Supporting Information

Molecular acceptor engineering to design precisely NIR type I photosensitizer for efficient PDT-based synergistic therapy

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1 Experimental

1.1 Materials and measurements

All solvents and reagents used in this work were analytically pure. Phenothiazine (PHE), 1-bromohexane, triphenylamine borate, 1,3-indanedione, and malononitrile were purchased from Aladdin. Biochemical reagents containing dihydrorhodamine 123 (DHR123), and vitamin C were purchased from Sigma-Aldrich. ROS indicators 9,10-anthracenediyl-bis(methylene)-dipropanedioic acid (ABDA), 2,7-dichlorodihydroluciferin diacetate (H_2DCF-DA), and hydroxyphenylfluorescein (HPF) were supplied by Aladdin. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV 500 spectrometer at room temperature. UV-visible absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer. PL spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. Fluorescence quantum yields were measured using a Hamamatsu Absolute PL Quantum Yield Spectrometer C11347 Quantaurus_QY. High resolution mass spectrometry (MS) data were performed using the LTQ Orbit rap XL in the instrument. Confocal laser scanning microscopy (CLSM) images were performed on an Olympus FV1000-IX81 confocal laser scanning microscope. All solvents and reagents used in this work were analytically pure.

1.2 Synthesis and characterization

The target products were prepared by nucleophilic addition mechanism as shown in **Figure S1**, C6PHE-CHO-TPA was synthesized in good yields by one-pot reaction with 1,3 indandione（IN）, 3-(Dicyanomethylene) inden-1-one (IC), 1,3-Bis(dicyanomethylene) indan (ID) respectively. Spectroscopic techniques (e.g. ^{1}H/^{13}C NMR) were used to fully characterize the purified intermediates and the target products and to propose the correct chemical structures. Detailed data were provided in the supporting information (**Figure S9-20**).

1.2.1 Preparation of IC

1,3-Indandione (1.75 g, 12 mmol), malononitrile (792 mg, 12 mmol) and sodium acetate (1.18 g, 14.4 mmol) were dissolved in ethanol (40 mL). The reaction was stirred at room temperature for 24 h. The reaction solution was plunged into cold water and the product was obtained by filtration. The crude product was purified by fast column chromatography (silica gel), eluting with petroleum ether/dichloroethane (1:1 by volume) to give the product as a purple solid. Yield: 75%.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.94 (s, 1H), 7.42 (s, 1H), 5.43 (s, 1H), 4.22 (s, 2H).

1.2.2 Preparation of ID

1,3-Indandione (1.75 g, 12 mmol), malononitrile (1.98 g, 30 mmol) and sodium acetate (2.95 g, 36 mmol) were dissolved in ethanol (40 mL). The reaction was stirred at reflux for 24 h. The reaction solution was plunged into cold water and the product was filtered. The crude product was purified by fast column chromatography (silica gel), eluting with petroleum ether/dichloroethane (1:1 by volume) to give the product as a purple solid. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.38 (s, 1H), 5.67 (s, 1H), 4.93 (s, 1H), 2.48 (s, 2H).

1.2.3 Preparation of C6PHE

Phenothiazine (10 g, 50 mmol) and bromohexane (9.84 g, 60 mmol) and sodium hydride (20 g, 100 mmol) were dissolved in DMF (60 mL) and the reaction was allowed to proceed for 24 hours at 25 degrees Celsius under ambient stirring, and then the reaction was terminated by spotting the plate tracer, and the raw materials basically disappeared. The reaction was terminated by adding a large amount of water, extracting with dichloromethane (100 mL) and washing with water for 6 times. Finally purified by column. Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.04 (m, 4H), 6.96 (t, J = 7.5 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 3.89 (t, J = 7.1 Hz, 2H), 1.92-1.86 (m, 1H), 1.84 (d, J = 7.6 Hz, 1H), 1.49 (dd, J = 14.1, 6.7 Hz, 2H), 1.37 (dd, J = 8.0, 5.4 Hz, 4H), 0.96 (t, J = 6.4 Hz, 3H).

1.2.4 Preparation of C6PHE-CHO

N, N-Dimethylformamide (2.6 mL, 6.7 mmol) and 1,2-dichloroethane (8 mL) were first stirred at 0 °C for 10 min. $POCl_3$ (2.5 mL, 5 mmol) was then injected into the reaction vial and C6PHE (5.7 g, 20 mmol) was dissolved in 1,2-dichloroethane (8 mL) and subsequently injected into the reaction vial and refluxed for 12 hours. The product was fluorescent and the reaction was followed by spot plate. At the end of the reaction, aqueous sodium bicarbonate was added to neutralise the acidic gases in the reaction solution (there were bubbles, so be careful to pour slowly). Extract with dichloromethane and purify by passing through a column. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (s, 1H), 7.61 (dt, J = 8.6, 2.2 Hz, 1H), 7.55 (t, J = 2.1 Hz, 1H), 7.24 (d, J = 2.3 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 7.7 Hz, 1H), 6.94 (d, J = 7.7 Hz, 1H), 6.85 (d, J = 7.1 Hz, 1H), 3.86 (t, J = 7.0 Hz, 2H), 1.82 – 1.60 (m, 2H), 1.42 (q, J = 7.6 Hz, 2H), 1.33 – 1.19 (m, 4H), 0.85 (q, J = 4.7 Hz, 3H).

1.2.5 Preparation of C6PHE-CHO-Br

C6PHE-CHO (1.8 g, 5 mmol) was dissolved in DMF (30 ml) under ice cooling and N-Bromosuccinimide, abbreviated as NBS (3.5 g, 20 mmol) was dissolved in DMF (20 mL) and added

dropwise to the reactor slowly, then returned to room temperature and reacted for 2 hours. The reaction was then followed by spotting the plate. At the end of the reaction, the DMF was extracted with water several times to remove the DMF and finally purified by passing through a column. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.57 (s, 1H), 7.23 (d, J = 2.0 Hz, 1H), 7.11 (s, 1H), 6.90 (d, J = 8.3 Hz, 1H), 6.71 (d, J = 8.6 Hz, 1H), 3.98 – 3.67 (m, 2H), 1.77 (d, J = 7.4 Hz, 2H), 1.40 (d, J = 16.8 Hz, 2H), 1.27 (d, J = 16.2 Hz, 4H), 0.86 (d, J = 7.0 Hz, 3H).

1.2.6 Preparation of C6PHE-CHO-TPA

C6PHE-CHO-Br (3.89 g, 10 mmol) was reacted with triphenylamine boronic acid (3.47 g, 12 mmol), palladium tetrakis (triphenylphosphine) (231 mg, 0.2 mmol), and potassium carbonate (2.4 g) under nitrogen. Tetrahydrofuran (36 mL) and water (12 mL) were injected into the reaction vial and the reaction refluxed overnight. The plate was spotted, the raw material disappeared and purified by passing through the column. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 7.87 – 7.48 (m, 3H), 7.36 (dd, J = 17.5, 7.7 Hz, 3H), 7.32 – 7.16 (m, 5H), 7.15 – 7.06 (m, 4H), 7.03 (t, J = 7.3 Hz, 2H), 6.90 (d, J = 8.5 Hz, 3H), 3.90 (t, J = 7.1 Hz, 2H), 1.92 – 1.76 (m, 2H), 1.55 (s, 2H), 1.33 – 1.16 (m, 4H), 0.90 – 0.80 (m, 3H).

1.2.7 Preparation of TP1

C6PHE-CHO-TPA (554 mg, 1 mmol) and 1,3 indandione (175 mg, 1.2 mmol) were dissolved in chloroform (20 mL) and pyridine (0.5 mL) was added. After the reaction is finished, the mixture was then extracted with water and dichloromethane (4 × 15 mL). The received organic phase was then dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by fast column chromatography (silica gel), eluting with petroleum ether/ethyl acetate (2:1 by volume) to give the product as a black solid (360 mg, 80% yield). Yield: 75%. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.50 (s, 1H), 8.25 (d, J = 8.5 Hz, 1H), 7.89 (d, J = 15.2 Hz, 3H), 7.62 (s, 2H), 7.53 (d, J = 7.3 Hz, 2H), 7.42 (d, J = 7.6 Hz, 1H), 7.37 (s, 1H), 7.33 – 7.19 (m, 4H), 7.01 (td, J = 18.2, 16.0, 8.0 Hz, 9H), 3.92 (s, 2H), 1.68 (s, 2H), 1.34 (d, J = 28.3 Hz, 3H), 1.23 (s, 4H), 0.80 (s, 3H), ^{13}C NMR (101 MHz, DMSO-d6) δ 191.36, 158.77, 152.41, 148.30, 147.59, 145.68, 139.25, 131.76, 130.90, 130.84, 130.67, 130.51, 130.20, 128.89, 127.55, 127.45, 127.16, 126.49, 125.30, 124.91, 123.16, 120.83, 118.94, 116.37, 108.29.

