Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

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1. General Information

1.1 Materials.

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na_2HPO_4 water solution and 0.05mol/L KH_2PO_4 water solution with the volume ratio 4:1. MitoTracker-Green was gained by Shanghai Beyotime Co., Ltd. (Shanghai, China). All chemicals and solvents used were of

analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

1.2 Instruments

TLC analysis was performed using precoated silica plates. Using a pH meter (METTLER TOLEDO, Switzerland) to measure pH. Ultraviolet–visible (UV–vis) spectra were recorded on U-3900 UV-Visible spectrophotometer. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with an Thermo Scientific Q Exactive. And the cells imaging experiments were measured the Zeiss LSM810 Airyscan confocal laser scanning microscope.

2. Experimental Section

2.1 Synthesis and characterization

Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 were synthesized as follows.

Synthesis of **Compound 1**

2-methylphenothiazine (3.44 g, 15 mmol) and potassium tert-butoxide (2.52 g, 22.5 mmol) were added to N,N-Dimethylformamide (40 mL), stirred and heated to reflux for 1 hour, then bromoethane (3.36 mL, 45 mmol) was added to the reaction mixture,

stirred at room temperature for 24 h. Then the reaction mixture was poured into water and continued stirring. After standing, a solid precipitated out. After suction filtration, the obtained solid was dried. The residue was further purified by silica gel chromatography using petroleum ether/ethyl acetate (v/v, 5:1) as an eluent to give pure **compound 1** (1.89 g, 49% yield) as a pale pink solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.19 (t, J = 7.7 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 7.01 (dd, J = 11.6, 8.1 Hz, 2H), 6.93 (t, J = 7.4 Hz, 1H), 6.58 – 6.53 (m, 2H), 3.91 (q, J = 6.9 Hz, 2H), 3.74 (s, 3H), 1.29 (t, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 146.39 (s), 127.86 (d, J = 4.3 Hz), 127.39 (s), 114.27 (s), 103.28 (s), 55.80 (s).

Synthesis of **Compound 2**

N,N-Dimethylformamide (0.17 mL, 2.4 mmol) was added to phosphorous oxychloride (0.2 mL, 2.4 mmol) at 0℃.The resulting mixture was allowed to stirring at this temperature for 15 minutes. Then a portion of compound 1 (0.518 g, 2 mmol) (dissolved in 2 mL anhydrous N,N-Dimethylformamide) was added to the cooled reagent with stirring. The mixture warmed to 60 ℃ and stirred for 4 h, then poured into ice water (100 mL).The clear solution obtained was neutralized by $NaHCO_3$ solution (10%). The resulting sticky mass was extracted with dichloromethane (3×100 mL). The organic layers were separated, combined and washed successively with brine and water, dried over anhydrous Na_2SO_4 and vacuum evaporated. The residue was further purified by silica gel chromatography using petroleum ether/ethyl acetate (v/v, 3:1) as an eluent to give pure **compound 2** (0.434 g, 76% yield) as orange powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.55 (s, 1H), 7.12 (d, J = 7.5

Hz, 2H), 6.90 (t, J = 16.0 Hz, 2H), 6.39 (s, 1H), 4.06 (s, 2H), 3.94 (s, 3H), 1.48 (t, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 187.14 (s), 162.65 (s), 151.99 (s), 142.68 (s), 127.46 (s), 127.28 (s), 126.79 (s), 124.24 (s), 123.52 (s), 119.67 (s), 115.67 (s), 115.42 (s), 98.37 (s), 55.79 (s), 42.62 (s), 13.03 (s).

Synthesis of **Compound 3**

Aluminium powder (0.0675 g, 2.5 mmol) was added to anhydrous acetonitrile (5 mL) and stirred at room temperature for 5 minutes. To the slurry, iodine (0.394 g, 1.55 mmol) was added in small portions and stirred under nitrogen atmosphere till the colour changed to yellow. Compound 2 (0.285 g, 1 mmol) was dissolved in anhydrous acetonitrile (4 mL) and added to the slurry dropwise. The reaction mixture was then gently refluxed for 6 h, cooled to room temperature and poured into ice water (80 mL). The mixture was extracted with ethyl acetate (3×60 mL). Combined ethyl acetate extracts were washed with water, dried over anhydrous sodium sulphate. After removal of the solvent, the residue was further purified by silica gel chromatography using petroleum ether/dichloromethane (v/v, 2:1) as an eluent to afford **compound 3** (0.147 g, 54% yield) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.40 (s, 1H), 9.63 (s, 1H), 7.20 – 7.16 (m, 2H), 7.12 (d, *J* = 7.6 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 6.42 (s, 1H), 3.98 (q, *J* = 7.0 Hz, 2H), 1.48 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 185.96 (s), 156.43 (s), 156.09 (s), 154.04 (s), 141.54 (s), 139.12 (s), 134.07 (s), 130.09 (s), 122.29 (s), 118.63 (s), 115.23 (s), 109.78 (s), 103.55 (s), 44.71 (s), 12.74 (s).

Synthesis of **Compound 4**

To a solution of compound 3 (0.271 g, 1 mmol) in ethanol (3 mL) was added diethyl malonate (0.320 g, 2 mmol) and piperidine, and the reaction mixture was refluxed for 3 h. Then the reaction mixture cooled to room temperature. The precipitate was isolated by vacuum filtration and washed with ethanol. The obtained crude product was purified by silica gel flash chromatography using petroleum ether/ethyl acetate = 3/1 as the eluent to give **compound** 4 as an orange solid (0.231 g, 63% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.23 – 7.19 (m, 2H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 6.75 (s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 3.98 (d, *J* = 6.9 Hz, 2H), 1.49 (t, *J* = 7.0 Hz, 3H), 1.42 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.47 (s), 157.15 (s), 156.70 (s), 150.54 (s), 147.72 (s), 141.96 (s), 127.82 (s), 127.41 (s), 126.01 (s), 123.94 (s), 122.58 (s), 120.70 (s), 115.85 (s), 113.62 (s), 112.76 (s), 101.77 (s), 61.68 (s), 43.07 (s), 14.29 (s), 12.51 (s).

Synthesis of **Compound 5**

To a solution of compound 4 (0.735 g, 2 mmol) in methanol (20 mL) was added NaOH (0.240 g, 6 mmol), and the reaction mixture was refluxed for 1 h. Then the solvent was removed under a reduced pressure and the residue was dissolved in dichloromethane (50 mL) and was acidified to pH 3-4 with 10% HCl. After washing with brine and water, the organic layer was dried over anhydrous sodium sulfate and was concentrated in vacuum to give **compound 5** as an oxblood red solid (0.618 g, 91% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.30 (s, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.81 (s, 1H), 4.03 (q, *J* = 7.0 Hz, 2H), 1.52 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$)

δ 164.55 (s), 163.19 (s), 156.36 (s), 151.61 (s), 149.56 (s), 141.22 (s), 128.02 (s), 127.52 (s), 126.46 (s), 124.51 (s), 122.46 (s), 122.20 (s), 116.06 (s), 113.39 (s), 110.04 (s), 101.51 (s), 43.38 (s), 12.46 (s).

