Supplementary Materials

Materials and methods

Synthesis of compound PVIS-Ir

Compound **PVIS-OH** (65 mg, 0.21 mmol) and EDCI (59 mg, 0.31 mmol) were dissolved in dried DMF (10 mL) and stirred for 10 min, then **Ir** (100 mg, 0.17 mmol) and DMAP (38 mg, 0.31 mmol) were added to the mixture. The resulting solution was stirred overnight at room temperature and monitored by TLC. After completion of reaction, the solution was diluted with CH_2Cl_2 (120 mL) and washed with water (150 mL), dried over with anhydrous Na_2SO_4. The crude product was purified by column chromatograph using dichloromethane/methanol (30:1 v/v) as the eluent. The product was collected to give a yellow solid (87 mg, 51.7%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.68 (d, J = 1.7 Hz, 1H), 8.46 (dd, J = 4.7, 1.3 Hz, 1H), 8.32 (d, J = 9.1 Hz, 1H), 8.25 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 2.3 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.62 (dd, J = 9.1, 2.4 Hz, 1H), 7.55 (s, 1H), 7.50 (s, 1H), 7.27 (d, J = 6.7 Hz, 1H), 7.18 – 7.11 (m, 2H), 7.08 (d, J = 16.5 Hz, 1H), 6.65 (t, J = 7.9 Hz, 1H), 5.68 (d, J = 16.9 Hz, 1H), 5.38 (d, J = 16.9 Hz, 1H), 5.22 – 5.12 (m, 2H), 4.45 (dd, J = 59.9, 12.9 Hz, 2H), 3.37 (ddd, J = 15.5, 9.3, 5.2 Hz, 1H), 3.26 – 3.17 (m, 2H), 3.12 (dd, J = 13.3, 7.5 Hz, 2H), 2.98 – 2.91 (m, 2H), 2.73 (s, 6H), 2.28 (dd, J = 14.1, 7.4 Hz, 1H), 2.17 (dd, J = 14.2, 7.4 Hz, 1H), 2.10 (d, J = 11.0 Hz, 1H), 2.04 (d, J = 8.0 Hz, 1H), 1.76 (s, 6H), 1.53 (s, 2H), 1.37 (t, J = 7.6 Hz, 3H), 1.03 (t, J = 7.5 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.55, 169.15, 167.70, 157.38, 153.16, 151.61, 150.47, 148.42, 148.19, 147.17, 146.73, 146.29, 145.23, 136.36, 133.09, 132.39, 131.81, 128.13, 127.51, 127.18, 125.98, 125.82, 125.47, 124.03, 123.59, 122.70, 121.61, 120.43, 119.64, 119.31, 116.80, 114.62, 96.76, 76.55, 66.99, 62.34, 50.22, 49.27, 44.43, 44.09, 31.59, 30.60, 29.70, 28.35, 28.21, 27.49, 26.05, 24.54, 23.15, 14.00, 7.67. HRMS (ESI) m/z calculated for $C_{52}H_{52}N_6O_8$ $[M+H]^+$: 889.37726, found: 889.37752. Elemental analysis calcd (%) for $C_{52}H_{52}N_6O_8$: C, 70.25; H, 5.90; N, 9.45; found: C, 70.08; H, 5.98; N, 9.35.

Synthesis of compound PVIG-Ir

According to the general procedure of **PVIS-Ir**, **Ir** was treated with **PVIG-OH**, and then purified on silica to give compound **PVIG-Ir** as a yellow solid (92 mg, 43.4%). ^{1}H NMR (600