1.2.8 Preparation of TP2

C6PHE-CHO-TPA (554 mg, 1 mmol) and IC (233 mg, 1.2 mmol) were dissolved in chloroform (20 mL) and pyridine (0.5 mL) was added. After the reaction is finished, the mixture was then extracted with water and dichloromethane (4 × 15 mL). After the reaction is finished, the mixture was then extracted with water and dichloromethane (3 × 30 mL). The received organic phase was then dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by fast column chromatography (silica gel), eluting with petroleum ether/ethyl acetate (2:1 by volume) to give the product as a black solid (360 mg, 80% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.45 (d, J = 8.0 Hz, 1H), 8.31 (s, 1H), 8.26 – 8.17 (m, 1H), 8.05 (s, 1H), 7.92 (dt, J = 13.5, 7.7 Hz, 1H), 7.81 (d, J = 30.8 Hz, 1H), 7.75 – 7.64 (m, 1H), 7.63 – 7.47 (m, 3H), 7.47 – 7.42 (m, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.29 (t, J = 7.6 Hz, 4H), 7.10 (dd, J = 19.5, 8.8 Hz, 2H), 7.05 – 6.88 (m, 6H), 6.85 (s, 1H), 4.00 – 3.86 (m, 2H), 1.66 (q, J = 7.4 Hz, 2H), 1.35 (d, J = 20.2 Hz, 2H), 1.25 – 1.19 (m, 4H), 0.83 – 0.75 (m, 3H), ^{13}C NMR (101 MHz, DMSO-d6) δ 190.98, 150.16, 147.44, 147.00, 135.37, 131.21, 130.03, 127.49, 124.93, 124.59, 124.54, 123.71, 117.20, 31.20, 30.79, 26.48, 26.10, 22.47, 14.25.

1.2.9 Preparation of TP3

A mixture of compound 4 (554 mg, 1 mmol) and ID (291 mg, 1.2 mmol) was placed in a 50 mL round-bottomed flask having acetic anhydride (10 mL) and it was stirred for more than 24 hours at 90°C under N_2. After cooling to room temperature, 20 mL of water was added to the reaction and stirred for 2 hours. The mixture was then extracted with water and dichloromethane (4 × 15 mL). The received organic phase was then dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by fast column chromatography (silica gel), eluting with petroleum ether/ethyl acetate (1:1 by volume) to give the product as a black solid (360 mg, 80% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.40 (s, 1H), 8.20 (s, 1H), 7.97 (s, 1H), 7.87 (q, J = 2.9, 2.5 Hz, 4H), 7.83 – 7.74 (m, 1H), 7.70 (dd, J = 17.1, 5.8 Hz, 1H), 7.58 (s, 1H), 7.50 (d, J = 8.3 Hz, 1H), 7.37 (dq, J = 7.1, 4.5, 3.8 Hz, 6H), 7.27 (t, J = 8.2 Hz, 2H), 7.17 (d, J = 14.7 Hz, 1H), 6.99 (td, J = 14.6, 14.0, 6.4 Hz, 4H), 5.66 (d, J = 2.5 Hz, 1H), 3.59 (d, J = 19.2 Hz, 2H), 1.61 (d, J = 10.3 Hz, 2H), 1.39 (d, J = 32.5 Hz, 2H), 1.19 (d, J = 16.1 Hz, 4H), 0.81 (dd, J = 17.2, 7.8 Hz, 3H), ^{13}C NMR (101 MHz, DMSO-d6) δ 158.57, 138.27, 136.63, 130.52, 129.99, 128.02, 127.44, 124.67, 124.49, 123.77, 123.61, 121.98, 121.22, 118.35, 118.26, 103.18, 50.81, 22.49.

1.2.10 Preparation of TP1 and TP3 NPs

Firstly, the photosensitizer (1 mg) and DSPE-PEG_{2000} (1,2-distearoyl-sn-glycero-3-phospho ethanolamine-N-[methoxy(polyethylene glycol)-2000]) (2 mg) were completely dissolved in THF (1 mL), and then a centrifuge tube with 10 mL of deionized water was prepared and placed in an ultrasonic machine (maximum power) to sonicate for 1 min, and then the THF solution from the dissolved samples was slowly added to 10 mL of deionized water and sonicated for 10 min in a sonicated state, and finally the mixed solution was placed on a stirrer for 24 h. The THF solution was removed to obtain an aqueous solution of NPs[32, 33].

1.3 ROS detection

Measurement of ROS produced by 6 μL TP1-3 (1 μM) and 750 μL H_2DCF-DA (10 μM) were added to a cuvette containing DMSO (2245 μL) or H_2O (2245 μL), respectively. Then, the cuvette was irradiated with a white light source (50 mW/cm^2) for different times and the PL spectra were measured from 500 to 650 nm.

1.3.1 Singlet oxygen detection

The $^1O_2$ produced by TP1-3 after light triggering was measured using a $^1O_2$ trapping agent (9,10-anthracenediyl-bis(methylene)-dipropanedioic acid, ABDA). PS (5 μM) and ABDA (10 μM) were added to a cuvette containing PBS solution (2 mL). The cuvette was then irradiated with a white light source (50 mW/cm^2) for different times and absorption spectra were collected from 320 to 420 nm.

2.2.10 Superoxide anion radical detection

Herein, superoxide anion radicals ($O_2^{\bullet-}$) produced by TP1-3 were detected by combining dihydrorhodamine 123 (DHR 123). First, 3μL PSs (1 μM) and 3μL DHR123 (1 μM) were added to a cuvette in the mixture of DMSO (0.7 mg/mL) / H_2O (3 mL). Then, the cuvette was irradiated with a white light source (50 mW/cm^2) for different times and the PL spectra were measured from 500 to 650 nm.

1.3.2 hydroxyl radical detection

The ·OH produced by TP1-3 after light triggering was measured using a ·OH trapping agent (3'-p-(Hydroxyphenyl) fluorescein, HPF). 3μL PS (2 μM) and 3μL HPF (1 μM) were added to a cuvette containing H_2O (3 mL). The cuvette was then irradiated with a white light source (50 mW/cm^2) for different times and absorption spectra were collected from 500 to 650 nm.

1.4 Cell imaging

4T1 cells were cultured at 37°C in a humidified environment containing 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum. TP1 and TP3 NPs (20 μg/ml) solution was cultured with 4T1 cells for 8 hours, then the residual PSs were washed with PBS to prepare for further cell imaging.