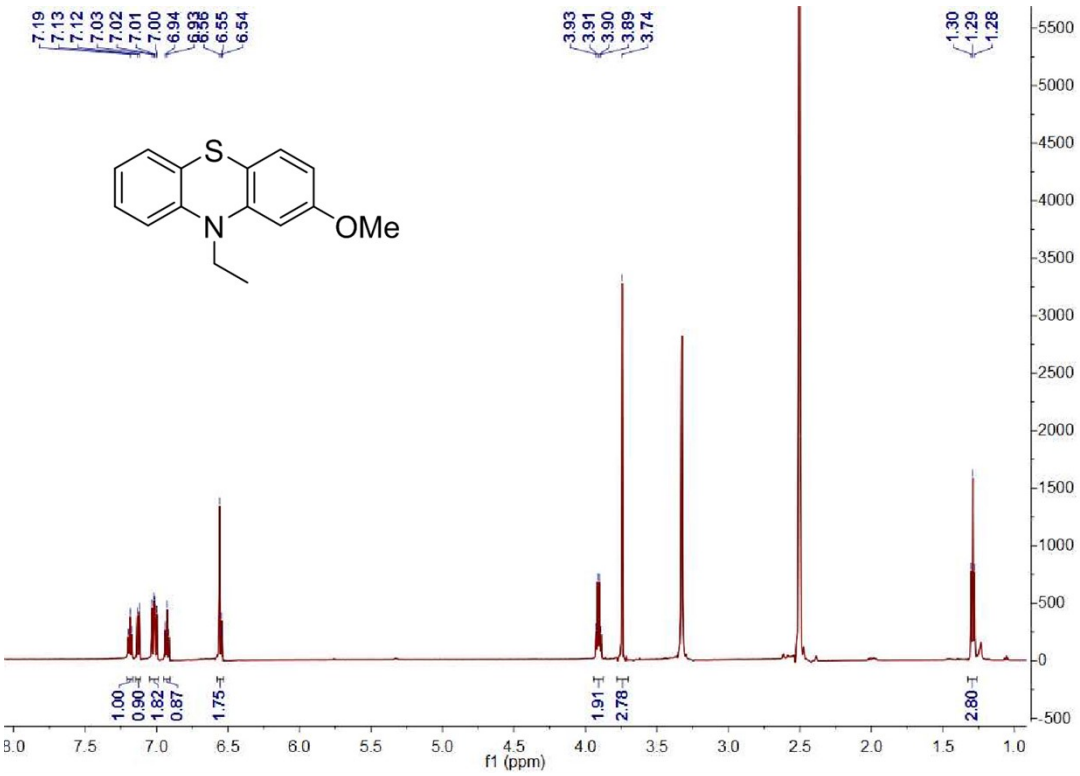
Synthesis of **Compound 6**

Compound 6 was synthesized according to the reported method[1].

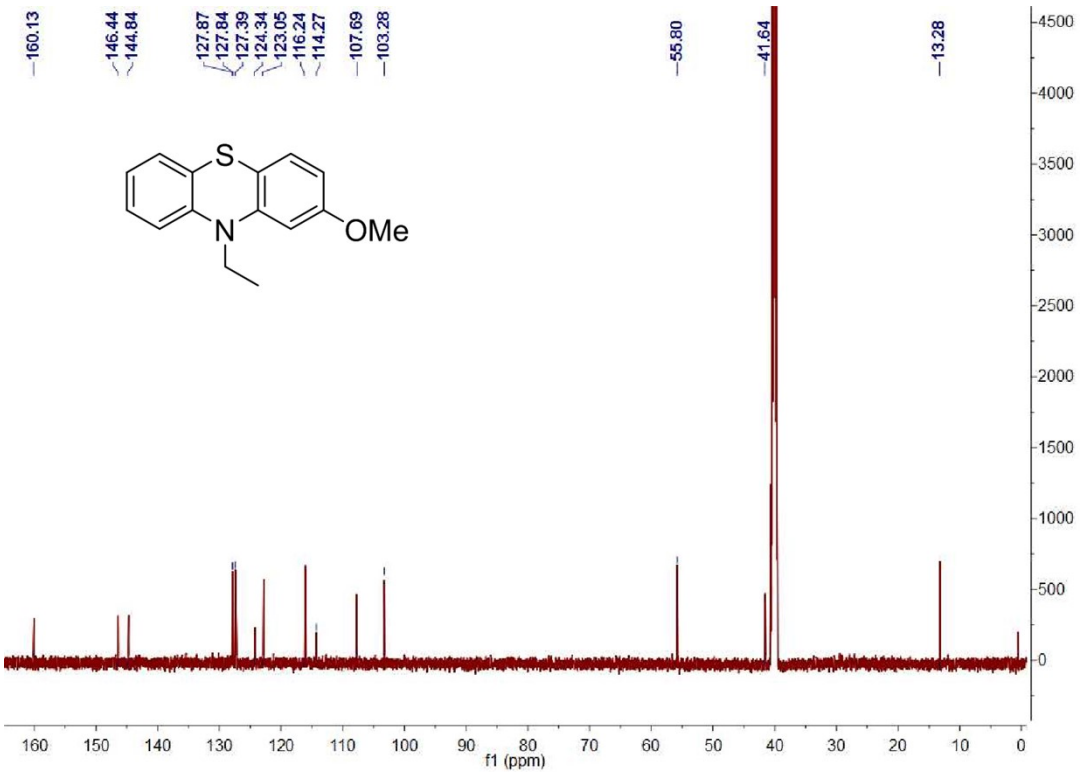
Synthesis of **Compound 7**

Compound 5 (0.339 g, 1 mmol), compound 6 (0.190 g, 1 mmol), EDCl (0.191 g, 1 mmol) and DMAP (0.015 g, 0.12 mmol) were added into dichloromethane (30 mL) and stirred at room temperature until the starting materials were consumed. Dichloromethane (3×30 mL) was added to the mixture which was then washed with brine for three times. Organic layer was separated and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (petroleum ether/ethyl acetate = 1/1) to afford compound 7 as an orange solid (0.440 g, 86 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.87 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.23 – 7.19 (m, 2H), 7.13 (d, *J* = 7.6 Hz, 1H), 7.01 (dd, *J* = 7.6, 3.9 Hz, 3H), 6.96 (d, *J* = 8.2 Hz, 1H), 6.77 (s, 1H), 4.00 (dd, *J* = 14.2, 7.1 Hz, 3H), 3.63 – 3.50 (m, 7H), 1.49 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.45 (s), 164.17 (s), 158.38 (s), 149.57 (s), 143.90 (s), 142.25 (s), 131.92 (s), 127.85 (s), 127.46 (s), 125.39 (s), 123.85 (s), 122.63 (s), 121.28 – 121.15 (m), 120.21 (s), 115.80 (s), 114.57 (s), 113.06 (s), 102.05 (s), 47.88 (s), 47.40 (s), 46.46 (s), 42.94 (s), 41.79 (s), 12.53 (s).

