Supplementary Information (SI) for ChemComm.
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Supplementary Information

Overcoming Multidrug Resistance by a Singlet Oxygen Releasing Camptothecin-Endoperoxide

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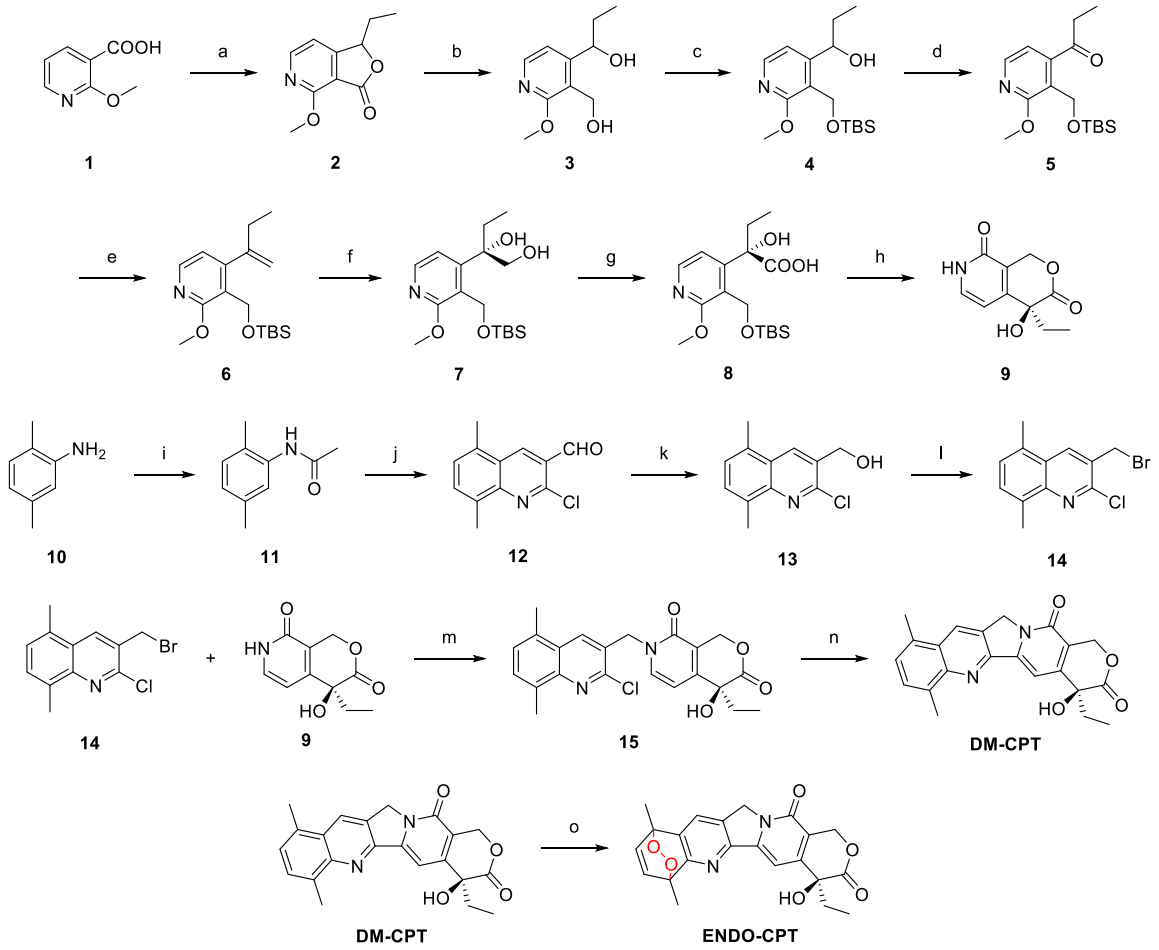
Synthesis of Intermediates



Reagents and conditions: (a) TMP, *n*-BuLi, propanal, THF, -78 °C to rt; (b) $LiAlH_4$, THF, 0 °C to rt; (c) TBSOTf, 2,6-lutidine, DCM, 0 °C; (d) PCC, DCM, rt; (e) CH_3Ph_3PBr, *n*-BuLi, toluene, rt to reflux; (f) $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, $K_2OsO_4{\cdot}2H_2O$, $MsNH_2$, H_2O, *t*-BuOH, rt to 0 °C; (g) TEMPO, NaClO, $NaClO_2$, H_2O, MeCN, PBS, 0 °C; (h) 3 N HCl, reflux; (i) Ac_2O, Et_3N, DCM, 0 °C to rt; (j) DMF, $POCl_3$, rt to reflux; (k) $NaBH_4$, MeOH, rt; (l) PBr_3, $CHCl_3$, 0 °C; (m) K_2CO_3, DMF, 50 °C; (n) $Pd(PPh_3)_2(OAc)_2$, AcOK, MeCN, 100 °C; (o) methylene blue, $CDCl_3$, 0 °C.

Synthesis procedures

Synthesis of **2:** *n*-BuLi (20.0 mL, 2.5 M in n-Hex) was added to a solution of 2,2,6,6-tetramethylpiperidine (6.0 mL, 35.6 mmol) in anhydrous THF (30 mL) at -78 °C under nitrogen. The mixture was stirred for 30 min before 2-methoxynicotinic acid (**1**, 2.0 g, 13.1 mmol) in anhydrous THF (40 mL) was added. After the mixture was stirred for 30 min at -78℃, propanal (3.0 mL, 41.6 mmol) in anhydrous THF (20 mL) was added and the mixture stirred for another 30 min. Then the mixture was slowly allowed to reach room temperature and quenched with 1 N HCl (50 mL). The mixture was extracted with EA (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated to afford crude product, which was further purified by column chromatography (Hex: EA=3:1, v/v) to afford **2** (59.4% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 5.2 Hz, 1H), 6.94 (d, J = 5.2 Hz, 1H), 5.34 – 5.30 (m, 1H), 4.05 (s, 3H), 2.09 – 1.98 (m, 1H), 1.80 – 1.68 (m, 1H), 0.91 (t, J = 8.0 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.7, 162.8, 161.6, 152.5, 110.4, 108.8, 81.1, 54.3, 27.0, 8.6.

Synthesis of **3:** The solution of **2** (3.0 g, 15.5 mmol) in anhydrous THF (30 mL) was added dropwise to $LiAlH_4$ (12.0 mL, 2.5 M in THF) at 0 ℃ under nitrogen atmosphere. The mixture was allowed to reached room temperature over a period of 3 h, and then it was cooled to 0 ℃ and quenched with 1 N NaOH (15 mL). The mixture was diluted with THF (30 mL), 1 N NaOH was added until the precipitation completely disappeared. The aqueous phase was extracted with EA (3 × 50 mL), the combined organic layers were dried over anhydrous Na_2SO_4, and concentrated to afford crude product, which was further purified by column chromatography (Hex: EA=1:1, v/v) to afford **3** (80.0% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 5.4 Hz, 1H), 6.90 (d, J = 5.4 Hz, 1H), 4.72 (t, J

= 8.0 Hz, 1H), 4.64 (d, *J* = 12.2 Hz, 1H), 4.53 (d, *J* = 12.2 Hz, 1H), 3.97 (s, 1H), 3.88 (s, 3H), 3.56 (s, 1H), 1.72 – 1.53 (m, 2H), 0.86 (t, *J* = 8.0 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 162.2, 154.2, 146.2, 119.5, 114.9, 71.2, 55.5, 53.8, 30.7, 10.3.

Synthesis of **4:** *tert*-Butyldimethylsilyl triflate (0.5 mL, 2.2 mmol) in anhydrous DCM (20 mL) was added dropwise to a solution of **3** (440 mg, 2.2 mmol) and 2,6-lutidine (0.5 mL, 4.4 mmol) in anhydrous DCM (20 mL) at 0 ℃ over 2 min. After 30 min at this temperature, saturated $NaHCO_3$ solution (50 mL) was added, the aqueous phase was extracted with DCM (3 × 30 mL). The combined organic layers were washed with 1 N HCl, saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated to afford crude product, which was further purified by column chromatography (Hex: EA=3:1, v/v) to afford **4** (43.1% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 5.3 Hz, 1H), 6.94 (d, *J* = 5.3 Hz, 1H), 4.84 (t, *J* = 8.0 Hz, 1H), 4.84 – 4.75 (m, 2H), 3.92 (s, 3H), 3.49 (s, 1H), 1.85 – 1.74 (m, 2H), 0.97 (t, *J* = 8.0 Hz, 3H), 0.86 (s, 9H), 0.07 (d, *J* = 8.0 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 161.8, 155.2, 146.3, 119.8, 114.8, 71.4, 56.1, 53.7, 29.6, 26.0, 18.4, 10.6, -5.2, -5.3.

Synthesis of **5:** PCC (660 mg, 3.0 mmol) was added to a solution of **4** (470 mg, 1.5 mmol) in DCM (20 mL), and the suspension was stirred at room temperature for 12 h. After quenched with MeOH (10 mL), the organic phase was filtered and the filter was concentrated under reduced pressure to afford crude product, which was further purified by column chromatography (Hex: EA=4:1, v/v) to afford **5** (57.0% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 5.1 Hz, 1H), 6.70 (d, *J* = 5.1 Hz, 1H), 4.76 (s, 2H), 3.94 (s, 3H), 2.83 – 2.77 (m, 2H), 1.14 (t, *J* = 7.3 Hz, 3H), 0.86 (s, 9H), 0.05 (s, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 206.2, 161.3, 150.4, 145.9, 119.7, 114.1, 57.4, 53.8, 36.5, 26.0, 18.7, 7.8, -5.5.

Synthesis of **6:** *n*-BuLi (3.0 mL, 2.5 M in *n*-Hex) was added to a suspension of CH_3Ph_3PBr (2.1 g, 6.0 mmol) in toluene (30 mL) under nitrogen atmosphere. The mixture was stirred at room temperature for 30 min. Then, **5** (620 mg, 2.0 mmol) was added and the resulting mixture was refluxed until completely by TLC detection. The mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (Hex: EA=10:1, v/v) to afford **6** (63.1% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 5.2 Hz, 1H), 6.66 (d, J = 5.3 Hz, 1H), 5.21 – 5.20 (m, 1H), 5.04 – 5.03 (m, 1H), 4.60 (s, 2H), 3.95 (s, 3H), 2.42 – 2.37 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H), 0.89 (s, 8H), 0.09 (s, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 163.1, 154.2, 148.4, 145.4, 120.0, 117.0, 114.1, 57.4, 53.4, 30.5, 26.0, 18.6, 12.4, 1.2, -5.2.

Synthesis of **7:** $(DHQD)_2PHAL$ (70.9 mg, 0.09 mmol), $K_3Fe(CN)_6$ (4.5 g, 13.7 mmol), K_2CO_3 (1.9 g, 13.7 mmol), $K_2OsO_4{\cdot}2H_2O$ (6.7 mg, 0.018 mmol) and $MsNH_2$ (430 mg, 4.5 mmol) were dissolved in H_2O/*t*-BuOH (v/v = 1:1, 50 mL). The mixture was stirred at room temperature for 30 min and then cooled to 0 °C. **6** (1.4 g, 4.5 mmol) was added to the mixture in 1 portion and the resulting mixture was stirred for 40 h at 0 °C. The reaction was quenched at this temperature by Na_2SO_3 (5.0 g), then the mixture was warmed to room temperature and stirred for 30 min. DCM (50 mL) and H_2O (20 mL) were added, and the aqueous layer was further extracted with DCM (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (Hex: EA=2:1, v/v) to afford **7** (96.5% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 5.5 Hz, 1H), 6.74 (d, J = 5.5 Hz, 1H), 5.42 (s, 1H), 5.00

(d, J = 11.4 Hz, 1H), 4.93 (d, J = 11.4 Hz, 1H), 3.88 (s, 3H), 3.76 (d, J = 11.3 Hz, 1H), 3.63 (d, J = 11.3 Hz, 1H), 2.83 (s, 1H), 1.88 – 1.69 (m, 2H), 0.84 (s, 9H), 0.73 (t, J = 7.4 Hz, 3H), 0.06 (d, J = 1.7 Hz, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 162.4, 154.5, 145.8, 120.4, 116.3, 80.0, 71.1, 56.8, 53.7, 31.8, 25.8, 18.2, 7.7, -5.2, -5.3.

Synthesis of **8:** $NaClO_2$ solution (1.7 g, in 12 mL H_2O) and bleach (10%, 4.0 mL) were added to a mixture of **7** (1 g, 2.93 mmol), TEMPO (91.5 mg, 0.59 mmol) and PBS (20 mL, pH=7.4) in MeCN (40 mL) at 0 °C. The mixture was stirred at 0 °C until completely by TLC detection and then the pH was adjusted to 6.0 with 1 N HCl. The reaction was quenched with saturated Na_2SO_3 solution (20 mL) and the mixture was extracted with DCM (3 × 50 mL). The combined organic layers were dried by Na_2SO_4, concentrated under reduced pressure and the residue was purified by column chromatography (DCM: MeOH=50:1, v/v) to afford **8** (68.4% yield) as a colorless oil. TLC-MS (ESI, m/z): $[M+H]^+$ calcd for $C_{17}H_{30}NO_5Si$, 356.2; found, 356.7.

Synthesis of **9: 8** (940 mg, 2.64 mmol) was suspended in 3 N HCl (12 mL). The mixture was refluxed for 12 h, then cooled to room temperature, and concentrated under reduced pressure. The crude product was purified by column chromatography (EA: MeOH=10:1, v/v) to afford **9** (54.9% yield) as a white solid. ^{1}H-NMR (400 MHz, CD_3OD) δ. 7.48 (d, J = 6.8 Hz, 1H), 6.64 (d, J = 6.8 Hz, 1H), 5.42 (d, J = 16.1 Hz, 1H), 5.23 (d, J = 16.1 Hz, 1H), 1.93 – 1.79 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 174.8, 161.4, 153.0, 135.7, 120.6, 105.0, 73.8, 66.6, 32.1, 8.0.

Synthesis of **11:** Ac_2O (2.0 mL, 21.3 mmol) was added to a solution of 2,5-dimethylaniline (**10**, 2.5 mL, 20.0 mmol) and TEA (3.1 mL, 22.5 mmol) in anhydrous DCM (20 mL) at 0 °C under nitrogen atmosphere. The mixture was stirred overnight, and then the mixture

was poured into ice and stirred vigorously for another 30 min. The organic layer was washed with 1 N HCl, saturated $NaHCO_3$ solution and brine. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated to afford **11** (97.2% yield) as a white solid which without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ. 7.45 (s, 1H), 7.34 (s, 1H), 7.03 (d, J = 7.7 Hz, 1H), 6.87 (d, J = 7.7 Hz, 1H), 2.28 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 168.8, 136.3, 135.5, 130.3, 127.0, 126.3, 124.6, 24.1, 21.1, 17.4.