MHz, $CDCl_3$) δ 8.67 (d, J = 1.7 Hz, 1H), 8.45 (dd, J = 4.7, 1.3 Hz, 1H), 8.32 (d, J = 9.1 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 2.4 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.63 (dd, J = 9.1, 2.4 Hz, 1H), 7.55 (s, 1H), 7.50 (s, 1H), 7.28 – 7.24 (m, 1H), 7.19 – 7.12 (m, 2H), 7.08 (d, J = 16.5 Hz, 1H), 6.70 – 6.58 (m, 1H), 5.67 (d, J = 16.9 Hz, 1H), 5.38 (d, J = 16.9 Hz, 1H), 5.17 (dd, J = 45.1, 18.6 Hz, 2H), 4.48 (d, J = 13.0 Hz, 1H), 4.38 (d, J = 12.6 Hz, 1H), 3.41 – 3.31 (m, 1H), 3.27 – 3.16 (m, 2H), 3.12 (dd, J = 13.6, 6.9 Hz, 2H), 2.98 – 2.89 (m, 2H), 2.61 (s, 5H), 2.45 (s, 3H), 2.29 – 2.24 (m, 1H), 2.16 (dd, J = 14.2, 7.4 Hz, 1H), 1.99 (t, J = 14.3 Hz, 2H), 1.69 – 1.60 (m, 6H), 1.49 (s, 2H), 1.37 (t, J = 7.7 Hz, 3H), 1.03 (t, J = 7.5 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.56, 169.15, 167.70, 157.37, 153.08, 151.67, 150.31, 148.38, 148.15, 147.20, 146.71, 146.28, 145.26, 136.35, 133.11, 132.42, 131.86, 128.13, 127.49, 127.21, 125.87, 125.80, 125.45, 124.03, 123.62, 122.73, 121.62, 120.41, 119.65, 119.33, 116.79, 114.69, 96.77, 76.54, 66.99, 62.75, 50.08, 49.27, 44.05, 43.69, 31.57, 31.44, 30.61, 30.19, 29.70, 28.36, 27.55, 26.73, 24.80, 23.77, 23.15, 14.01, 7.68. HRMS (ESI) m/z calculated for $C_{53}H_{54}N_6O_8$ $[M+H]^+$: 903.37545, found: 903.37503. Elemental analysis calcd (%) for $C_{53}H_{54}N_6O_8$: C, 70.49; H, 6.03; N, 9.31; found: C, 70.38; H, 6.21; N, 9.21.

HPLC analysis of hydrolysis behavior of a typical compound (PVIS-Ir)

The compounds were dissolved in PBS (pH=5.0) or a solvent of 60.0% acetonitrile and 40.0% water, and analyzed at different time by HPLC. Reversed phase HPLC was carried out on a 250×4.5 mm ODS column and the HPLC profiles were recorded on UV detection at 245 nm. Mobile phase consisted of acetonitrile and water (60:40, v/v), and flow rate was 1.0 mL/min. All the solutions were filtrated by 0.45 mm filter before taken for HPLC analysis.