Fig. S1: The characterization data of **Compound 1**

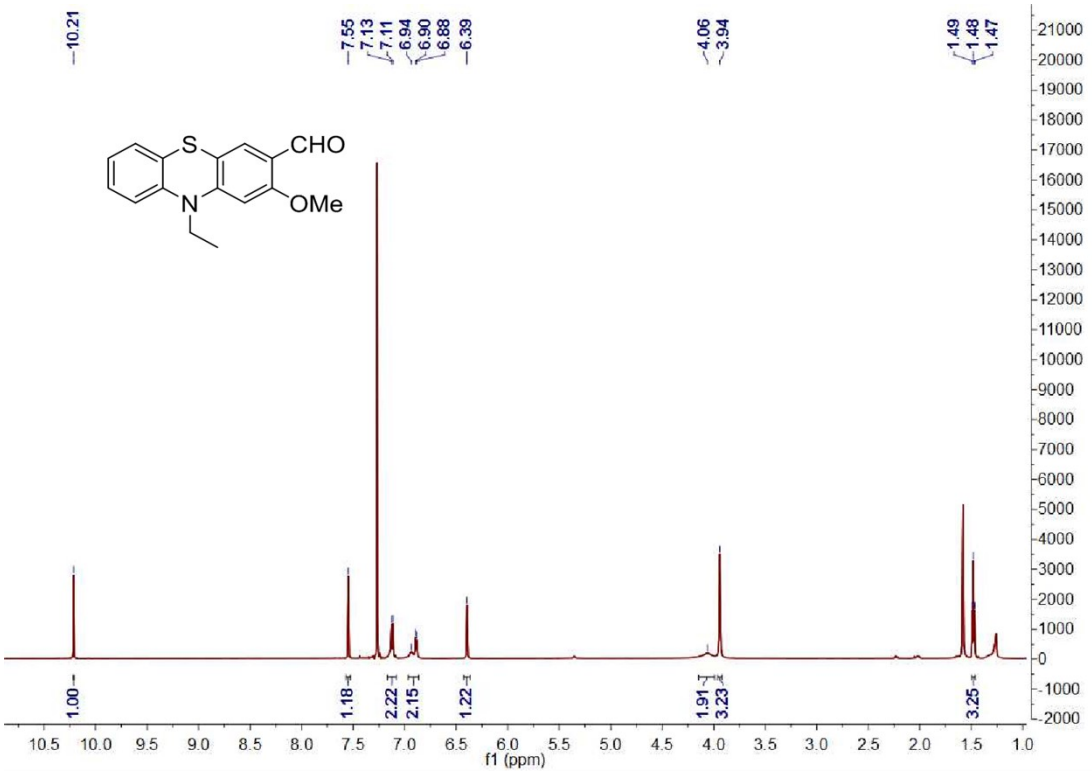


The ^{1}H MNR (600 MHz) spectra of **Compound 1** in DMSO-d_6.

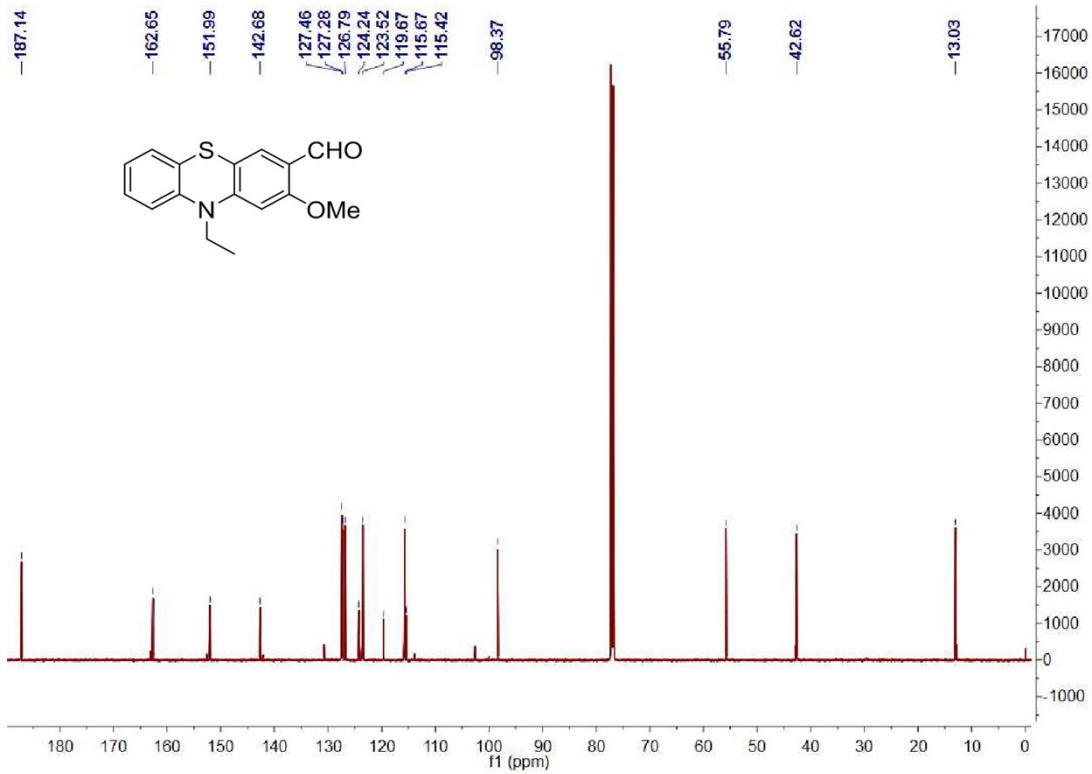


The ^{13}C NMR (151 MHz) spectra of **Compound 1** in DMSO-d_6.

Fig. S2: The characterization data of **Compound 2**

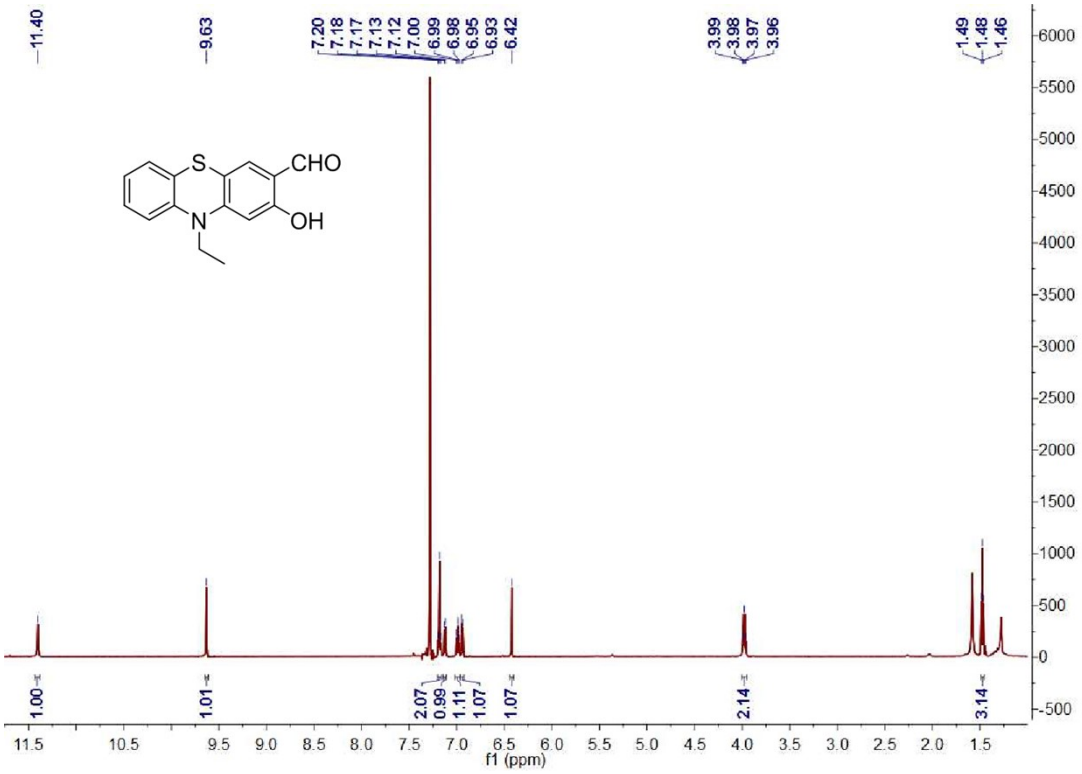


The ^{1}H MNR (600 MHz) spectra of **Compound 2** in DMSO-d_6.