Synthesis of **12:** $POCl_3$ (14.0 mL, 140.0 mmol) was added dropwise to DMF (4.0 mL, 50.0 mmol) over 30 min at room temperature, then the reaction solution was stirred for another 30 min. **11** (3.3 g, 20 mmol) was added to the reaction solution over 10 min, and the reaction solution was refluxed for 10 h. The mixture was poured into ice (500 g) and stirred for another 30 min. The pH was adjusted to 9.0 with solid Na_2CO_3 and the aqueous layer was extracted with DCM (3 × 200 mL). The combined organic layers were dried (Na_2SO_4), concentrated under reduced pressure and the residue was purified by column chromatography (Hex: EA=4:1, v/v) to afford **12** (49.5% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ. 10.53 (s, 1H), 8.81 (s, 1H), 7.56 (d, J = 7.2 Hz, 1H), 7.30 (d, J = 7.2 Hz, 1H), 2.70 (s, 3H), 2.67 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.8, 149.4, 148.9, 137.0, 135.0, 134.8, 133.6, 128.3, 126.2, 125.4, 18.7, 17.8.

Synthesis of **13:** $NaBH_4$ (150.0 mg, 4.0 mmol) was added to the solution of **12** (435.2 mg, 2.0 mmol) in anhydrous MeOH (20 mL). The reaction was stirred for 4 h, then the mixture was poured into water (100 mL) and extracted with EA (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), concentrated to afford **13** (98.7% yield) as a white solid which without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.43 (d, J = 7.2

Hz, 1H), 7.25 (d, J = 7.2 Hz, 1H), 4.91 (s, 2H), 2.71 (s, 3H), 2.63 (s, 3H), 2.26 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 147.8, 146.7, 134.4, 133.4, 132.4, 131.3, 130.2, 127.5, 126.9, 62.5, 18.8, 18.0.

Synthesis of **14:** PBr_3 (1 mL) was added to a solution of **13** (1.76 g, 7.9 mmol) in $CHCl_3$ (50 mL) at 0 °C. The mixture was stirred at this temperature for 1 h and quenched by saturated $NaHCO_3$ solution (100 mL). The aqueous layer was extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give crude product. The residue was purified by column chromatography (Hex: DCM=4:1, v/v) to afford **14** (78.3% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ. 8.33 (s, 1H), 7.46 (d, J = 7.2 Hz, 1H), 7.27 (d, J = 7.2 Hz, 1H), 4.75 (s, 2H), 2.71 (s, 3H), 2.64 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 148.7, 147.2, 136.4, 134.6, 132.4, 131.0, 128.7, 127.8, 126.8, 30.6, 18.7, 17.9.

Synthesis of **15:** K_2CO_3 (276.4 mg, 2.0 mmol) was added to the solution of **9** (209.2 mg, 1.0 mmol) and **14** (316.5 mg, 1.1 mmol) in DMF (5 mL) under nitrogen atmosphere. The mixture was heated for 10 h at 50 °C, then poured into 1 N HCl solution (25 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were dried with anhydrous Na_2SO_4, and evaporated to dryness to afford crude product. The residue was purified by column chromatography (DCM: MeOH=50:1, v/v) to afford **15** (80.0% yield) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.62 (d, J = 7.1 Hz, 1H), 7.45 (d, J = 7.2 Hz, 1H), 7.26 (d, J = 7.2 Hz, 1H), 6.55 (d, J = 7.1 Hz, 1H), 5.57 (d, J = 16.3 Hz, 1H), 5.45 – 5.32 (m, 2H), 5.16 (d, J = 16.3 Hz, 1H), 2.69 (s, 3H), 2.59 (s, 3H), 1.88 – 1.68 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). HRMS (ESI, m/z): $[M+H]^+$ calcd for $C_{22}H_{22}ClN_2O_4$, 413.1263; found, 413.1255.

Synthesis of **DM-CPT:** The mixture of **15** (84.3 mg, 0.2 mmol), KOAc (60.1 mg, 0.6 mmol) and $Pd(PPh_3)_2(OAc)_2$ (30.6 mg, 0.04 mmol) in anhydrous MeCN (10 mL) was stirred at 100 °C for 12 h under nitrogen atmosphere. 25% NaOH solution (20 mL) was added to the mixture, then the organic phase was separated and the aqueous layer was extracted with MeOH-$CHCl_3$ (1:10, 2 × 20 mL). Concentrated HCl solution was added dropwise to the aqueous layer until pH=2, then the aqueous layer was extracted with MeOH-$CHCl_3$ (1:10, 2 × 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, the residue was recrystallized (1,4-dioxane) to give **DM-CPT** (32.5% yield) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.63 (s, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.36 (d, J = 7.1 Hz, 1H), 5.76 (d, J = 16.3 Hz, 1H), 5.36 – 5.28 (m, 3H), 2.85 (s, 3H), 2.71 (s, 3H), 2.00 – 1.85 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 174.1, 157.9, 150.8, 150.2, 148.6, 147.4, 136.3, 132.4, 130.3, 128.4, 127.9, 127.8, 127.7, 118.3, 97.6, 73.0, 66.6, 50.4, 29.8, 19.0, 18.2, 8.0.

Synthesis of **ENDO-CPT: DM-CPT** (8.0 mg, 0.95 mmol) was dissolved in $CHCl_3$ (5 mL). The reaction mixture was cooled to 0 ℃ in an ice bath. Methylene blue (0.25 mg) was added to the solution and the mixture was stirred for 6 h under oxygen atmosphere. During the reaction process, 18W, 630 nm red light was used to irradiate the reaction solution. Activated carbon (100 mg) was added to the reaction solution, filtered, and the filtrate was concentrated to give **ENDO-CPT** (98% yield) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.45 (s, 1H), 6.84 – 6.71 (m, 2H), 5.71 (d, J = 16.3 Hz, 1H), 5.28 (d, J = 16.2 Hz, 1H), 5.13 (d, J = 7.4 Hz, 2H), 2.01 (s, 3H), 1.95 (s, 3H), 1.93 – 1.80 (m, 2H), 1.03 (t, J = 7.0 Hz, 3H).

Reaction Rate Experiments

The half-life calculations were done in accordance to the first-order reaction rate equations. Cycloreversion reaction rate of **ENDO-CPT** was calculated based on the ^{1}H-NMR integral ratios and peak integrals between 7.7 to 8.7 ppm were used for this calculation.

The equations are given below:

$$\ln[A] = -kt + \ln[A_0]\,, t_{0.5} = 0.693/k$$

Singlet Oxygen Trap Experiments

In singlet oxygen generation experiments, 1,3-Diphenylisobenzofuran (DPBF) was used as chemical singlet oxygen trap molecule in DMF. The solution of DPBF (100 μM) was mixed with the DMF solution of **ENDO-CPT** and **DM-CPT** (200 μM) in same volume. Measurements were taken at 10 minutes intervals at 37 ℃ in dark conditions. Absorbance decrease of trap molecules at 414 nm was monitored to reveal singlet oxygen generation.

Time-Dependent UV-vis Absorption and Fluorescence Spectra Experiments

The solution of **ENDO-CPT** (100 μM) in DMF was prepared, measurements were taken at 37 ℃ in dark conditions. The changes in UV-vis absorption and fluorescence (E_x=366 nm) were monitored to reveal the conversion of **ENDO-CPT** to **DM-CPT**.

Intracellular Imaging

The ROS generation ability of **ENDO-CPT** was evaluated via **High Content imaging (HCIS)** with DCFH-DA as probe. 1×10^4 per dish Hela cells were seeded with DMEM medium containing 10% FBS and 1% penicillin on Nest 96 Well Plate. After 1 day, the cells were treated with 128 μM of **ENDO-CPT**, **DM-CPT** and CPT (dissolved in medium),

and DCFH-DA was co-incubated[1] with the drugs at a concentration of 10 μM. After 5 h, cells were washed with PBS (× 3), following observed by using HCIS.

Co-localization imaging experiment of ROS and **DM-CPT** was evaluated via HCIS with DCFH-DA as probe. Hela cells were plated at 96-well plates (1×10^4 cells per well). After 1 day, the cells were treated with 64 μM of **ENDO-CPT** (dissolved in medium), and DCFH-DA was co-incubated with the drugs at a concentration of 10 μM. After 1 day, cells were washed with PBS (× 3), following observed by using HCIS.

Calcein-AM/PI live/dead cell dual staining experiment was evaluated via HCIS. MCF-7 ADR cells were plated at 96-well plates (1×10^4 cells per well). After 1 day, the cells were treated with 32 μM of **ENDO-CPT**, **DM-CPT** and CPT (dissolved in medium) for 2 h. The cells were incubated with Calcein-AM solution for 30 min, then incubated with PI solution for 5 min, washed with PBS (× 3), following observed by using HCIS.

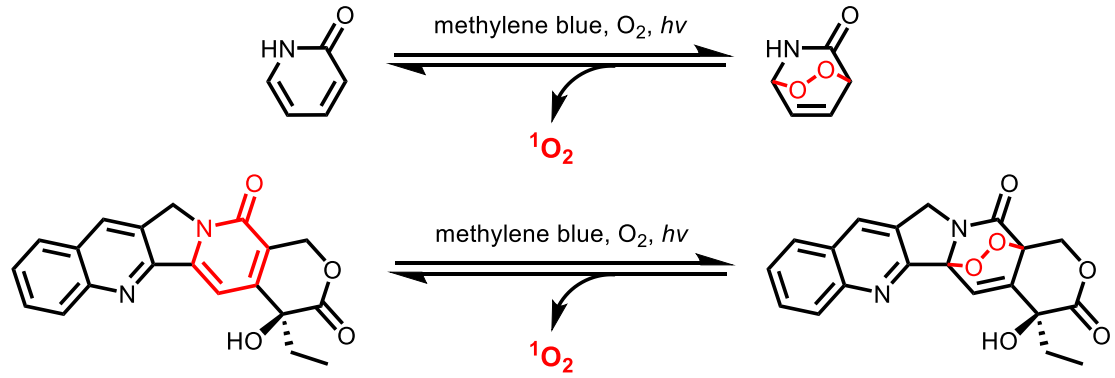
Western Blotting Analysis

1×10^7 per dish MCF-7 ADR cells were seeded on Nest 100 mm Culture Dishes with 1640 medium. After 1 day, the cells were treated with 64 μM of **ENDO-CPT**, **DM-CPT** and CPT (dissolved in medium) for 1 day. Then, the cells were washed with PBS, scraped off from the culture dish and collected in multiple PE tubes. All samples were treated with RIPA lysis buffer and placed on ice for about 30 min. Thereafter, all samples were centrifuged in a cryogenic centrifuge (12000 rpm for 10 min), and the protein supernatant was obtained. Finally, the obtained samples were analyzed by Western blotting.

MTT Assay

4T1 / MCF-7 / MCF-7 ADR / 3T3 / LO2 cells were plated at 96-well plates (7×10^3 cells per well), then incubated at 37℃ for 1 day. The cells were treated with **ENDO-CPT**, **DM-CPT** and CPT (dissolved in medium) at different concentrations for 1 day. Then, MTT solution was added to each well and the cells were incubated at 37 ℃ for 4 h. Subsequently, the medium was removed and 150 μL DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm with a microplate reader.

Supporting Figure



Scheme S1. Attempted synthesis of the endoperoxide unmodified camptothecin, based on the reactivity of 2-pyridone.

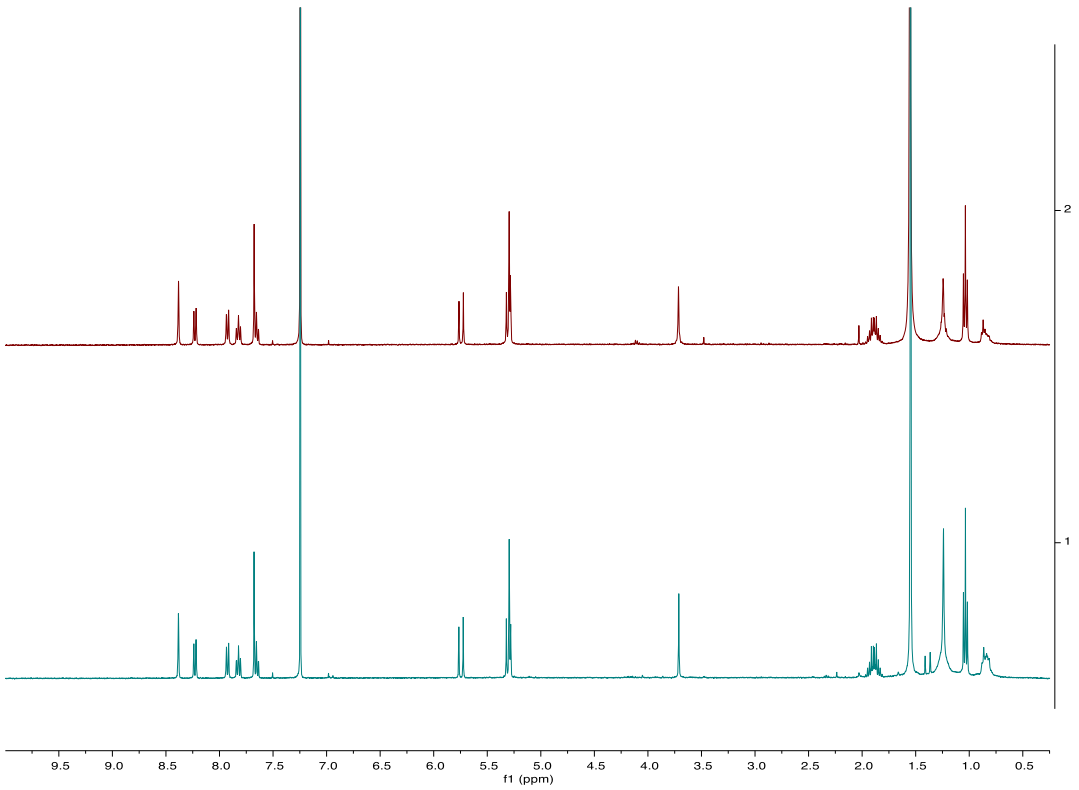


Figure S1. The unmodified camptothecin was subjected to endoperoxide formation conditions, and the ^{1}H-NMR spectra (in $CDCl_3$) before and after irradiation were obtained (Spectra 1- before the test, Spectra 2- after the test).