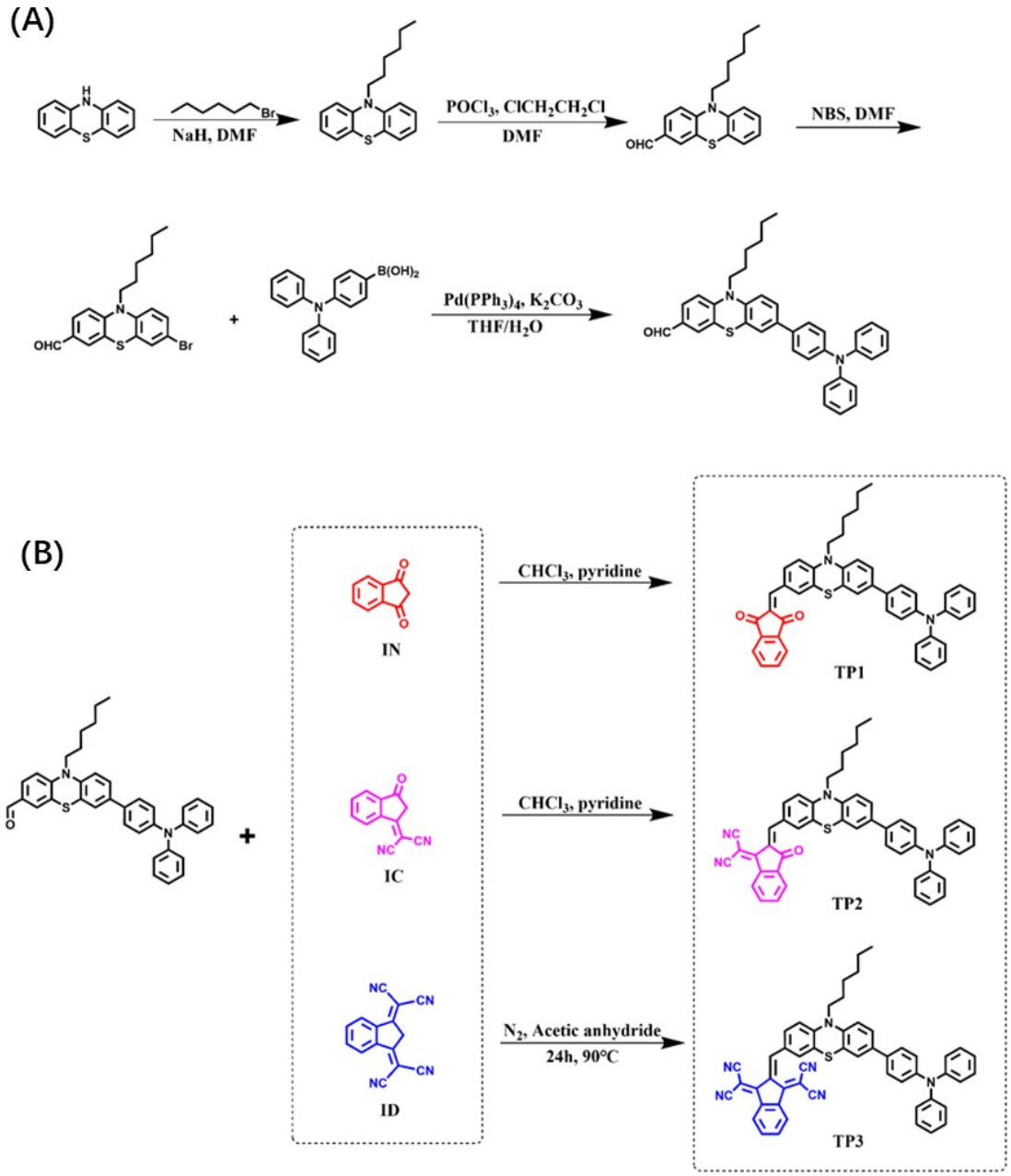
1.5 Cell viability

We tested the cytotoxicity of TP1 and TP3 NPs using 3-(4, 5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT). The cells were cultured in an incubator at room temperature at 2% oxygen and 5% carbon dioxide to simulate a hypoxic environment, and the oxygen content was monitored with an oxygen detector (Nuvair, O_2 Qucikstick). The 4T1 cells were first inoculated into DMEM in 96-well plates at a density of 1 × 104 cells per well and incubated for 24 h. The medium was then replaced with different concentrations of PS (0, 2, 4, 6, 8, and 10 μM) and the cells were incubated for another 24 h. The cells were then incubated with PS (0, 2, 4, 6, 8, and 10 μM). After incubation, the medium was removed and 100 μL of new medium containing MTT (0.5 mg/mL) was added to each well and incubated for another 4 h. The cells were then incubated for 24 h with different concentrations of TP1 and TP3 NPs (0, 2, 4, 6, 8, and 10 μM). The medium was then discarded, and 100 μL of DMSO was added to each well. The OD490 value (Abs.) of each well was immediately determined by an enzyme marker. The viability of the cells incubated with TP1 and TP3 NPs suspensions was measured by comparing the OD490 value of the cells incubated with medium alone with the OD490 value of the cells incubated with TP1 and TP3 NPs suspensions.

1.6 Live-dead cell staining

After 4T1 cells were incubated with or without NPs for 6 h, then cells were washed with PBS twice. After that, these cells were treated upon white laser (30 mW/cm^2) irradiation for 10 min. 18 h later, the cells were then incubated with Calcein-AM (2 μM) and Homodimer-1 (4 μM) for 30 min. After washing, the cells were imaged by a confocal microscope.

2 Results



Scheme S1 Synthetic routes of donor (**A**) and target molecule (**B**).

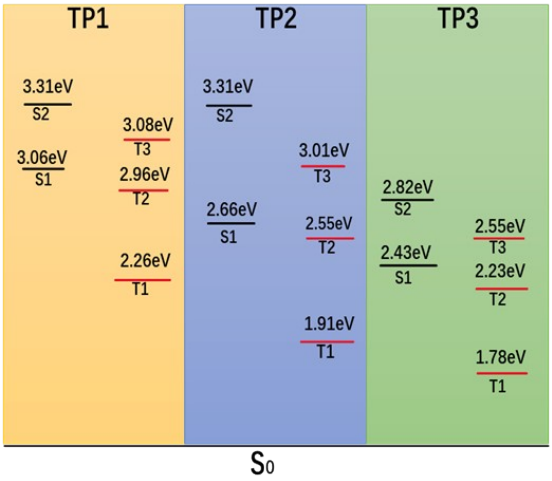


Fig. S1 Theoretical calculations of excited-state energy levels for TP1, TP2, and TP3 at the M062X/6-31G (d, p) level. TP1 (S1=3.06 eV, S2=3.31 eV, T1=2.26 eV, T2=2.96 eV, T3=3.08 eV), TP2 (S1=2.66 eV, S2=3.31 eV, T1=1.91 eV, T2=2.55 eV, T3=3.01 eV), TP3 (S1=2.43 eV, S2=2.82 eV, T1=1.78 eV, T2=2.23 eV, T3=2.55 eV).

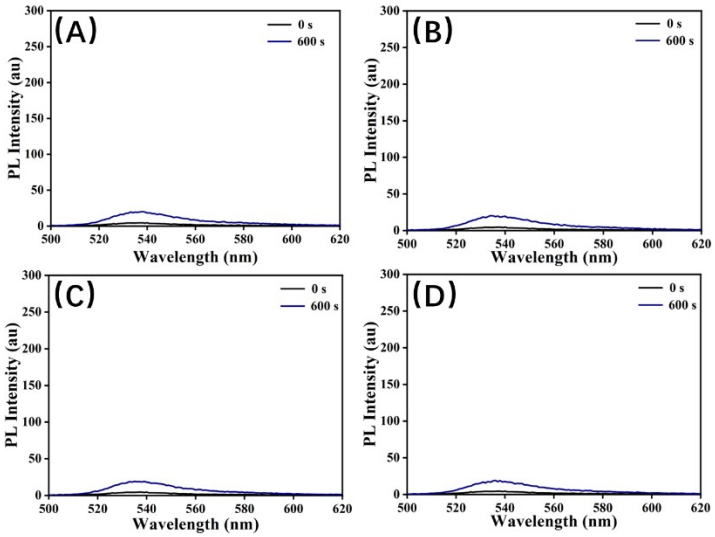


Fig. S2 PL spectra of (A) Blank, (B) TP1, (C) TP2 and (D) TP3 during 0-600s, respectively. [PS = 6 μl, H_2DCF-DA = 750 μl, DMSO = 2245 μl, [PS] = 1 μM, [DCFH] = 10 μM, slit: 1.5, 1.0]; white light irradiation (50 mW/cm^2).

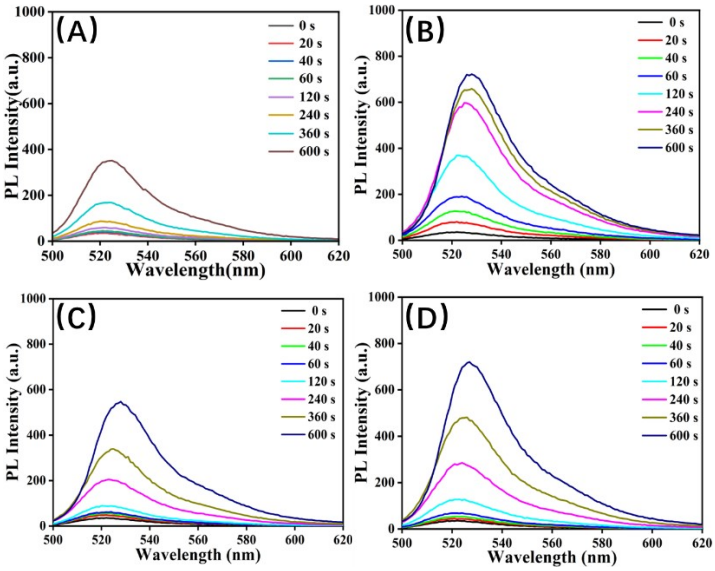


Fig. S3 PL spectra of (**A**) Blank, (**B**) TP1, (**C**) TP2 and (**D**) TP3 during 0-600 s, respectively. [PS = 6 μl, H_2DCF-DA = 750 μl, H_2O = 2245 μl, [PS] = 1 μM, [DCFH] = 10 μM, slit: 1.5, 1.0]; white light irradiation (50 mW/cm^2).

