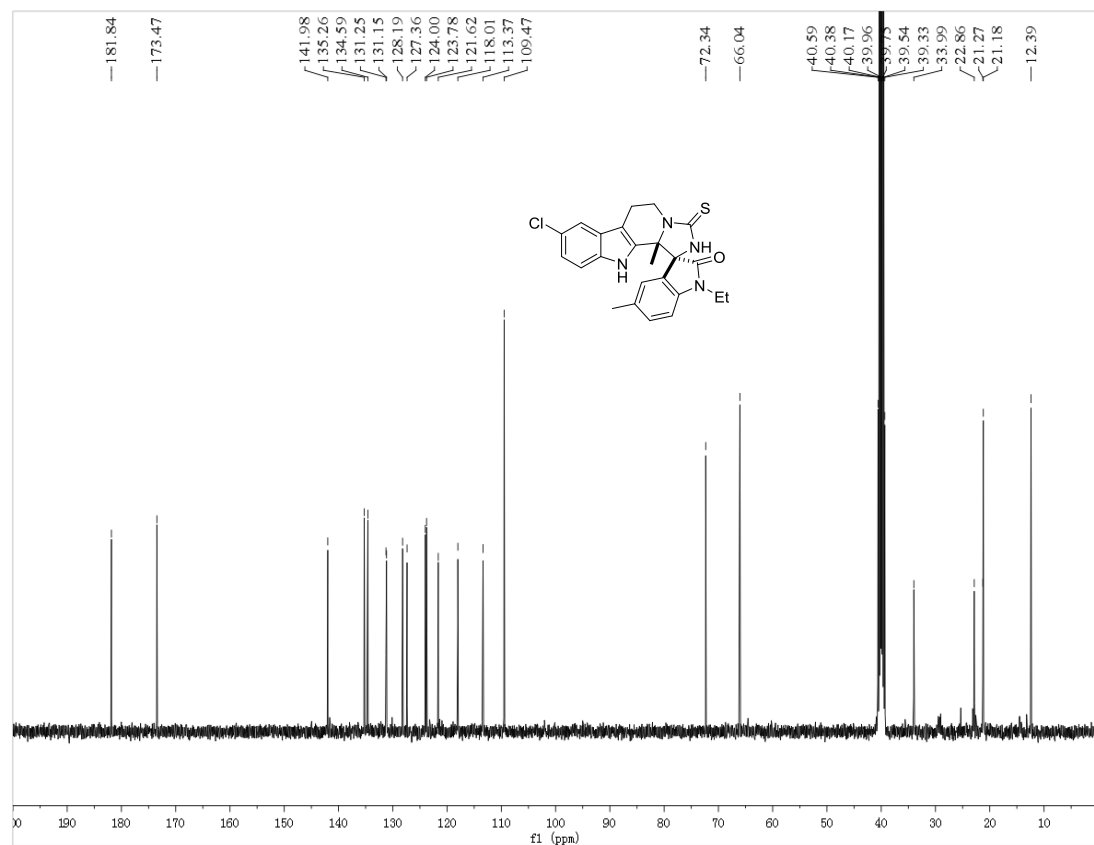
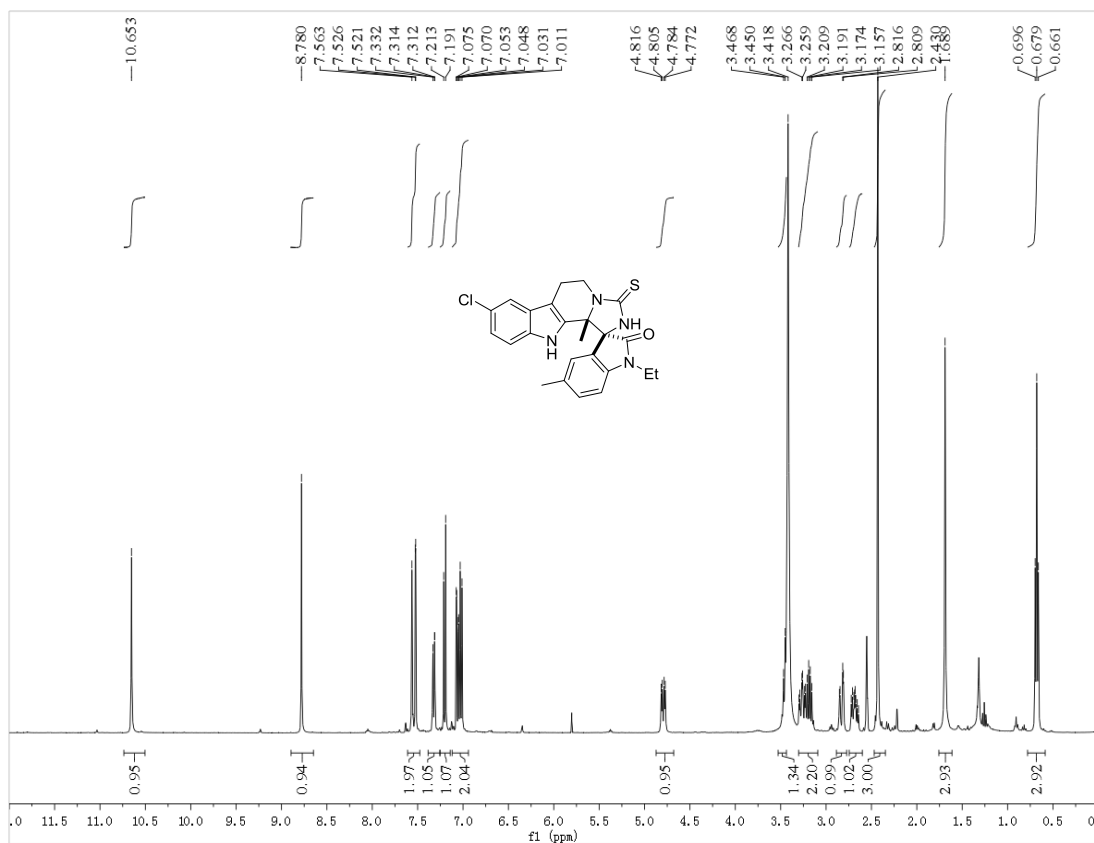
HPLC of racemic 3b



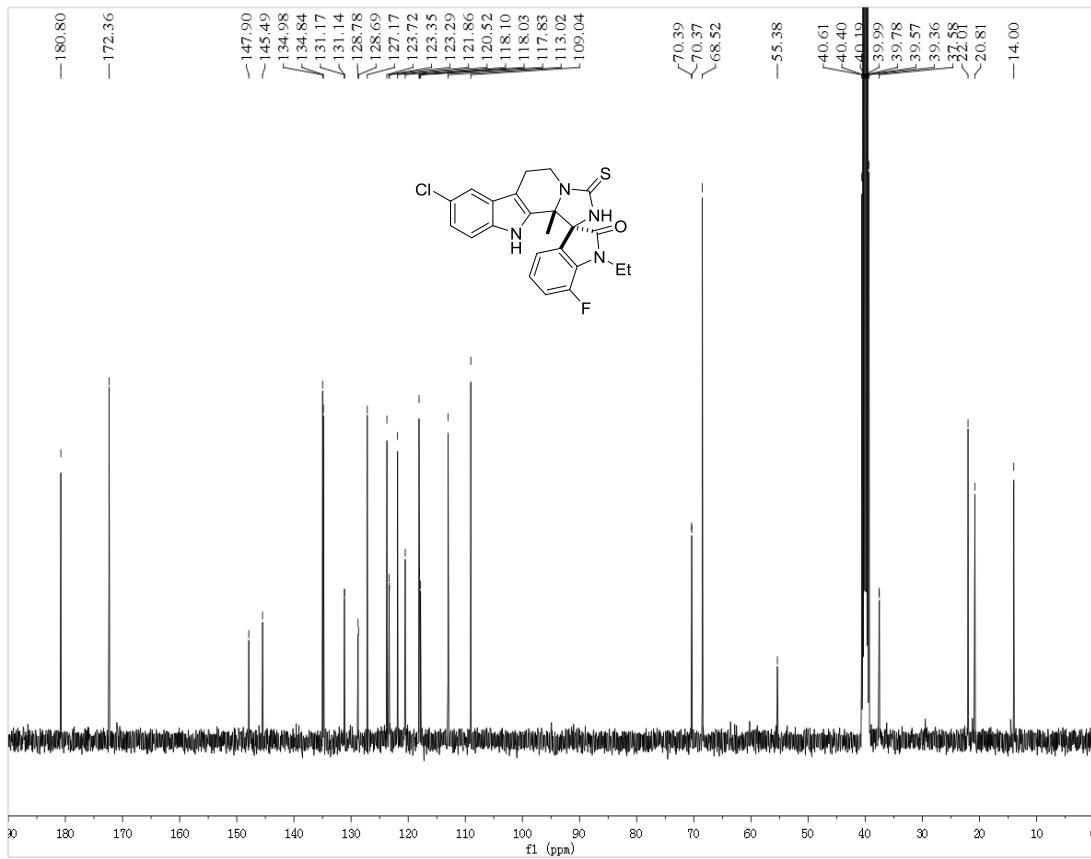
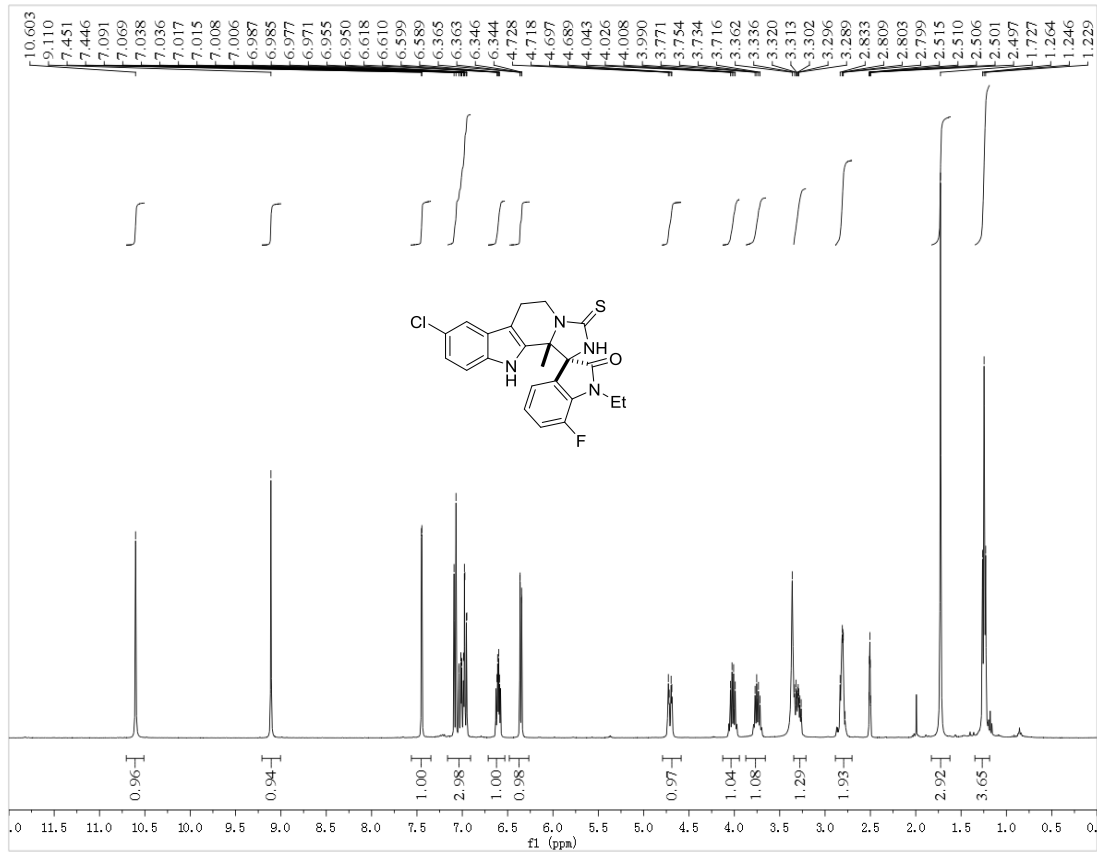
^1H and ^{13}C NMR of 3cc