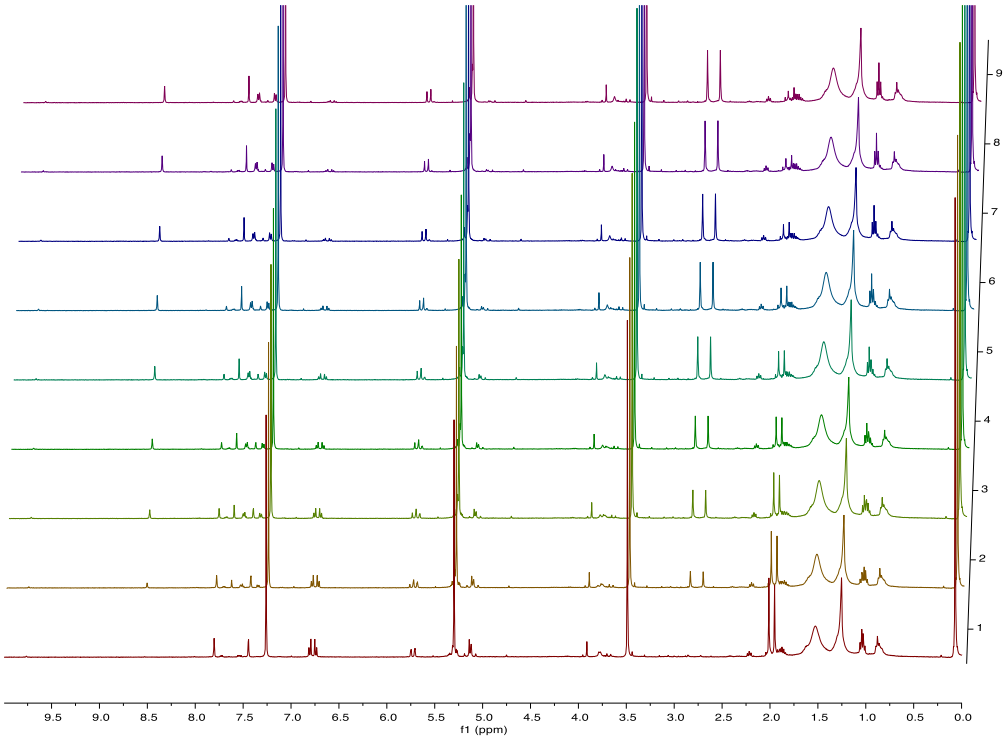


Figure S2. Evolution of the ^{1}H-NMR spectra of **ENDO-CPT** with time at 37 ℃ in $CDCl_3$ as the solvent. (Spectra 1- 0 min, Spectra 2- 30 min, Spectra 3- 60 min, Spectra 4- 90 min, Spectra 5- 120 min, Spectra 6- 150 min, Spectra 7- 180 min, Spectra 8- 210 min, Spectra 9- 240 min).

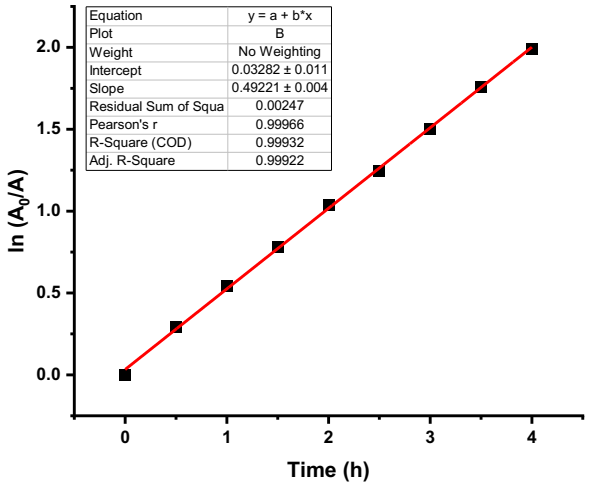


Figure S3. Half-life calculation of **ENDO-CPT**: 1.4 hours (at 37 ℃ in $CDCl_3$).

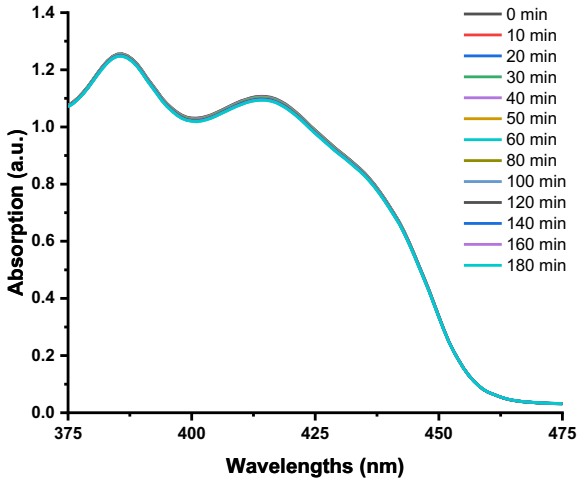


Figure S4. The decrease in the absorption peak of DPBF (50 μM) in the presence of **DM-CPT** (100 μM) in DMF in dark at 37 ℃.

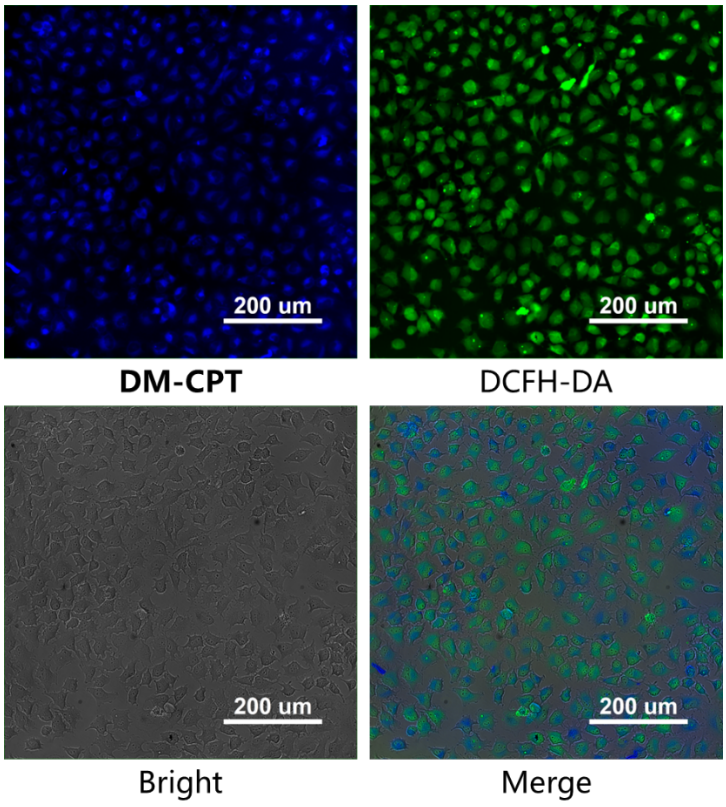


Figure S5. Co-localization HCIS images of intracellular ROS and **DM-CPT**.

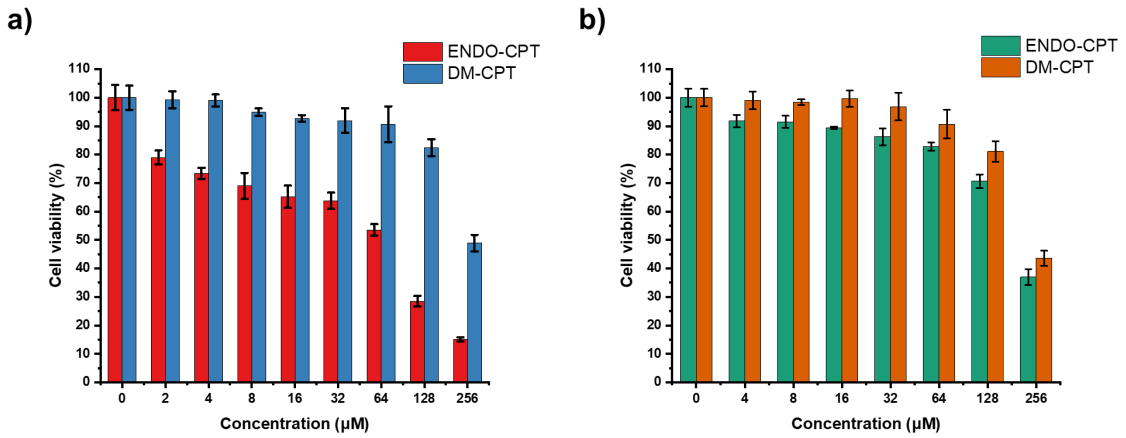


Figure S6. Cell viability of cells after incubating with different concentration of **ENDO-CPT** and **DM-CPT**. a) 4T1, b) MCF-7. (n = 6, mean ± SD).

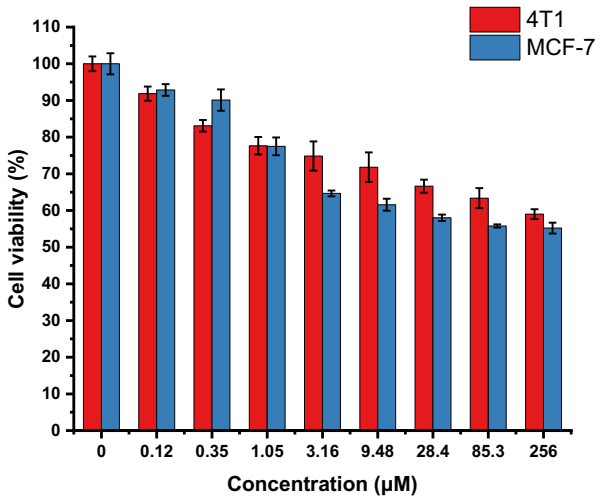


Figure S7. Cell viability of cells after incubating with different concentration of CPT in 4T1 and MCF-7 cells. (n = 6, mean ± SD).

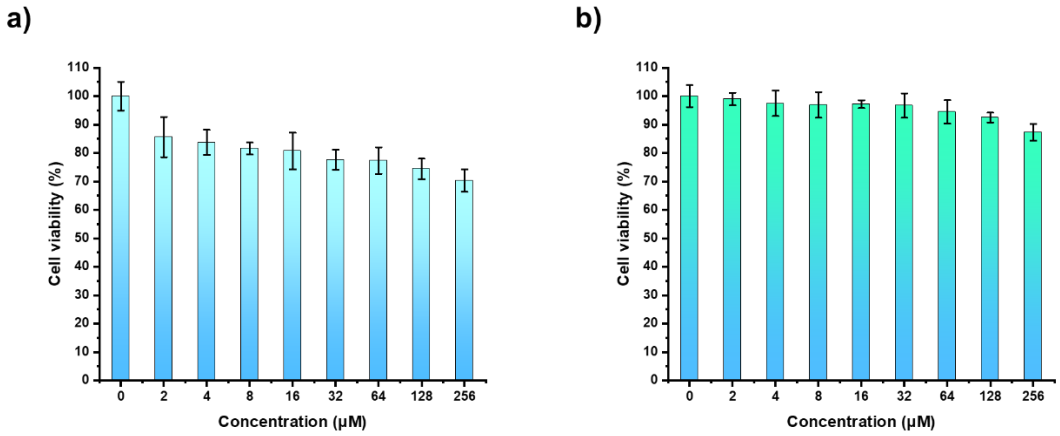


Figure S8. Cell viability of cells after incubating with different concentration of a) CPT and b) 5,8-dimethylquinoline endoperoxide in MCF-7 ADR cells. (n = 6, mean ± SD).

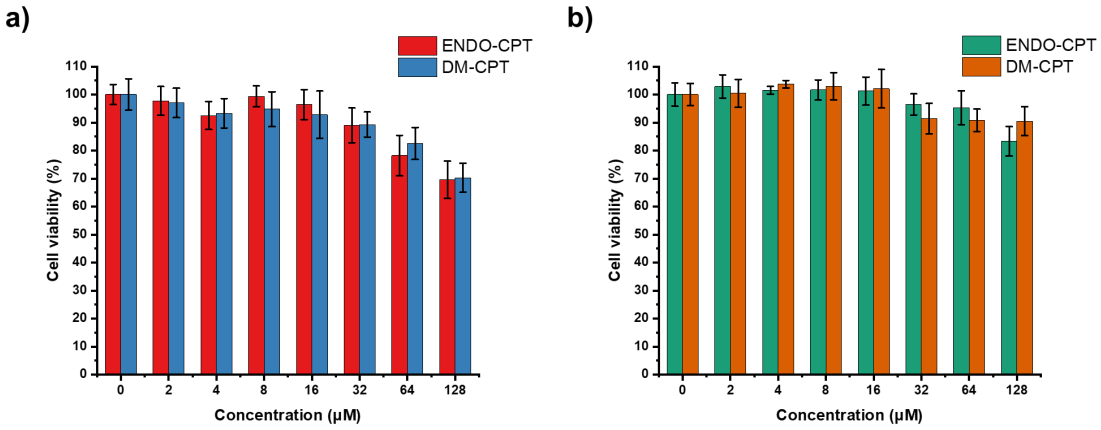


Figure S9. Cell viability of cells after incubating with different concentration of **ENDO-CPT** and **DM-CPT**. a) 3T3, b) LO2. (n = 6, mean ± SD).