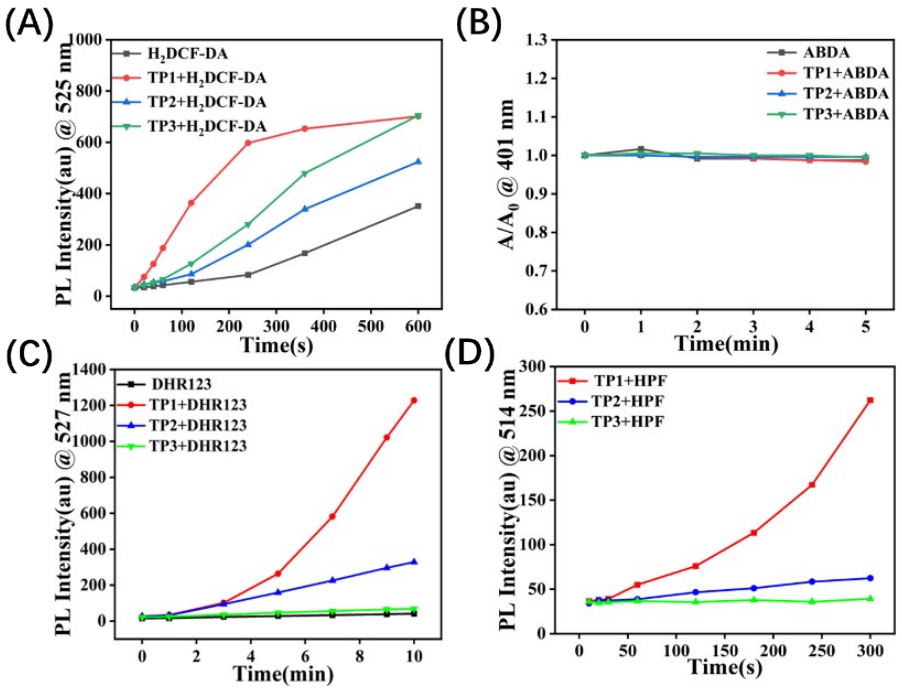


Fig. S4 (**A**) ROS generation by the three PSs (1 μM) at 525 nm versus the irradiation time upon white light irradiation using H_2DCF-DA (750 μl, 10 μM) as an indicator in H_2O (2245 μl); (**B**) Absorbance intensity of ABDA (10 μM) solution at 401 nm containing three PSs (5 μM) versus the irradiation time; (**C**) Fluorescence intensity of DHR123 (1 μM) solution at 527 nm containing three PSs (1 μM) versus the irradiation time; (**D**) Fluorescence intensity of a HPF (1 μM) solution at 514 nm containing three PSs (2 μM) versus the irradiation time; White light: 50 mW cm^{-2}.

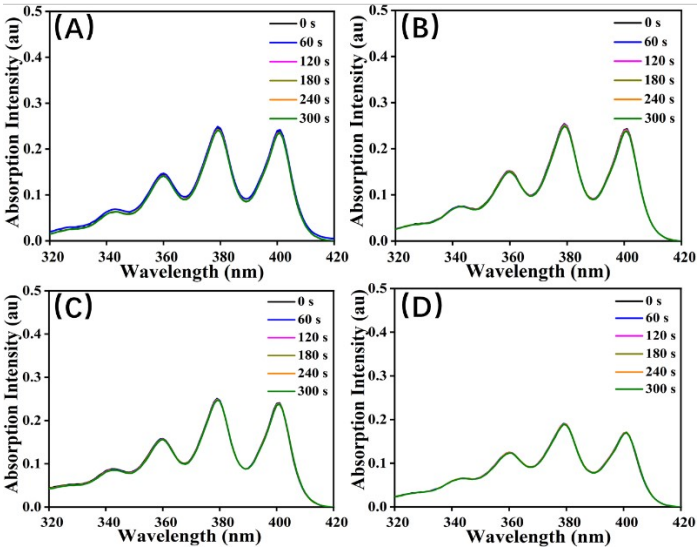


Fig. S5 The UV-visible absorption spectra of (**A**) Blank, (**B**) TP1, (**C**) TP2 and (**D**) TP3 at the initial time of 0-300 s, respectively. [PS] = 5 μM, [ABDA] = 10 μM.

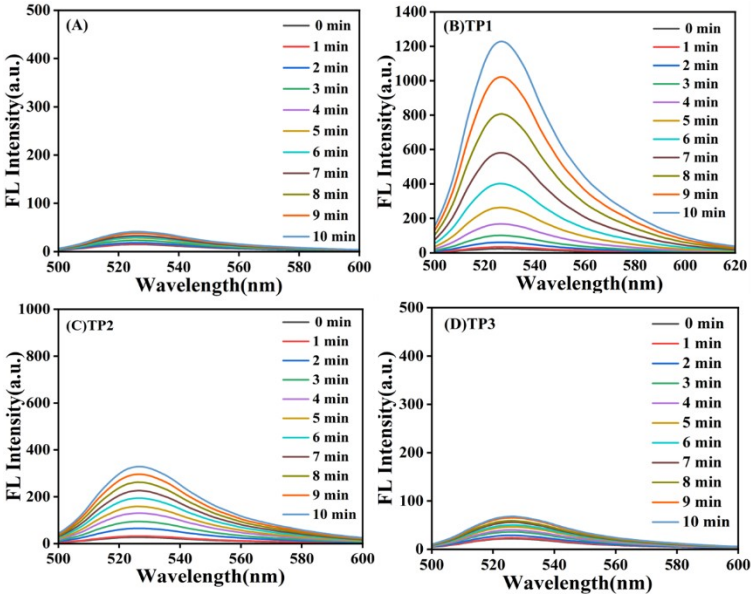


Fig. S6 PL spectra of (**A**) Blank, (**B**) TP1, (**C**) TP2, (**D**) TP3 during 0-10 min, respectively. 15 μl (**B**) TP1, (**C**) TP2, (**D**) TP3 in the mixture of DHR123 (15 μl)/PBS (2970 μl), respectively. [Excitation wavelength: 480 nm, Emission wavelength: 500-650 nm, PMT:400V, Excitation bandwidth: 10 nm, Emission bandwidth: 10 nm. [PS] =1μM, [DHR123] = 1μM; white light irradiation (50 mW cm^{-2}).

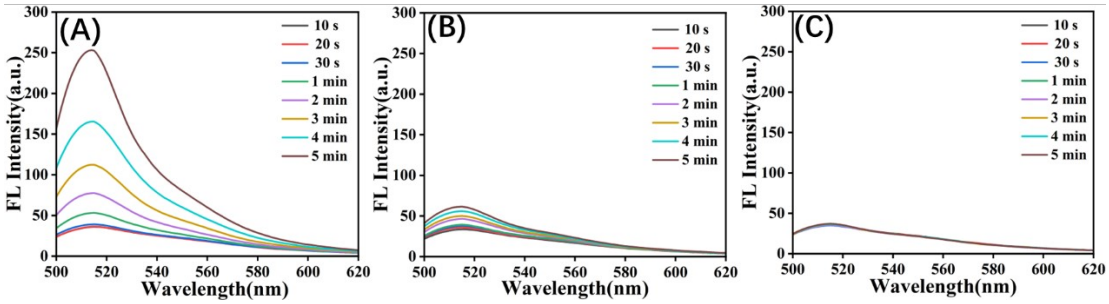


Fig. S7 PL spectra of (**A**) TP1, (**B**) TP2, (**C**) TP3 during 10 s-5 min, respectively. 3 μl (**A**) TP1, (**B**) TP2, (**C**) TP3 in the mixture of HPF (3 μl)/H_2O (3000 μl), respectively. [Excitation wavelength: 490 nm, Emission wavelength:500-650 nm, [PS] = 2 μM, [HPF] = 1 μM, slit: 1.5, 1.0; white light irradiation (50 mW/cm^2).

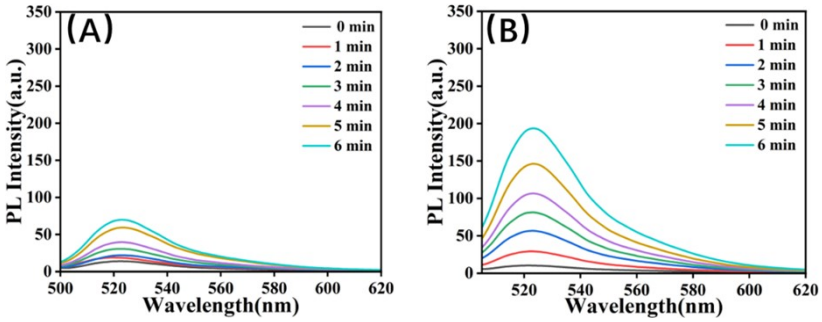


Fig. S8 PL spectra of H_2DCF-DA in the absence and presence of PS NPs under irradiation with different time in DMSO/PBS (v/v=1/99): (**A**) Blank, (**B**) TP3 NPs. Excitation wavelength: 488 nm, Emission wavelength:500-650 nm, PMT:400V, Excitation bandwidth: 10 nm, Emission bandwidth: 10 nm. [PS] =100 μg/ml, [DCFH] = 1μM; white light irradiation (50 mW cm^{-2}).