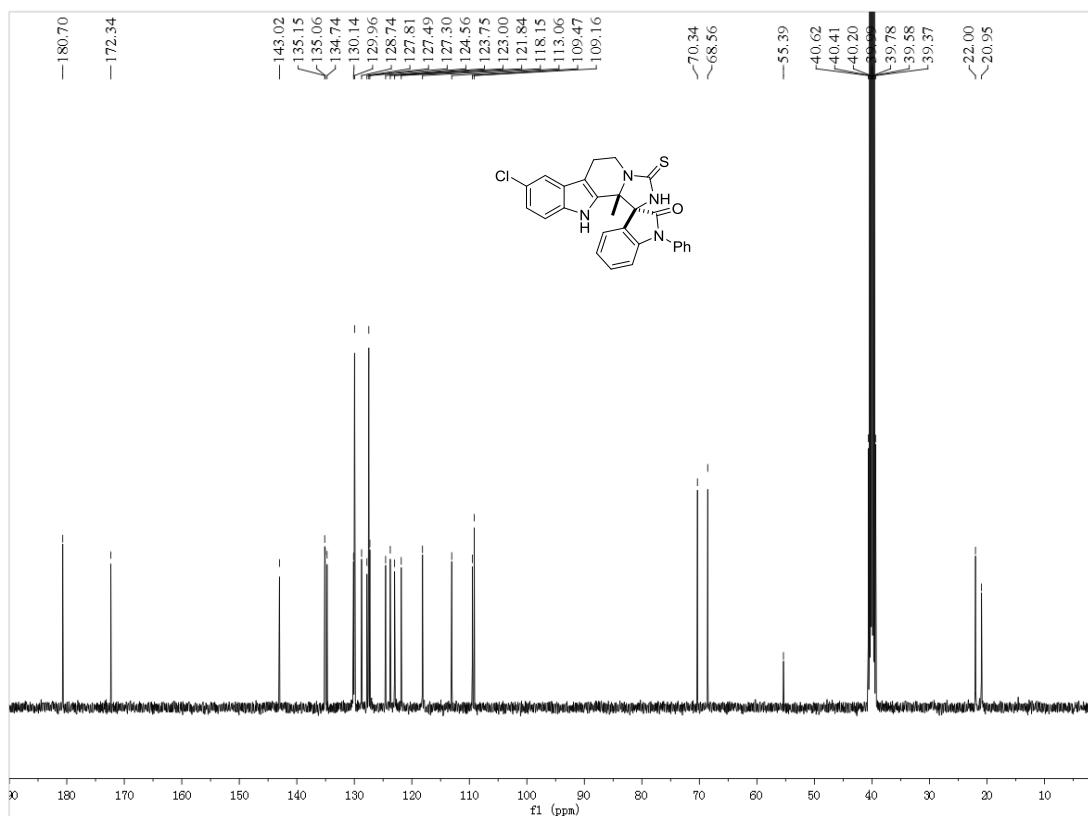
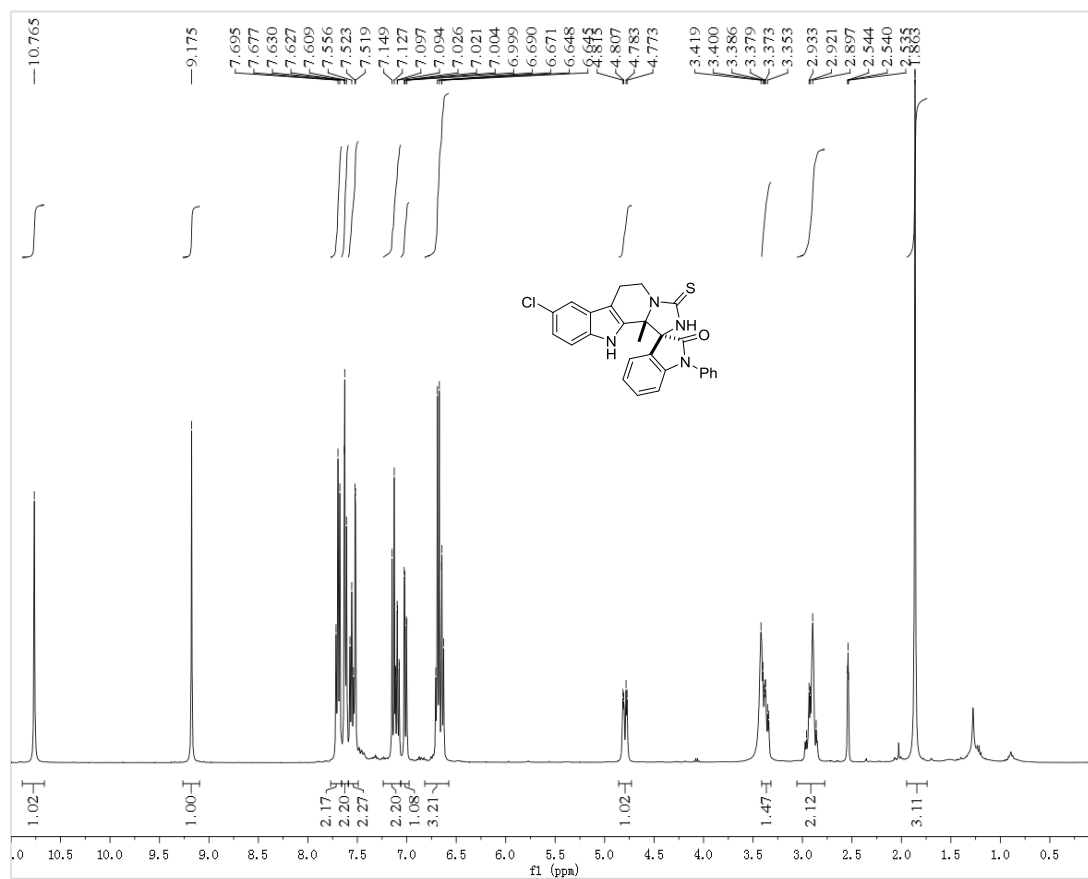
¹H and ¹³C NMR of 3cd



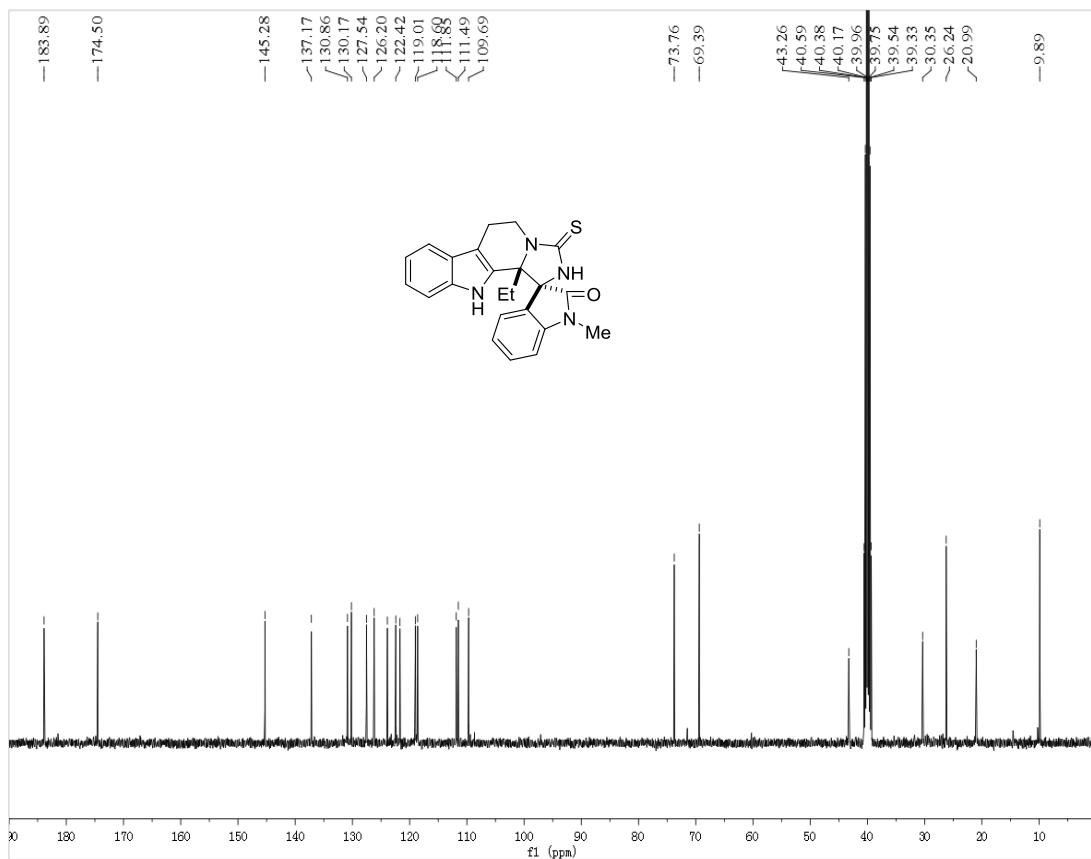
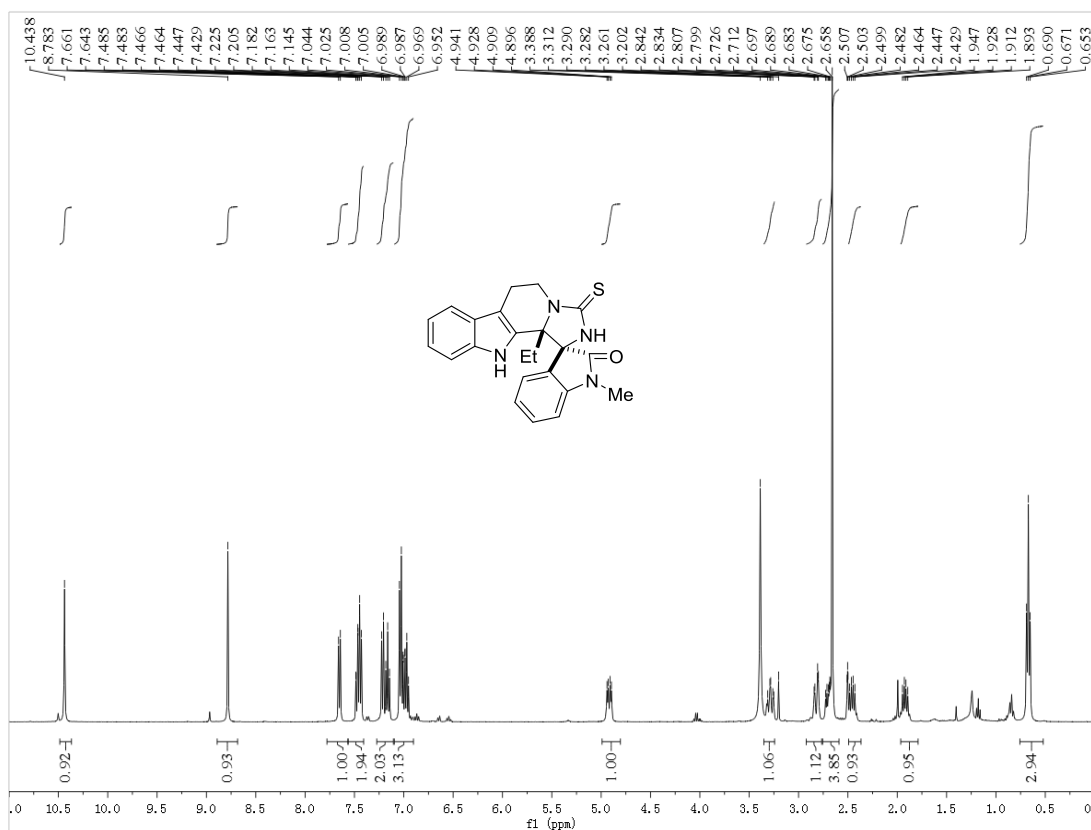
¹H and ¹³C NMR of 3ce



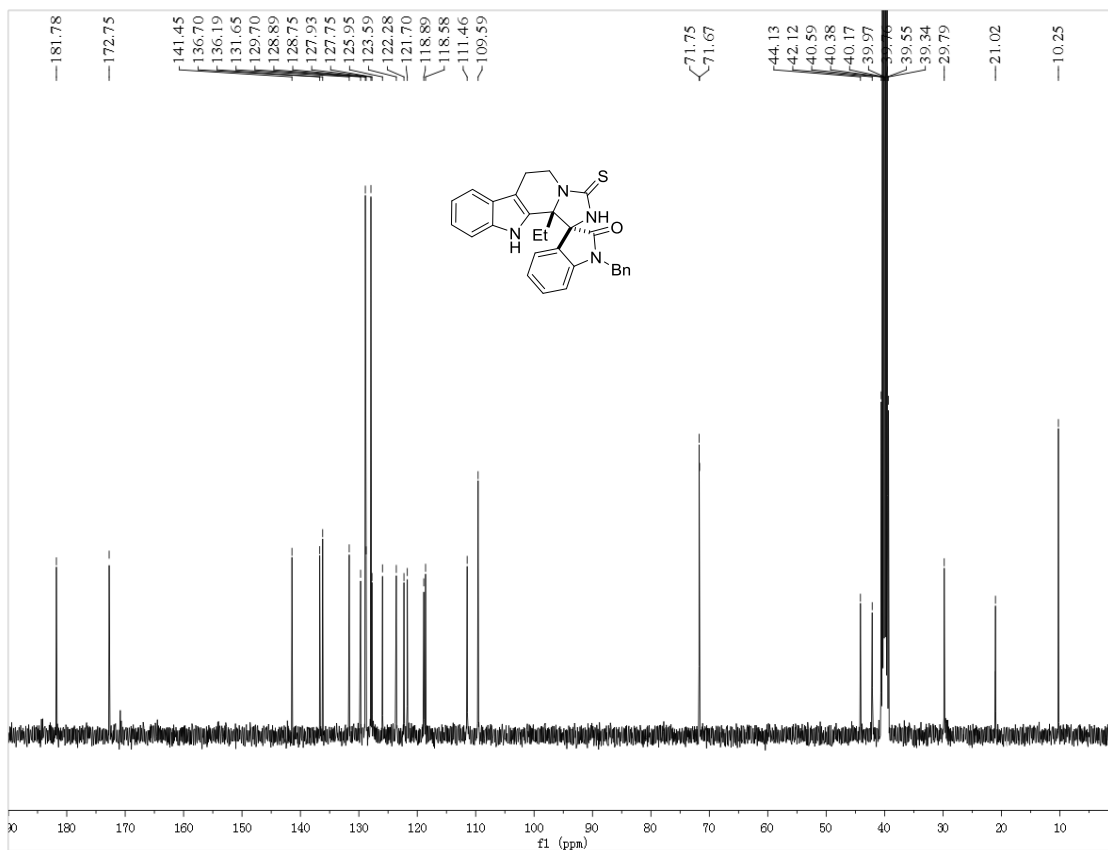
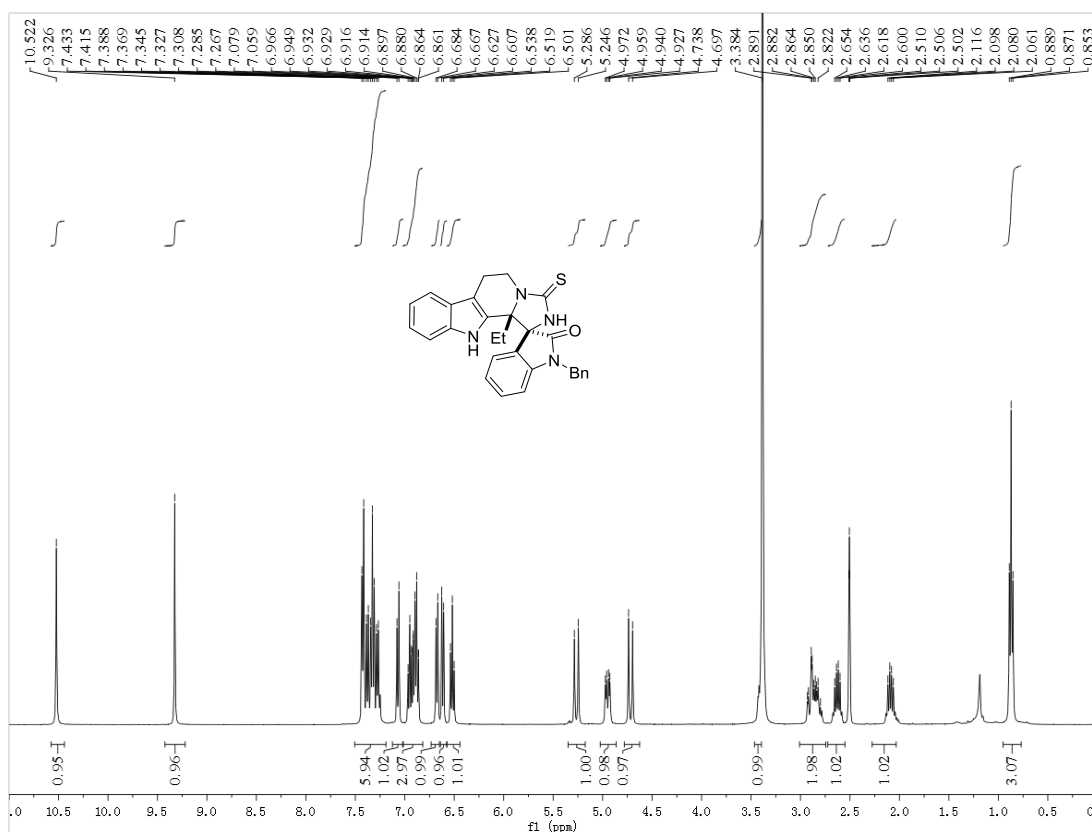
^1H and ^{13}C NMR of 3f