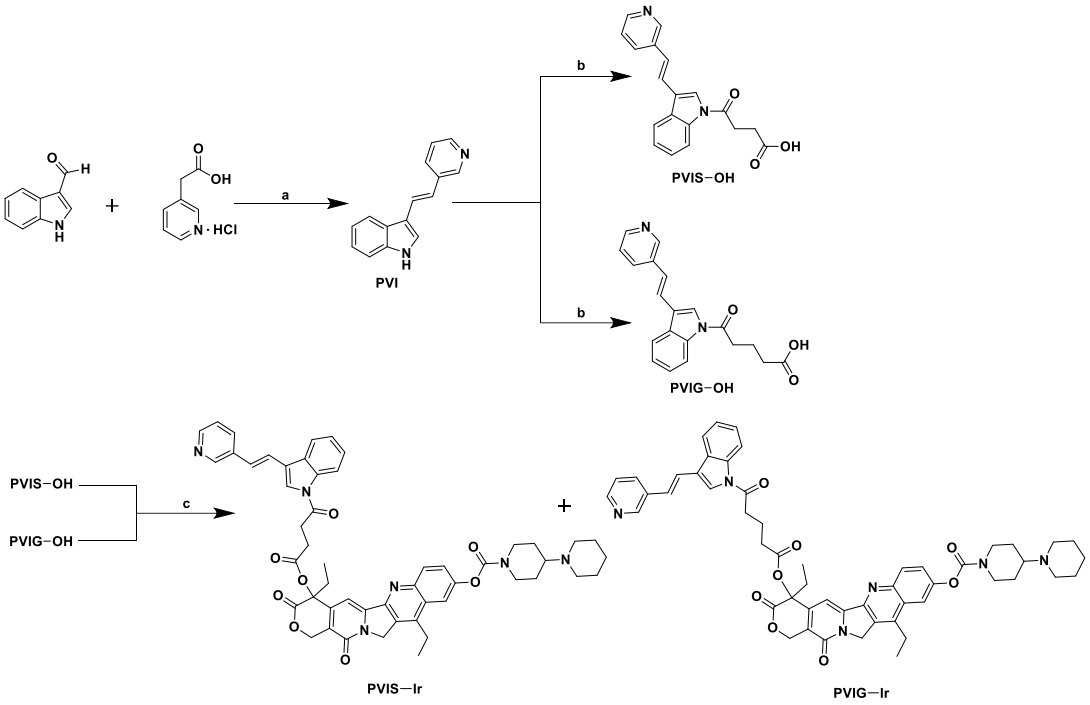


Fig. S1. Synthetic pathway to prepare target compounds **PVIS-Ir** and **PVIG-Ir**. Reagents and conditions: (a) dioxane, Et_3N, piperidine, 24 h; (b) succinic anhydride or glutaric anhydride, DMAP, Et_3N, CH_2Cl_2, 45 ^oC, 12 h; (c) EDCI, DMAP, DMF, room temperature, overnight.

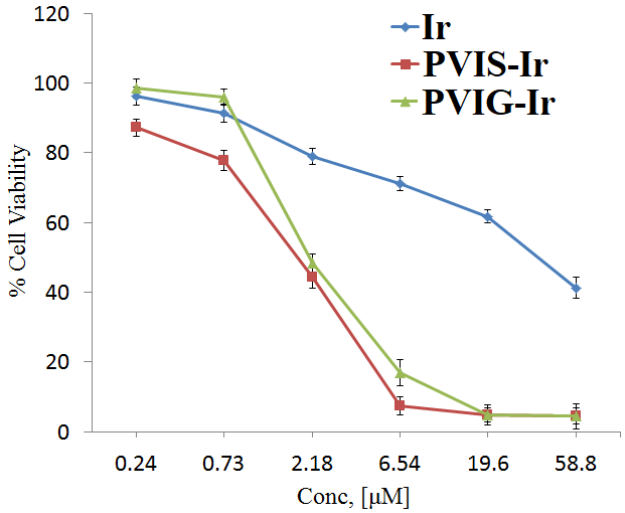


Fig. S2. Graphs show effect of increasing concentrations of **Ir**, **PVIS-Ir** and **PVIG-Ir** on viability of HepG2 cancer cells.