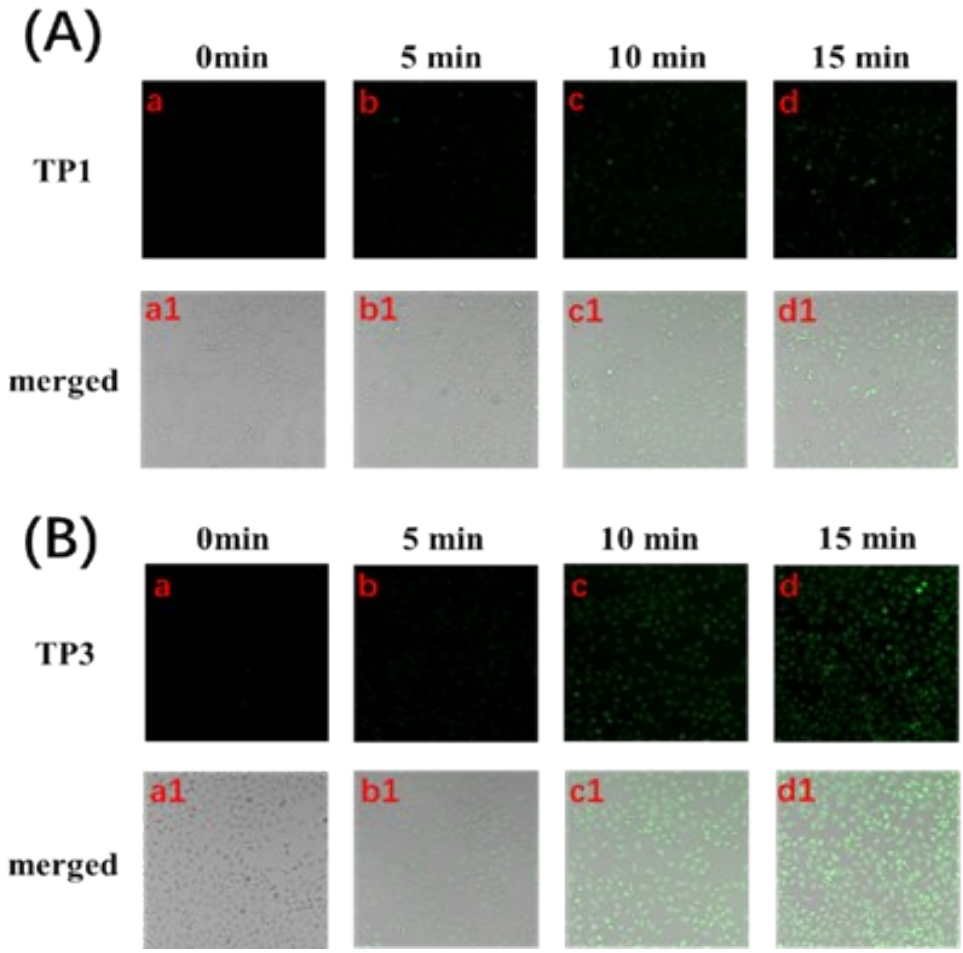


Fig. S9 CLSM observations of 4T1 cells incubated with 20 μg/mL of (**A**) TP1 NPs and (**B**) TP3 NPs for 15 min (a-d: fluorescence image and a1-d1: superimposed field image), scale bar: 100 μm.

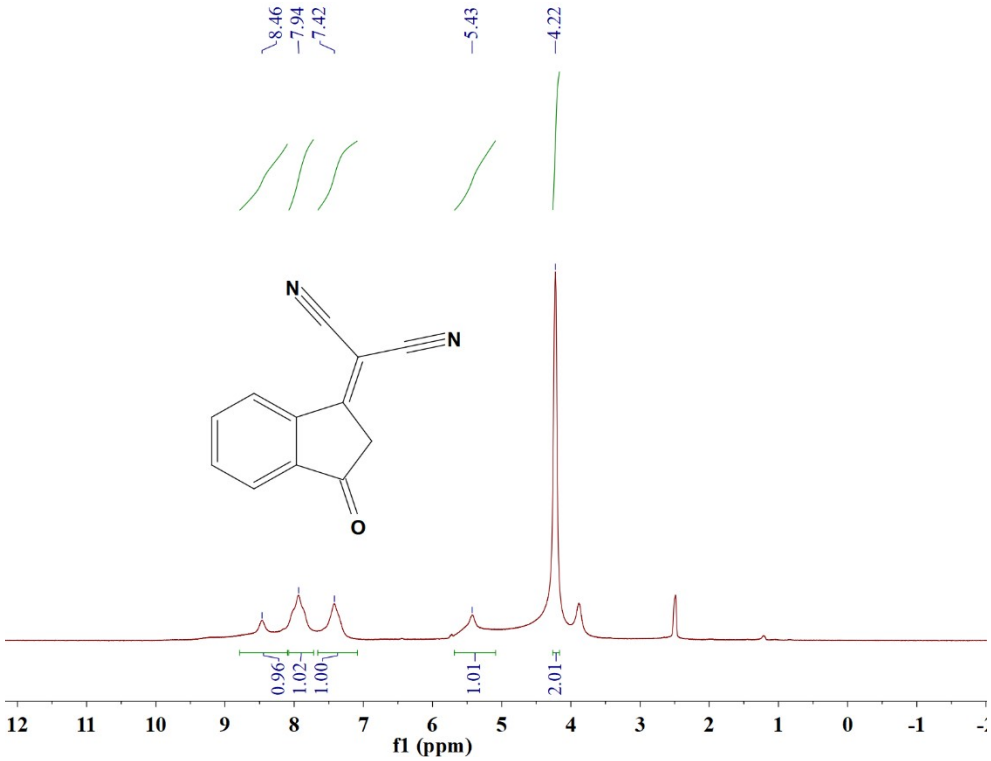


Fig. S10 ^{1}H NMR of IC.

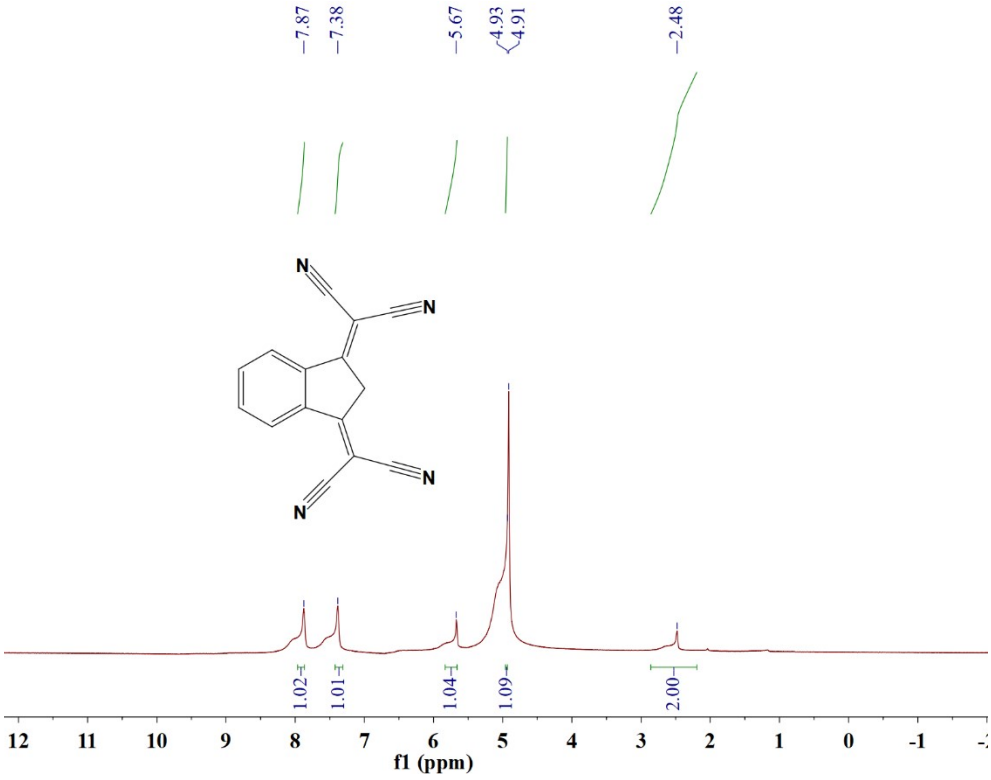


Fig. S11 ^{1}H NMR of ID.