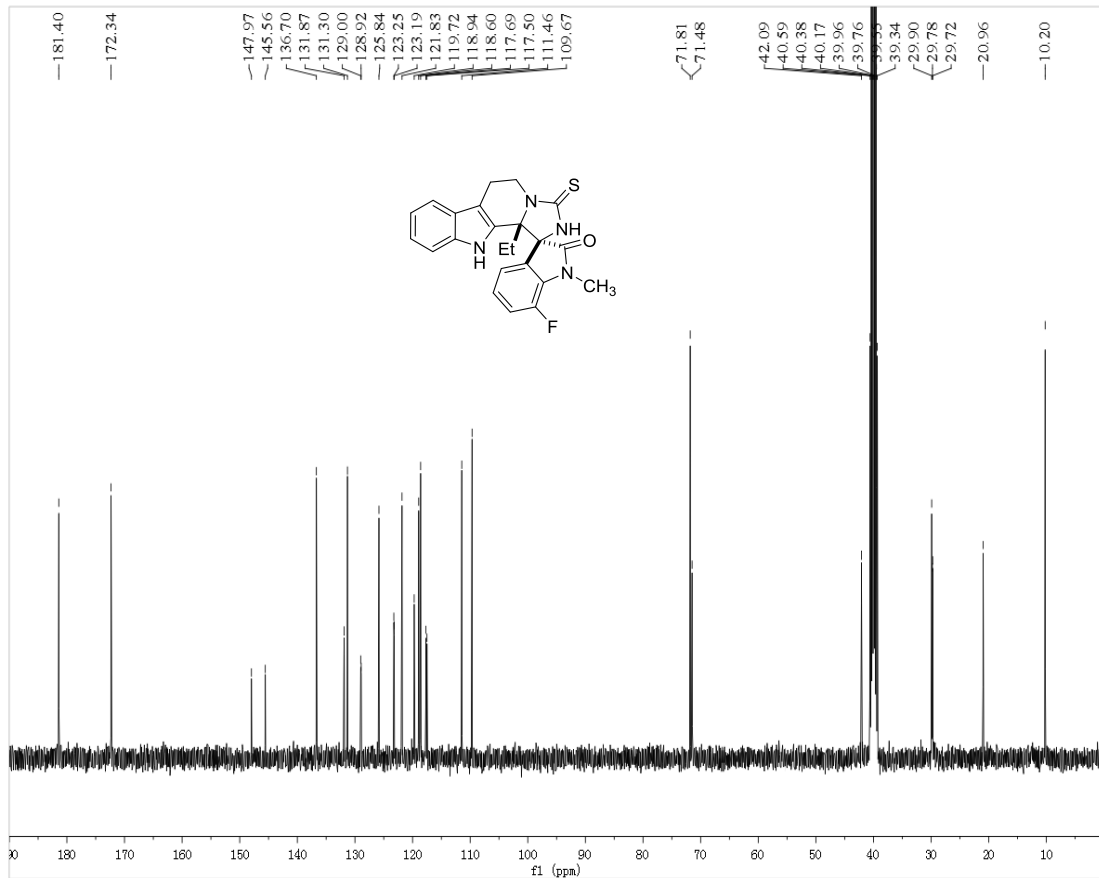
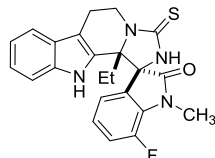
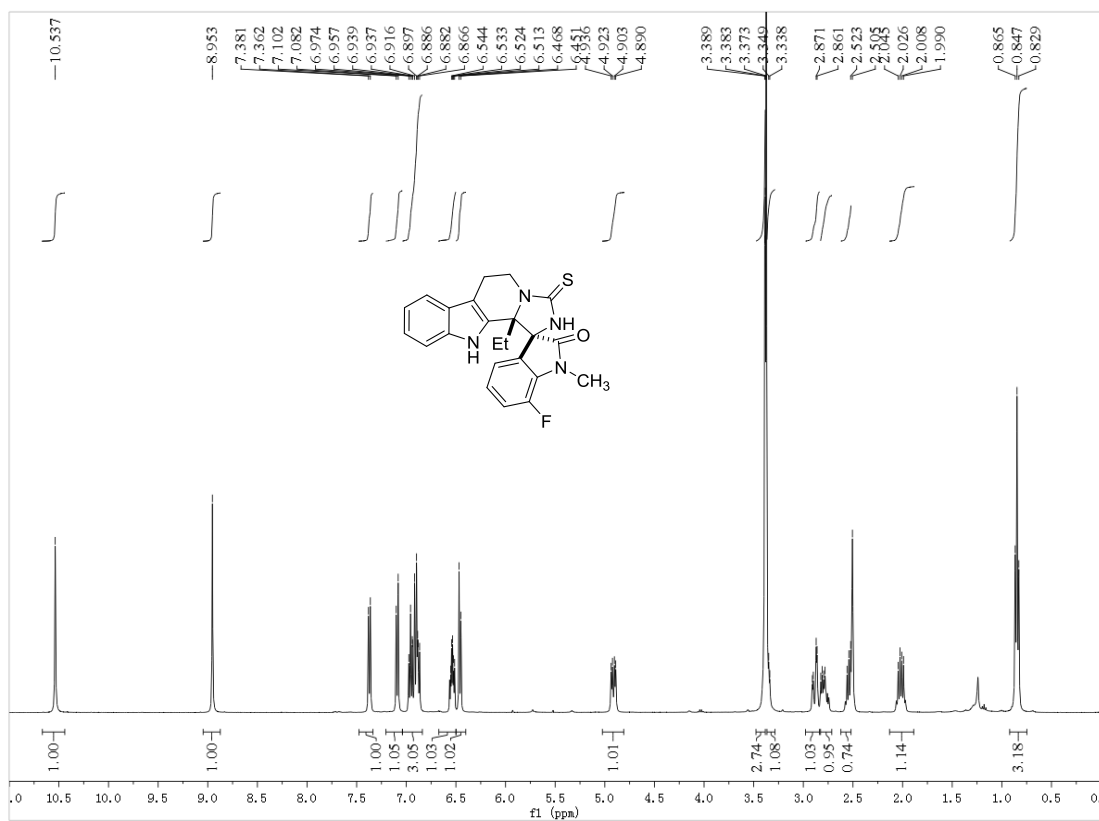
^1H and ^{13}C NMR of 3da



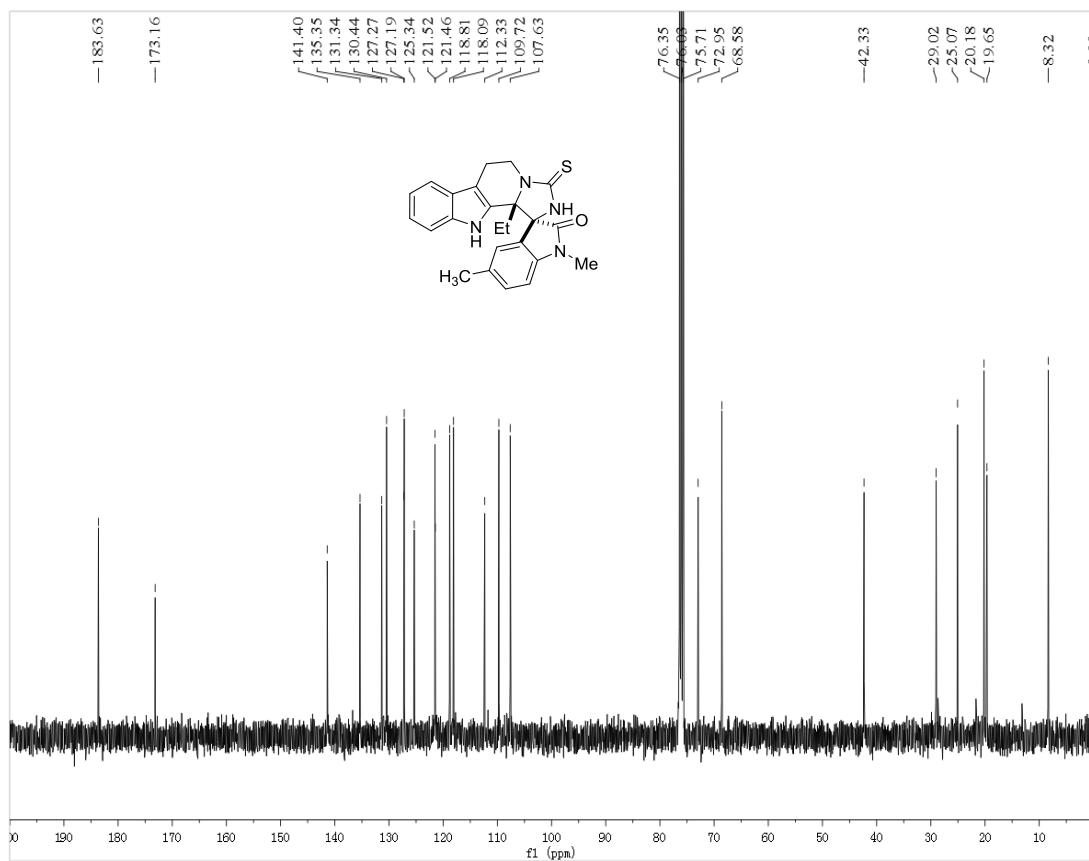
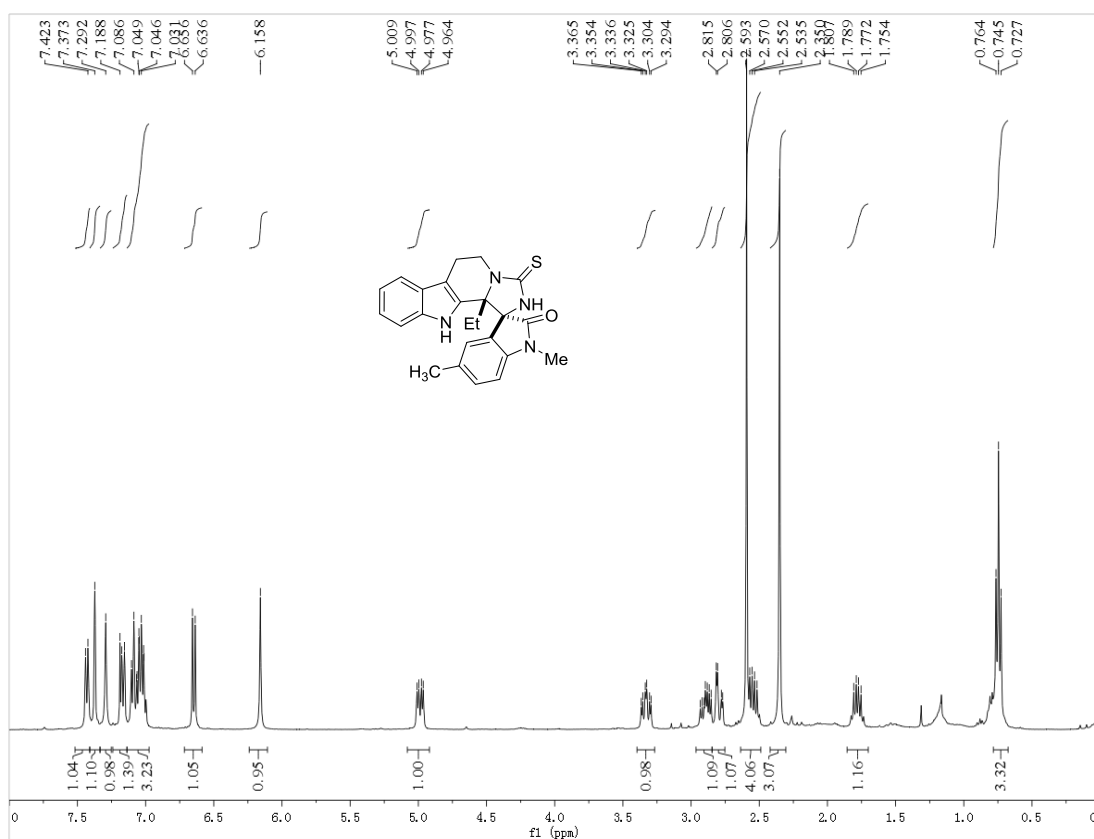
^1H and ^{13}C NMR of 3db