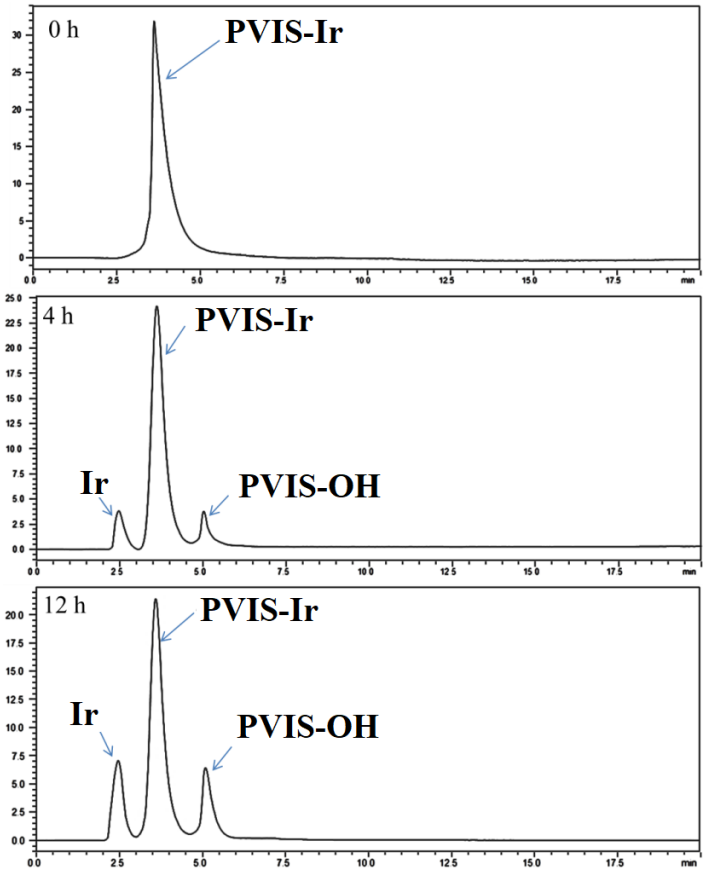


Fig. S3. The release of compound **PVIS-Ir** in PBS buffer (pH=5.0).

compounds	TDO inhibition IC_{50}（μM）
PVI	0.22±0.04
PVI-OH	0.68±0.02
PVIS-Ir	0.78±0.02

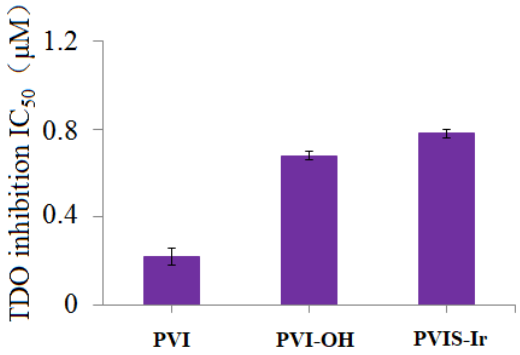


Fig. S4. TDO enzymatic activity inhibition by compounds **PVI**, **PVIS-OH**, and **PVIS-Ir**. Error bars indicate SD (n = 3).

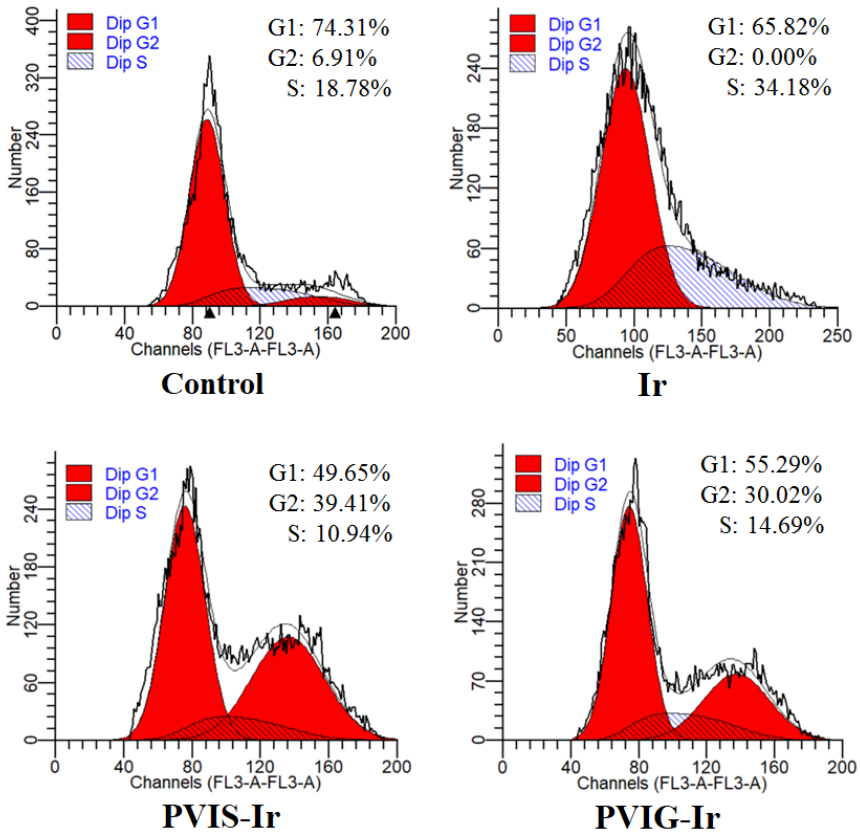


Fig. S5**.** Cell cycle distribution of HepG2 cells by flow cytometry analysis following the treatment of **PVIS-Ir** and **PVIG-Ir** at 10 μM for 24 h. **Ir** was used as a positive control. The cells were trypsinized, harvested and washed three times with ice-PBS for PI-stained DNA content detected by flow cytometry.