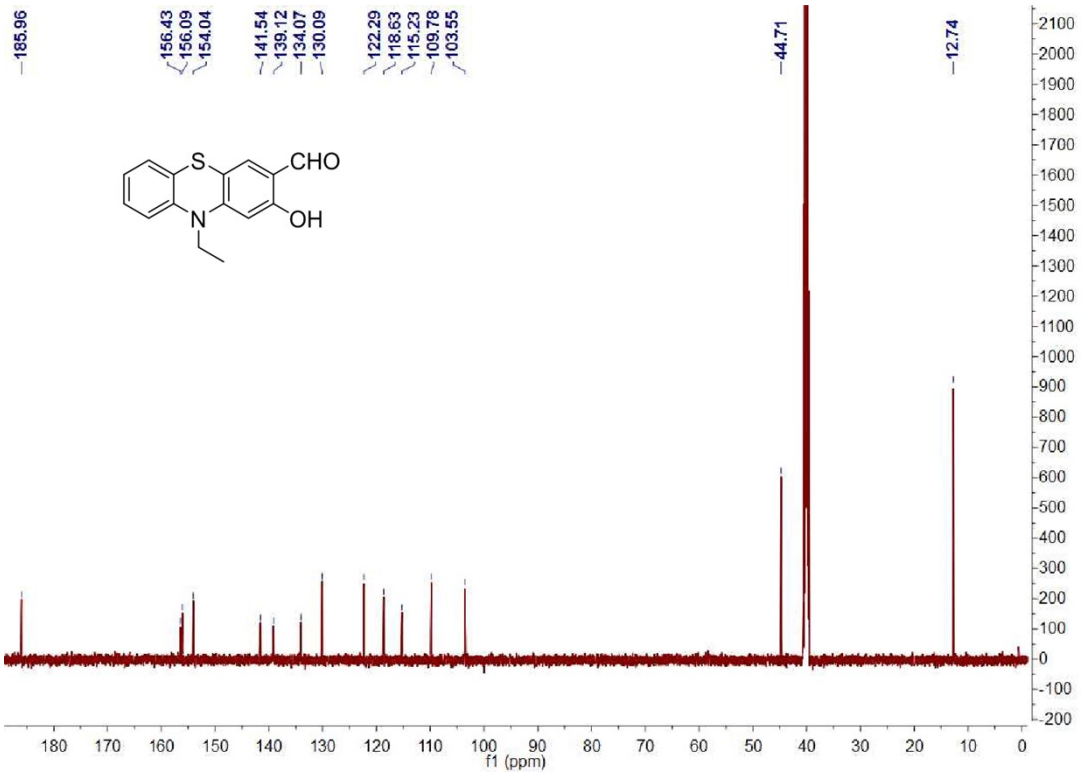


The ^{13}C NMR (151 MHz) spectra of **Compound 2** in $CDCl_3$.

Fig. S3: The characterization data of **Compound 3**

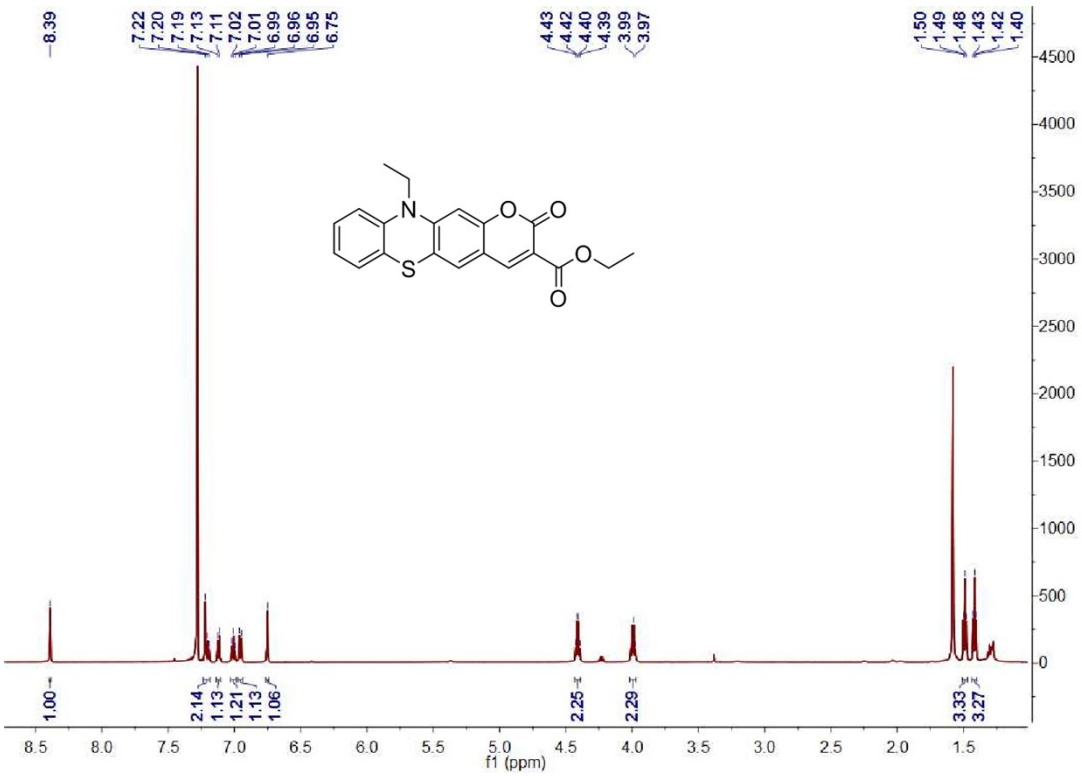


The ^{1}H MNR (600 MHz) spectra of **Compound 3** in $CDCl_3$.