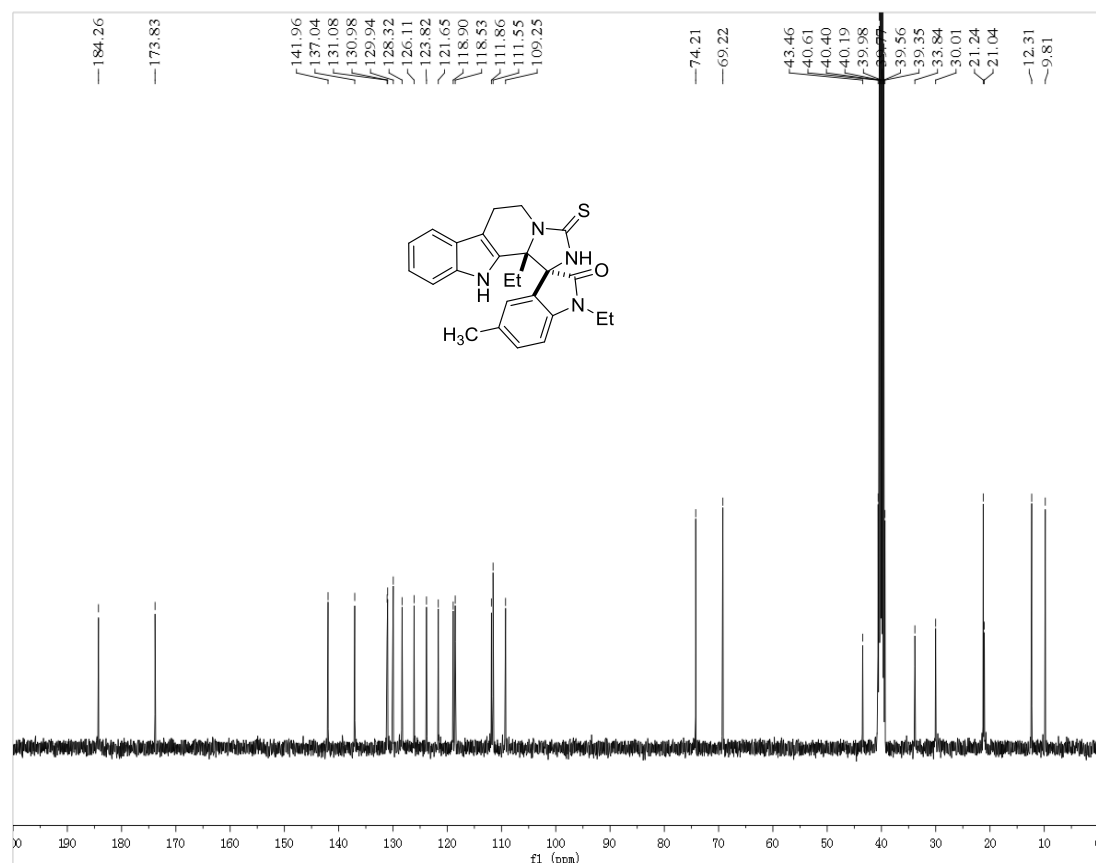
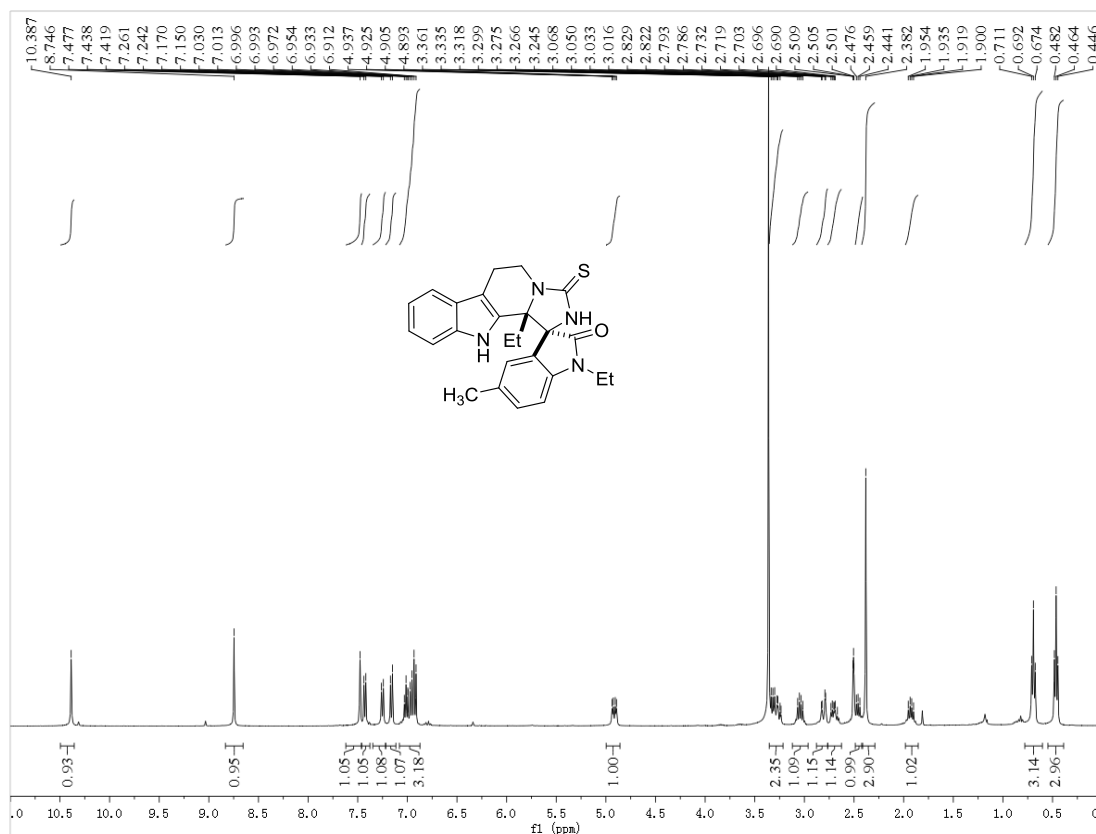
¹H and ¹³C NMR of 3dc



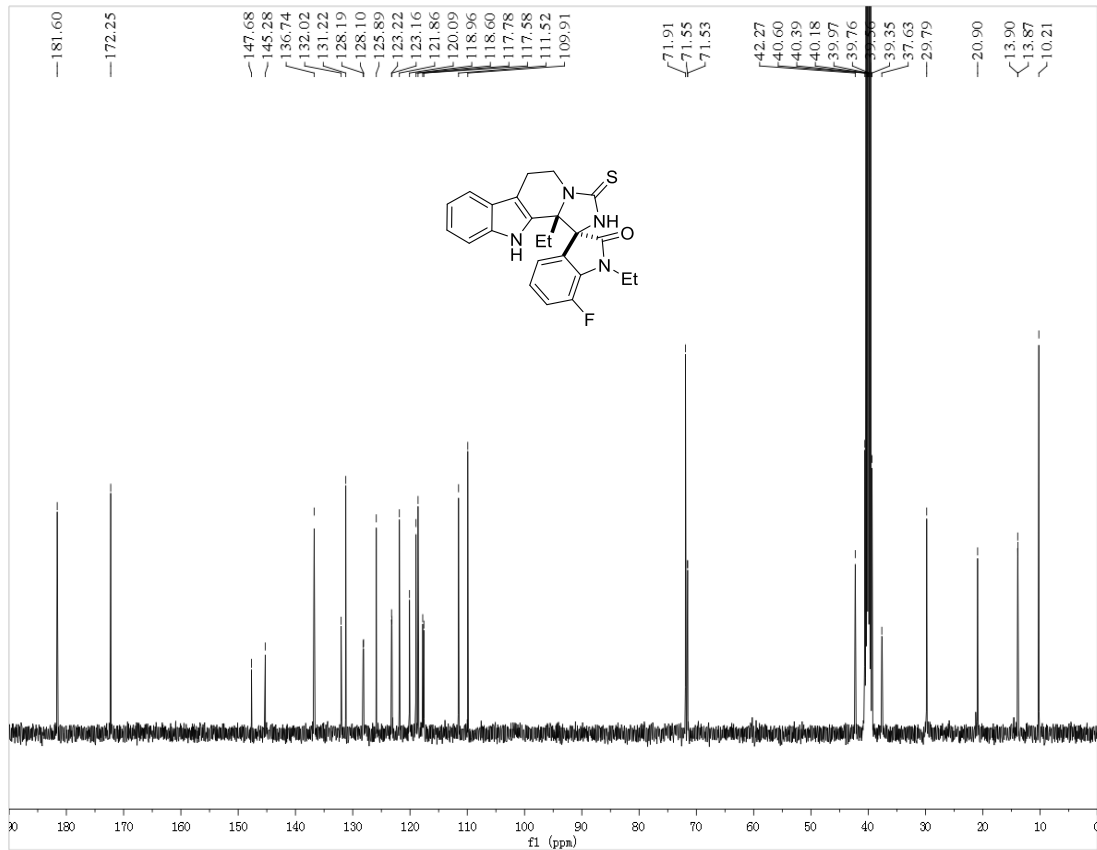
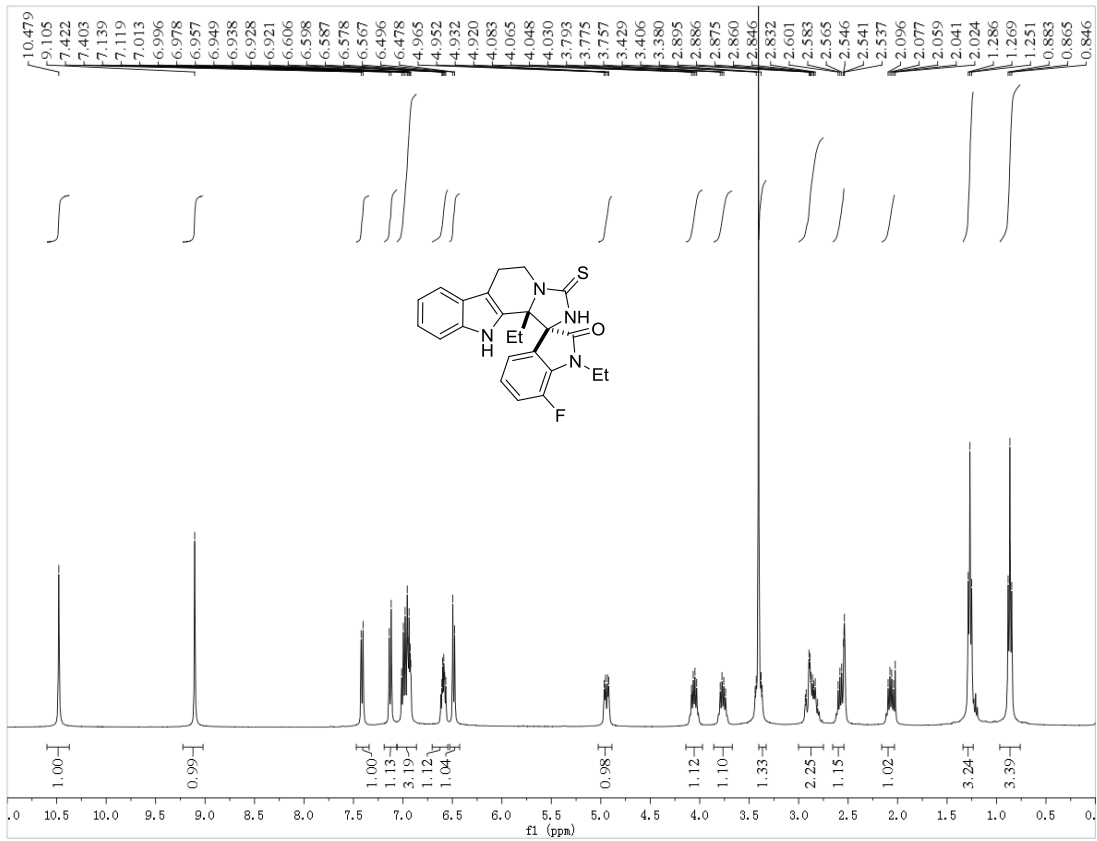
^1H and ^{13}C NMR of 3dd