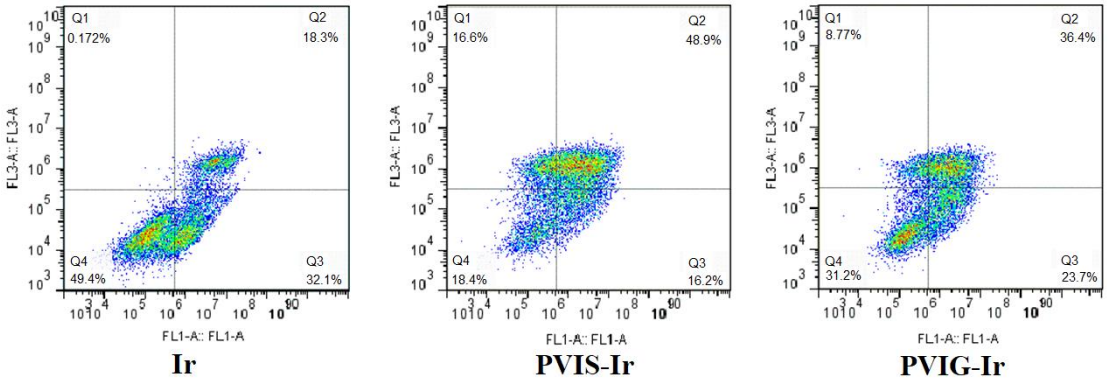
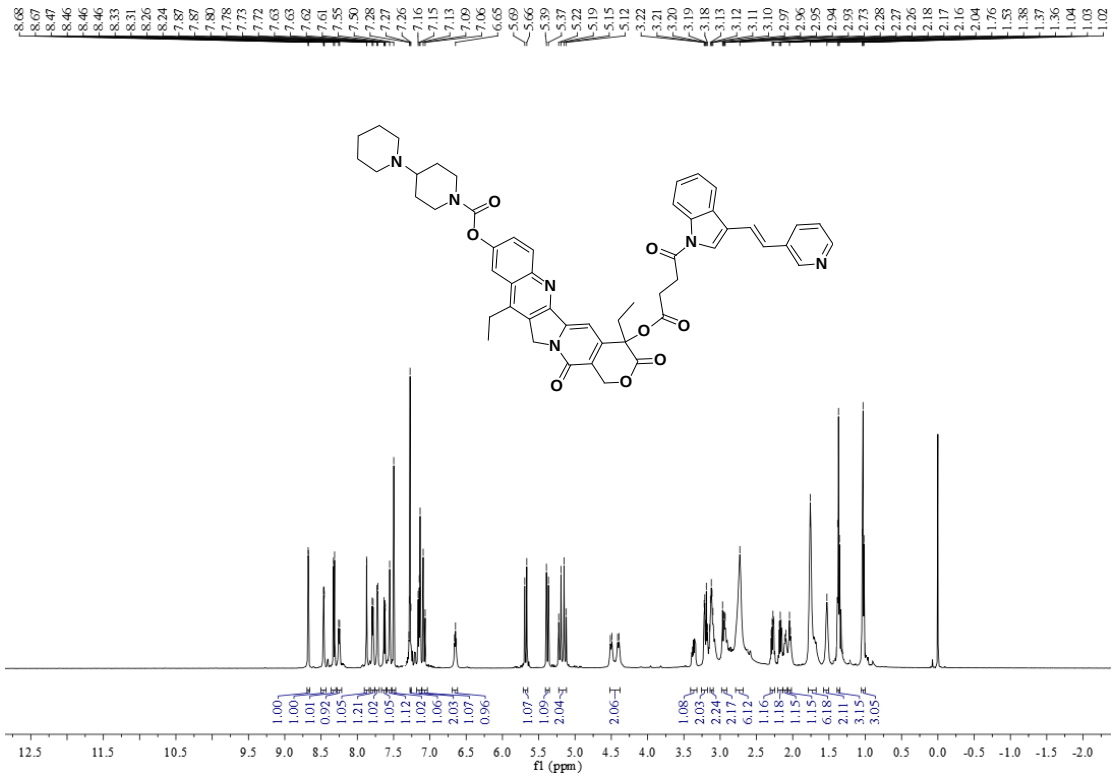