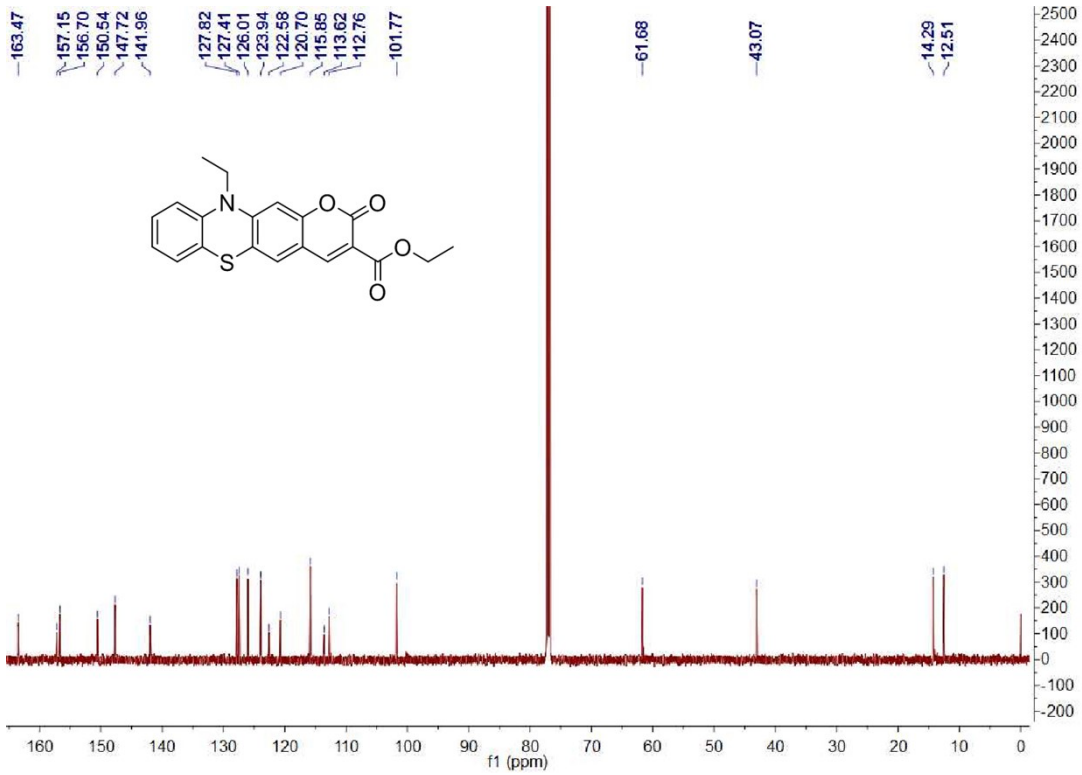


The ^{13}C NMR (151 MHz) spectra of **Compound 3** in DMSO-d_6.

Fig. S4: The characterization data of **Compound 4**

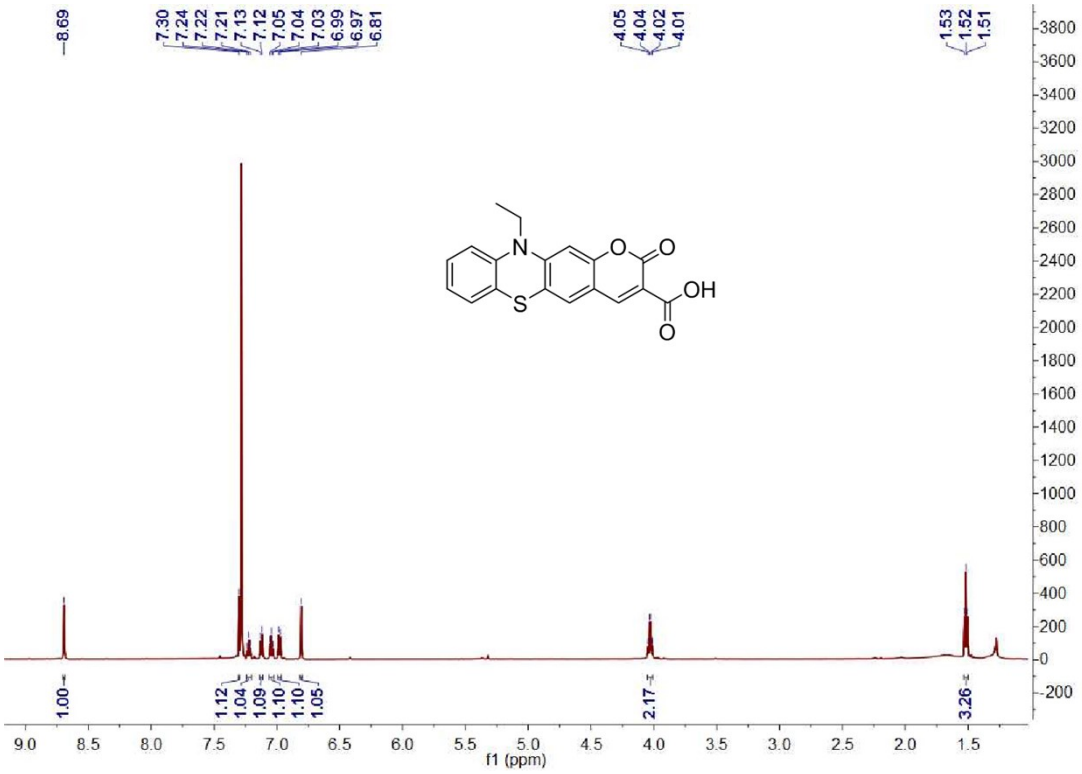


The ^{1}H MNR (600 MHz) spectra of **Compound 4** in $CDCl_3$.

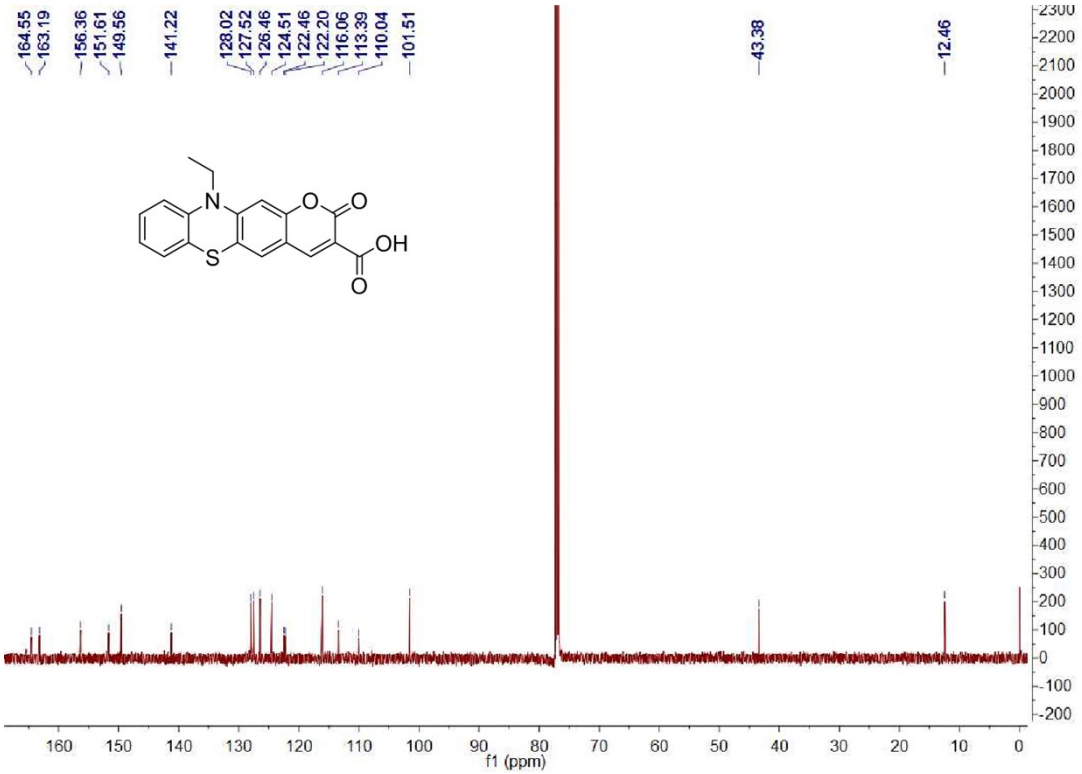


The ^{13}C NMR (151 MHz) spectra of **Compound 4** in $CDCl_3$.