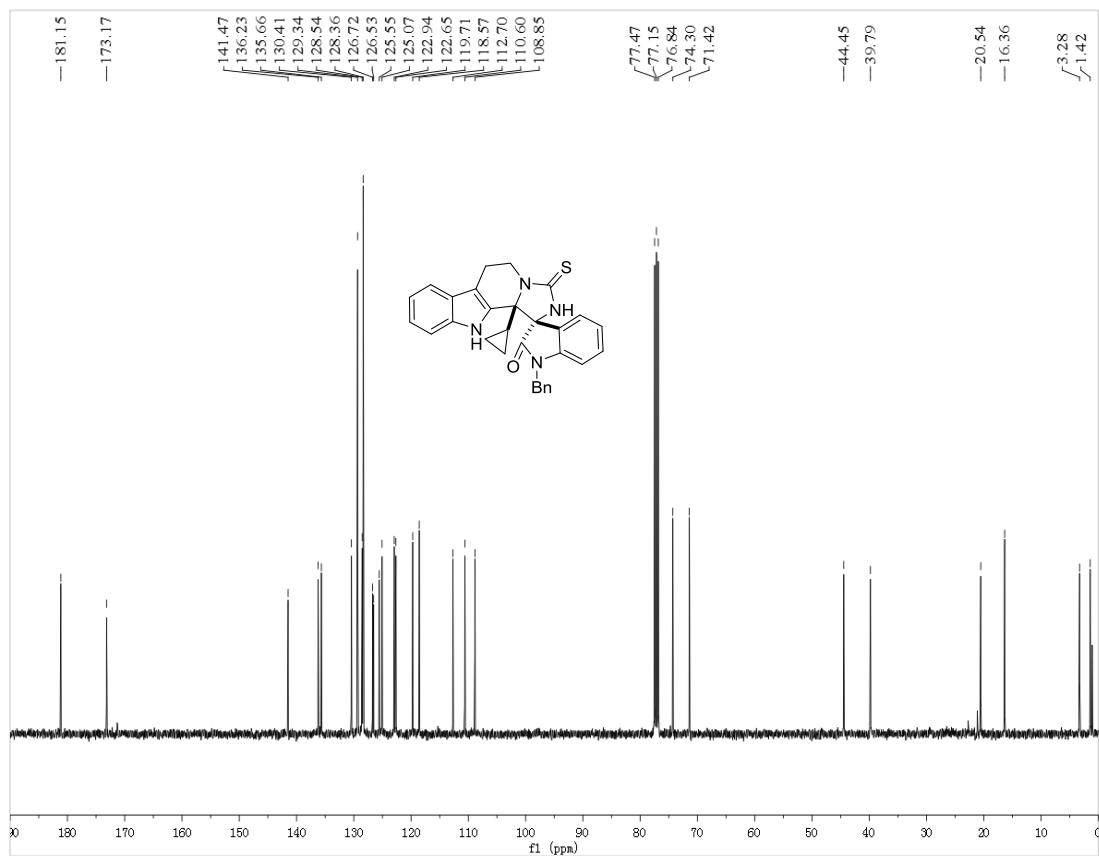
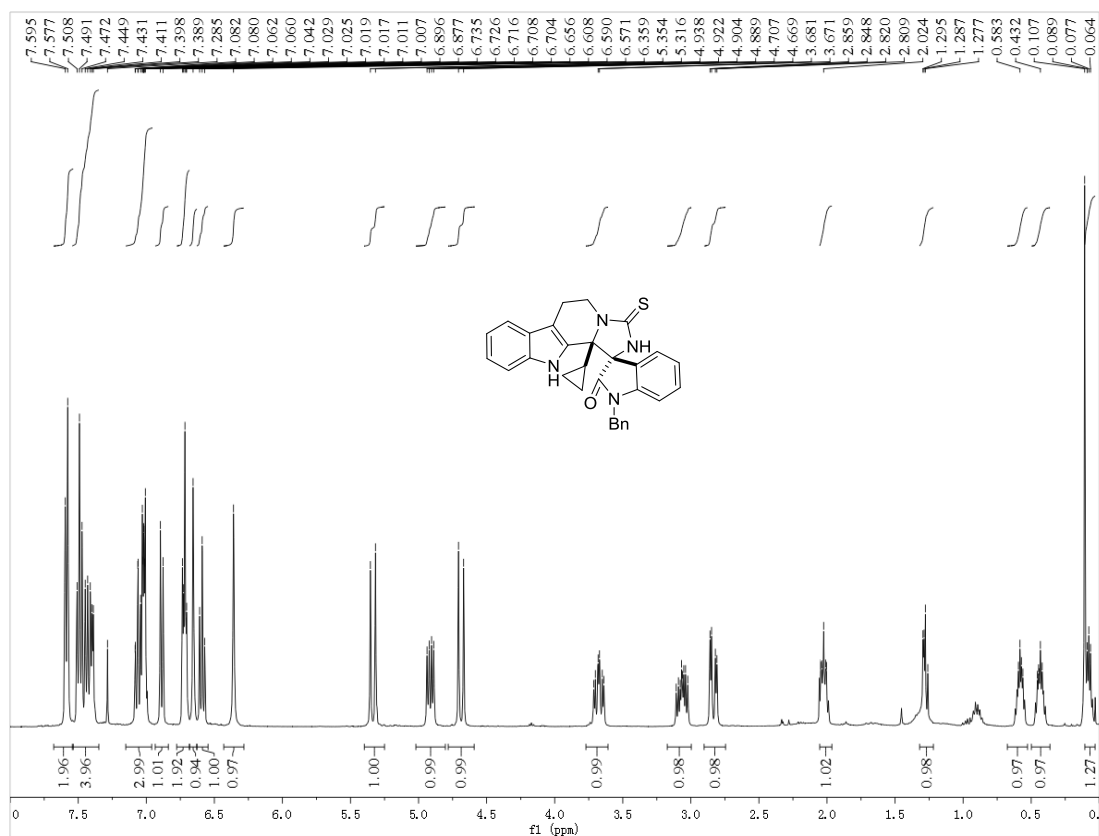
¹H and ¹³C NMR of 3de



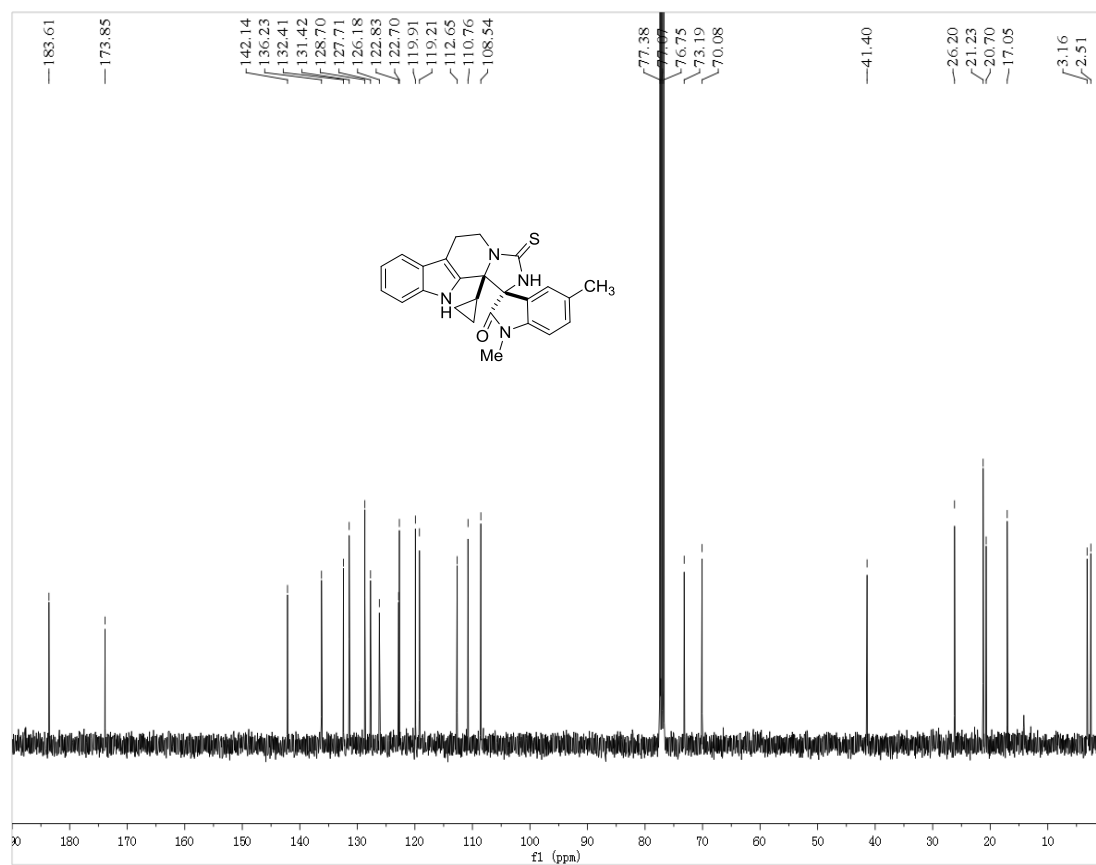
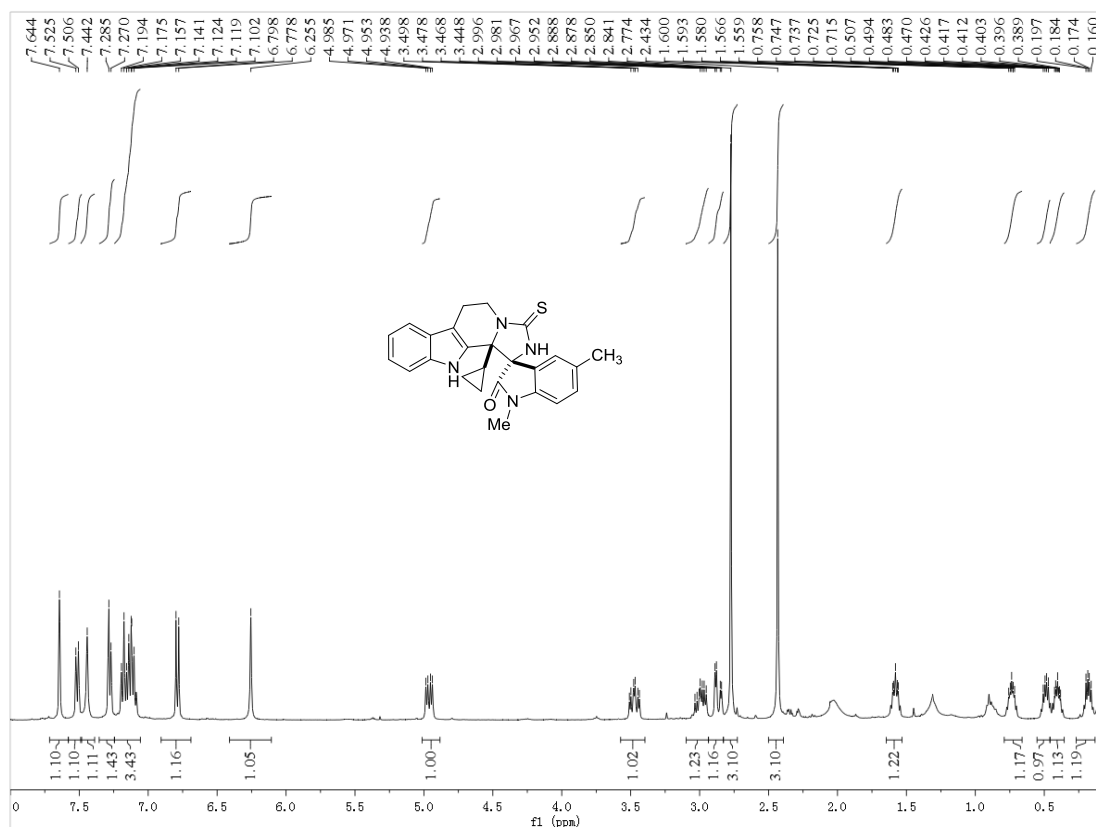
¹H and ¹³C NMR of 3df