NMR spectra (all in CDCl3)

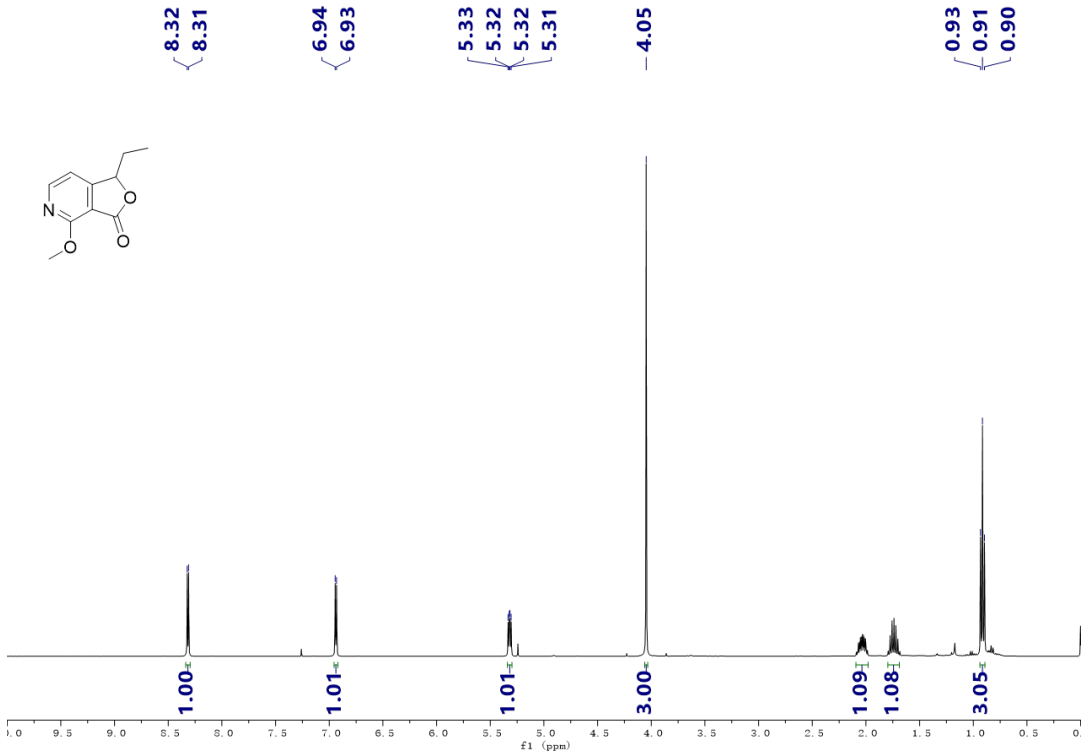


Figure S10. ^{1}H-NMR spectrum of compound **2**

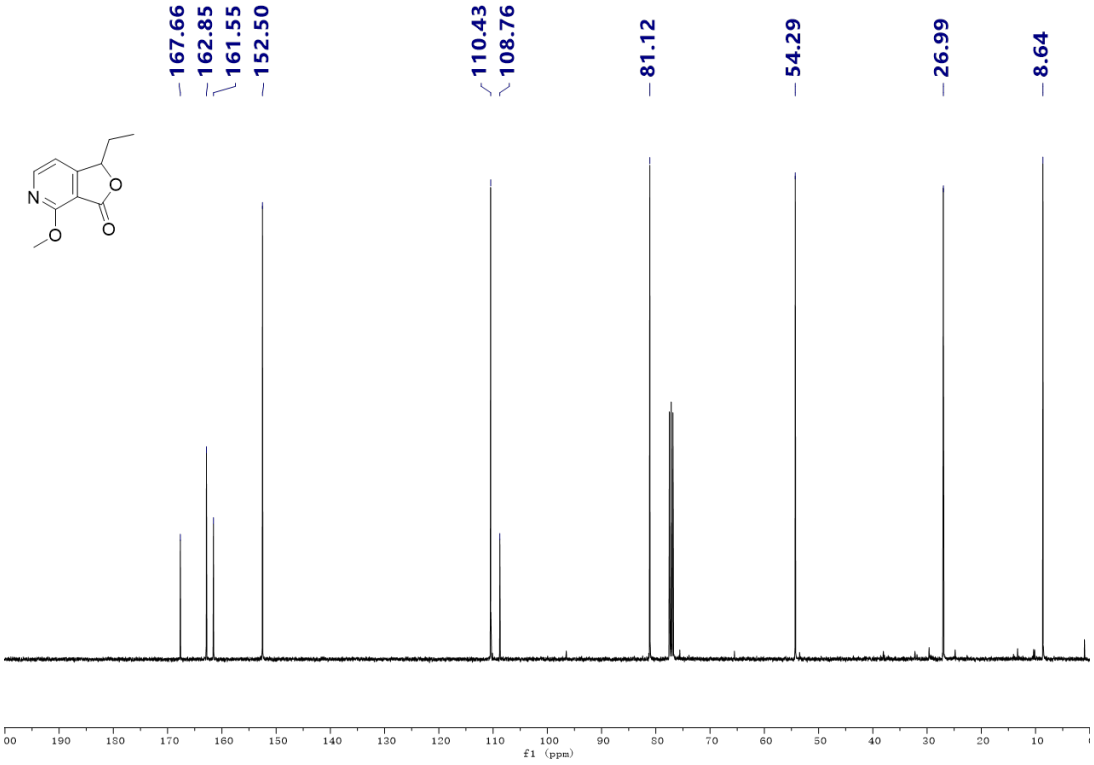


Figure S11. ^{13}C-NMR spectrum of compound **2**

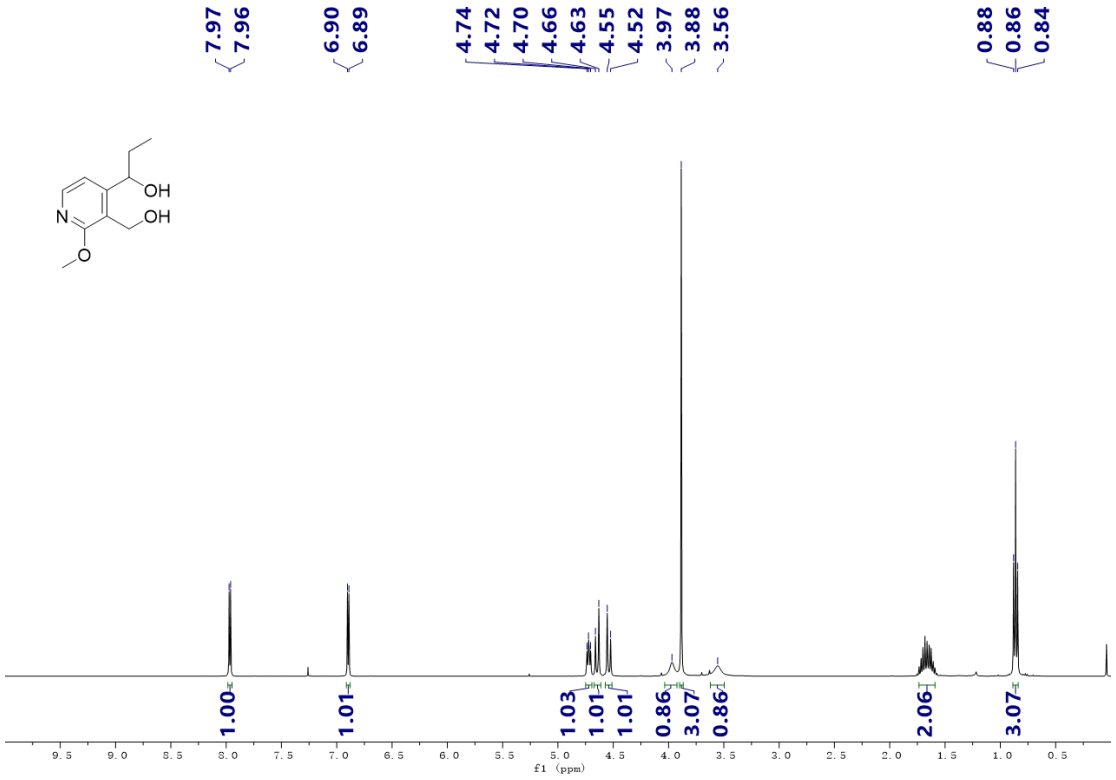


Figure S12. ^{1}H-NMR spectrum of compound **3**

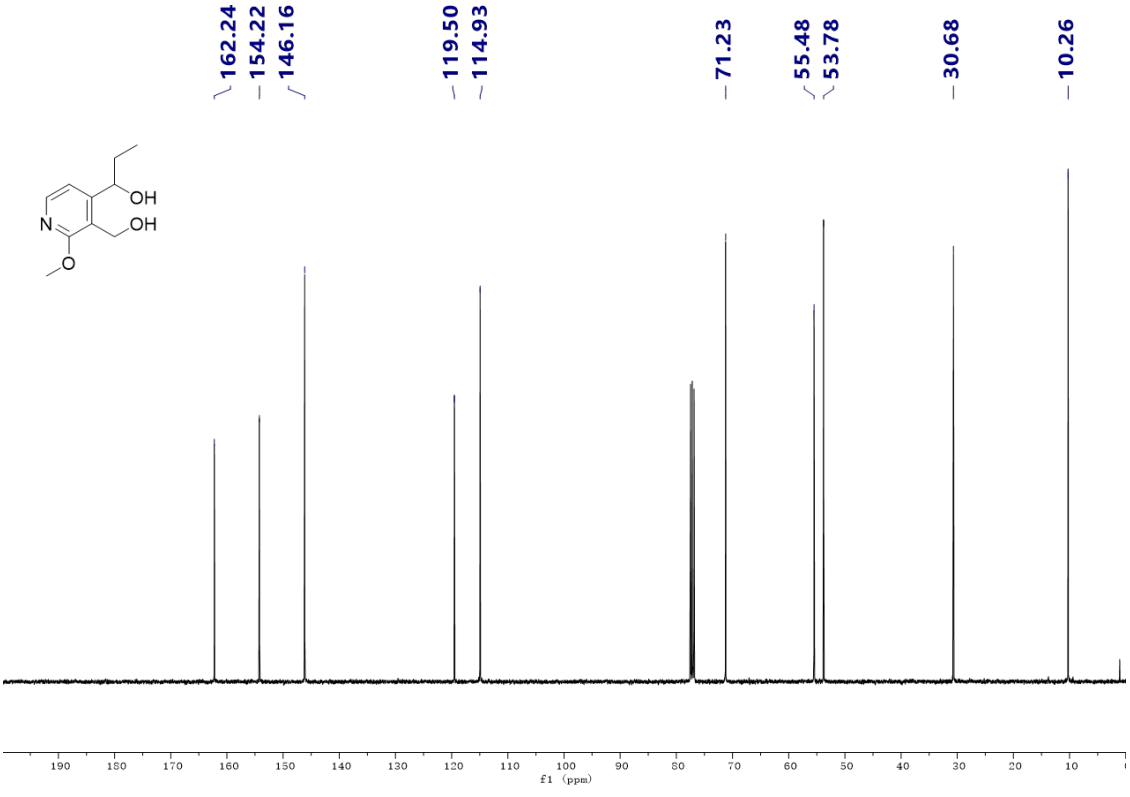


Figure S13. ^{13}C-NMR spectrum of compound **3**

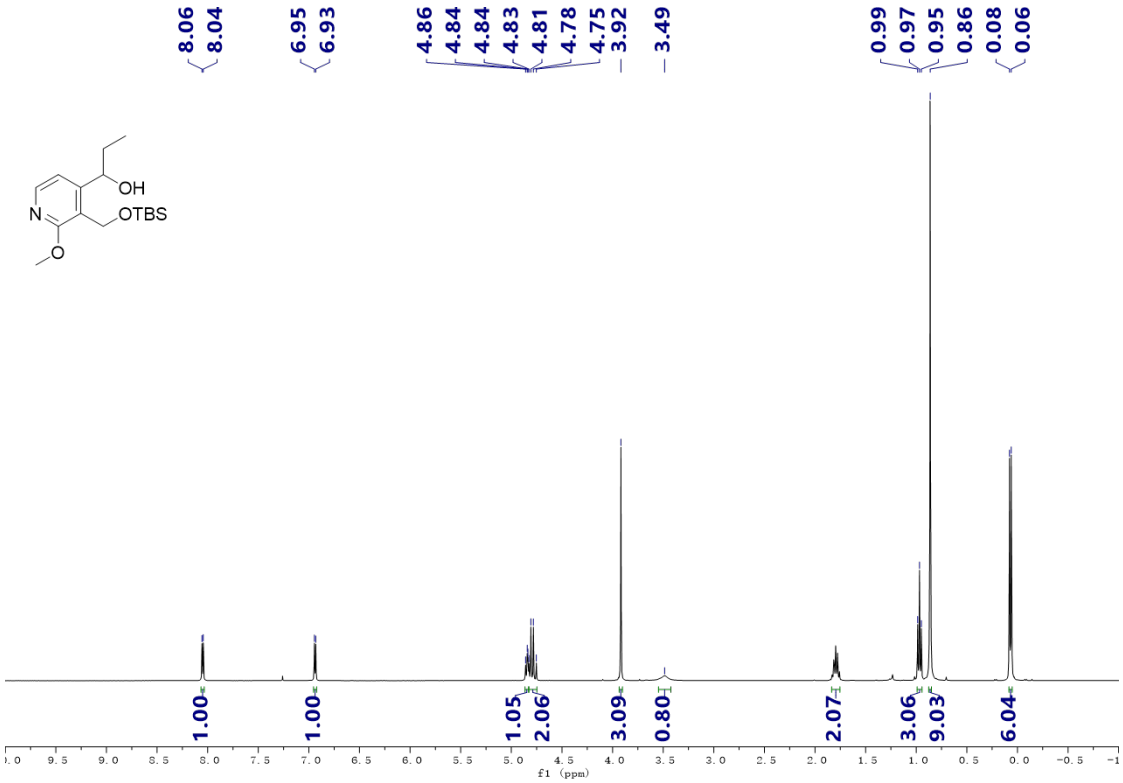


Figure S14. ^{1}H-NMR spectrum of compound **4**

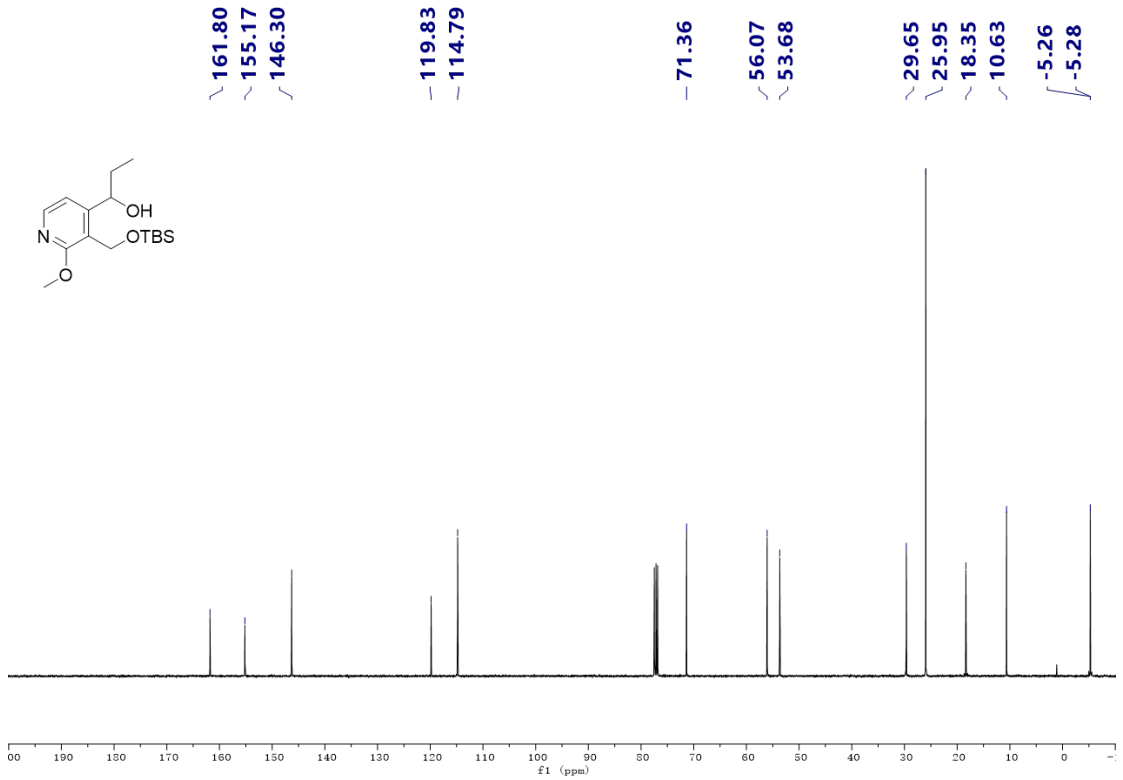