Fig. S5: The characterization data of **Compound 5**

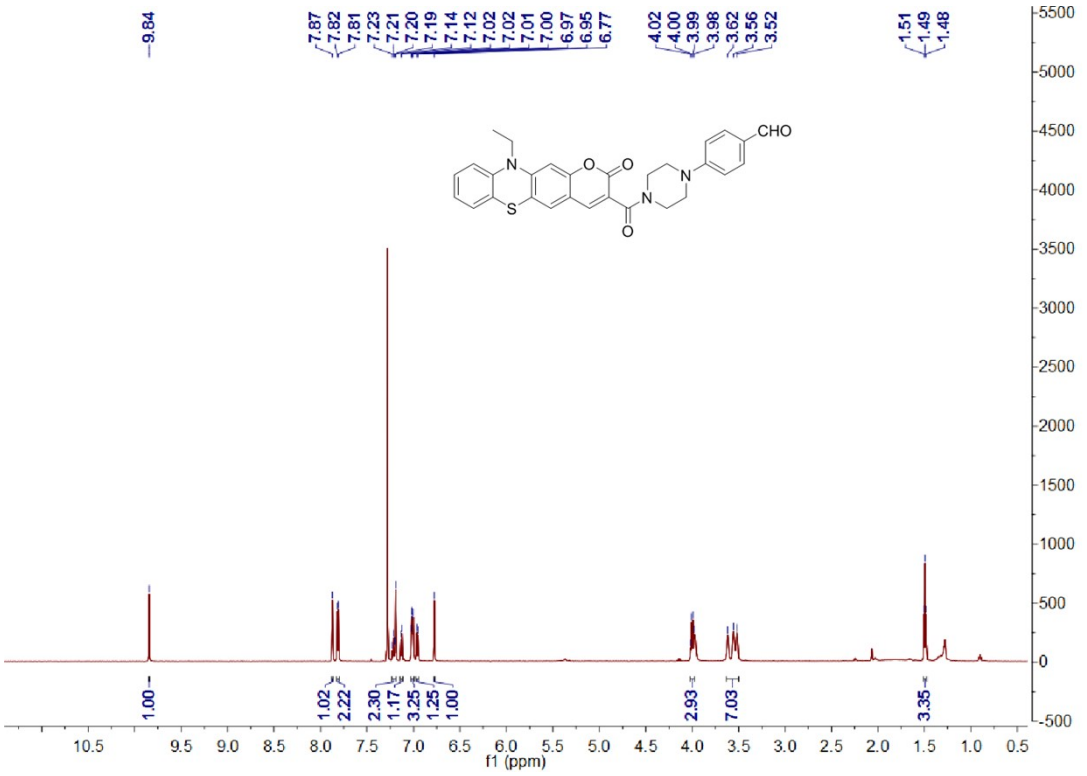


The ^{1}H MNR (600 MHz) spectra of **Compound 5** in $CDCl_3$.

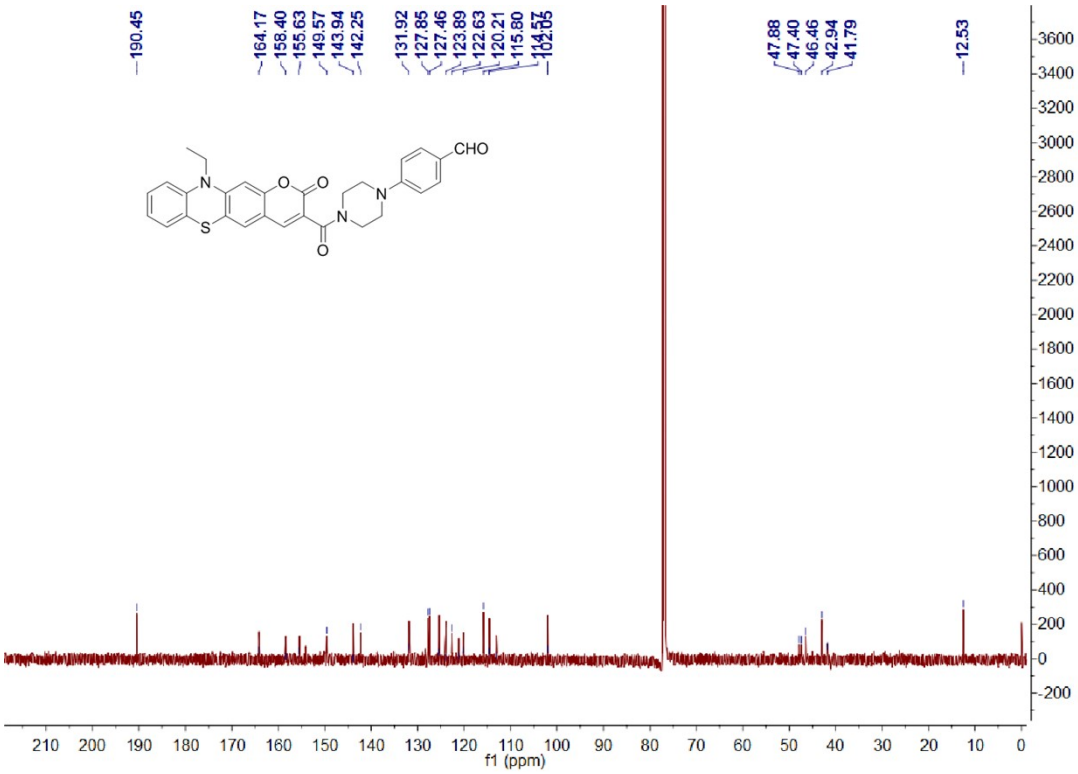


The ^{13}C NMR (151 MHz) spectra of **Compound 5** in $CDCl_3$.