Fig. S6**.** Flow cytometry analysis for apoptosis of HepG2 cells induced by compounds at the concentration of 10 μM for 24 h. The cells were harvested, stained with Annexin V-FITC and PI, and analyzed by flow cytometry (apoptosis cells included early and late apoptosis cells).

A



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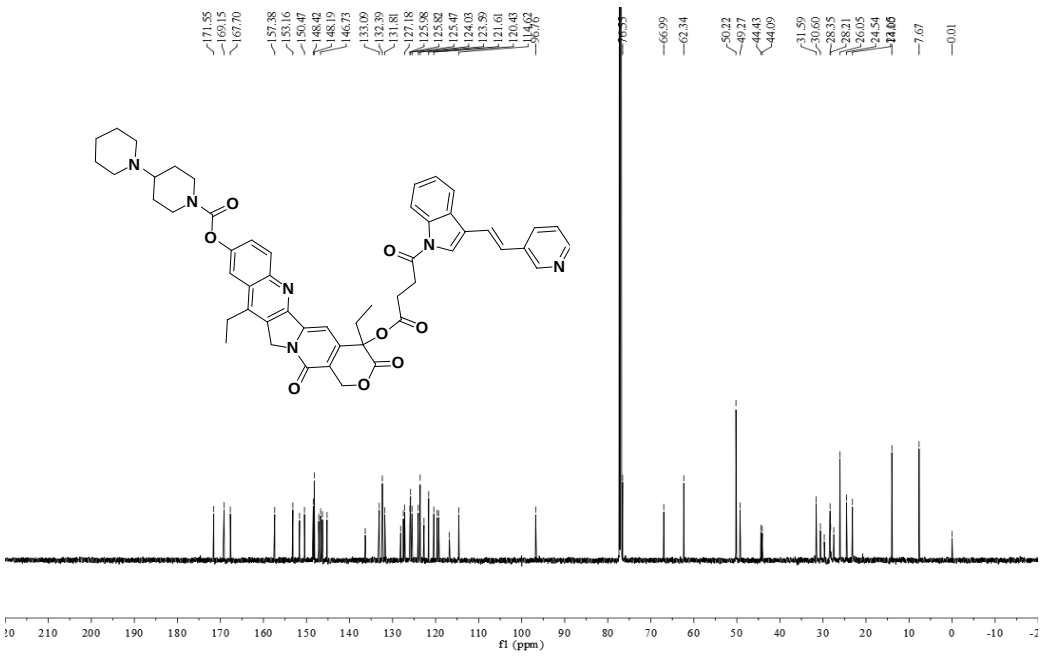