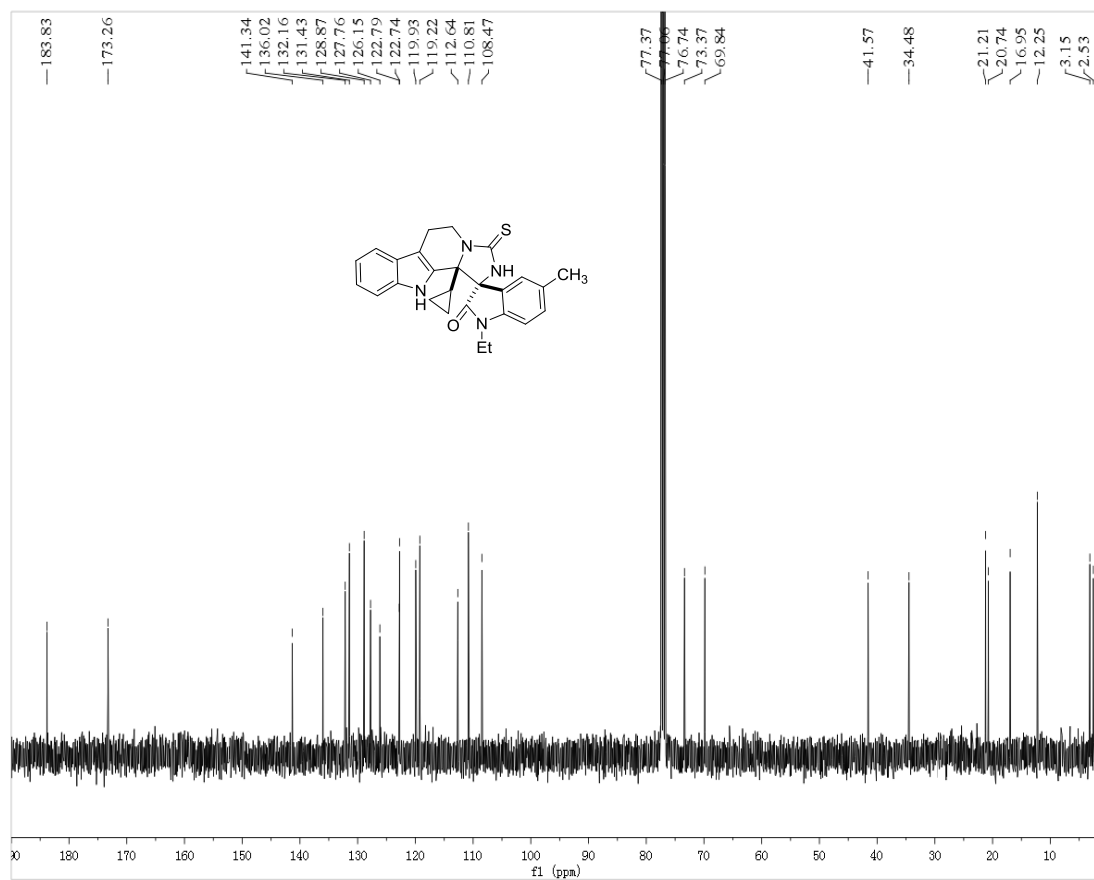
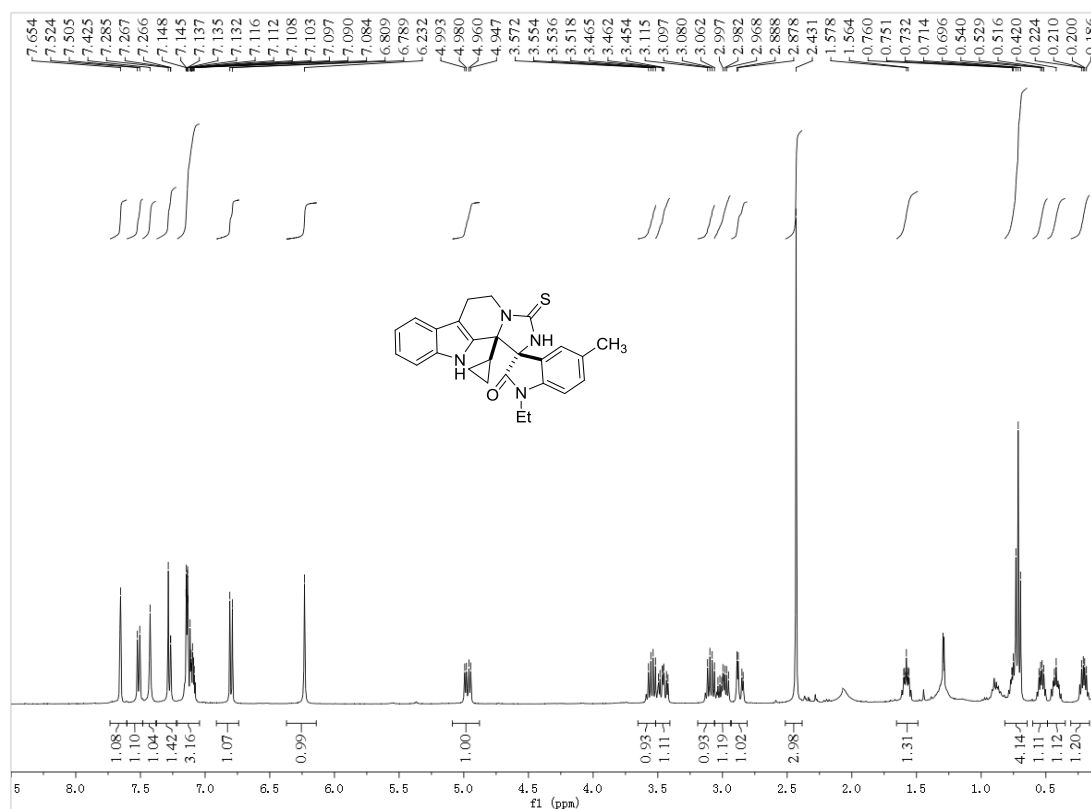
^1H and ^{13}C NMR of 3ea



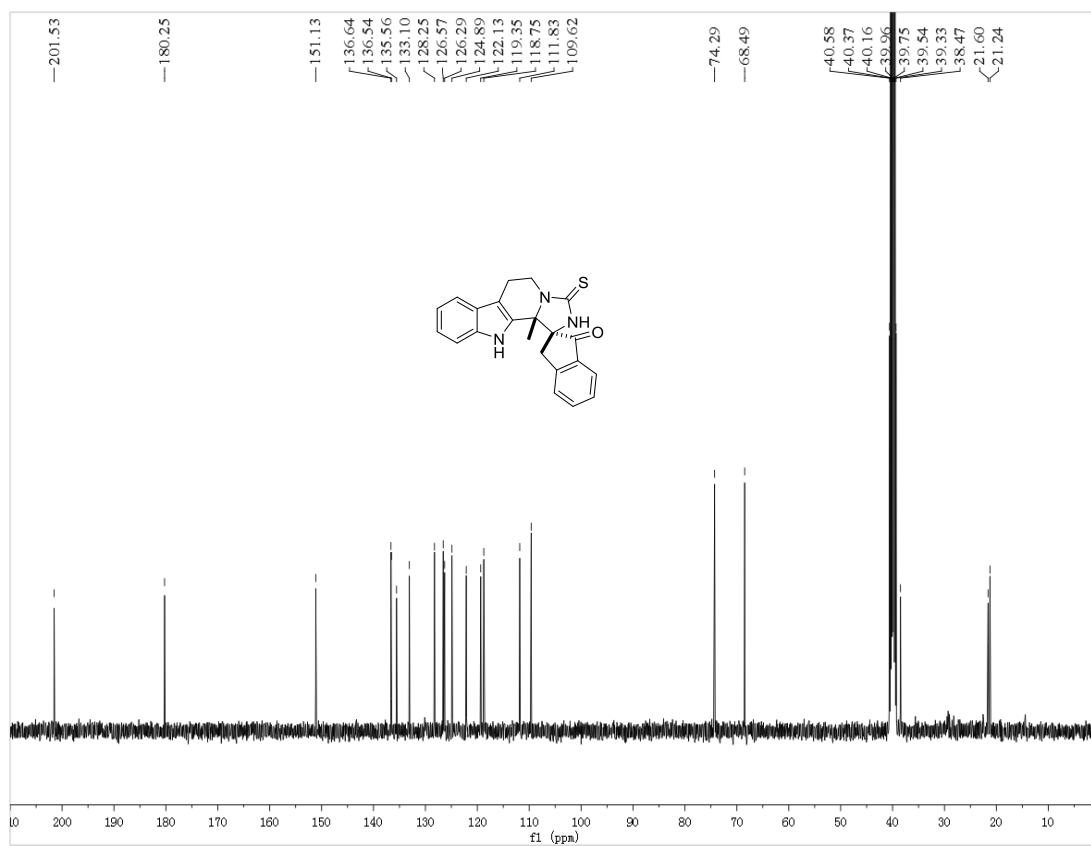
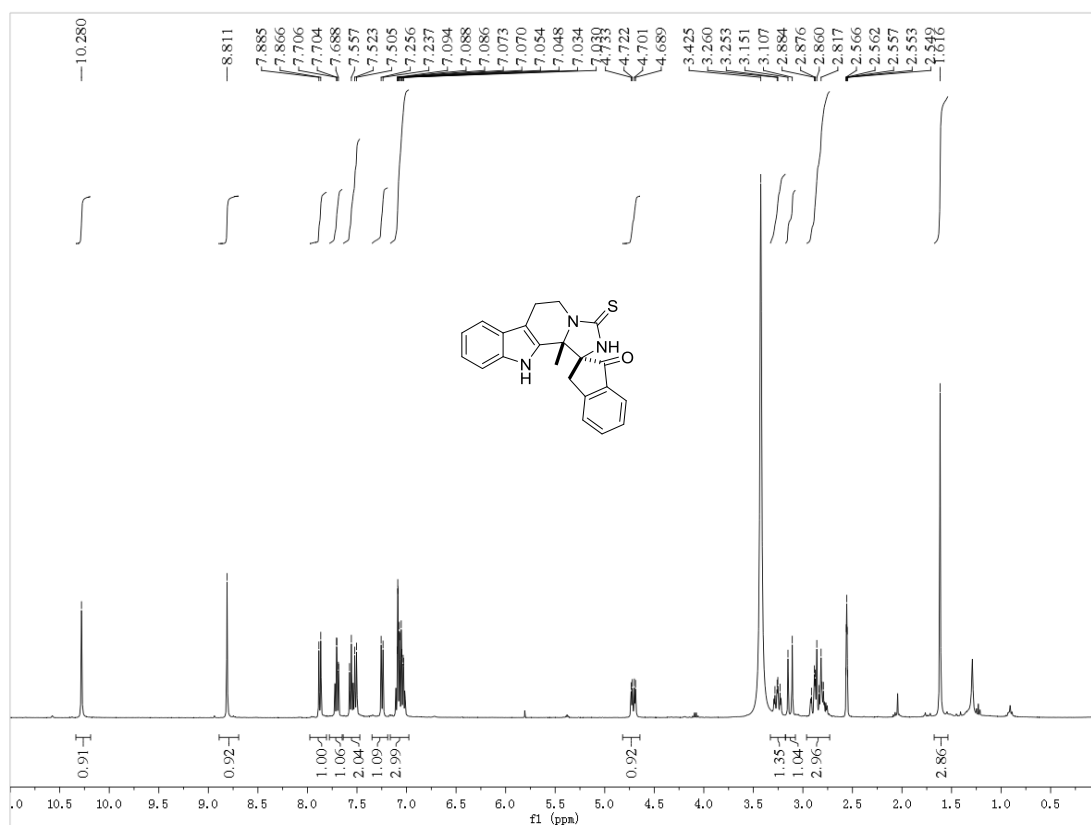
¹H and ¹³C NMR of 3eb