Fig. S6: The characterization data of **Compound 7**

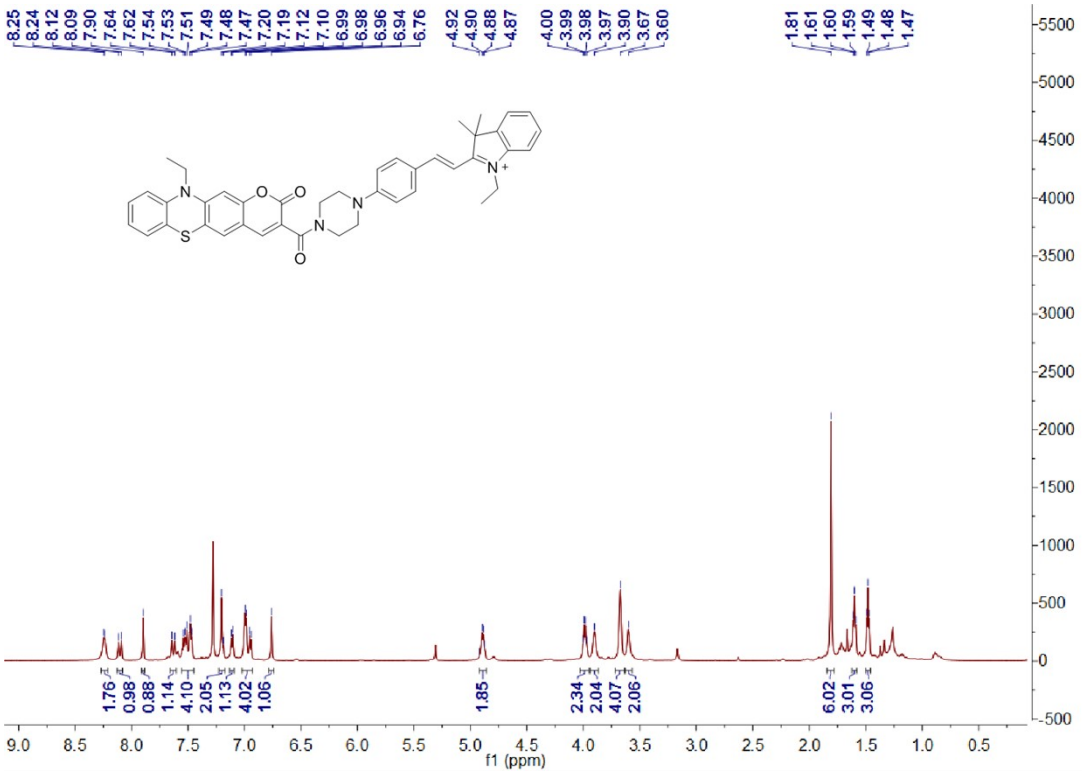


The ^{1}H MNR (600 MHz) spectra of **Compound 7** in $CDCl_3$.

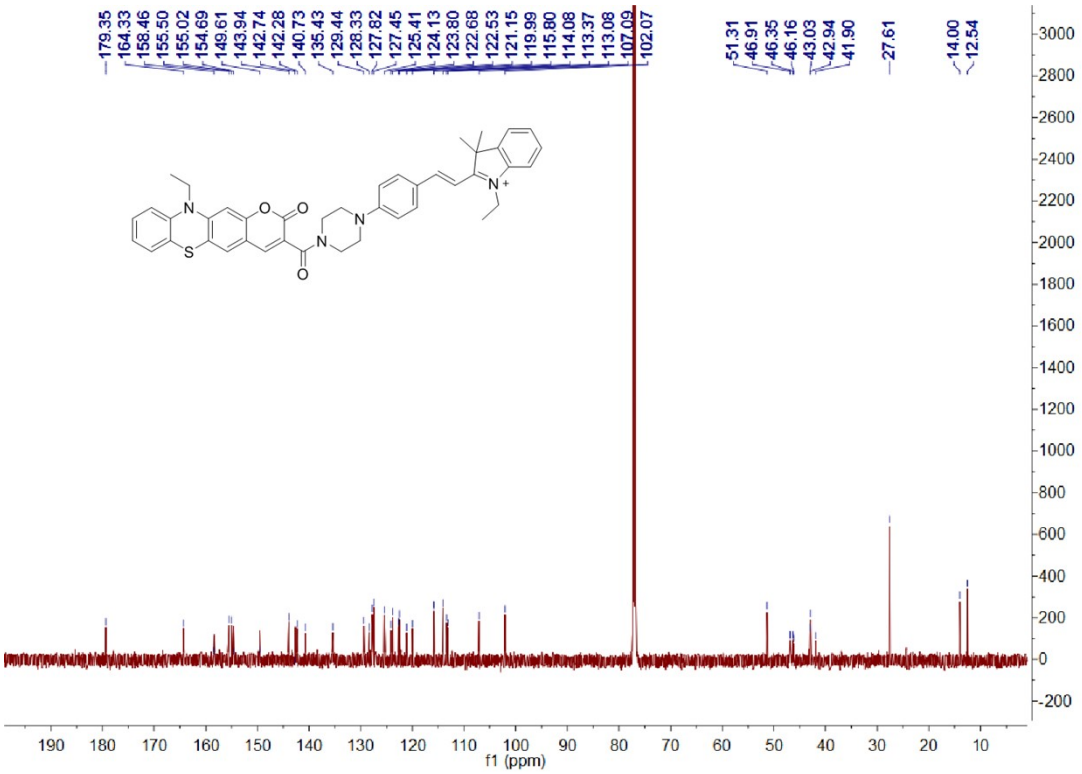


The ^{13}C NMR (151 MHz) spectra of **Compound 7** in $CDCl_3$.

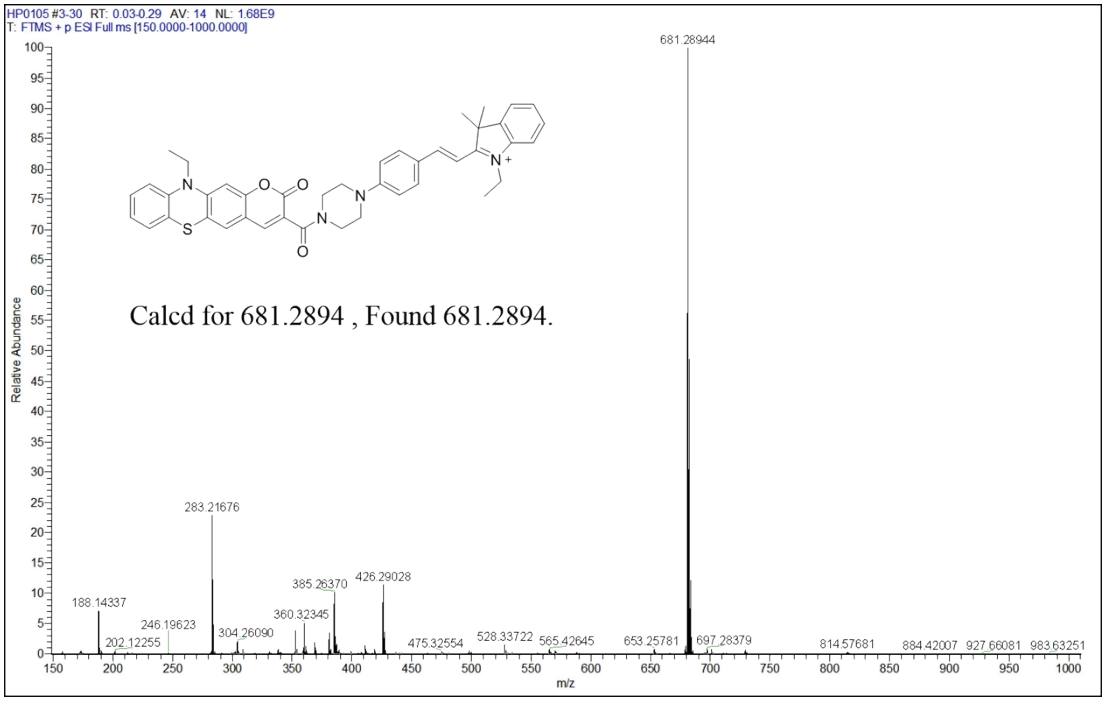
Fig. S7: The characterization data of probe ***PC***



The ^{1}H MNR (600 MHz) spectra of probe ***PC*** in $CDCl_3$.