Figure S15. ^{13}C-NMR spectrum of compound **4**

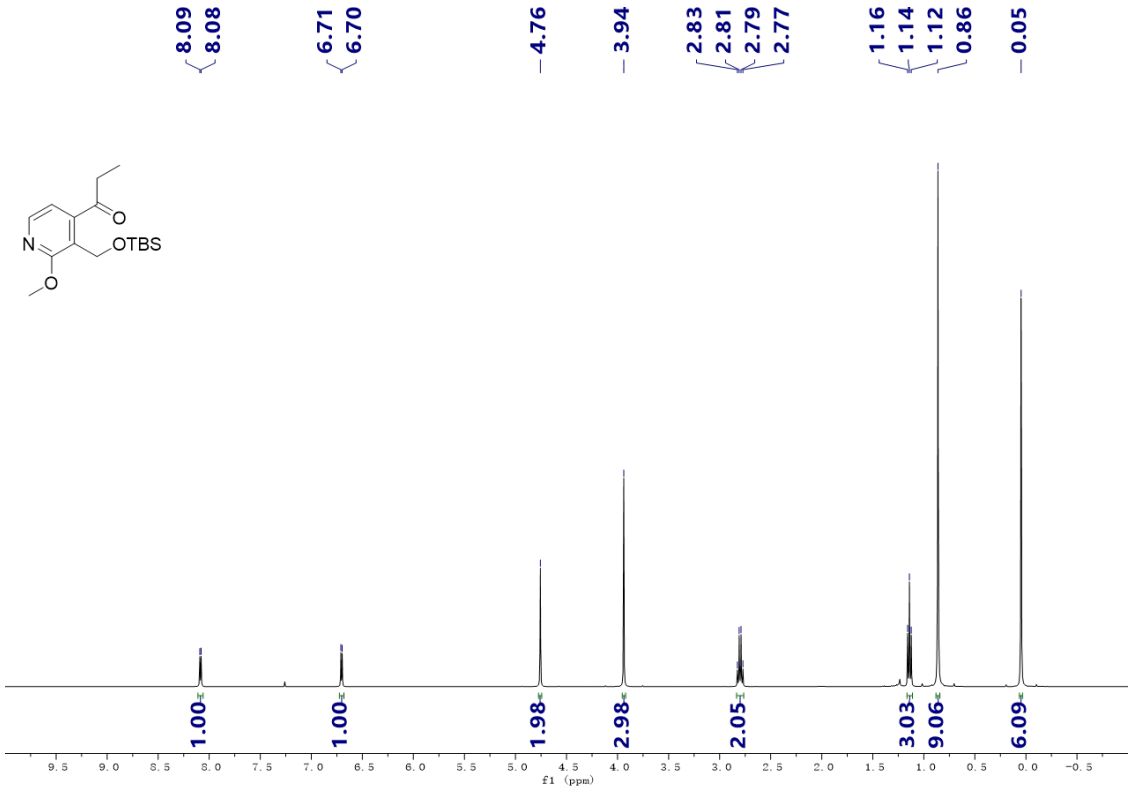


Figure S16. ^{1}H-NMR spectrum of compound **5**

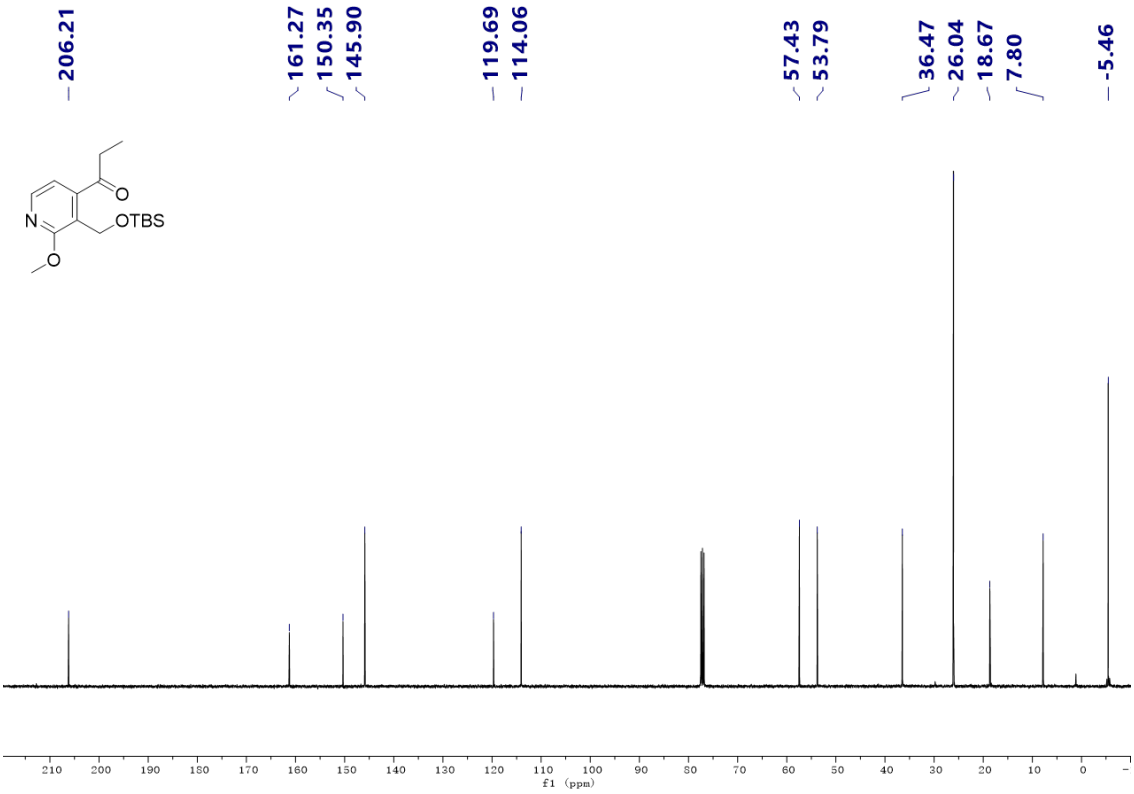


Figure S17. ^{13}C-NMR spectrum of compound **5**

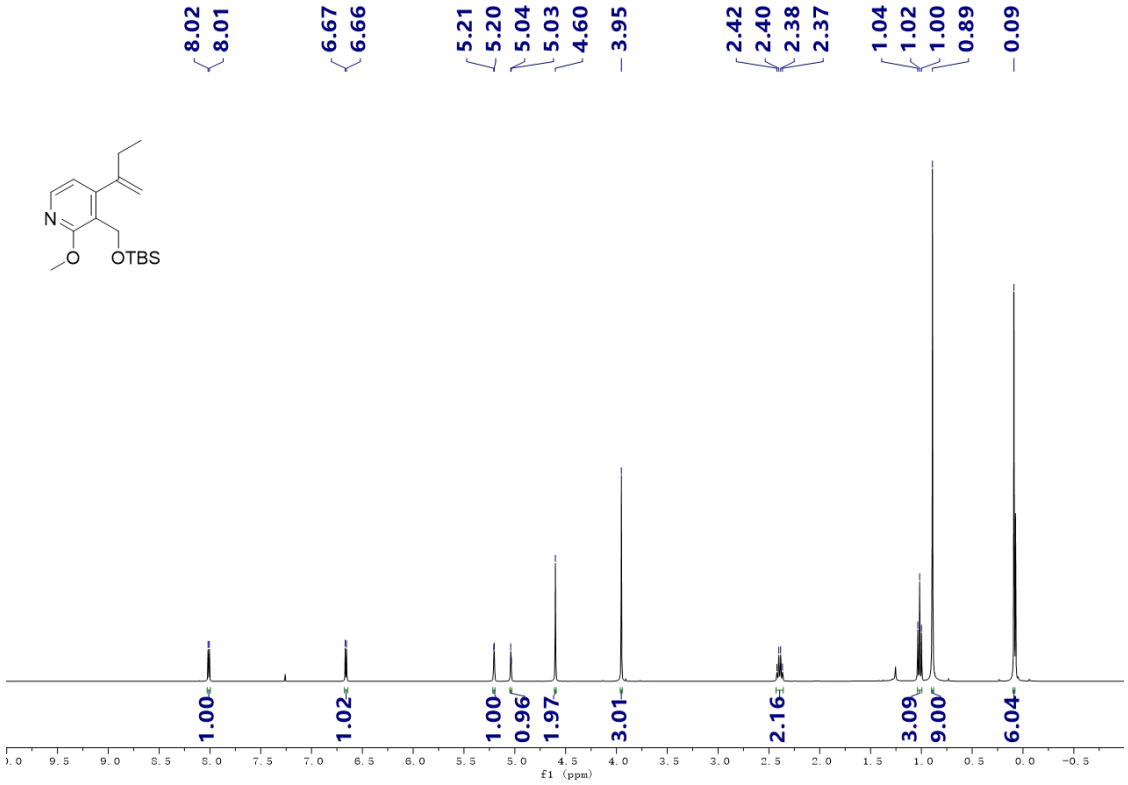


Figure S18. ^{1}H-NMR spectrum of compound **6**

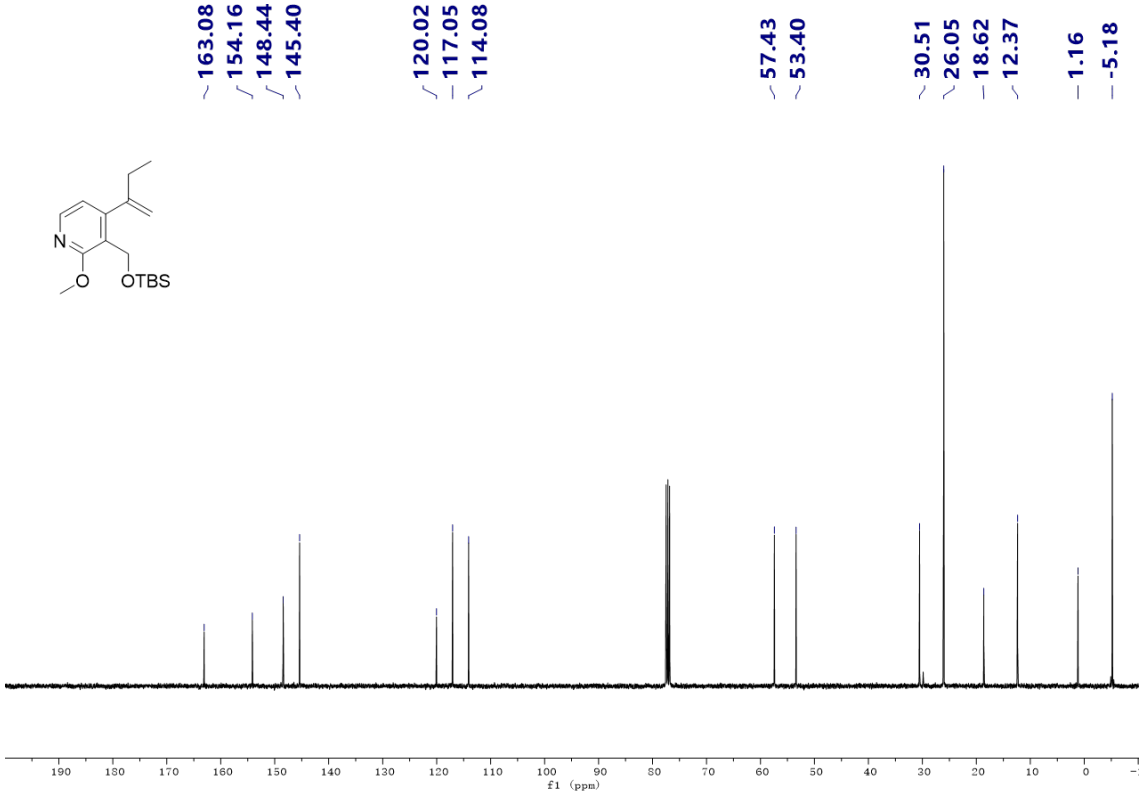


Figure S19. ^{13}C-NMR spectrum of compound **6**

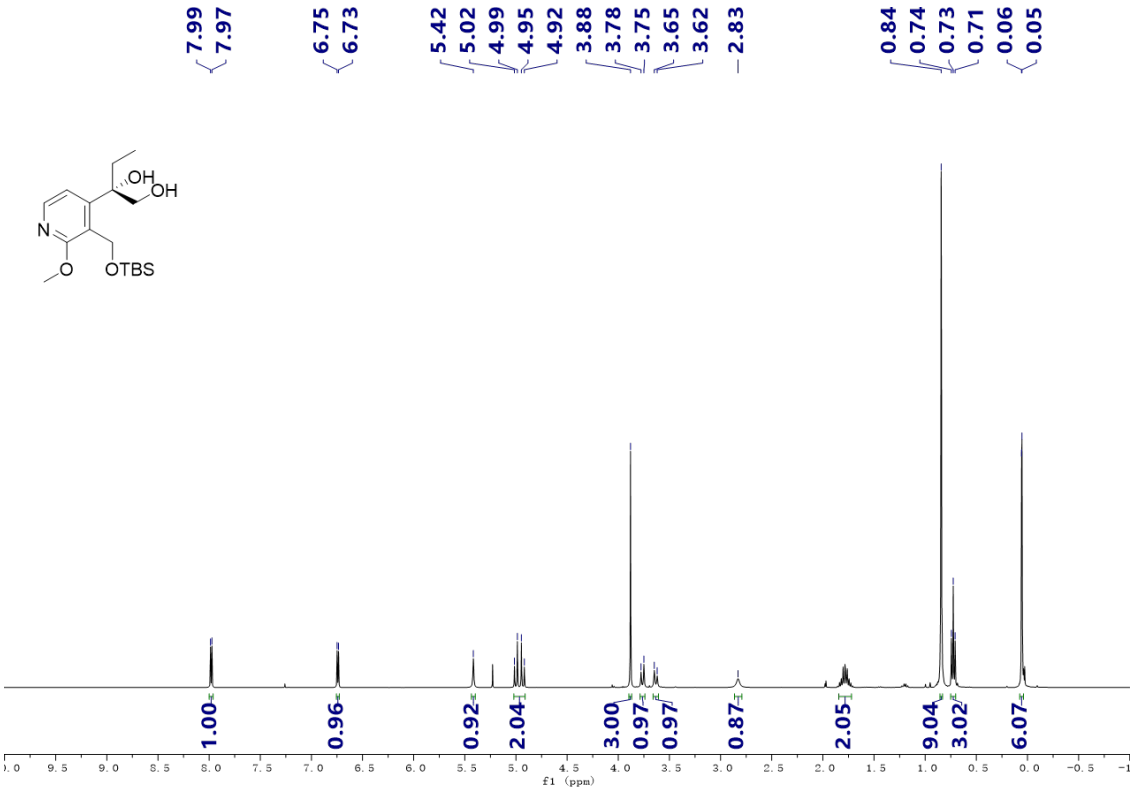


Figure S20. ^{1}H-NMR spectrum of compound **7**