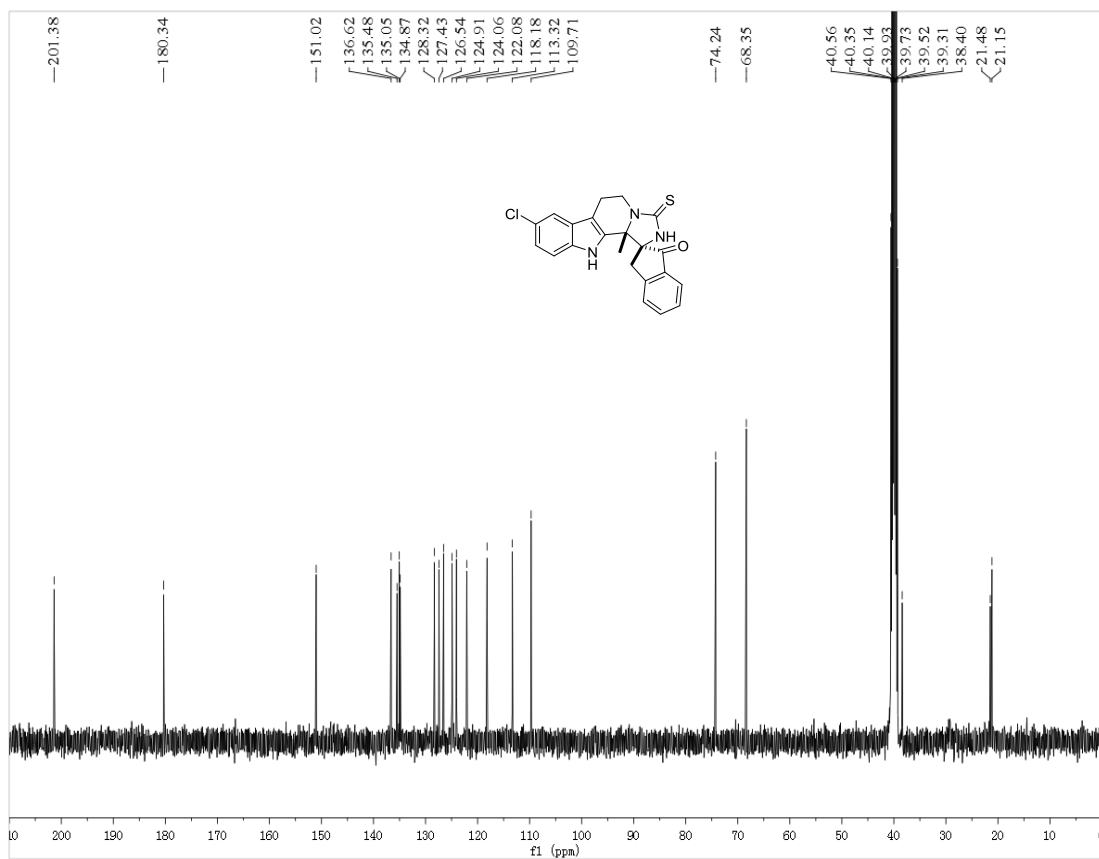
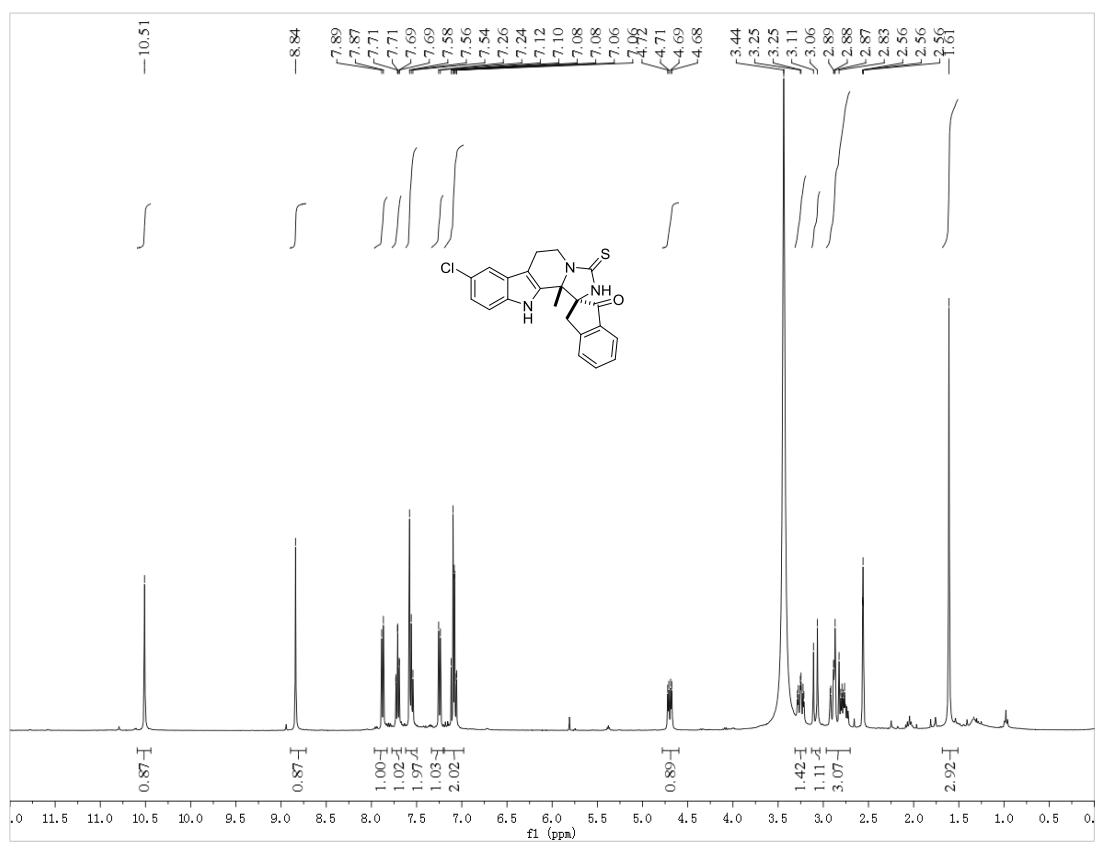
^1H and ^{13}C NMR of 3ec



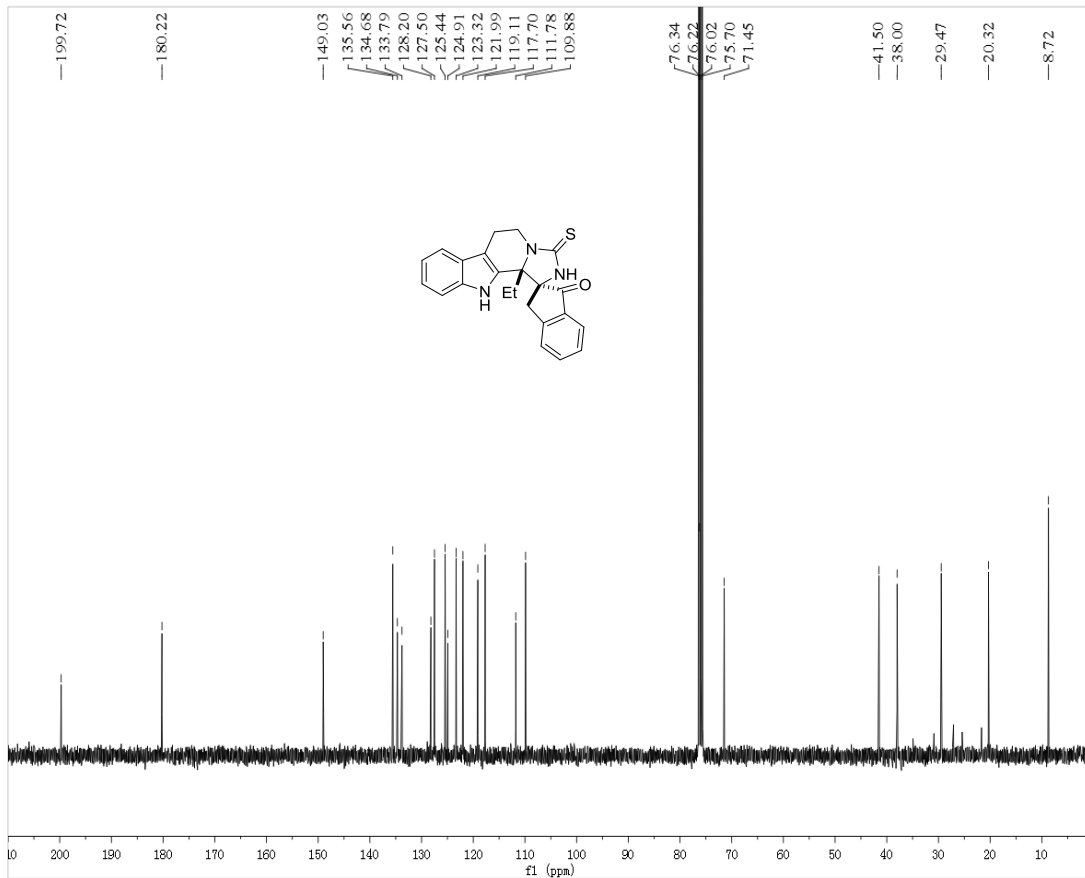
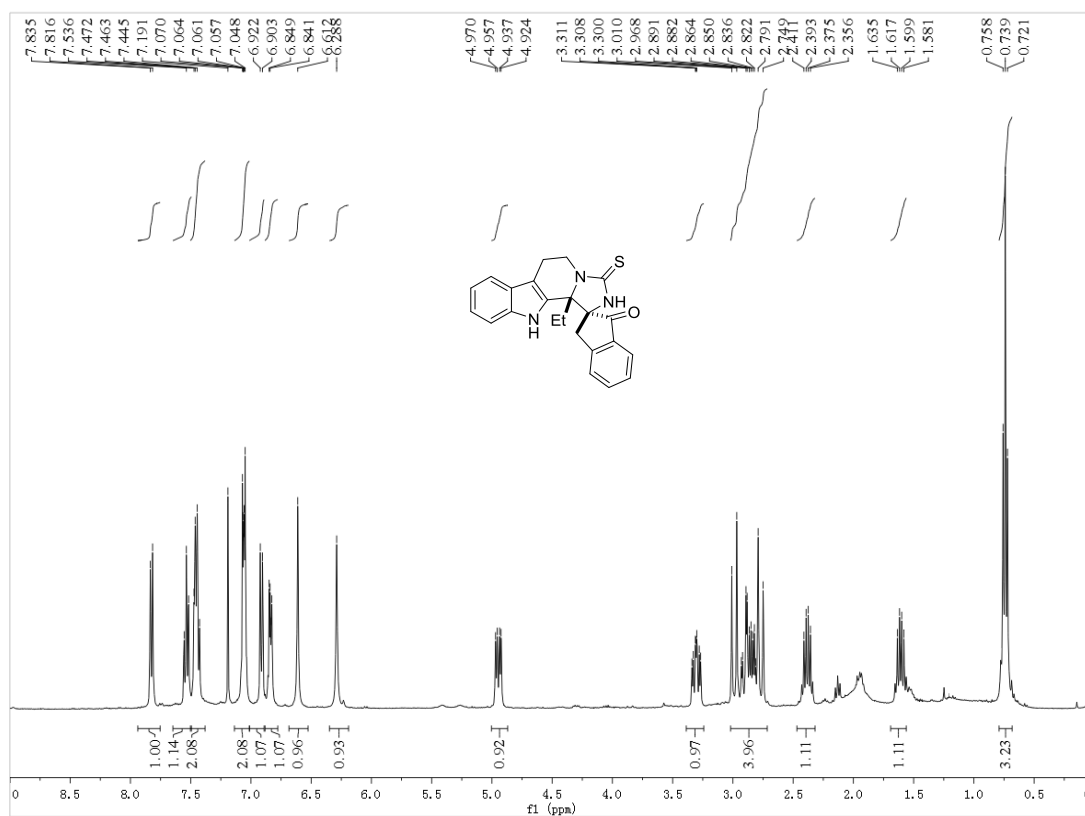
^1H and ^{13}C NMR of 3fa



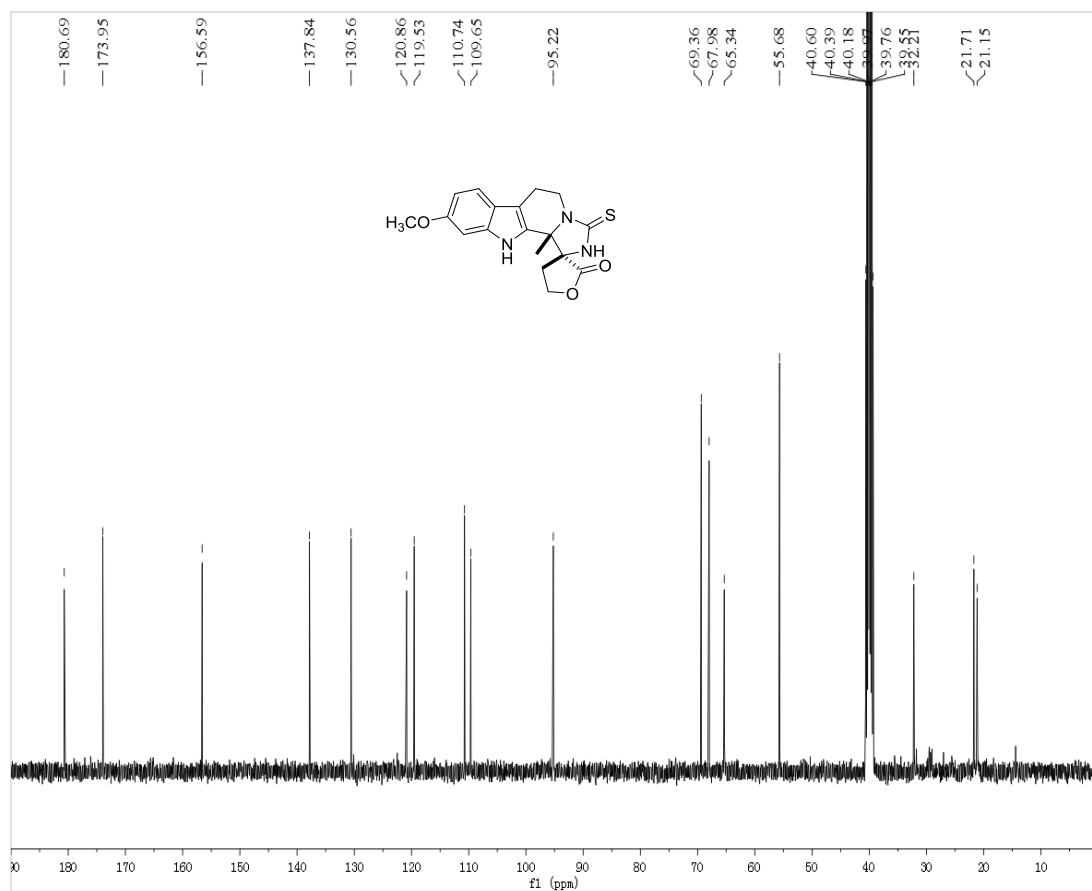
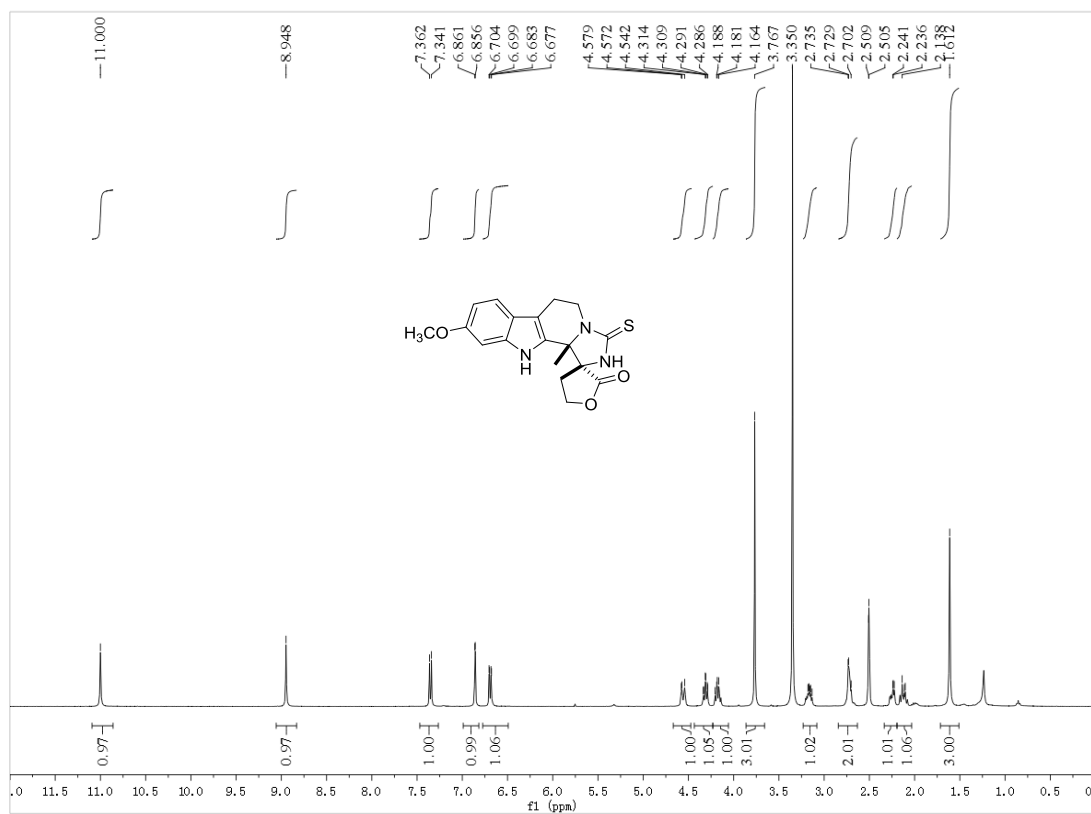
¹H and ¹³C NMR of 3ga