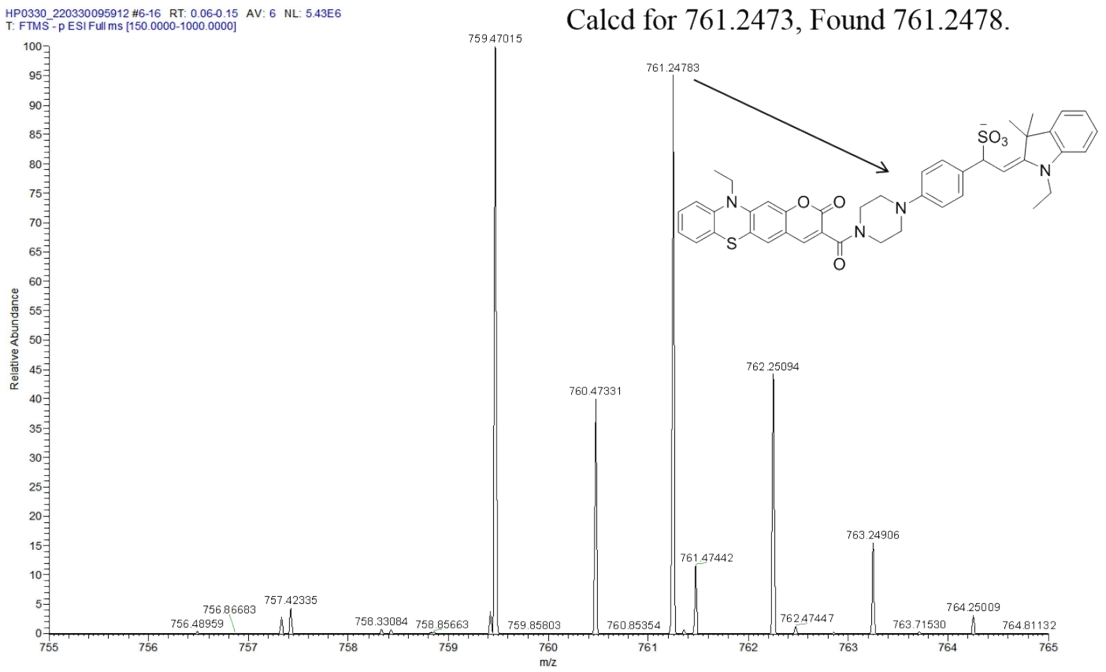


The ^{13}C NMR (151 MHz) spectra of probe ***PC*** in $CDCl_3$.



ESI-MS of probe ***PC***.

Fig. S8:ESI-MS analysis of probe ***PC***+ $\mathbf{SO_3^{2-}}$.



ESI-MS analysis of probe ***PC***+ $\mathbf{SO_3^{2-}}$

2.2 Cell Viability

CCK-8 test: HeLa cells growing at logarithmic phase were cultured in a 96-well plate. Cell density was adjusted to 5 × 10^4 cells/well. After adherence, the cells were incubated with 0，1，2.5，5，7.5，10，15，20，30 and 50 μM probe ***PC***. Incubation times was 10 hours. Cell viability was measured via CCK-8 assay. In brief,

the cells were incubated with 10 μL CCK-8 for 1 h. The amount of CCK-8 formazan was determined at the reference wavelength of 450 nm by Microplate Reader.

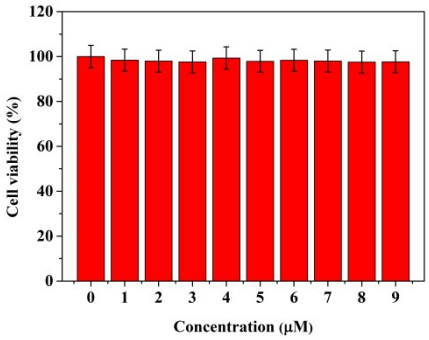
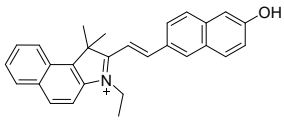
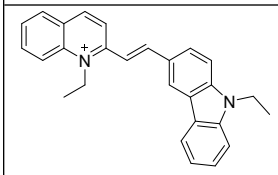
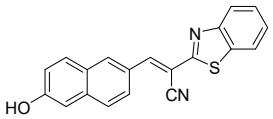
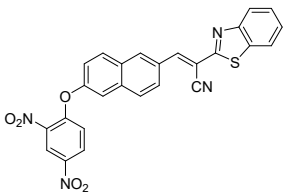
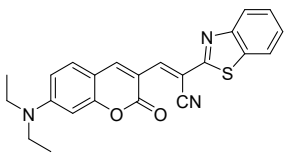
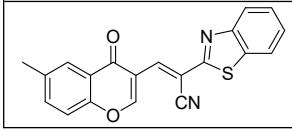
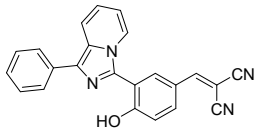
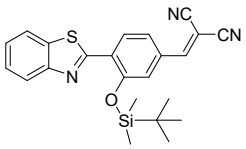
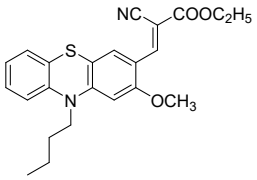


Fig. S9 CCK-8 assay of Hela cells incubated in the presence of the probe ***PC*** (0-50 μM) at 37 ℃ for 10 h.

3. Supporting tables

Probes	$\lambda_{ex}/\lambda_{em}$(nm)	Detection medium	Detection limit	Applications	Ref.
	404/593 404/467	PBS(pH=7.4)	3.6 nm	Mitochondria-targeted, cell	[2]
	460/598	PBS(pH=7.4, containing 10% CH_3CN)	18.1nM	Wine and sugar samples, live adult zebrafish and nude mouse	[3]
	398/525	PBS(pH=6, containing 33% DMSO)	0.1μM	Sugar, Red Wine	[4]
	363/458	DMSO	5.53μM	Water,Granulated/Crystal Sugar/Water	[5]
	460/502	PBS(pH=7.4, containing2 % DMSO)	0.08μM	Crystal Sugar	[6]
	460/530	CH_3CN/HEPES=1/9，pH=7.4	0.45μM	Granulated Sugar	[7]

	340/476	DMSO/PBS =1/9, pH=7.4	70nM	White/Red Wine, Beer, Mineral Water	[8]
	340/498 340/371	CH_3CN/HEPES=8/2, pH=7.0	8.16nM	Crustal/Granulated Sugar	[9]
	340,440/398,535	DMSO/PBS =3/7, pH=7.4	12nM	Granulated/Crystal Sugar	[10]

1 X. Feng, T. Zhang, J. Liu, J. Miao, B. Zhao, *Chem. Commun.,* 2016, **52**, 3131-3134.
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