C

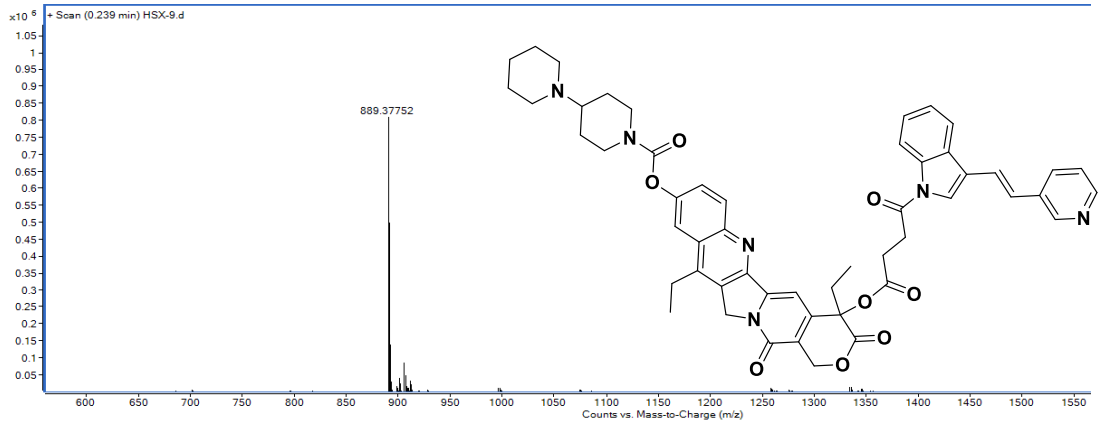
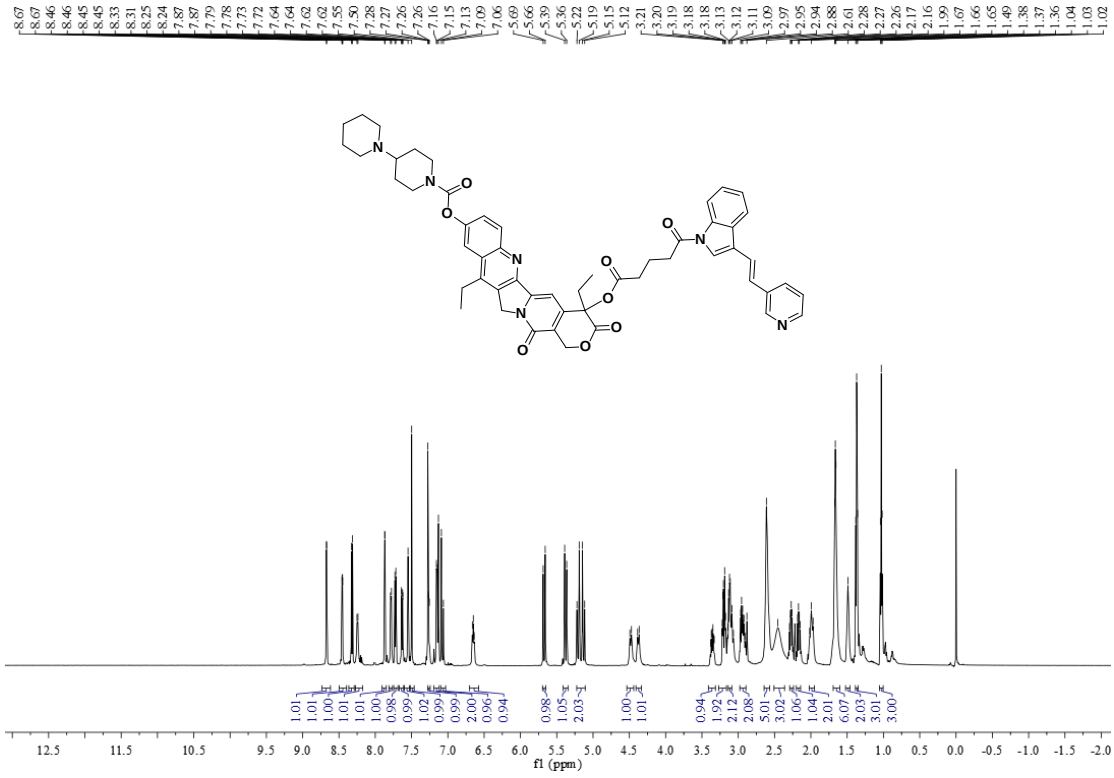


Fig. S7. Characterization of compound **PVIS-Ir**. A) ^{1}H NMR spectrum of compound **PVIS-Ir**. B) ^{13}C NMR spectrum of compound **PVIS-Ir**, and C) HR-MS spectrum of compound **PVIS-Ir**.