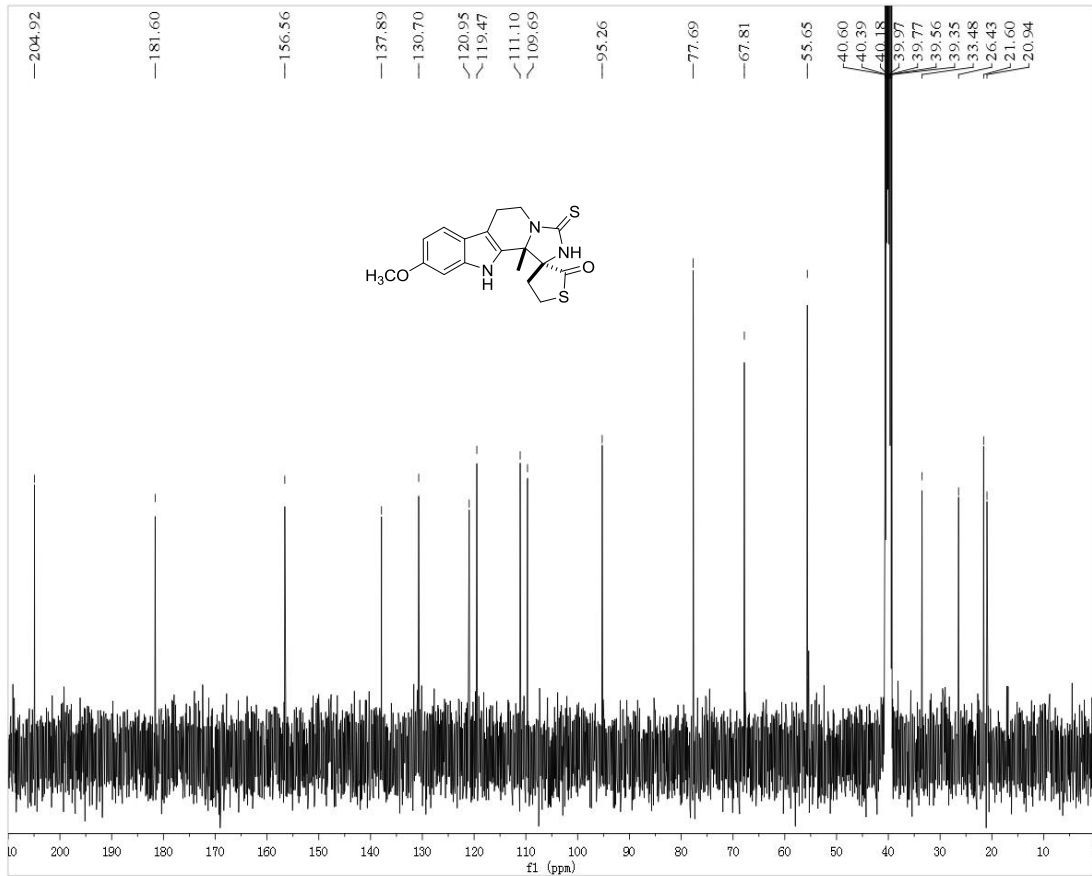
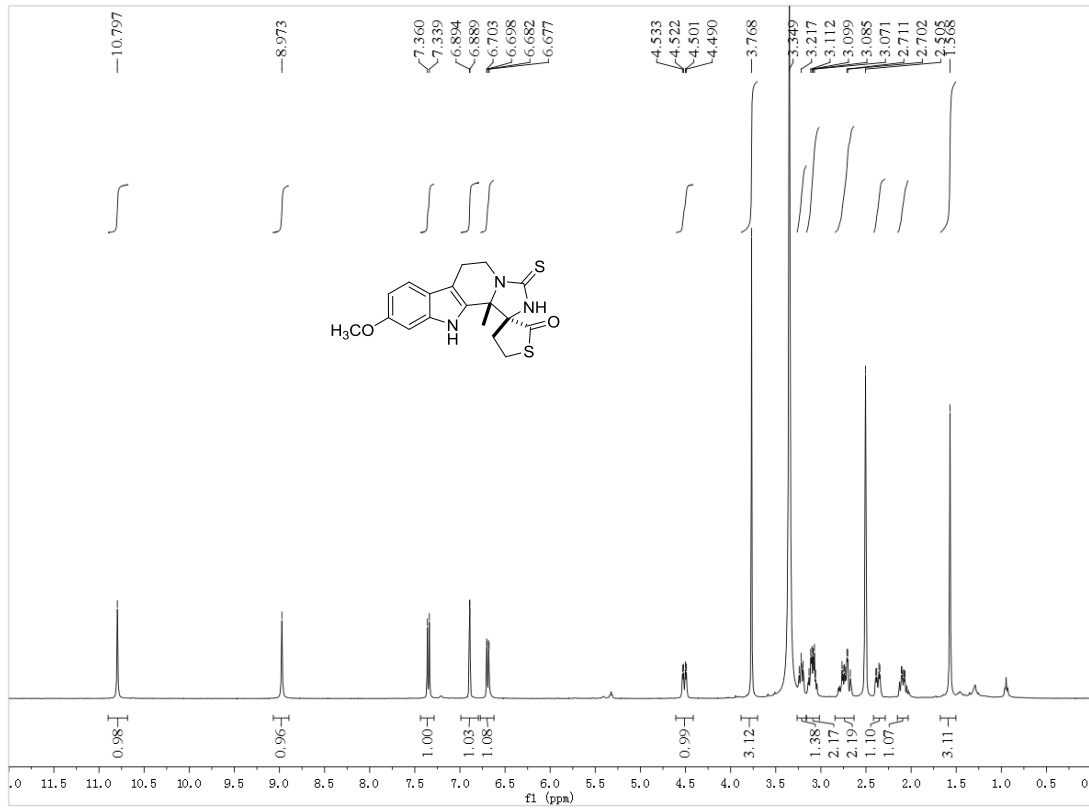
¹H and ¹³C NMR of 3ha



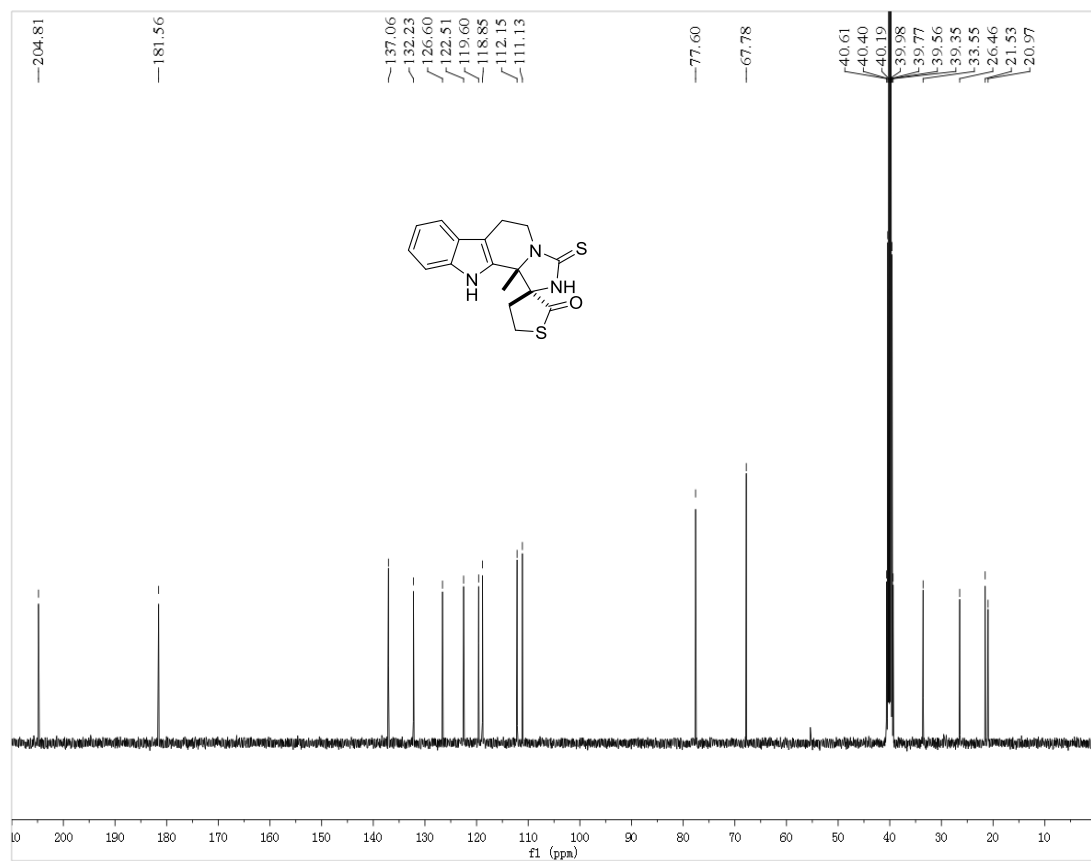
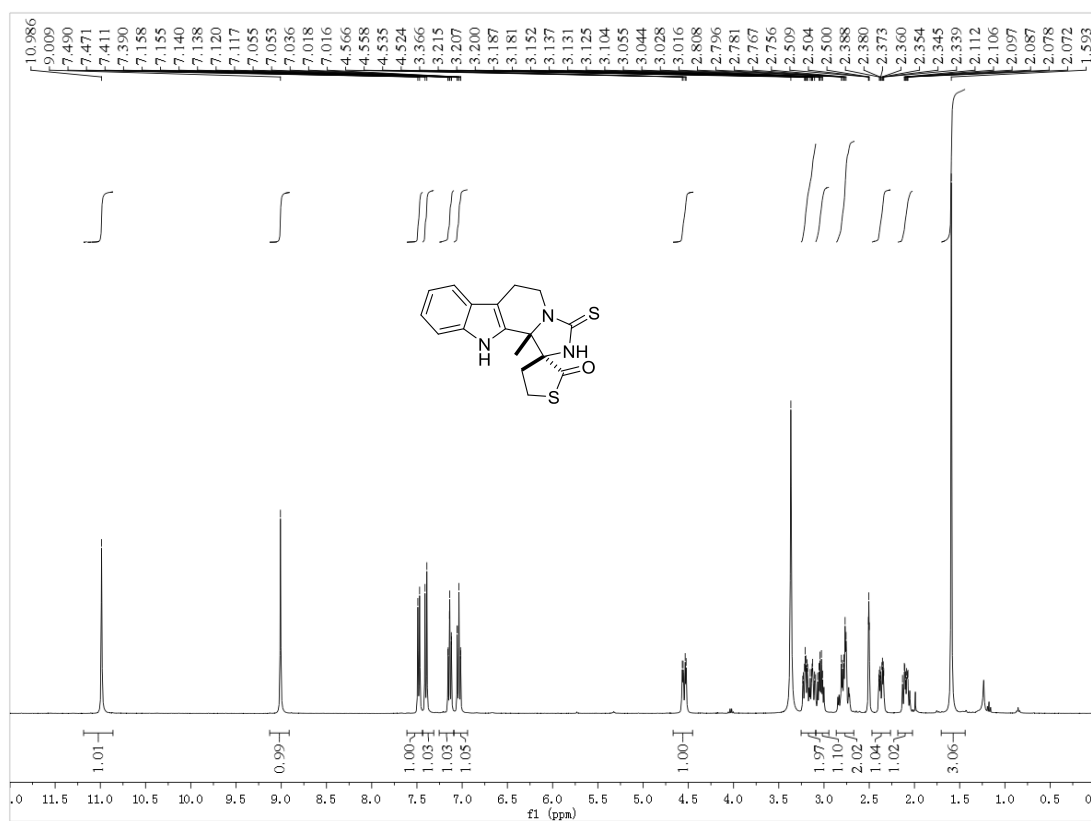
¹H and ¹³C NMR of 3ia



¹H and ¹³C NMR of 3ib



¹H and ¹³C NMR of 3ja



^1H and ^{13}C NMR of 3ka