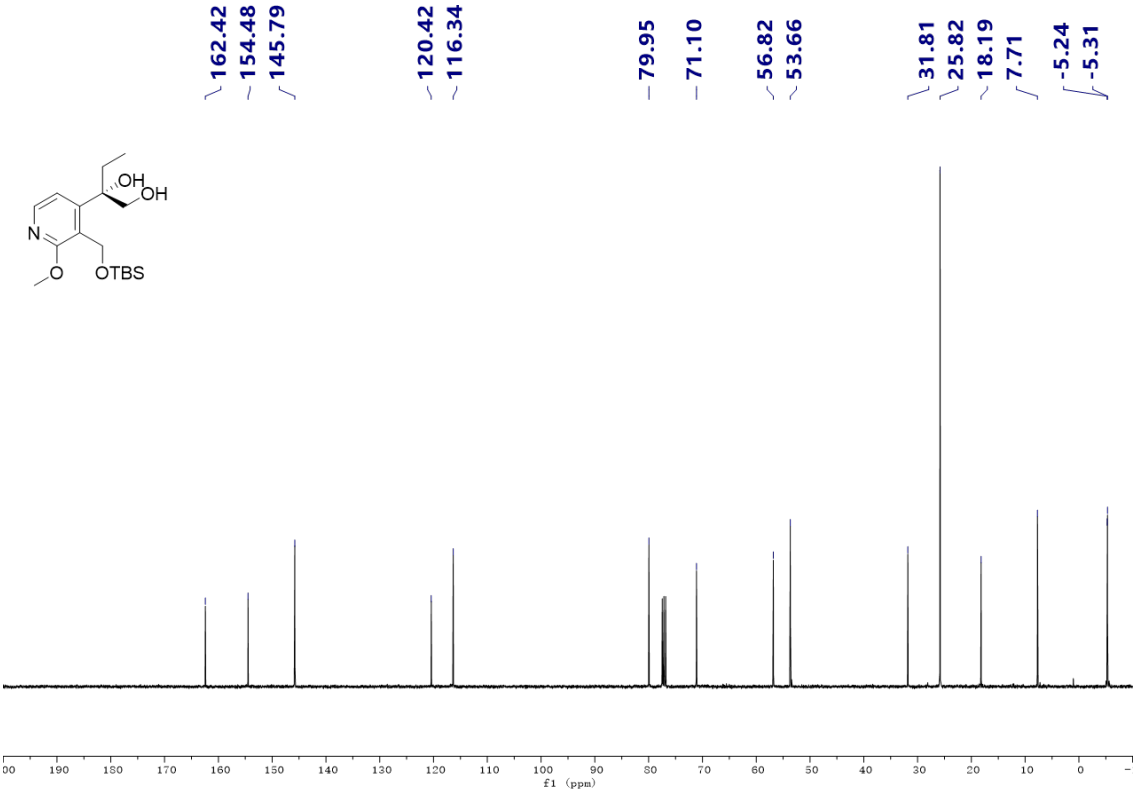


Figure S21. ^{13}C-NMR spectrum of compound **7**

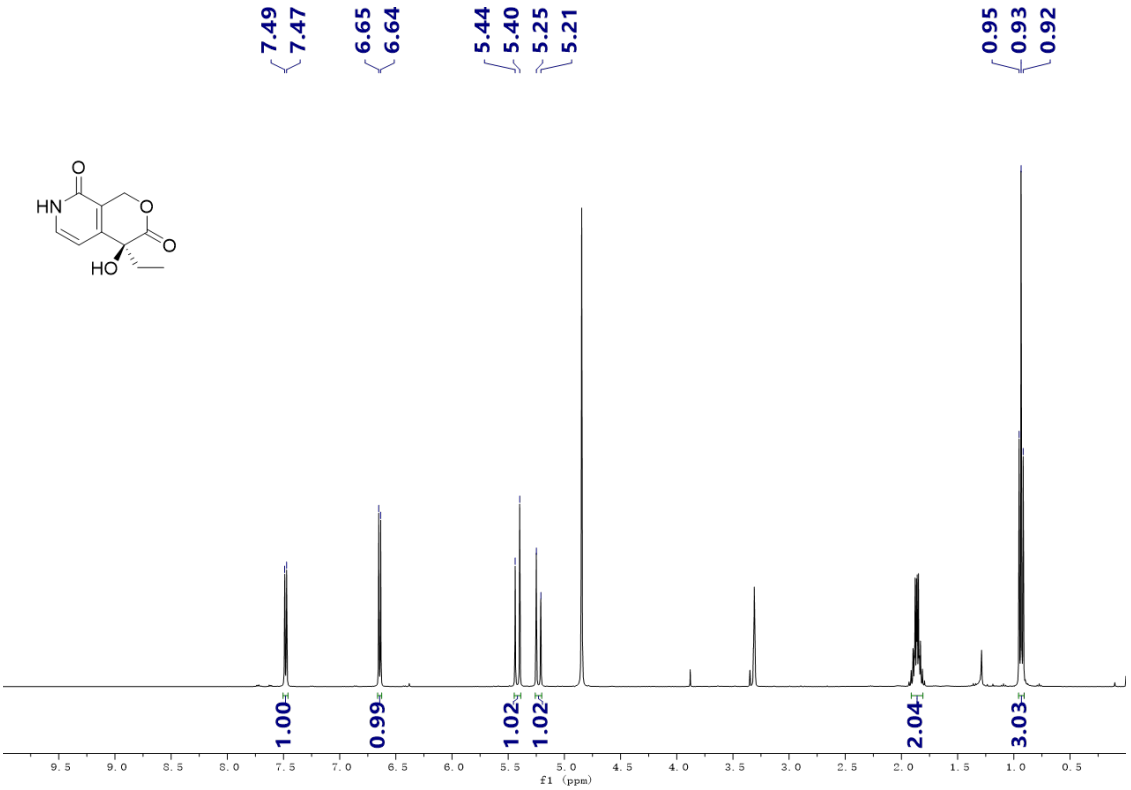


Figure S22. ^{1}H-NMR spectrum of compound **9**

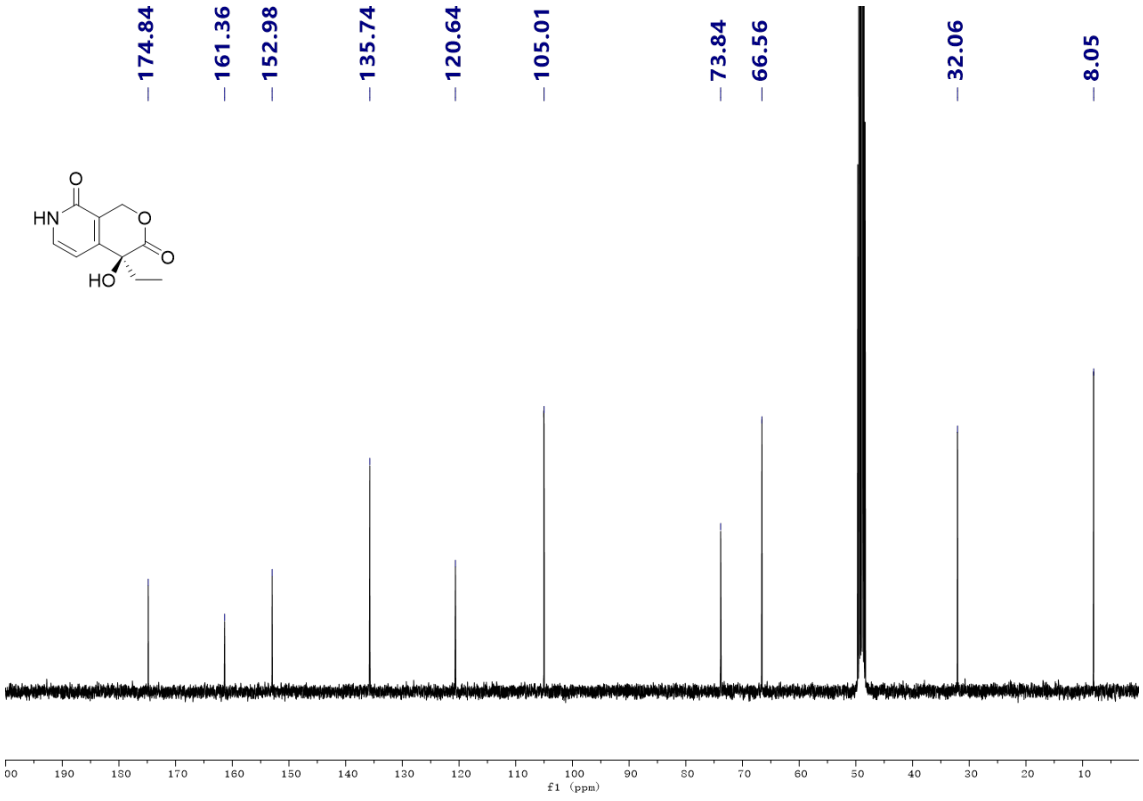


Figure S23. ^{13}C-NMR spectrum of compound **9**

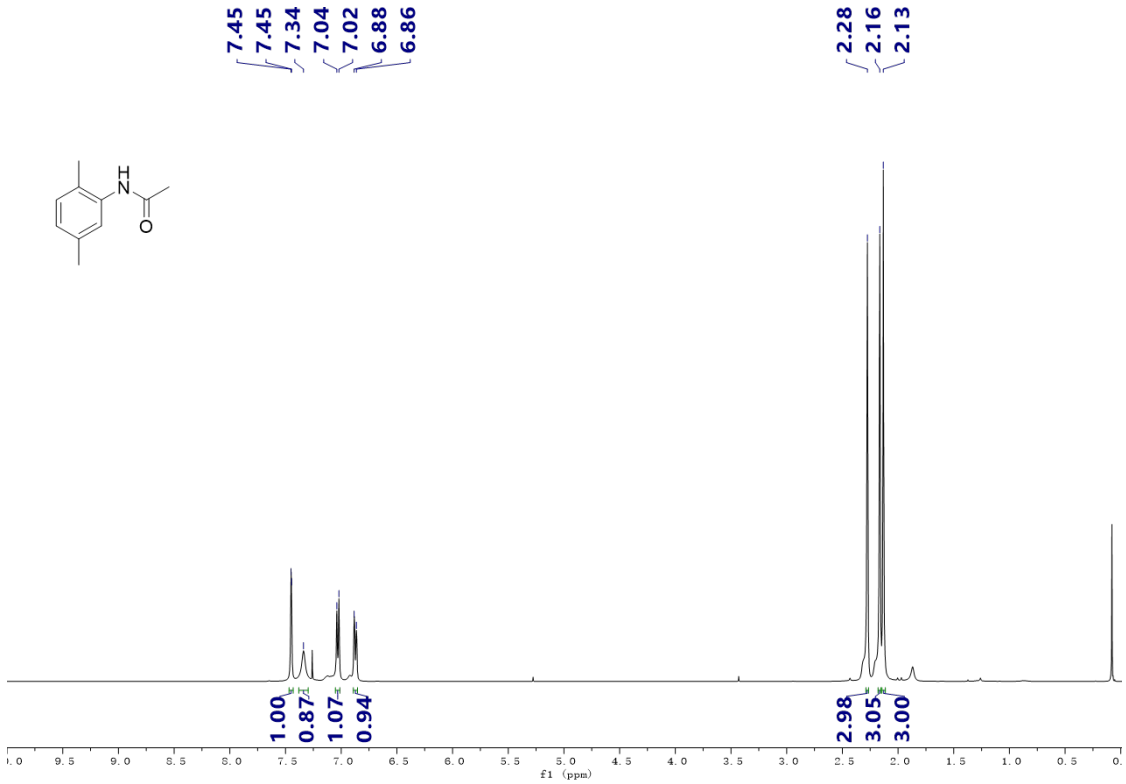


Figure S24. [1]H-NMR spectrum of compound **11**

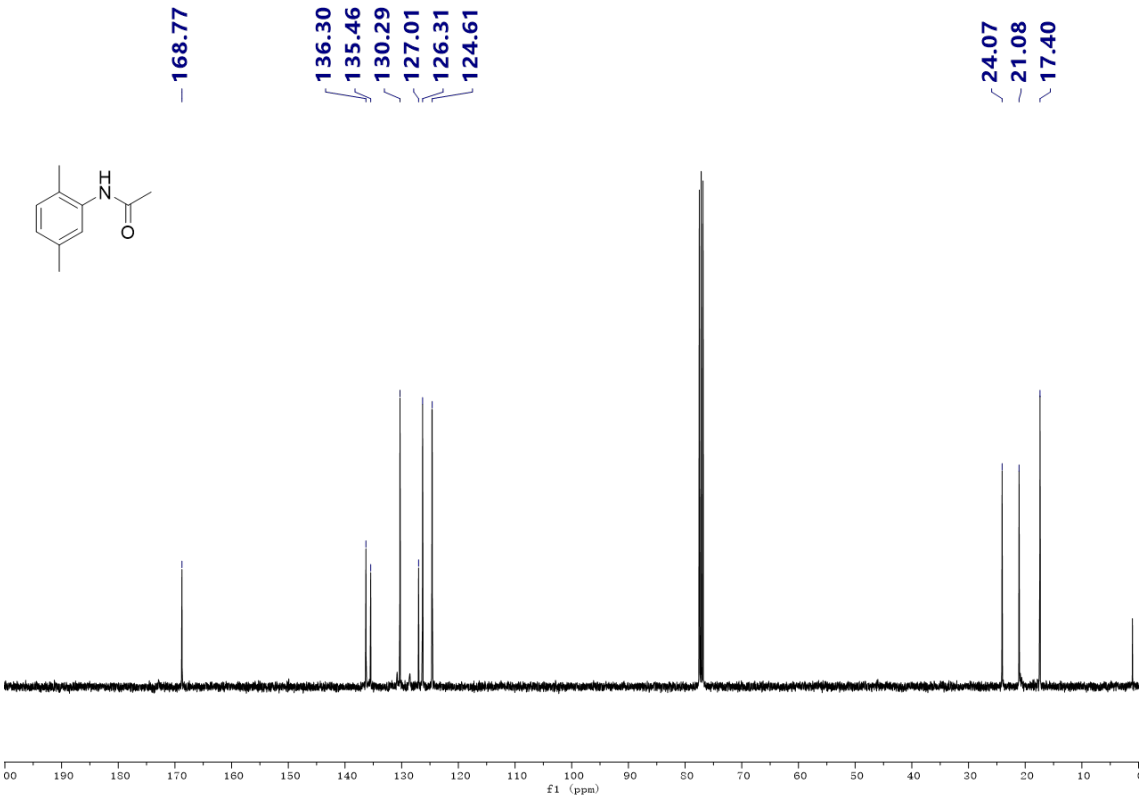


Figure S25. [13]C-NMR spectrum of compound **11**

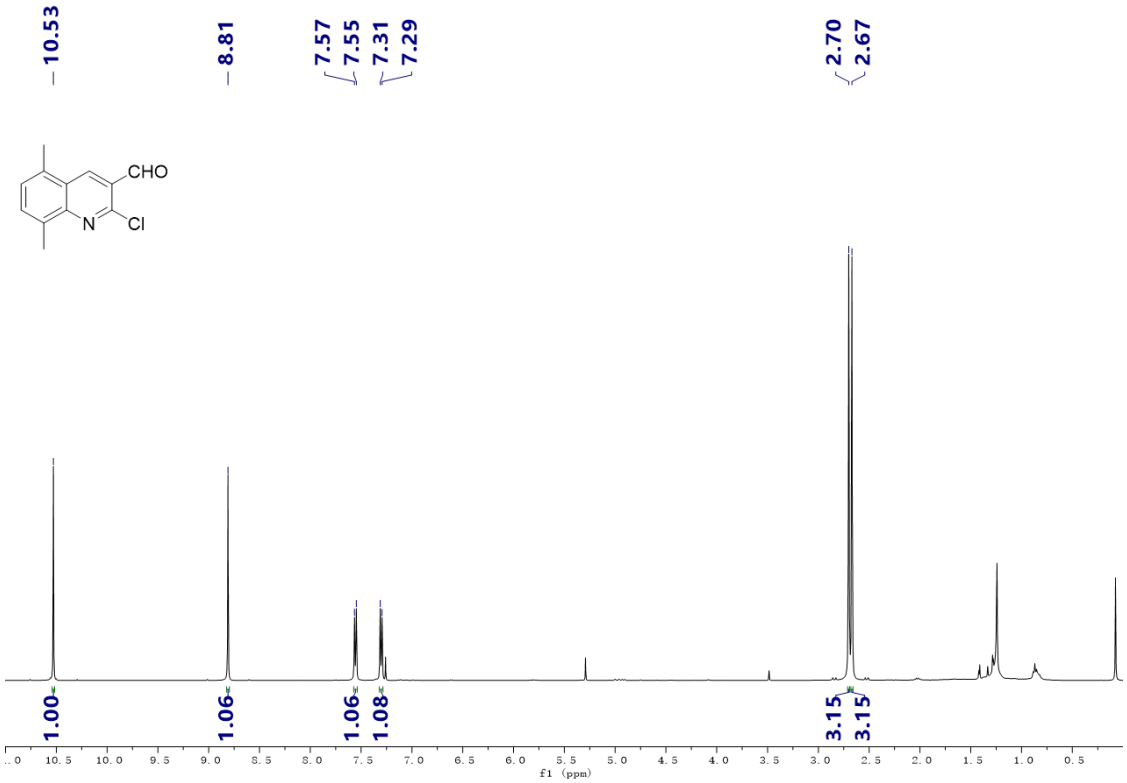


Figure S26. ^{1}H-NMR spectrum of compound **12**