A



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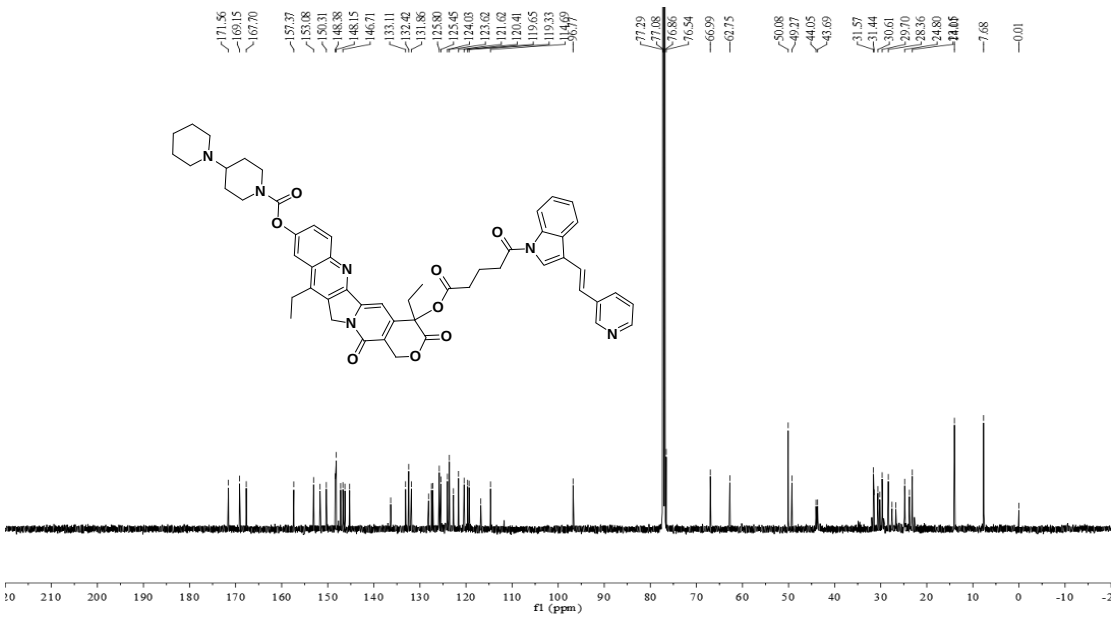

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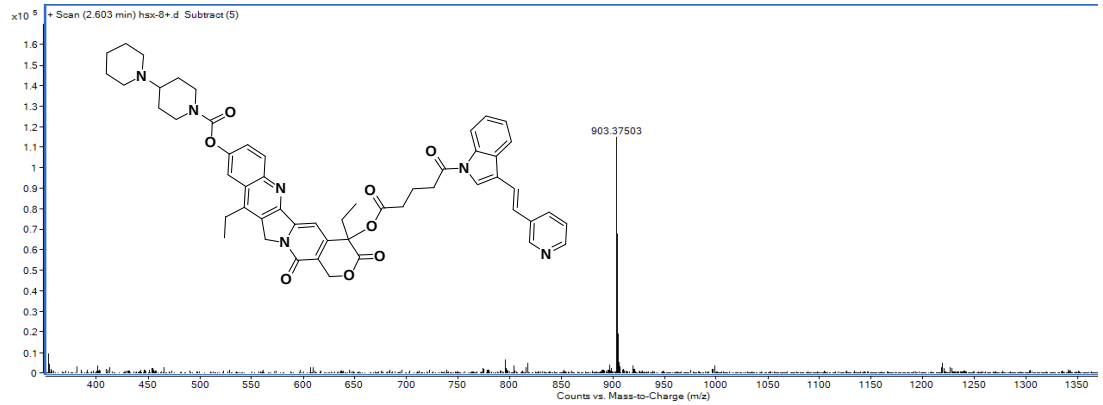

Fig. S8. Characterization of compound **PVIG-Ir**. A) ^{1}H NMR spectrum of compound **PVIG-Ir**. B) ^{13}C NMR spectrum of compound **PVIG-Ir**, and C) HR-MS spectrum of compound **PVIG-Ir**.