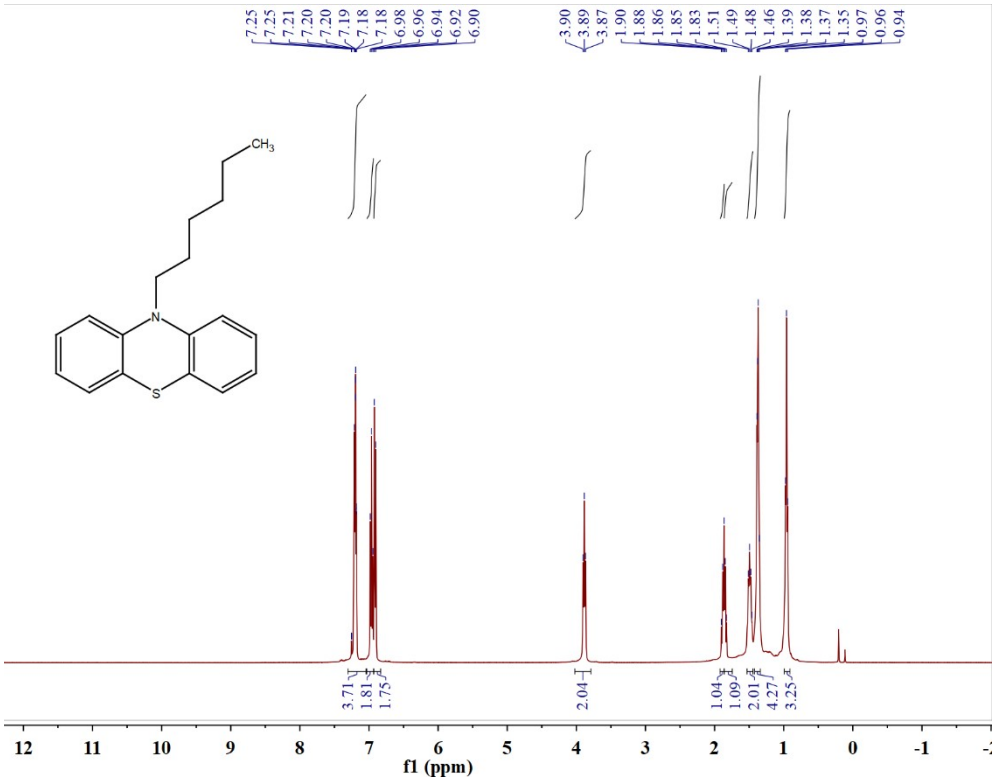


Fig. S12 [1]H NMR of C6PHE.

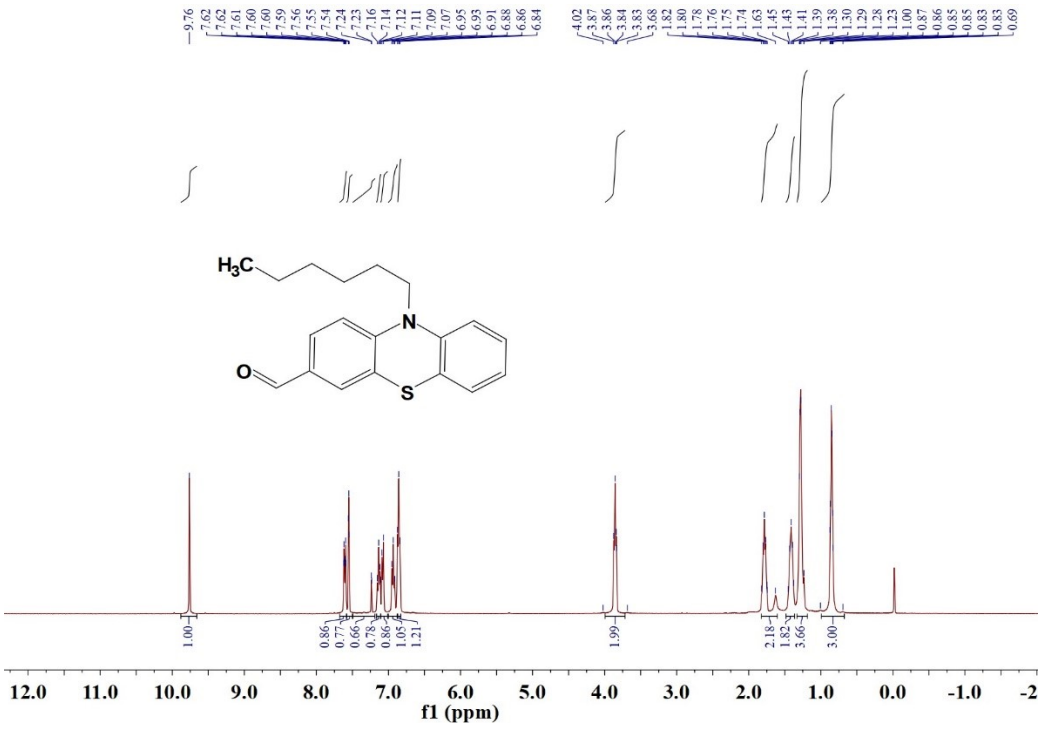


Fig. S13 [1]H NMR of C6PHE-CHO.

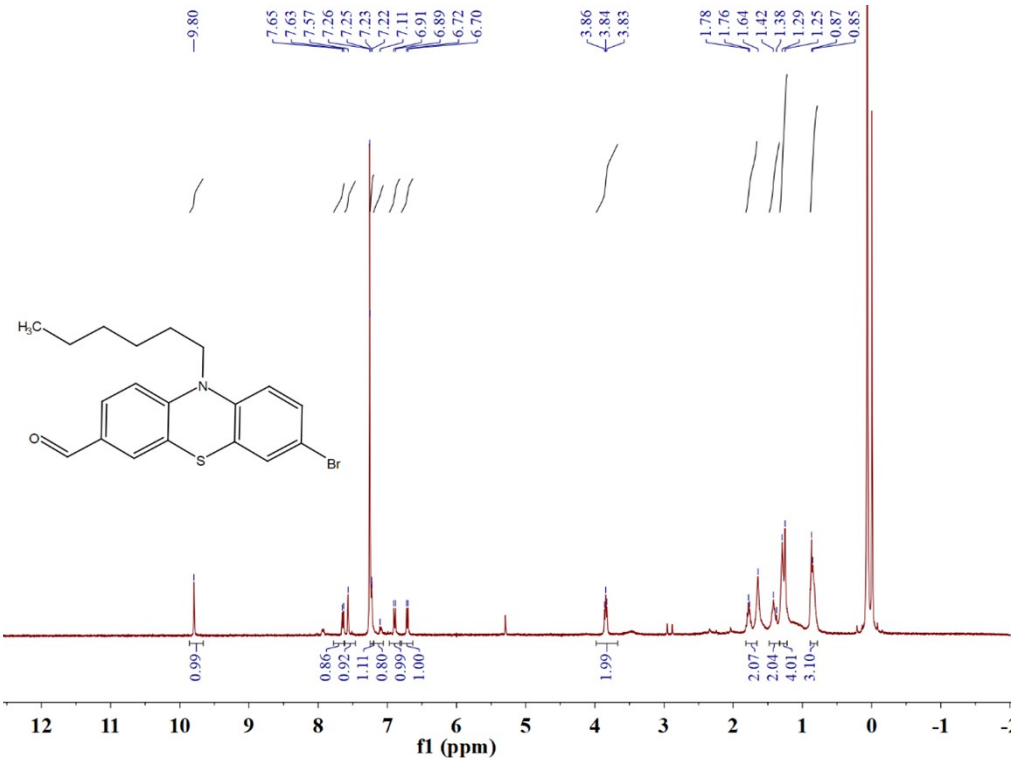


Fig. S14 [1]H NMR of C6PHE-CHO-Br.