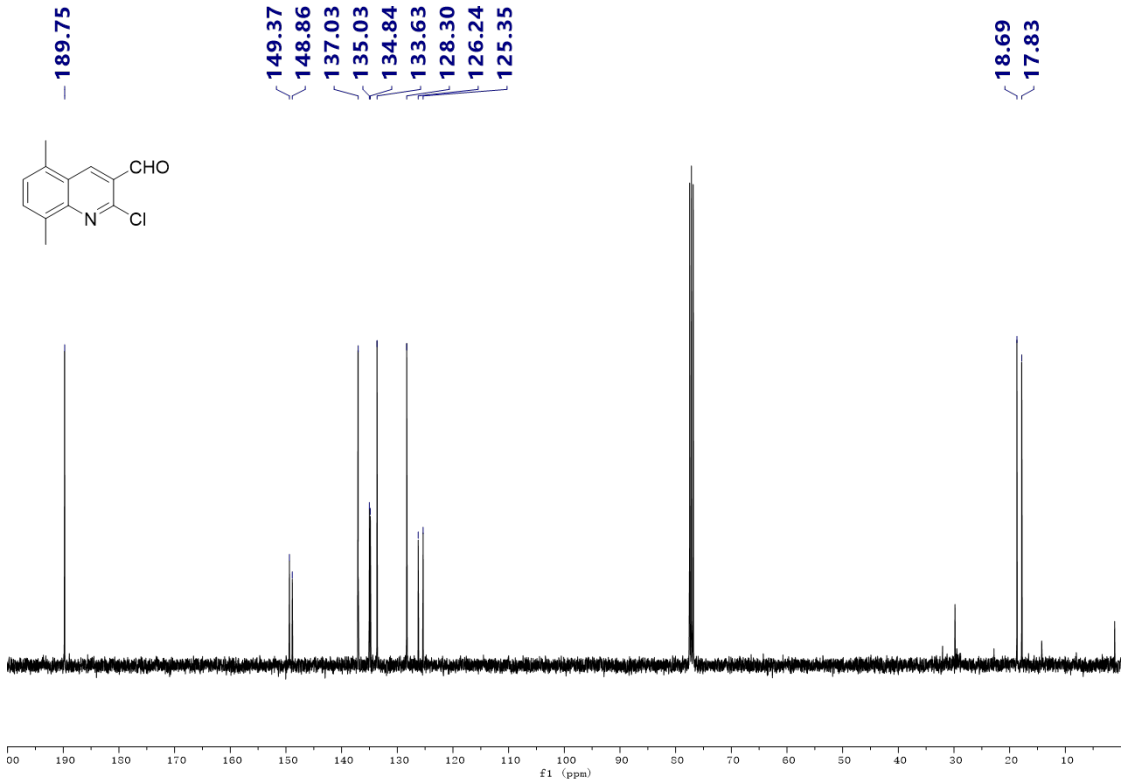


Figure S27. ^{13}C-NMR spectrum of compound **12**

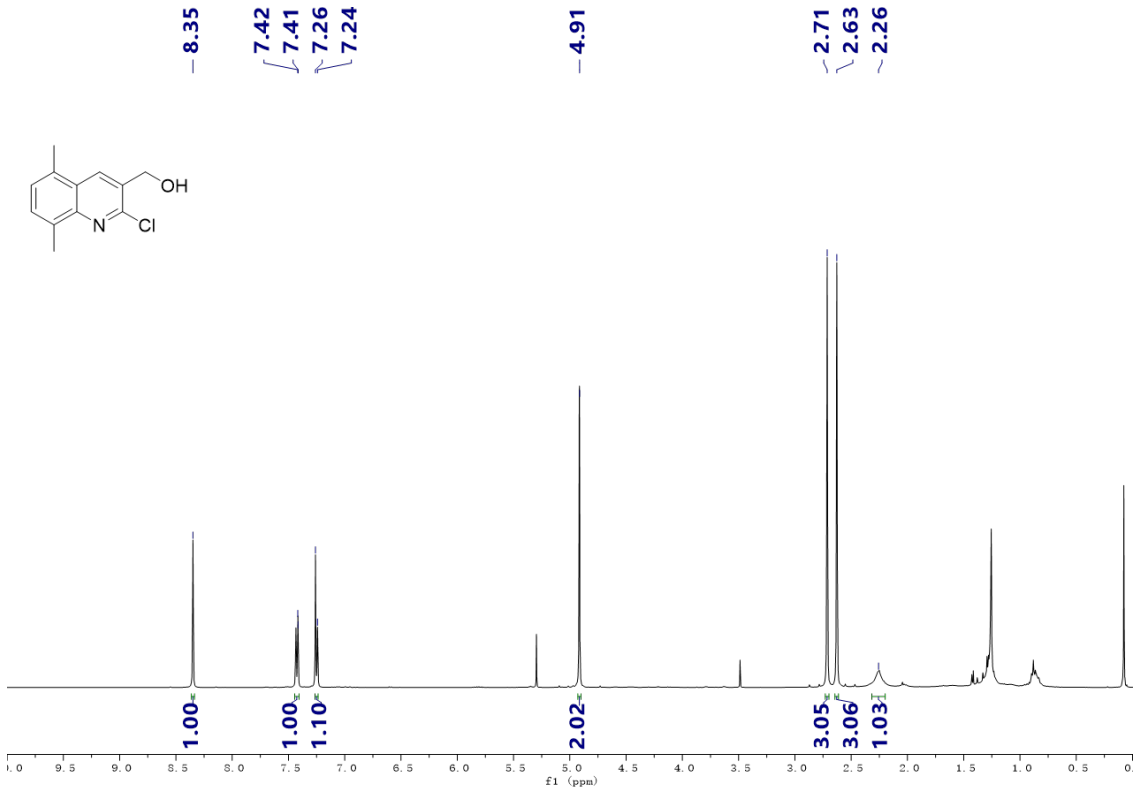


Figure S28. ^{1}H-NMR spectrum of compound **13**

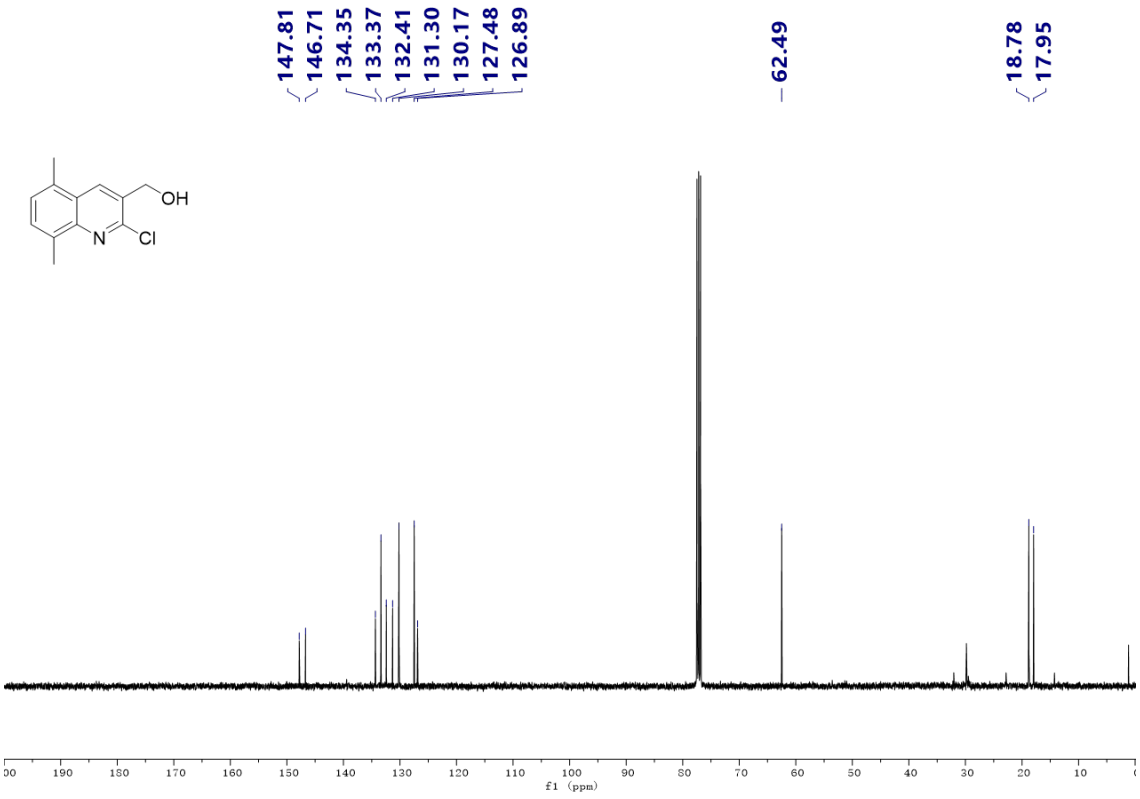


Figure S29. ^{13}C-NMR spectrum of compound **13**

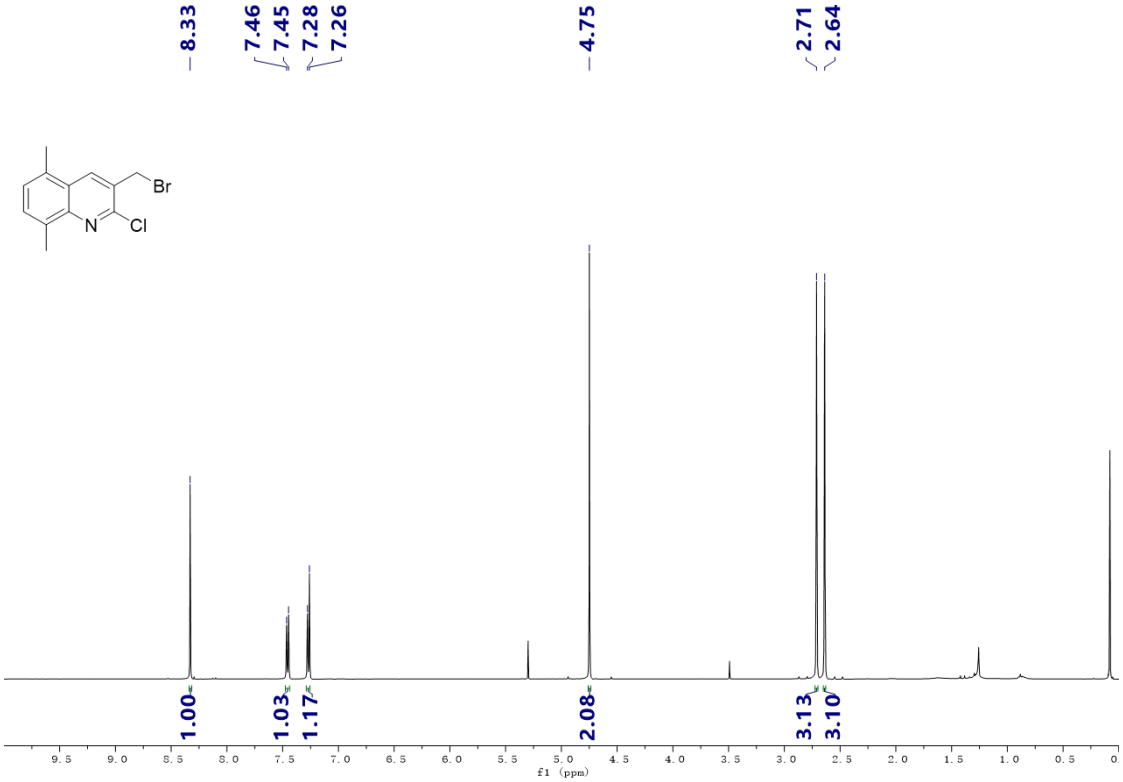


Figure S30. ^{1}H-NMR spectrum of compound **14**

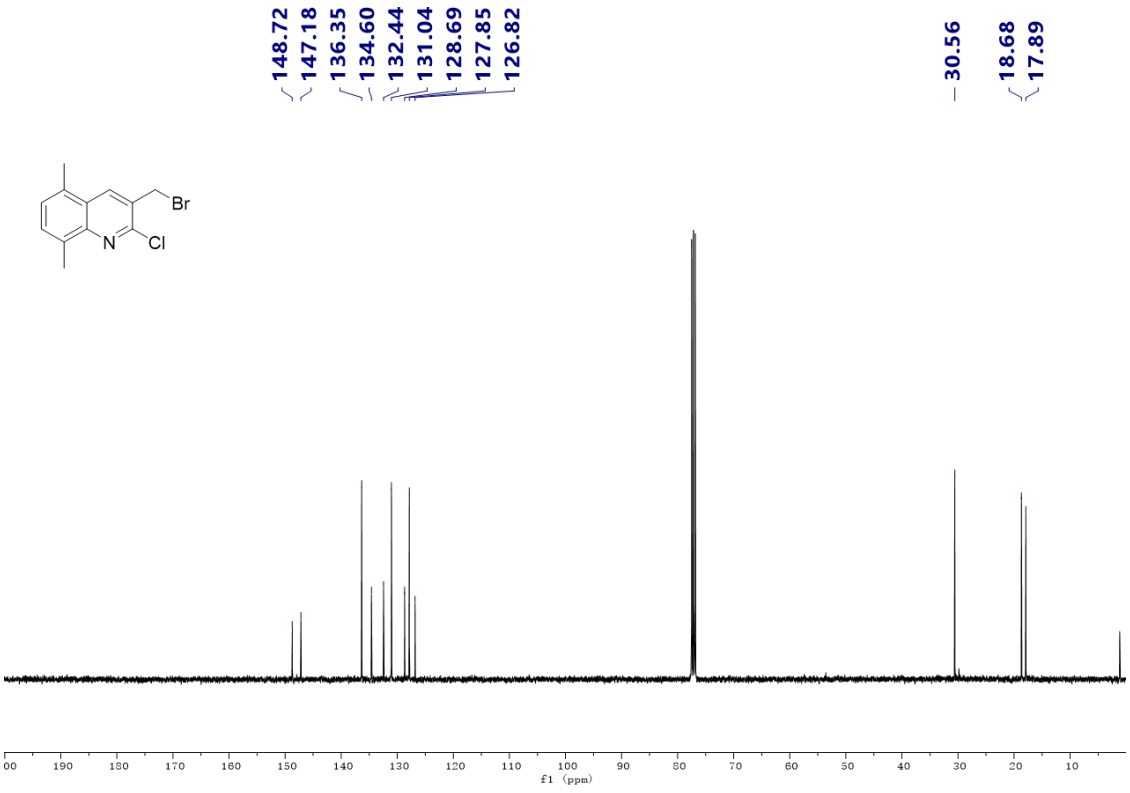


Figure S31. ^{13}C-NMR spectrum of compound **14**

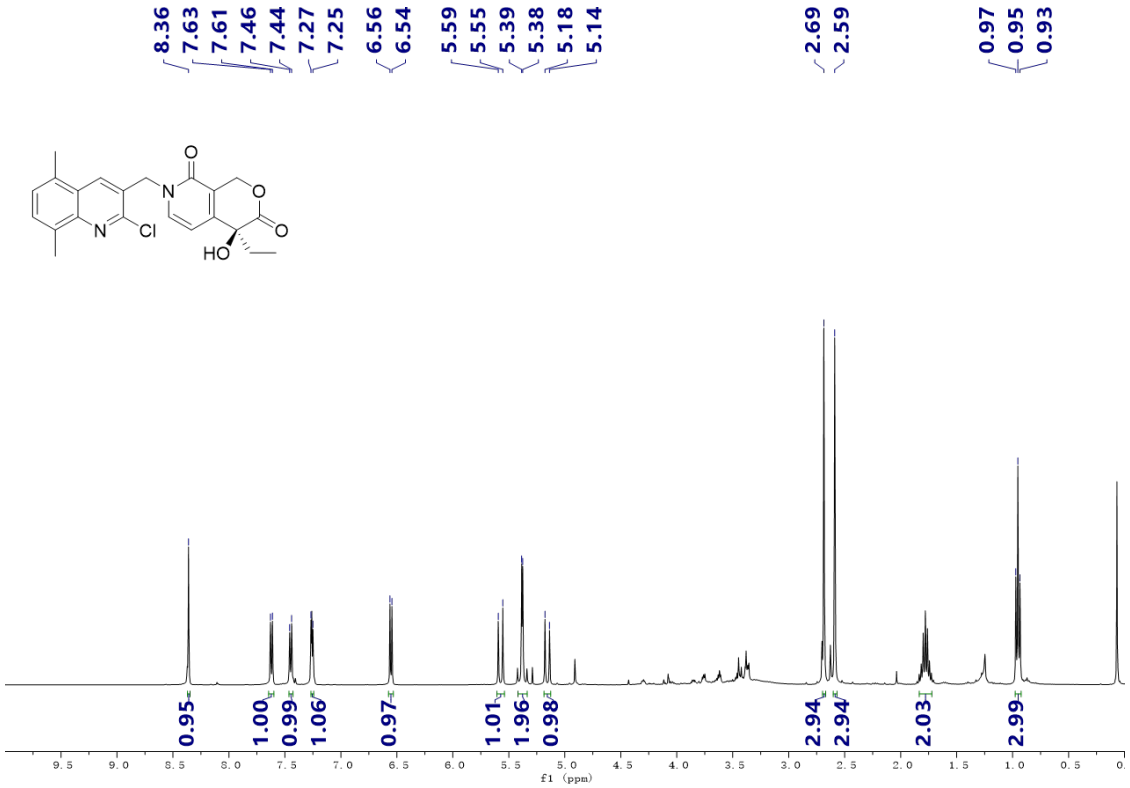


Figure S32. [1]H-NMR spectrum of compound **15**

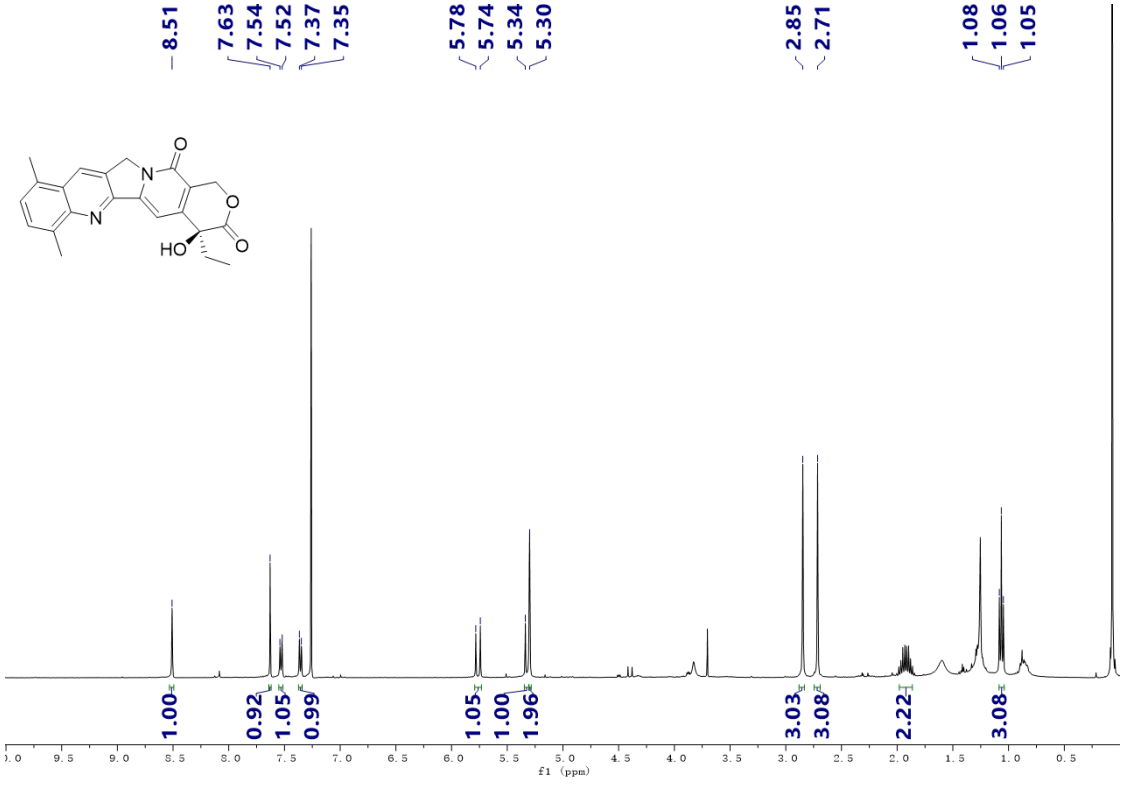


Figure S33. [1]H-NMR spectrum of compound **DM-CPT**

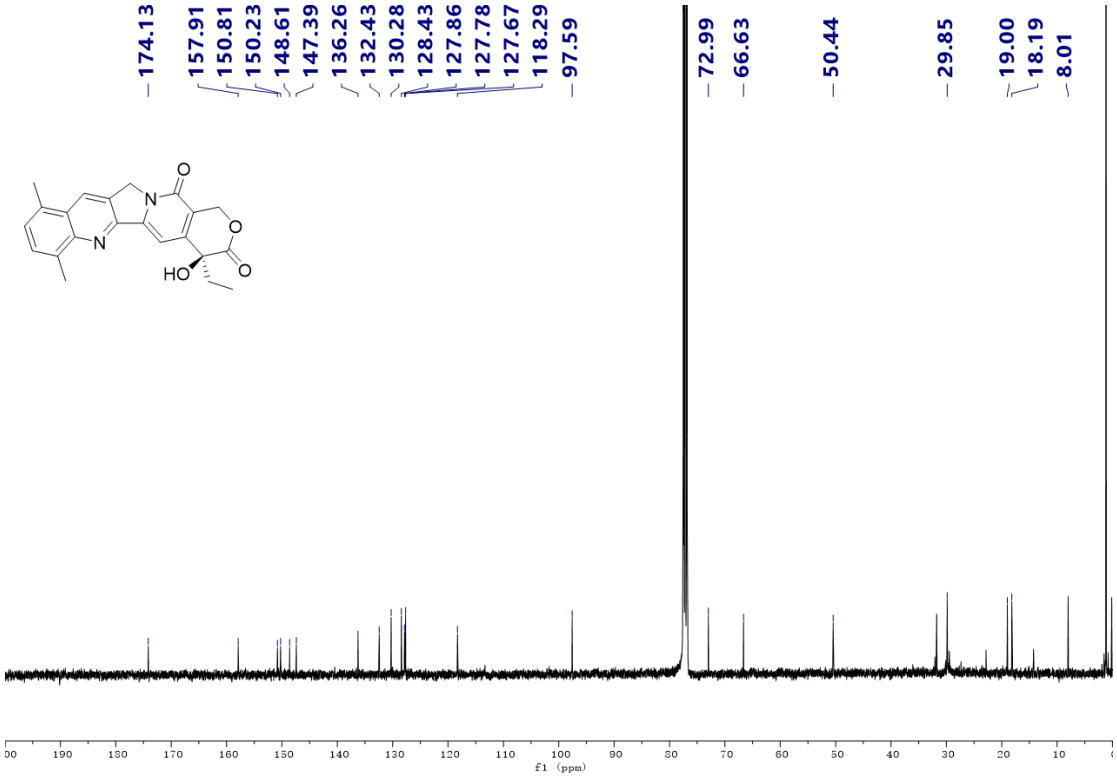


Figure S34. ^{13}C-NMR spectrum of compound **DM-CPT**

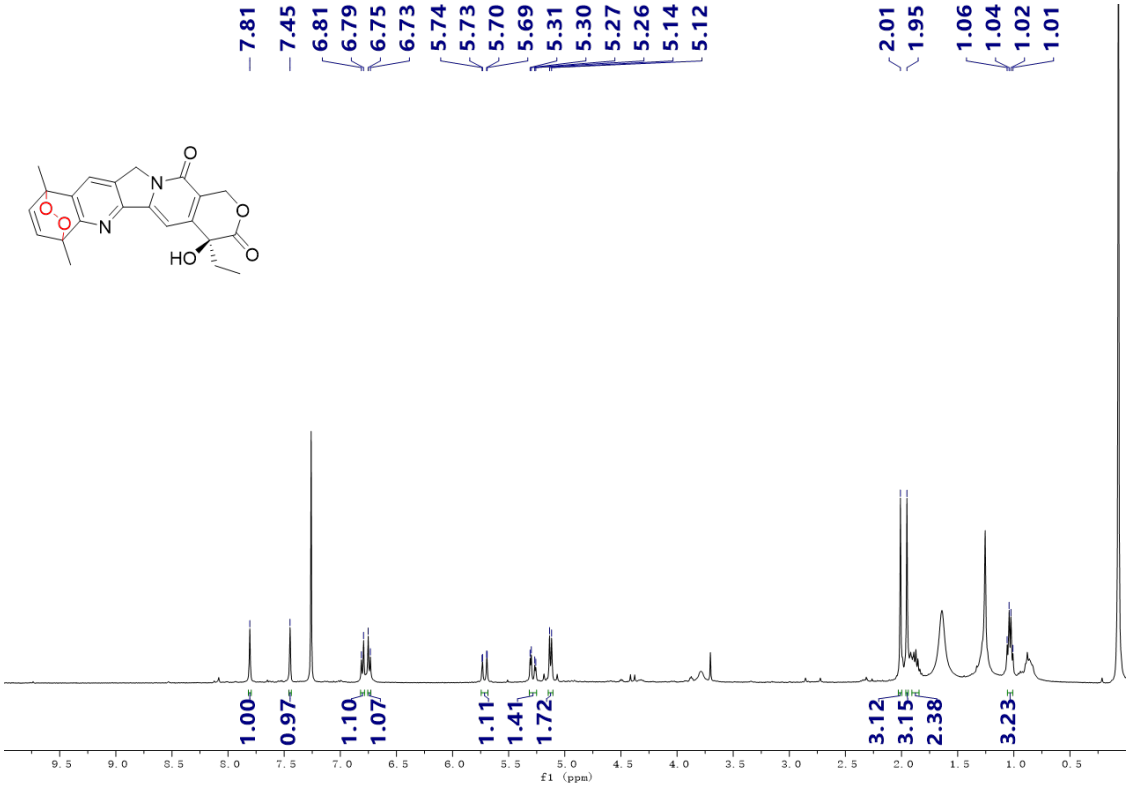


Figure S35. ^{1}H-NMR spectrum of compound **ENDO-CPT**

A

B

Figure S36. Uncropped gel images of β-actin (A) and P-gp (B).

References:

1- Wu, Y. et. al., Communications Chemistry. 2023, 6, 241. https://doi.org/10.1038/s42004-023-01043-9.