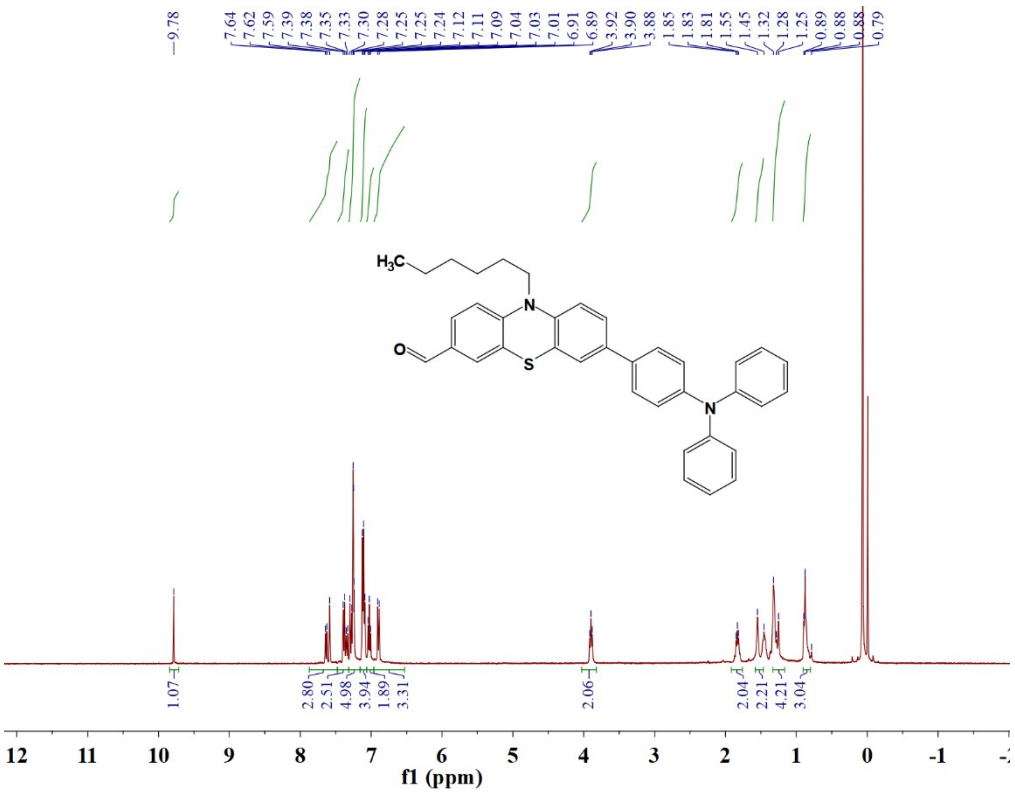


Fig. S15 [1]H NMR of C6PHE-CHO-TPA.

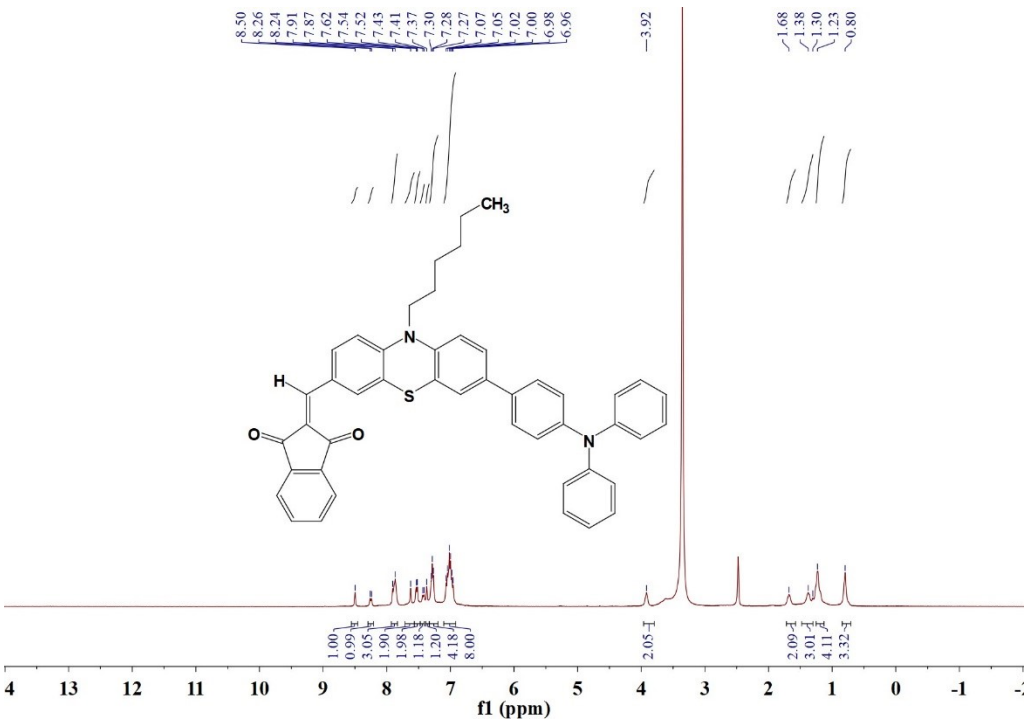


Fig. S16 ^{1}H NMR of TP1.

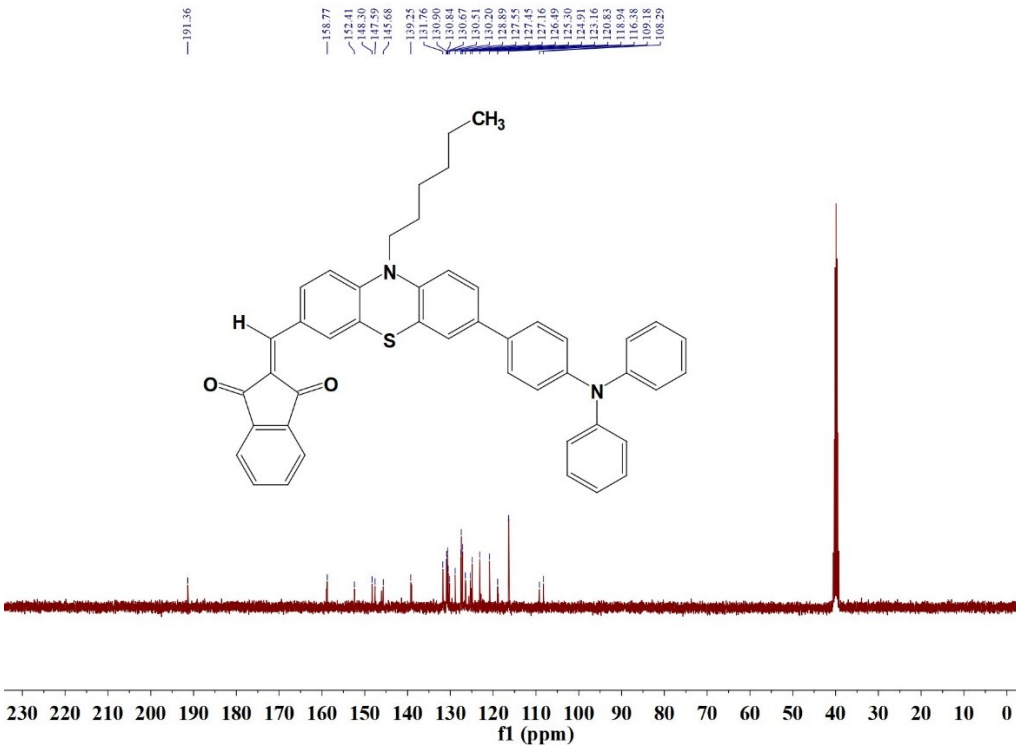


Fig. S17 ^{13}C NMR of TP1.

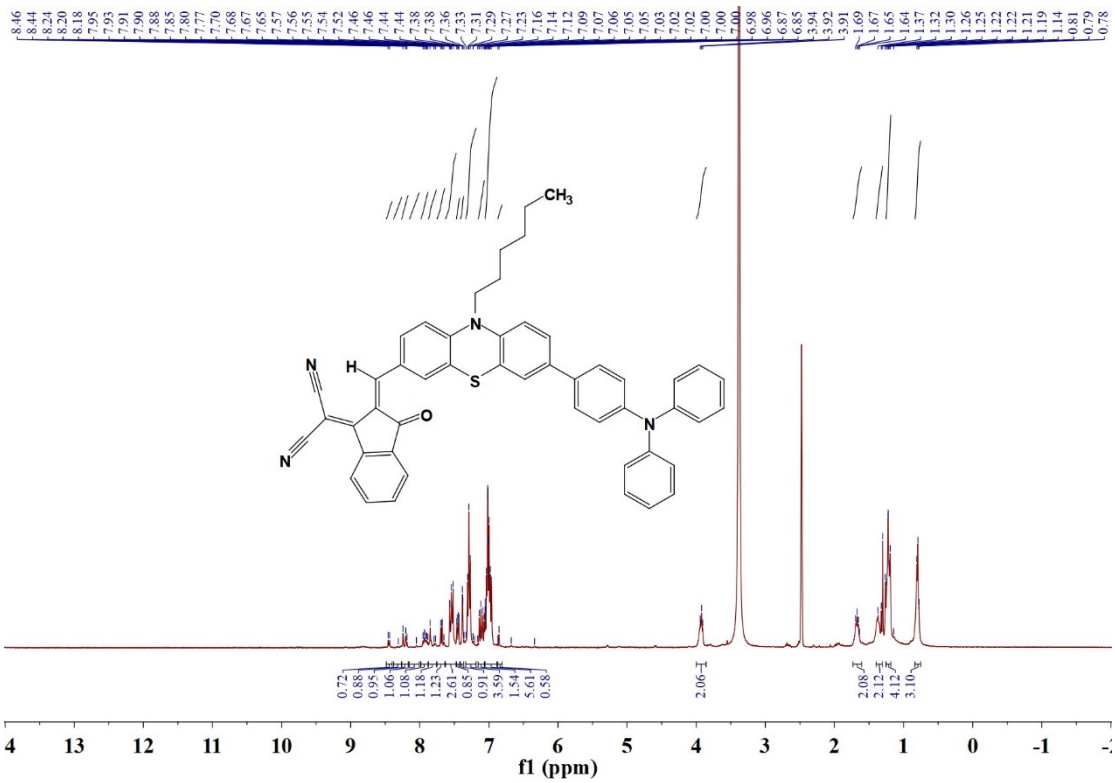


Fig. S18 ^{1}H NMR of TP2.

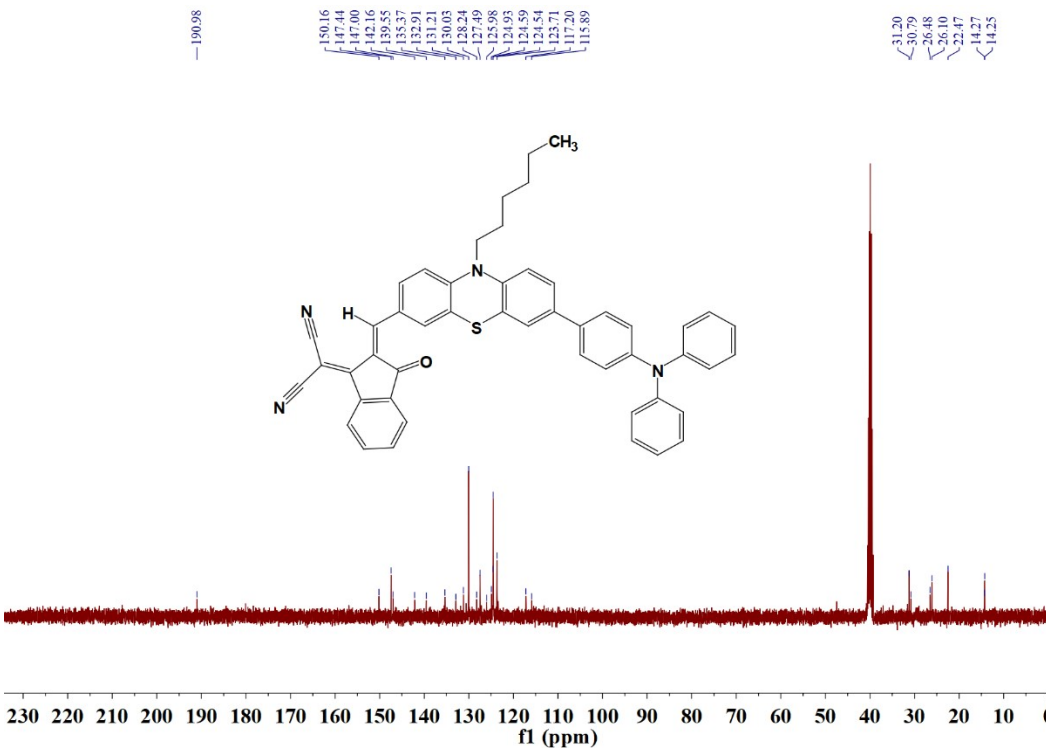


Fig. S19 ^{13}C NMR of TP2.

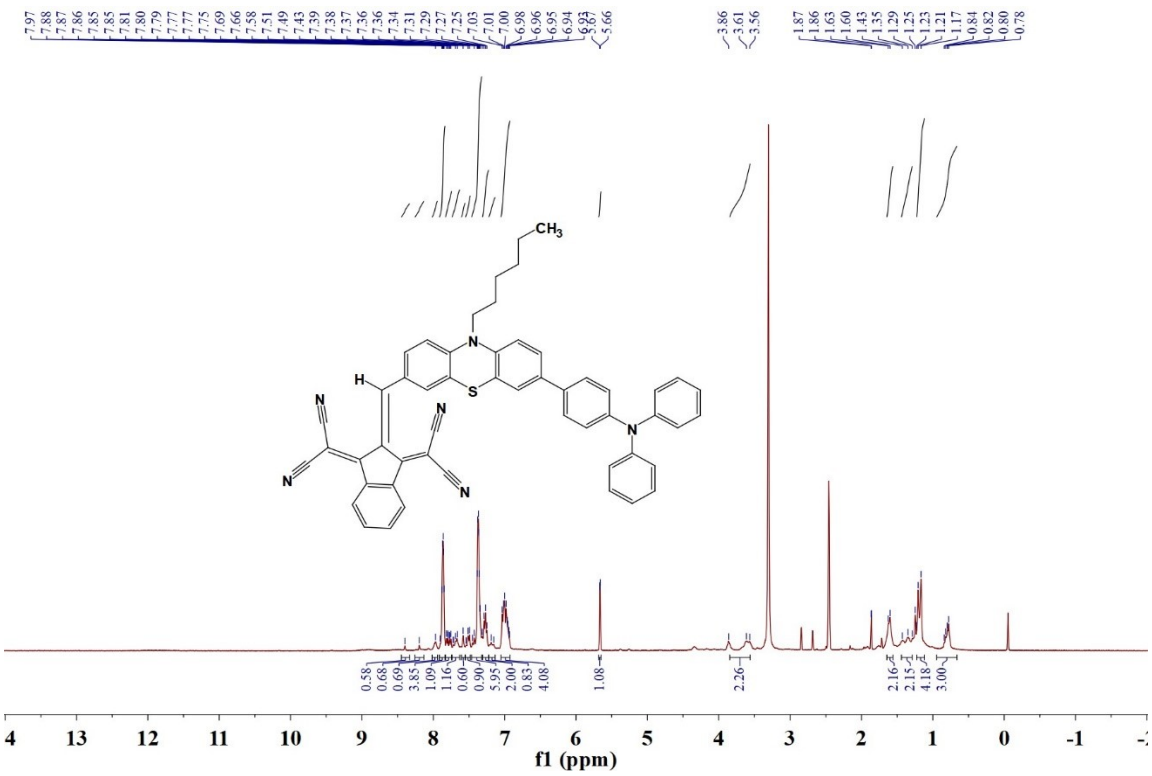


Fig. S20 ^{1}H NMR of TP3.

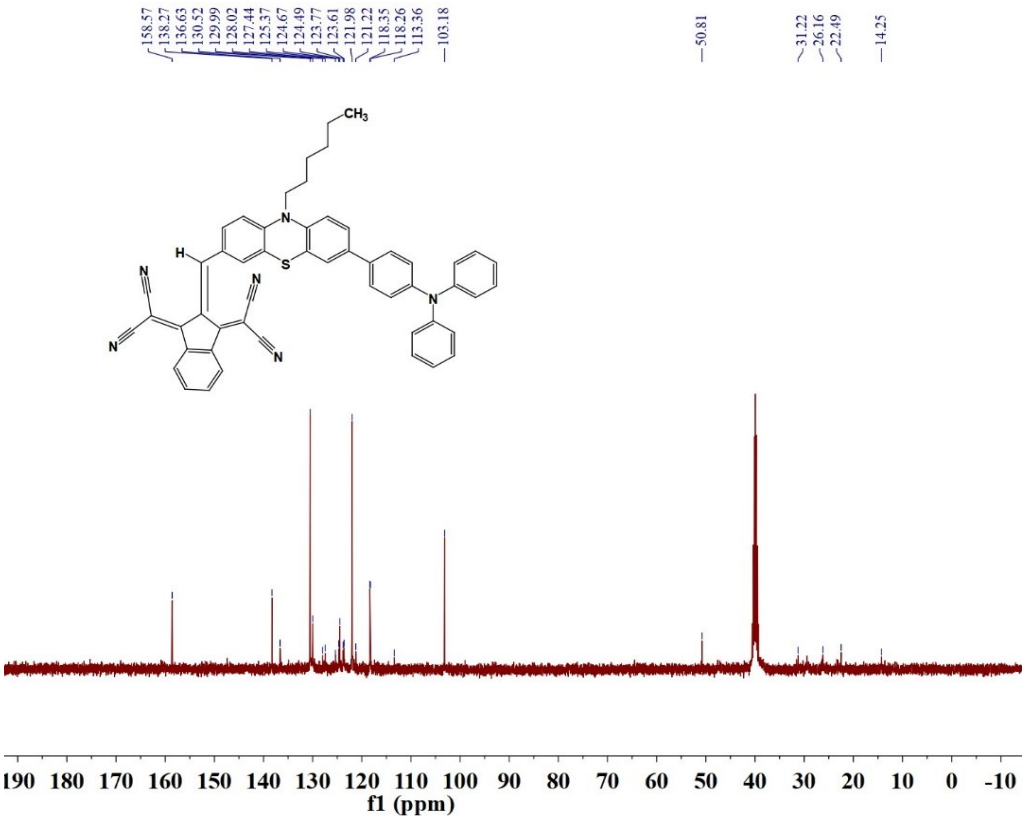


Fig. S21 ^{13}C NMR of TP3.